Seroprevalence and humoral immune durability of anti-SARS-CoV-2 antibodies in Wuhan, China: a longitudinal, population-level, cross-sectional study

Zhenyu He*, Lili Ren*, Juntao Yang*, Li Guo*, Luzhao Feng*, Chao Ma, Xia Wang, Zhiwei Leng, Xunliang Tong, Wang Zhou, Geng Wang, Ting Zhang, Yan Guo, Chao Wu, Qing Wang, Manqing Liu, Conghui Wang, Mengmeng Jia, Xuejiao Hu, Ying Wang, Xingxing Zhang, Rong Hu, Jingchuan Zhong, Jin Yang, Juan Dai, Lan Chen, Xiaoqi Zhou, Jianwei Wang†, Weizhong Yang†, Chen Wang†

Summary

Background Wuhan was the epicentre of the COVID-19 outbreak in China. We aimed to determine the seroprevalence and kinetics of anti-SARS-CoV-2 antibodies at population level in Wuhan to inform the development of vaccination strategies.

Methods In this longitudinal cross-sectional study, we used a multistage, population-stratified, cluster random sampling method to systematically select 100 communities from the 13 districts of Wuhan. Households were systematically selected from each community and all family members were invited to community health-care centres to participate. Eligible individuals were those who had lived in Wuhan for at least 14 days since Dec 1, 2019. All eligible participants who consented to participate completed a standardised electronic questionnaire of demographic and clinical questions and self-reported any symptoms associated with COVID-19 or previous diagnosis of COVID-19. A venous blood sample was taken for immunological testing on April 14–15, 2020. Blood samples were tested for the presence of pan-immunoglobulins, IgM, IgA, and IgG antibodies against SARS-CoV-2 nucleocapsid protein and neutralising antibodies were assessed. We did two successive follow-ups between June 11 and June 13, and between Oct 9 and Dec 5, 2020, at which blood samples were taken.

Findings Of 4600 households randomly selected, 3599 families (78·2%) with 9702 individuals attended the baseline visit. 9542 individuals from 3556 families had sufficient samples for analyses. 532 (5·6%) of 9542 participants were positive for pan-immunoglobulins against SARS-CoV-2, with a baseline adjusted seroprevalence of 6·92% (95% CI 6·41–7·43) in the population. 437 (82·1%) of 532 participants who were positive for pan-immunoglobulins were asymptomatic. 69 (13·0%) of 532 individuals were positive for IgM antibodies, 84 (15·8%) were positive for IgA antibodies, 532 (100%) were positive for IgG antibodies, and 212 (39·8%) were positive for neutralising antibodies at baseline. The proportion of individuals who were positive for pan-immunoglobulins who had neutralising antibodies in April remained stable for the two follow-up visits (162 [44·6%] of 363 in June, 2020, and 187 [41·2%] of 454 in October–December, 2020). On the basis of data from 335 individuals who attended all three follow-up visits and who were positive for pan-immunoglobulins, neutralising antibody levels did not significantly decrease over the study period (median 1/5·6 [IQR 1/2·0 to 1/14·0] at baseline vs 1/5·6 [1/4·0 to 1/11·2] at first follow-up [p=1·0] and 1/6·3 [1/2·0 to 1/12·6] at second follow-up [p=0·29]). However, neutralising antibody titres were lower in asymptomatic individuals than in confirmed cases and symptomatic individuals. Although titres of IgG decreased over time, the proportion of individuals who had IgG antibodies did not decrease substantially (from 30 [100%] of 30 at baseline to 26 [89·7%] of 29 at second follow-up among confirmed cases, 65 [100%] of 65 at baseline to 58 [92·1%] of 63 at second follow-up among symptomatic individuals, and 437 [100%] of 437 at baseline to 329 [90·9%] of 362 at second follow-up among asymptomatic individuals).

Interpretation 6·92% of a cross-sectional sample of the population of Wuhan developed antibodies against SARS-CoV-2, with 39·8% of this population seroconverting to have neutralising antibodies. Our durability data on humoral responses indicate that mass vaccination is needed to effect herd protection to prevent the resurgence of the epidemic.

