Seroprevalence of SARS-CoV-2 in Hong Kong and in residents evacuated from Hubei province, China: a multicohort study

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Summary

Background The role of subclinical severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections in perpetuating the COVID-19 pandemic is unknown because population seroprevalence data are absent. We aimed to establish the sensitivity and specificity of our enzyme immunoassay and microneutralisation assay, and the seroprevalence of SARS-CoV-2 in Hong Kong before and after the pandemic, as well as in Hong Kong residents evacuated from Hubei province, China.

Methods We did a multicohort study in a hospital and university in Hong Kong. We evaluated the sensitivity of our enzyme immunoassay and microneutralisation assay with RT-PCR data from patients positive for SARS-CoV-2 and the specificity of our enzyme immunoassay and microneutralisation assay with archived serum samples collected before 2019. We compared the seropositivity of the general population of Hong Kong before and after the pandemic had begun, and determined the seropositivity of Hong Kong residents evacuated from Hubei province, China, in March, 2020.

Findings Between Feb 26 and March 18, 2020, we assessed RT-PCR samples from 45 patients who had recovered from COVID-19 to establish the sensitivity of our enzyme immunoassay and microneutralisation assay. To establish the specificity of these assays, we retrieved archived serum. The sensitivity was 91·1% (41 of 45 [95% CI 78·8–97·5]) for the microneutralisation assay, 57·8% (26 of 45 [42·2–72·3]) for anti-nucleoprotein IgG, 66·7% (30 of 45 [51·1–80·0]) for anti-spike protein receptor binding domain (RBD) IgG, and 73·3% (33 of 45 [58·1–85·4]) for enzyme immunoassay (either positive for anti-nucleoprotein or anti-RBD IgG). The specificity was 100% (152 of 152 [95% CI 97·6–100·0]) for both the enzyme immunoassay and microneutralisation assay. Among the Hong Kong general population, 53 (2·7%) of 1938 were enzyme immunoassay positive, but of those who were positive, all 53 were microneutralisation negative, and no significant increase was seen in the seroprevalence between April 12, 2018, and Feb 13, 2020. Among asymptomatic Hubei returnees, 17 (4%) of 452 were seropositive with the enzyme immunoassay or the microneutralisation assay, with 15 (88%) of 17 seropositive with the microneutralisation assay, and two familial clusters were identified.

Interpretation Our serological data suggest that SARS-CoV-2 is a new emerging virus. The seropositivity rate in Hubei returnees indicates that RT-PCR-confirmed patients only represent a small proportion of the total number of cases. The low seroprevalence suggests that most of the Hong Kong and Hubei population remain susceptible to COVID-19. Future waves of the outbreak are inevitable without a vaccine or antiviral prophylaxis. The role of age-related cross reactive non-neutralising antibodies in the pathogenesis of COVID-19 warrants further investigation.

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Introduction

COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first reported in Wuhan, Hubei province, China, in December, 2019.[1,2] The first case in Hong Kong was reported on Jan 22, 2020. Due to the rapidly progressing epidemic, the Chinese Government banned all travel to and from Wuhan on Jan 23, 2020, and soon extended the travel ban to the entire province of Hubei.[3] Although the epidemic subsided after control measures were taken in China, SARS-CoV-2 continues to spread globally with more than 5 million laboratory-confirmed cases. An analysis of more than 70 000 patients showed that most patients (87%) were aged between 30 and 79 years, and only 2% of patients were younger than 20 years.[4] The overall case fatality rate was 2·3%, but was much higher among patients aged 70 years or older and those with underlying health conditions.[4] Most patients present with respiratory symptoms, although diarrhoea has been reported in 3–10% of patients.[5]

Population serological data are essential for understanding the prevalence of subclinical infections and the

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Research in context

Evidence before this study

We searched PubMed on April 5, 2020, with no limitations by start date or language, with the terms "COVID-19", "SARS-CoV-2", "antibody", "seroprevalence", and "seroepidemiology". Our search did not retrieve any reports on seroprevalence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). We did not find other reports or evidence on the seroprevalence of COVID-19 at that time.

Added value of this study

With use of serology tests, we showed that SARS-CoV-2 was not circulating in the Hong Kong population before the COVID-19 pandemic, and there was no increase in the seroprevalence of SARS-CoV-2 from 2018 up until the second month of the COVID-19 outbreak in Hong Kong. Our Hong Kong returnees evacuated from Hubei province in March, 2020, showed a SARS-CoV-2 seropositivity rate of 4%, which indicates that a large amount of cases of subclinical COVID-19 were not detected during the epidemic period in Hubei. Children younger than 10 years had the lowest absorbance values in antibody tested by enzyme immunoassay among all age groups.

Implications of all the available evidence

Despite the high transmissibility of SARS-CoV-2, the control measures implemented in Hong Kong and Hubei province had successfully restricted the spread of SARS-CoV-2 in these areas as of May, 2020. However, the relatively low seroprevalence indicates a lack of herd immunity, suggesting that both Hong Kong and Hubei province are susceptible to a resurgence of the SARS-CoV-2 epidemic if public health measures are relaxed. Pragmatic control measures without too much disruption to society are warranted. The possibility of pre-existing, disease-enhancing, cross-reactive antibodies, which lead to worse disease severity in older age groups should be further studied.

population's herd immunity against SARS-CoV-2. Such data would affect decision making on epidemiological control measures and risk assessments of the epidemic trajectory.[6] Previous studies of influenza showed that population serology can determine the susceptibility of the population for antigenically drifted virus.[7] Population serological data can also reveal the hidden burden of infection by identifying subclinical infections.[8,9] This factor is especially important for COVID-19, because asymptomatic infections are common.[1,10,11] Furthermore, serology data for samples collected before the current outbreak can help to establish whether the virus was circulating in humans before its discovery.[12]

Previously, we have shown that enzyme immunoassay antibodies against the SARS-CoV-2 nucleoprotein and spike protein receptor binding domain (RBD) can be found in most patients within the third week after symptom onset, and that the enzyme immunoassay antibody amount correlates with the miconeutralisation assay antibody titre.[13] Here, we comprehensively evaluated our serology assays using samples from patients with COVID-19 and from the general population, and used these assays to establish the seroprevalence in Hong Kong before and after the COVID-19 pandemic, and the seroprevalence among returnees evacuated from Hubei in March, 2020.

Methods

Study design and participants

We did a multicohort study in a hospital and university in Hong Kong. For assessment of specificity, we retrieved serum samples from live poultry market and slaughterhouse workers from Hong Kong that were collected during a seroprevalence study for avian influenza viruses in 2013 and 2014,[8] and serum samples collected between 2016 and 2018 from potential organ donors for a study on hepatitis E in Hong Kong. For assessment of sensitivity, we collected convalescent serum samples from patients with laboratory-confirmed COVID-19 at Queen Mary Hospital (Hong Kong). Patients with COVID-19 were diagnosed by real-time RT-PCR targeting the SARS-CoV-2 E gene or the RNA-dependent-RNA-polymerase-helicase gene region, as described previously.[13] Serum samples from Hong Kong residents who were evacuated from Hubei on March 4–5, 2020, were also included.

The study was approved by the institutional review board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (UW 18-141, UW 13-265, UW 13-372, and UW 18-074). Written informed consent was obtained from patients with COVID-19. The analysis of Hubei returnees and suspected COVID-19 cases in January, 2020, were part of the public health response and were considered exempt from institutional review board approval.

Procedures

Our enzyme immunoassay and microneutralisation assay are described in detail in the appendix (pp 1–2). To set the cutoff for the enzyme immunoassay, we retrieved archived anonymous serum samples from the clinical biochemistry laboratory of Queen Mary Hospital collected between April 12 and July 3, 2018, which were used in our previous study.[7] To establish potential cross-reactivity, we retrieved serum samples from patients suspected to have COVID-19 based on clinical and epidemiological criteria as outlined by the hospital authority of Hong Kong between Jan 4 and Jan 28, 2020, but negative for SARS-CoV-2 by RT-PCR, as well as archived serum samples from patients infected with SARS during the 2003 outbreak.

For the seroprevalence study, we retrieved archived anonymous serum samples from the clinical biochemistry laboratory and microbiology laboratory that were collected between Jan 2, 2019, and Feb 13, 2020. Furthermore, as part of public health service, we collected serum samples from Hong Kong residents who were evacuated from Hubei on March 4–5, 2020.

Posterior oropharyngeal saliva samples of Hubei returnees were collected 1 day after returning from Hubei and were tested by real-time RT-PCR targeting the E gene at the Public Health Laboratory Service in Hong Kong.

SARS-CoV-2 recombinant nucleoprotein and spike protein RBD were generated as described previously.[13] Enzyme immunoassays for SARS-CoV-2 nucleoprotein and spike protein RBD were done as described previously with modifications (appendix p 1).[13] Known positive and negative serum samples were included in each run as controls. To determine the cutoff value for positivity, we first calculated the mean optical density (OD) values and SD of 295 archived anonymous serum samples from 2018, and the cutoff OD value for a positive result was set as the mean OD value plus 3 SDs.

The microneutralisation assay and virus culture were done as described previously (appendix pp 1–2).[14] The microneutralisation assay antibody titre was the highest dilution with 50% inhibition of the cytopathic effect. A microneutralisation assay titre of 20 or greater was considered positive, as described previously.[15] Viral culture of SARS-CoV-2 and the microneutralisation assay was done in a biosafety level-3 facility at Queen Mary Hospital.