Funding Chinese Academy of Medical Sciences & Peking Union Medical College, National Natural Science Foundation, and Chinese Ministry of Science and Technology.

Introduction

The emergence of COVID-19, caused by SARS-CoV-2, has led to an unprecedented global public health crisis.[1] Almost 60% of confirmed cases in mainland China were from Wuhan, the early epicentre of the COVID-19 outbreak.[2] Through unprecedented lockdown action, which started on Jan 23 and ran until April 8, 2020, transmission of SARS-CoV-2 in Wuhan was rapidly

Articles

National Clinical Research
Center for Respiratory Diseases,
China-Japan Friendship
Hospital, Beijing, China
(Ch Wang); National Center for
Respiratory Medicine, Beijing,
China (Ch Wang); Chinese
Academy of Engineering,
Beijing, China (Ch Wang)

Correspondence to:
Dr Chen Wang, Chinese Academy
of Medical Sciences & Peking
Union Medical College,
Beijing 100730, China
wangchen@pumc.edu.cn

or

Dr Weizhong Yang, Chinese
Academy of Medical Sciences &
Peking Union Medical College,
Beijing 100730, China
yangweizhong@cams.cn

or

Dr Jianwei Wang, Chinese
Academy of Medical Sciences &
Peking Union Medical College,
Beijing 100730, China
wangjw28@163.com

For the WHO COVID-19
dashboard see https://covid19.
who.int/

Research in context

Evidence before this study

We searched PubMed for articles published in English from database inception until Dec 15, 2020, using the keywords ("2019-nCoV" OR "novel coronavirus" OR "COVID-19" OR "SARS-CoV-2") AND ("antibody" OR "population" OR "humoral responses" OR "longitudinal") AND "seroprevalence". We identified seven papers published in peer-reviewed journals describing humoral responses against SARS-CoV-2 at the general population level. One study was done in Geneva, Switzerland, and it estimated 11·6 infections in the community for every reported confirmed case among the general population older than 5 years. Another study estimated seroprevalences of 5·0% using point-of-care tests and 4·6% using immunoassays in Spain. The third study estimated that 1·0–6·9% of individuals are positive for SARS-CoV-2 antibodies and the actual number of infections in the USA is 6–24 times higher than reported. The fourth study suggested that 0·9% of people in Iceland are infected with SARS-CoV-2 and that titres of antibodies against SARS-CoV-2 do not decrease within 4 months after diagnosis. The fifth study estimated that seropositivity in Wuhan is 3·2% in adults and the study did not have a rigorous statistical design. The sixth study estimated an overall seroprevalence of 2·8% (95% CI 2·1–3·7) in the general population of the Netherlands in the middle of the first epidemic wave (medium inclusion date was April 3, 2020). In the seventh study, the estimated seroprevalence was 9·3% (95% CI 8·8–9·9) in the US adult population. At present, little is understood of kinetic changes in antibodies against SARS-CoV-2 after natural infection, particularly in individuals with asymptomatic infections. To date, the durability of IgA, IgM, IgG, and neutralising antibodies against SARS-CoV-2 has not been assessed in a general population-level study in mainland China.

Added value of this study

To our knowledge, this is the first general longitudinal population-based study involving over 9000 community residents in Wuhan, China—the epicentre of the COVID-19 epidemic in China. We use a multistage, population-stratified, random sampling method to select participants. The adjusted seroprevalence was 6·92% (95% CI 6·41–7·43), and over 80% of antibody-positive individuals were asymptomatic. Neutralising antibodies were developed in approximately 40% of antibody-positive individuals. The proportion of participants who were positive for IgG and neutralising antibodies, and the titres of neutralising antibodies, did not significantly decrease between the middle of April and October–December, 2020. Additionally, families with more members who were antibody positive have an increased likelihood of contracting SARS-CoV-2. Our findings provide new insights into the mode of viral transmission and kinetics of humoral responses in natural SARS-CoV-2 infections.