Statistical analysis

We compared categorical variables using Fisher's exact test and continuous variables using the Mann-Whitney *U* test. A value of 10 was arbitrarily assigned to all microneutralisation assay titres less than 20. We used Spearman's correlation to assess the relationship between microneutralisation assay titre and anti-nucleoprotein or anti-RBD IgG. The OD values were compared between different age groups using one-way ANOVA with the Kruskal-Wallis test. A p value of less than 0·05 was considered to be significant. We used SPSS 26.0 or PRISM 6.0 for statistical analysis.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Our study took place between Feb 26 and April 5, 2020. We first determined the cutoff, sensitivity, and specificity of our enzyme immunoassay and microneutralisation assay. We determined the cutoff value for seropositivity using 295 anonymous archived serum samples collected in 2018. This 2018 cohort consisted of 32–38 serum

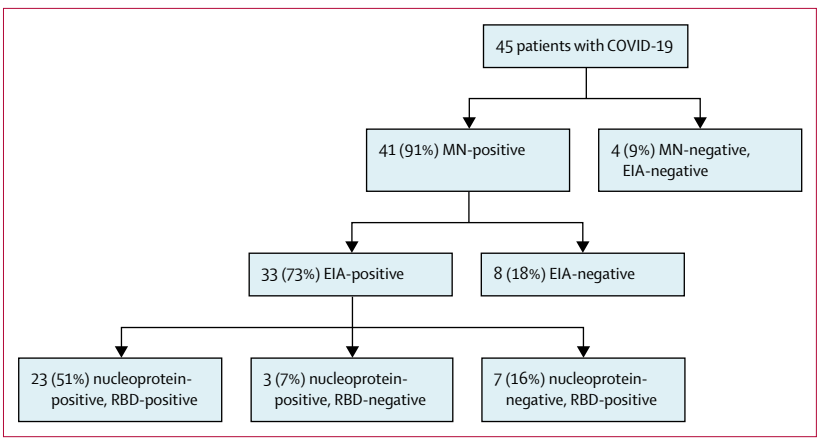

Figure 1: **Serological data for patients with COVID-19**
EIA=enzyme immunoassay. MN=microneutralisation assay. RBD=receptor binding domain.

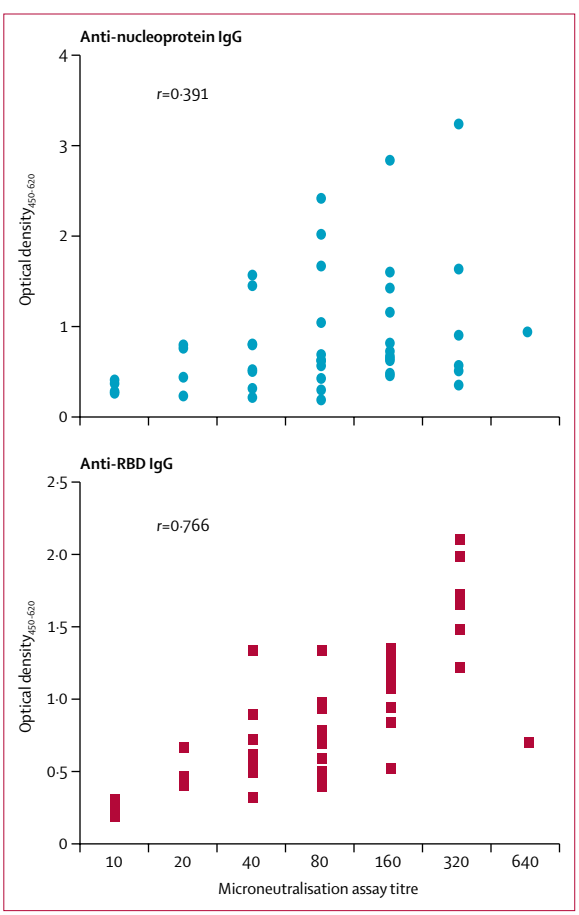

Figure 2: **Correlation between the microneutralisation assay with anti-nucleoprotein IgG or anti-RBD IgG**
A value of 10 was assigned to all microneutralisation assay titres less than 20. RBD=receptor binding domain.