Implications of all the available evidence

Our data suggest that most individuals remain susceptible to SARS-CoV-2 infection after the first-wave epidemic in Wuhan. These findings suggest that vaccinations will be required to effect herd immunity. The high proportion of asymptomatic infections after natural infection suggest that most individuals had mild disease and their symptoms are too mild to cause them to seek medical care. Our seroprevalence data will inform public policy for tackling the COVID-19 pandemic and be beneficial for vaccine development efforts.

contained. During lockdown, further action was taken to control the epidemic, including proactive case finding and isolation, tracing and quarantining of close contacts, traffic control and travel restrictions, physical distancing, closing of schools and factories, and home-based quarantine.[3] With the spreading of COVID-19 to other countries, the declaration of a global pandemic, and the development of vaccines, assessing the proportion of the population that have been infected and who are immune is of utmost importance for determining effective prevention and control strategies to reduce the likelihood of future resurgence of the pandemic.[4]

SARS-CoV-2 infections elicit detectable humoral responses,[5,6] and the proportion of cases that are asymptomatic is uncertain, with estimates between 6·3% and 96·0%.[7,8] Given that individuals with mild infections might not seek medical care and that asymptomatic individuals are not usually screened, the prevalence of SARS-CoV-2 might be largely under-estimated on the basis of the number of cases reported. Generally, measurement of the seroprevalence of antibodies, especially neutralising antibodies, against

SARS-CoV-2 from population-based seroepidemiological surveys is informative for the assessment of the proportion of the population who have at some point been infected with the virus and provides insight into the design of vaccination programmes.[9–11] Nevertheless, most studies on humoral responses in China have involved a small number of healthy participants or selected cohorts (eg, health-care workers, people living with HIV, hotel staff), which might not be indicative of the status of herd immunity in the general population.[10,11] Moreover, in these surveys, measurement of neutralising antibody concentrations has not been done simultaneously with testing for the presence of immunoglobulins at the individual level, because the procedures need to be done in biosafety level 3 facilities. Hence, our understanding of seroconversion to give protective antibodies in natural SARS-CoV-2 infections is restricted. Previous studies suggested that antibodies against SARS-CoV-2 were maintained for at least 4 months;[12,13] however, the durability of humoral responses against SARS-CoV-2 after natural infection without repeat exposure still needs to be further clarified with longer follow-up time.

We did a population-based longitudinal seroepidemiological study in Wuhan, China, starting in April, 2020, when the lockdown ended in Wuhan, and two successive follow-ups in June, and between October and December, 2020. Levels of pan-immunoglobulins, IgM, IgA, and IgG antibodies against SARS-CoV-2 nucleocapsid (N), and neutralising antibodies were determined.

Methods

Study design and participants

In this a population-level, longitudinal, cross-sectional study, we used a multistage, population-stratified, cluster random sampling method to enrol participants from Wuhan. Within all the 13 districts in Wuhan, 100 communities were selected using the probability-proportionate-to-size sampling method (appendix 2 p 1).[14] Households were selected using simple random sampling in each community, as identified through lists of households provided by the local government.

Family sizes ranged from one to more than six members. All family members of the selected households were invited to community health-care centres in batches via telephone calls or door-to-door visits on April 9–13, 2020. Participants were screened at an individual level, and eligible individuals were those who had lived in Wuhan for at least 14 days since Dec 1, 2019. Individuals who experienced serious disease other than COVID-19, including but not limited to advanced cancers and people with severe mental illness, were excluded. We further excluded those who refused to participate and who were not able to finish sample collection.

This study was approved by the Ethical Review Board of Institute of Pathogen Biology, Chinese Academy of Medical Sciences (IPB-2020-04). Written informed consent was obtained from all participants before they were enrolled. For individuals younger than 18 years, consent was provided by parents or a legal guardian.

Procedures

After written informed consent was obtained, all eligible participants completed a standardised electronic questionnaire (appendix 2 pp 2–4), with the help of trained research staff if necessary. For young children, the questionnaires were completed by their parents or guardians. Over April 14–15, 2020, venous blood samples were taken from each participant for immunological testing; serum separation was done at the laboratory in Wuhan Center for Disease Control & Prevention within 8 h of sample collection.

Confirmed COVID-19 cases were determined through self-report on the baseline questionnaire of a diagnosis according to Chinese Clinical Guidance, with quantitative PCR assay for SARS-CoV-2 and lung CT scan.[15] Individuals were determined to have symptomatic infection if they self-reported fever or respiratory symptoms (including but not limited to cough, anhelation, stuffy nose, rhinorrhoea, sore throat, and pneumonia [appendix 2 pp 2–4]) or both,

and were positive for SARS-CoV-2 antibodies. Individuals were determined to have asymptomatic infections if they were positive for SARS-CoV-2 antibodies and had no self-reported COVID-19-related symptoms since Dec 1, 2019. This definition of asymptomatic infection is different from that of the Chinese National Health Commission, where it is defined as respiratory tract samples being positive for SARS-CoV-2 by viral RNA detection but without any respiratory symptoms.[16] In this study, we determined the presence of infection on the basis of antibody detection alone because we were assessing the history of infection since Dec 1, 2019, and so were not able to secure such samples.

A family with one or more individuals who were positive for antibodies against SARS-CoV-2 at baseline was defined as a positive family. A negative family was defined as a household that lived next-door to a positive family and all family members tested negative for SARS-CoV-2. For each positive family included, two location-matched negative families were included at baseline. All positive and matched negative families were followed up between June 11 and 13, and between Oct 9 and Dec 5, 2020, at which point they were invited to the heath-care centres where venous blood samples were taken. A timeline of when confirmed cases started to be reported in Wuhan, which were retrieved from the daily report of the National Heath Commission of China, up to the end of second follow-up period is shown in figure 1.

All laboratory tests on the blood samples were done at Christophe Mérieux Laboratory, Institute of Pathogen Biology, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China. All serum samples

See **Online** for appendix 2

For the **National Health Commission of China's website** see http://www.nhc.gov.cn/xcs/yqtb/list_gzbd.shtml
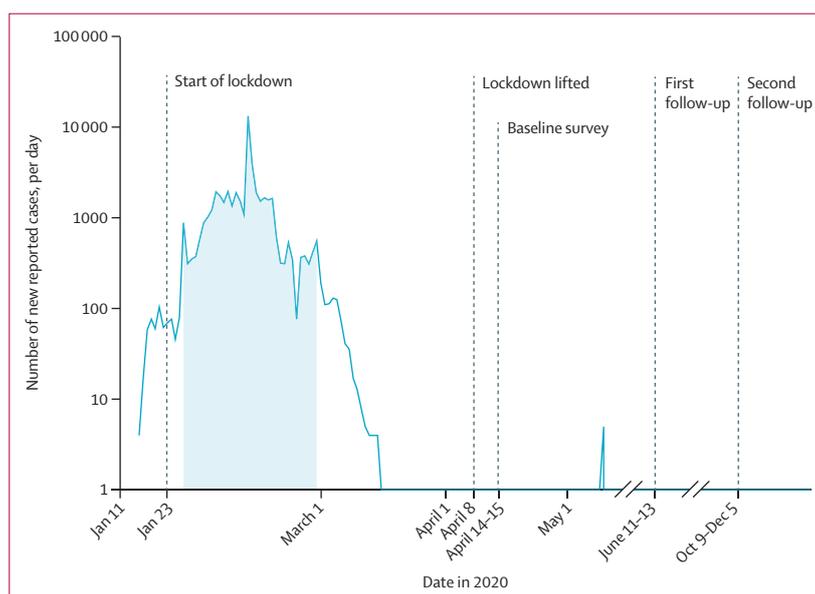
Figure 1: Timeline of new reported cases in Wuhan, China, and the follow-up period
The data on new reported COVID-19 cases were collected from Jan 11, 2020, and are available on the National Health Commission of China website. The shaded area shows the time period when 75% of confirmed cases were identified.