See Online for appendix

	Total number of samples	Seropositive samples, n (%, 95% CI)			p value*		
		Anti-nucleoprotein IgG	Anti-RBD IgG	Anti-nucleoprotein IgG or anti-RBD IgG	Anti-nucleoprotein IgG	Anti-RBD IgG	Anti-nucleoprotein IgG or anti-RBD IgG
April 12 to July 3, 2018	295	4 (1·36%, 0·37–3·44)	4 (1·36%, 0·37–3·44)	7 (2·37%, 0·96–4·83)	NA	NA	NA
Jan 2 to June 28, 2019	429	10 (2·33%, 1·12–4·25)	7 (1·63%, 0·66–3·33)	17 (3·96%, 2·33–6·27)	0·42	1·00	0·29
July 2 to Dec 31, 2019	401	8 (2·00%, 0·87–3·89)	7 (1·75%, 0·71–3·56)	13 (3·24%, 1·74–5·48)	0·57	0·77	0·65
Jan 1 to Jan 31, 2020	580	7 (1·21%, 0·49–2·47)	9 (1·55%, 0·71–2·93)	15 (2·59%, 1·46–4·23)	1·00	1·00	1·00
Feb 1 to Feb 13, 2020†	233	0 (0%, 0–1·57)	1 (0·43%, 0·01–2·37)	1 (0·43%, 0·01–2·37)	0·13	0·39	0·083
Total	1938	29 (1·50%, 1·00–2·14)	28 (1·44%, 0·96–2·08)	53 (2·73%, 2·06–3·56)	NA	NA	NA

Data are n (%, 95% CI) unless specified. NA=not applicable. RBD=receptor binding domain. SARS-CoV-2=severe acute respiratory syndrome coronavirus 2. *Compared with April to July, 2018. †Up to Feb 13, 2020.

Table: **Seropositivity rate of SARS-CoV-2 in serum collected between 2018 and 2020**

samples of each 10-year age cohort from 0 to 9 years to a cohort of those aged 80 years or older (appendix p 3). The cutoff OD values were 0·610 for anti-nucleoprotein IgG and 0·573 for anti-RBD IgG.

To establish the sensitivity of our enzyme immunoassay and microneutralisation assay, we recruited adult patients who had recovered from COVID-19. 45 patients were screened for eligibility between Feb 26 and March 18, 2020, at the follow-up outpatient clinic, and all were enrolled. 23 (51%) patients were female. The median age was 59 years (IQR 47–68). Serum samples were collected at a median of 29 days after symptom onset (IQR 20·5–38·0). The sensitivity was 91·1% (41 of 45 [95% CI 78·8–97·5]) for the microneutralisation assay, 73·3% (33 of 45 [58·1–85·4]) for the enzyme immunoassay (positive for either anti-nucleoprotein IgG or anti-RBD IgG), 57·8% (26 of 45 [42·2–72·3]) for anti-nucleoprotein IgG, and 66·7% (30 of 45 [51·1–80·0]) for anti-RBD IgG (figure 1). For the four patients who tested negative with the microneutralisation assay, all tested negative with the enzyme immunoassay for both anti-nucleoprotein IgG and anti-RBD IgG, and all had mild disease. The microneutralisation assay titre had a stronger correlation with anti-RBD IgG (Spearman's Rho=0·77 [95% CI 0·60–0·87]; p<0·0001) than with anti-nucleoprotein IgG (Spearman's Rho=0·39 [0·10–0·62]; p=0·0078; figure 2).

To establish the specificity of our enzyme immunoassay and microneutralisation assay, we retrieved archived serum collected from 99 animal handlers in 2013 and 2014, and from 53 potential organ donors between 2016 and 2018. The median age was 52 years (IQR 43–58), and 72 (47%) of 152 were female. All 152 serum samples tested negative for anti-nucleoprotein IgG, anti-RBD IgG, and microneutralisation assay. Hence, the specificity was 100·0% (152 of 152 [95% CI 97·6–100·0]) for anti-nucleoprotein IgG, anti-RBD IgG, and the microneutralisation assay.

Next, we evaluated the enzyme immunoassay using the serum samples of 53 patients suspected to have COVID-19. Patients included fulfilled the clinical and epidemiological criteria for testing on or before Jan 28, 2020, but were negative for SARS-CoV-2 with RT-PCR, when all serum samples from suspected COVID-19 cases from Hong Kong were sent to our laboratory (21 [40%] male and 32 [60%] female). The median age was 35 years (IQR 23–50). The most common symptom was fever (29 [55%] of 53), followed by cough (26 [49%] of 53), sore throat (19 [36%] of 53), chills (seven [13%] of 53), and myalgia (four [8%] of 53). All patients were seronegative for anti-nucleoprotein IgG and anti-RBD IgG.

As SARS-CoV and SARS-CoV-2 share substantial amino acid homology in their nucleoprotein (90%) and spike protein (76%),[16] antibodies from patients who had SARS might be cross-reactive with our enzyme immunoassay. We retrieved serum samples of 12 patients with RT-PCR confirmed 2003 SARS-CoV infection. Four (33%) of 12 patients were seropositive for anti-nucleoprotein IgG and two (17%) of 12 were seropositive for anti-RBD IgG of SARS-CoV-2. However, none of the patients were positive for both.