were inactivated at 56°C for 30 min before use. A recombinant N protein was used to determine the antibodies against SARS-CoV-2. Samples were first screened for pan-immunoglobulins with electrochemiluminescence immunoassay (ECLIA) kits according to the manufacturer's instructions (Roche Diagnostics, Rotkreuz, Switzerland) before antibody typing, because ECLIA is more sensitive than antibody typing.[17] Titres of IgA, IgM, and IgG antibodies against the SARS-CoV-2 N protein in serum samples were assessed with ELISA, as previously

reported.[6] Briefly, 10 ng of N protein was used as a coating protein. Serum samples were diluted 1/400 with 0·5% bovine serum albumin (BSA) and incubated for 1 h at 37°C. After washing, horseradish peroxidase-conjugated goat anti-human Fc5μ fragment specific polyclonal IgM (Jackson ImmunoResearch, West Grove, PA, USA), rabbit anti-human α chain specific polyclonal IgA (Jackson ImmunoResearch), and goat anti-human Fc specific polyclonal IgG (Sigma Aldrich, St Louis, MO, USA) antibodies were added to the plates at a dilution of 1/60000 with 0·5% BSA. After 1 h of incubation at 37°C, the plates were washed and developed with 100 μL substrate solutions A (3,3',5,5'-tetramethylbenzidine) and B (hydrogen peroxide) in each well (Wantai Biotech Corp, Beijing, China). The reaction was stopped by adding 50 μL of 2 M sulfuric acid. Optical density at 450 nm (OD_{450}) was determined with a multifunctional microplate reader SpectraMax M5 (Molecular Devices, Sunnyvale, CA, USA). The cutoff for IgM was 0·30, for IgA was 0·20, and IgG was 0·10, determined by calculating the mean OD_{450} of a negative serum sample plus 3 SDs. The reproducibility of the ECLIA and ELISA assays were validated using the following serum samples. 102 serum samples that were collected from volunteers in Wuhan health-care centres before 2019 and they were all negative for pan-immunoglobulins, IgA, IgM, and IgG in both assays, confirming the high specificity. We also tested 56 serum samples collected from patients with COVID-19 in hospitals, 46 from the Lotus cohort in Wuhan Jinyintan Hospital,[5] and ten recovered patients from Zhongnan Hospital of Wuhan University.[5] For all these serum samples, permission was granted by the study coordinators or individuals for their samples to be used in this study. All these patients were confirmed to be positive for SARS-CoV-2 infection by quantitative PCR assay before admission to hospital and they were all positive for pan-immunoglobulins and IgG antibodies (appendix 2 pp 5–7).

Presence of neutralising antibodies was assessed using in-house microneutralisation assays, as previously reported.[5] A serial two-fold dilution of serum samples (starting at 1:4) was preincubated with SARS-CoV-2 at 100 50% tissue culture infective doses for 2 h at 37°C, and the virus-serum mixture was added to Vero cells (American Type Culture Collection number CCL-81) and incubated for 1 h. The cytopathic effect was assessed 5 days after incubation. Four duplicate wells were used for each serum dilution. Neutralising antibody titres were calculated using the Reed-Muench method.[18] Viral back-titration was done, and serum samples known to be positive for neutralising antibodies were used as a positive control in each test. The cutoff for a positive neutralising antibody titre was 1/8.