To assess the prevalence of subclinical infection in Hong Kong, we compared the seropositivity rate of archived anonymous serum samples collected between April 12, 2018, and Feb 13, 2020. No significant increase was seen in the seropositivity rate of SARS-CoV-2 nucleoprotein IgG or RBD IgG in Jan 2 to June 28, 2019 (nucleoprotein 2·33%, RBD 1·63%), July 2 to Dec 31, 2019 (nucleoprotein 2·00%, RBD 1·75%), Jan 1 to Jan 31, 2020 (nucleoprotein 1·21%, RBD 1·55%), or Feb 1 to Feb 13, 2020 (nucleoprotein 0, RBD 0·43%) when compared with those in April 12 to July 3, 2018 (nucleoprotein 1·36%, RBD 1·36%; table).

Overall, 53 (2·73%) of 1938 serum samples were seropositive with the enzyme immunoassay for either anti-nucleoprotein IgG or anti-RBD IgG (table). The seropositivity rates were 1·5% (29 of 1938) for anti-nucleoprotein IgG and 1·4% (28 of 1938) for anti-RBD IgG. Four samples (0·2%) were positive for both anti-nucleoprotein IgG and anti-RBD IgG. Notably, of the 53 patients who tested positive with the enzyme immunoassay, all tested negative with the microneutralisation assay. Subgroup analysis showed that the 0–9-year-old age group had the lowest median $OD_{450–620}$,

and was significantly lower than all other age groups in Jan 1 to Jan 31, 2020, for anti-nucleoprotein IgG (figure 3, appendix p 4).

469 Hong Kong residents were evacuated from Hubei province on four different flights on March 4–5, 2020, and were quarantined at a housing estate. 1665 serum samples were collected from 452 returnees from Hubei province (of which 364 [80·5%] were from Wuhan) on day 1, day 5, day 9, or day 13 after returning from Hubei (appendix pp 5–7). 17 (4%) of 469 returnees refused to have their blood taken. The median age of returnees was 41 years and 45 (10%) of 452 were younger than 18 years. 265 (59%) were female. All 452 Hubei returnees were asymptomatic, and their posterior oropharyngeal saliva samples on day 1 after returning from Hubei tested negative by real-time RT-PCR. The enzyme immunoassay was done for all available samples on all days. The microneutralisation assay was done on the latest available samples, including 433 serum samples on day 13 after return, four on day 9, six on day 5, and nine on day 1. Among the 452 returnees, 17 (4%) were seropositive with either the microneutralisation assay or the enzyme immunoassay. Of the 17 seropositive returnees, 15 (88%) were seropositive with the microneutralisation assay, 13 (76%) were seropositive with the enzyme immunoassay, and 11 (65%) were seropositive with both the microneutralisation assay and enzyme immunoassay (figure 4). 16 individuals who were seropositive had been staying in Wuhan, and one had been staying in Jingzhou. The 17 returnees who were seropositive included six individuals from two family clusters. These six individuals were seropositive with the microneutralisation assay. For the four-member family cluster, two were anti-RBD IgG positive, but none were anti-nucleoprotein IgG positive. For the two-member family, both were anti-RBD IgG positive and one was anti-nucleoprotein IgG positive. For the 11 patients not within clusters, seven were positive with both the microneutralisation assay and enzyme immunoassay, two were positive with the microneutralisation assay only, and two were positive with the enzyme immunoassay only. Among the 433 returnees with samples collected on day 13, 16 (4%) returnees were positive with both the microneutralisation assay and enzyme immunoassay (appendix p 8).

Discussion

In this study, we generated SARS-CoV-2 seroepidemiological data for the general population in Hong Kong and Hong Kong residents who were evacuated from Hubei province using validated serological assays. Testing of 1938 serum samples collected from the general population did not identify any individuals who were seropositive for SARS-CoV-2 before and after the COVID-19 pandemic that could be confirmed by microneutralisation assay. Among 452 returnees evacuated from Hubei province in March, 2020, the seroprevalence was 4%, with the majority (88%) being confirmed

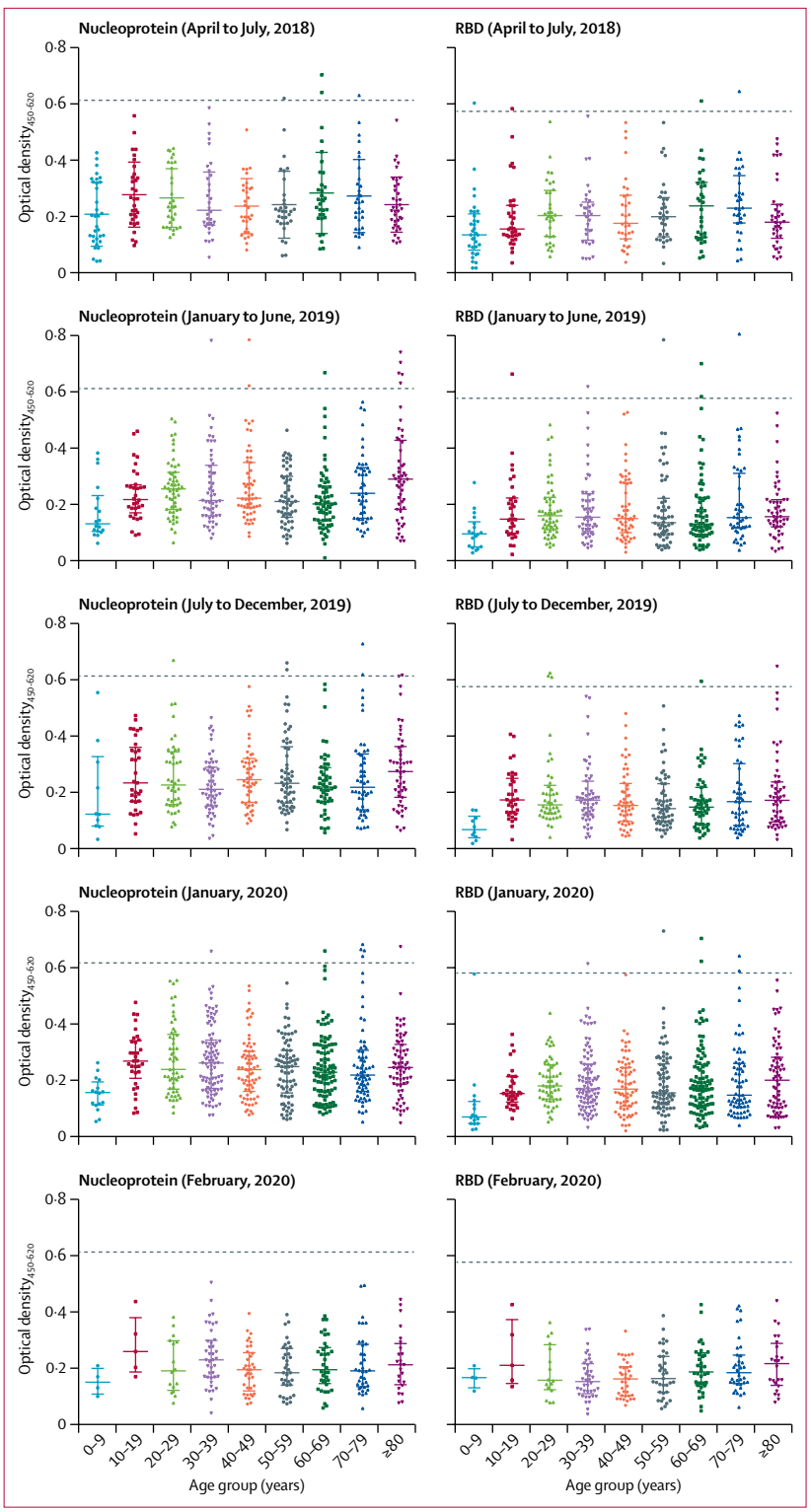

Figure 3: **Age-specific serological data for anonymised serum samples between 2018 and 2020**
The horizontal line represents the cutoff for seropositivity. The 0-9 year-old age group had the lowest median optical density$_{450-620}$. RBD=receptor binding domain.

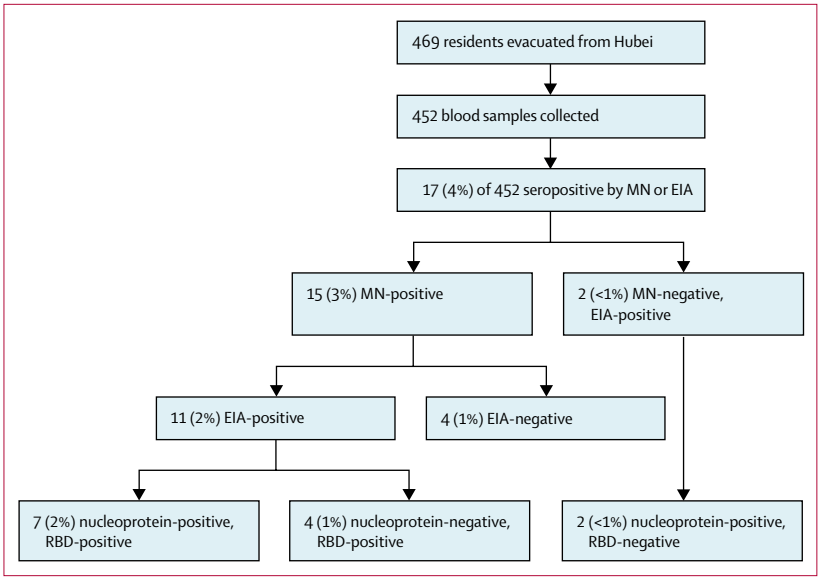

Figure 4: **Serological data for returnees evacuated from Hubei province**
EIA=enzyme immunoassay. MN=microneutralisation assay. RBD=receptor binding domain.