Statistical analysis

We did goodness of fit tests for our sampled population versus the actual population of Wuhan (with data provided by the Wuhan Public Security Bureau) using
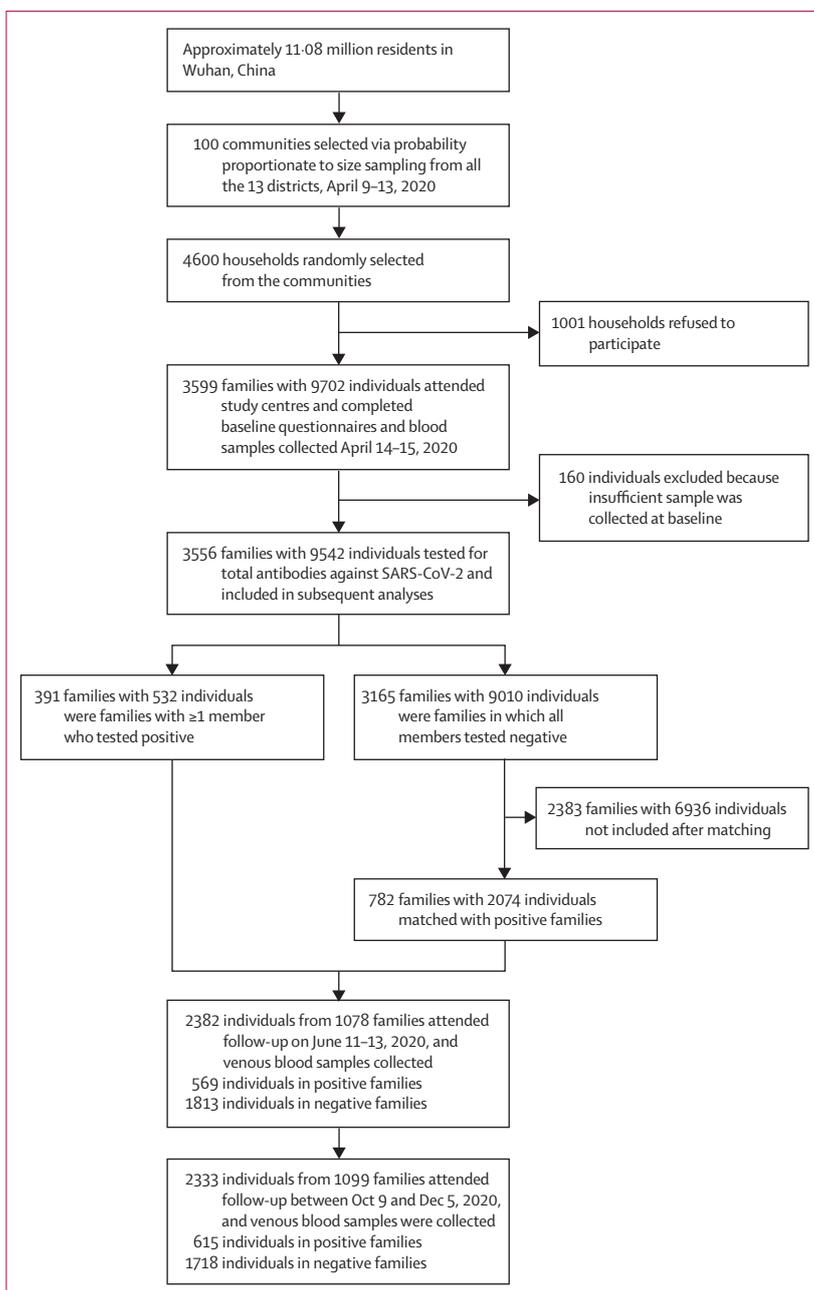
Figure 2: Study profile

the χ^2 test to estimate whether significant differences existed between the observed frequency and expected frequency caused by sampling error.

We compared continuous variables using the Kruskal-Wallis test. We compared categorical variables using the χ^2 test or Fisher's exact test for variables with low expected counts; data are described as n (%). We applied the individual sampling weight and post-stratification demographic weight, which were based on the population distribution of age, sex, and district in Wuhan (using data provided by the Wuhan Public Security Bureau), to the seroprevalence calculation. We adjusted our seroprevalence estimates by age group, sex, district, and the inverse probability of selection.

We used the matched negative families as the control population to confirm whether any new infections occurred in Wuhan during the study period, and to confirm there were no false-positive antibody detections.

A two-sided p value of less than 0·05 was considered to be statistically significant. We did all statistical analyses using SAS software (version 9.4).

Role of the funding source

The funders of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

Results

At the time of sampling, approximately 11·08 million residents lived in Wuhan. According to the size of communities among the 13 districts of Wuhan, 4600 households were randomly chosen to participate. Among these households, 3599 families (78·2%) with 9702 eligible individuals were enrolled (figure 2). 160 individuals were excluded because the volumes of their serum samples taken at baseline were insufficient to finish all the tests in our study. Hence, 9542 participants from 3556 families were ultimately investigated. Of 3556 families, 391 had at least one member who tested positive for SARS-CoV-2 and 3165 families had no members who tested positive. The positive families were location matched with 782 negative families with 2074 individuals and these matched families were invited for the first and second follow-up visits. 2382 individuals from 1078 families attended the first follow-up and 2333 individuals from 1099 families attended the second follow-up (figure 2). According to a goodness of fit test, the age and sex profiles of our baseline analysis population were consistent with the whole population of Wuhan. Although the goodness of fit test for the district distribution of our participants versus the actual population was significantly different (p<0·01; appendix 2 p 8), the sampling frame was consistent with the whole population after adjustment (data not shown).

532 (5·6%) of 9542 participants were positive for pan-immunoglobulins against SARS-CoV-2 at baseline,

with an adjusted seroprevalence of 6·92% (95% CI 6·41–7·43) in the population. Higher adjusted seroprevalence was found in female participants than in male participants (table 1). Adjusted seroprevalence was lower in those aged 12–17 years than in those aged 18 years and older. Among 83 health workers included at baseline, seven were positive for antibodies, and their seroprevalence was higher than that of community workers. Among all participants who tested positive, 437 (82·1%)

	Analysable population at baseline	Individuals who tested positive for pan-immunoglobulins	Adjusted seroprevalence (95% CI)*
Overall	9542 (100%)	532/9542 (5·6%)	6·92% (6·41–7·43)
Sex			
Male	4658 (48·8%)	217/4658 (4·7%)	6·22% (5·53–6·91)
Female	4884 (51·2%)	315/4884 (6·4%)	7·70% (6·95–8·45)
Age group, years			
0–5	303 (3·2%)	14/303 (4·6%)	5·33% (2·80–7·86)
6–11	682 (7·1%)	23/682 (3·4%)	4·72% (3·13–6·31)
12–17	485 (5·1%)	16/485 (3·3%)	3·22% (1·65–4·79)
18–44	3905 (40·9%)	214/3905 (5·5%)	6·65% (5·87–7·43)
45–65	3340 (35·0%)	202/3340 (6·0%)	7·71% (6·81–8·61)
≥66	827 (8·7%)	63/827 (7·6%)	9·51% (7·51–11·51)
Occupation			
Health workers	83 (0·9%)	7/83 (8·4%)	14·83% (7·18–22·48)
Community workers	829 (8·7%)	34/829 (4·1%)	4·37% (2·98–5·76)
Volunteers in pandemic†	719 (7·5%)	36/719 (5·0%)	6·26% (4·49–8·03)
Other	7911 (82·9%)	455/7911 (5·8%)	7·22% (6·65–7·79)
Underlying disease‡			
No	7840 (82·2%)	426/7840 (5·4%)	6·70% (6·15–7·25)
Yes	1702 (17·8%)	106/1702 (6·2%)	7·92% (6·64–9·20)
Self-reported symptom§			
No	9118 (95·6%)	437/9118 (4·8%)	5·99% (5·50–6·48)
Yes	424 (4·4%)	95/424 (22·4%)	26·13% (21·95–30·31)
Visited hospital in the past 5 months			
No	9281 (97·3%)	454/9281 (4·9%)	6·11% (5·62–6·60)
Yes	261 (2·7%)	78/261 (29·9%)	36·65% (30·80–42·50)
Known contact with an individual with COVID-19 in the past 5 months			
No	9289 (97·3%)	474/9287 (5·1%)	6·33% (5·83–6·83)
Yes	253 (2·7%)	58/253 (22·9%)	26·81% (21·35–32·27)
Known contact with people with respiratory infections before enrolment			
No	9023 (94·6%)	447/9023 (5·0%)	6·19% (5·69–6·69)
Yes	519 (5·4%)	85/519 (16·4%)	19·55% (16·14–22·96)
Family size (number of people)			
1	816 (8·6%)	64/816 (7·8%)	8·34% (6·44–10·24)
2–3	4839 (50·7%)	288/4839 (6·0%)	6·91% (6·17–7·65)
≥4	3887 (40·7%)	128/3887 (3·3%)	6·53% (5·73–7·33)

Data are n (%) or n/N (%) unless otherwise indicated. *Seroprevalence is adjusted for sex, age group, and district. †Volunteers in the pandemic included, but are not limited to, drivers, cleaners in medical facilities, and construction workers who were involved in the implementation of prevention and control measures. ‡Underlying diseases included hypertension, pulmonary disease, cancer (undergoing chemotherapy), diabetes, cardiovascular disease, chronic kidney disease, chronic liver disease, and immunodeficiency disease, among others. §Including fever or respiratory symptoms, or both.