by microneutralisation assay. We also identified two subclinical family clusters among the Hubei returnees.

It has been suggested that 50–60% of the population could become infected because of the lack of herd immunity against SARS-CoV-2.[6] Despite being the earliest COVID-19 affected areas, the seroprevalence among returnees from Hubei was only 4%, while there were no individuals who were microneutralisation assay-confirmed seropositive among the general population of Hong Kong. The relative lack of spread is probably attributable to the public health measures taken in both areas and the compliance of their citizens. Being the epicentre of COVID-19 in January, 2020, the Chinese Government imposed several unprecedented stringent public health measures in Hubei from Jan 23, 2020, including the ban on almost all transportation and travel to and from the province, limitation of people's movement within cities, suspension of public transport, closure and cessation of non-essential work and service premises, enforcement of compulsory wearing of face masks, aggressive case identification, testing and isolation, and rapid contract tracing and quarantine.[3] By contrast, taking into account the limited local spread in the city, the Hong Kong Government took a more pragmatic approach. Such measures included border control limiting travel from areas with COVID-19 epidemics, recommendation of voluntary community-wide wearing of face masks, hand hygiene and social distancing, isolation of suspected cases, and testing and quarantine of close contacts and travellers from epidemic areas. The measures taken in Hong Kong, although less stringent than in Hubei, have averted a total city lockdown thus far. Such pragmatic control measures should be continued until safe and effective antivirals and vaccines become available. As the pandemic continues, the population's fatigue toward the lockdown measures will affect the compliance of the general public with these stringent control measures. A delicate balance between effective epidemic control and physical health and livelihoods linked to socioeconomic activities and psychosocial health must be achieved.

Our seroprevalence data for Hubei returnees showed that RT-PCR confirmed infections grossly underestimated the actual prevalence of COVID-19. With a population of 59 million (Hubei province) and 11 million (Wuhan) people as of 2019,[17] our findings indicate that about 2·2 million people (3·8%, 95% CI 2·2–6·0) in Hubei and 0·5 million people (4·4%, 2·5–7·1) in Wuhan could have been infected. The number of laboratory-confirmed symptomatic patients in Hubei province was reported as 67 802 (3% of 2·2 million) as of March 31, 2020.[18] Thus 97% of infections in Hubei might have gone undiagnosed at that period of the epidemic. This approximation is much higher than the estimate of 86% from a previous study using mathematical modelling before Jan 23, 2020.[19] Furthermore, the number of deaths reported in Hubei was 3193 up to March 31, 2020, which is about 0·16% of the estimated 2·2 million seropositive individuals. This mortality is about nine-times higher than the mortality of 0·017% for the 2009 influenza pandemic based on seropositive individuals (number of respiratory and cardiovascular deaths 284 500[20]; global seropositive rate 24%[21]; global population in 2010 6·9 billion). A study of an outbreak in a long-term care facility showed that 57% of RT-PCR cases were asymptomatic at the time of testing.[11] Transmission of SARS-CoV-2 by asymptomatic individuals has also been reported.[22] Due to high transmission efficiency and high prevalence of subclinical infections, SARS-CoV-2 has disseminated rapidly into a pandemic. The large proportion of subclinical infection is compatible with our in-vitro study showing that SARS-CoV-2 has a high replication rate in cell culture while producing much less cell damage. Furthermore, our ex vivo SARS-CoV-2-infected human lung tissue explants showed less activation of the innate immune response. This finding was shown by lower induction of interferon and proinflammatory cytokines, when compared with those of SARS.[23,24] Our hamster model also showed that naive hamsters co-housed with SARS-CoV-2-infected hamsters readily acquire infection.[25] These in vitro and ex vivo findings corroborate the findings of a high incidence of asymptomatic infection (67%) in the infected patients on the Diamond Princess cruise ship,[26] and explained the rapid increase in viral load that peaked at symptom onset and presentation.

Analysis of the Hong Kong general population showed that the OD values of anti-nucleoprotein and anti-RBD IgG were lower for the 0–9-year-old age group when compared with older age groups. Because low amounts of antibody cross-reactivity in the enzyme immunoassay might be caused by previous exposures to other human coronaviruses, especially the genus *Betacoronavirus*, our

results suggest that most individuals in the 0–9-year age group could be immunologically naive to other human coronavirus infections, which is compatible with the results of a previous seroprevalence study of human coronavirus HKU1.[27] One intriguing phenomenon of the COVID-19 pandemic is that, unlike other respiratory viruses such as influenza virus and respiratory syncytial virus, adults have higher prevalence and severity of COVID-19 than children.[4] It has been speculated that cross-reactive non-neutralising antibodies from other human coronaviruses in adults might enhance COVID-19 severity. Previous studies have shown that monoclonal antibodies against the SARS-CoV spike protein cannot neutralise SARS-like coronaviruses in vitro and immunisation with inactivated whole SARS-CoV worsens the disease.[28] We have previously shown that anti-spike IgG actually stimulated the pulmonary proinflammatory response and could cause severe acute lung injury in a SARS-CoV macaque model.[29] The clinical significance of disease-enhancing cross-reactive antibody and even cell-mediated immunity should be further explored.

Our study has generated a baseline for SARS-CoV-2 seroprevalence in Hong Kong and Hubei province for ongoing serological surveillance. This baseline is important as reliance on case counts alone can overlook subclinical infections, which are common for SARS-CoV-2 and other respiratory viruses.[30] In addition to monitoring the prevalence of subclinical infections, continuous monitoring of seroprevalence will help to establish the level of herd immunity in the population.

Our study differs from other reports on returnees evacuated from Hubei province.[10,31] First, in our study, the returnees were evacuated from Hubei in early March, 2020, whereas in the other two studies, the participants were evacuated to Singapore and Germany in late-January or early-February, 2020.[10,31] Second, both antibody assays and RT-PCR were used in our study, whereas only RT-PCR was used in the other studies. As no serological data were reported in the other studies, the actual prevalence of previous SARS-CoV-2 infection in returnees up to early-February was unknown.

There are several limitations to our study. The 4% seropositivity rate among our Hubei returnees is likely to be an underestimate for a few reasons. First, as in all serology studies that use enzyme immunoassay, the sensitivity and specificity of the assay can be affected by the cutoff value chosen. Because the main aim of our study was to accurately determine the seroprevalence of COVID-19, we chose stringent assay cutoff values to maximise specificity. The stringent cutoff values have resulted in a relatively low sensitivity of enzyme immunoassay (73%), which is similar to the results of another study, in which the IgG sensitivity was only 65%.[32] Second, since the Hubei returnees were evacuated 3 months after the first reported laboratory-confirmed SARS-CoV-2 infection,[33] and 1·5 months after the travel ban in Hubei province, there is a possibility that the amount of anti-SARS-CoV-2 antibodies could have decreased below the cutoff OD value in returnees with previous SARS-CoV-2 infection during the early period of the outbreak. Third, as shown in our COVID-19 patient cohort, some patients with mild symptoms might mount a B-cell response that is undetectable by our conventional antibody assays. Another limitation is that we do not have serum samples of the Hubei population before the pandemic, and therefore we do not know the baseline seroepidemiology of Hubei. Finally, long-term serosurveillance with more serum samples is required to identify subclinical infections, especially after the second wave of infection in Hong Kong in March and April, 2020.

The static and low SARS-CoV-2 seroprevalence in Hong Kong provided support for the effectiveness of our pragmatic epidemiological control measures without city lockdown, and also the lack of population herd immunity. The 4% seroprevalence in Hubei returnees indicates that laboratory-confirmed symptomatic infections are a small proportion of the total cases. However, such a level of seroprevalence will not stop the progression of this pandemic once these effective epidemiological control measures are relaxed. Furthermore, the higher amount of possible disease-enhancing cross-reactive antibody in adults than in children, as evident by the OD value of the enzyme immunoassay, should be explored further as a possible explanation of more severe disease in adults.

Contributors

KK-WT, VC-CC, and K-YY had roles in the study design, data collection, data analysis, data interpretation, literature search, and writing of the manuscript. TW-HC and IF-NH had roles in recruitment, data collection, and clinical management. S-CW had roles in recruitment and data collection. J-PC, K-HC, L-LC, L-HW, C-YKC, C-HYF, A-CKN, L-L, C-TL, J-S, and G-SWK had roles in experiments, data collection, data analysis, and data interpretation. S-S, J-FWC, and K-HK had roles in data analysis, data interpretation, the literature search, and writing of the manuscript. C-YMF and V-WMC had roles in data collection, data interpretation, and writing of the manuscript. All authors reviewed and approved the final version of the manuscript.

Declaration of interests

We declare no competing interests.

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