Table 1: Baseline demographic and clinical characteristics and seroprevalence of antibodies against SARS-CoV-2 in the analysable population
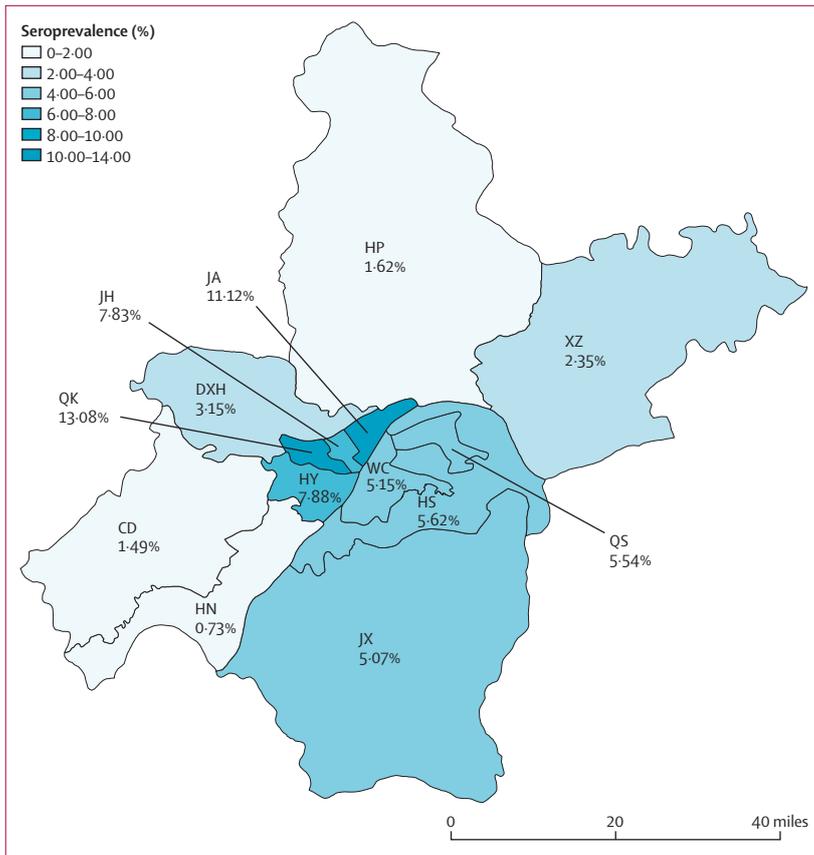
Figure 3: The seroprevalence of anti-SARS-CoV-2 antibodies in different areas of Wuhan, China, at baseline
Population densities of each district are shown on appendix 2 p 10. CD=Caidian. DXH=Dongxihu. HN=Hannan. HP=Huangpi. HS=Hongshan. HY=Hanyang. JA=Jiang'an. JH=JiangHan. JX=Jiangxia. QK=Qiaokou. QS=Qingshan. WC=Wuchang. XZ=Xinzhou.

SARS-CoV-2 (appendix 2 p 9). The estimates varied notably across districts, with seroprevalence ranging from 0·7% to 13·1% at baseline (figure 3; appendix 2 p 10).

All 532 individuals who were positive for pan-immunoglobulins at baseline were positive for IgG, 69 (13·0%) were positive for IgM, 84 (15·8%) were positive for IgA, and 212 (39·8%) were positive for neutralising antibodies (table 2). More female participants than male participants had IgG and neutralising antibodies (appendix 2 p 11). A lower proportion of participants aged 6–11 years and 12–17 years were positive for IgG antibodies than in the other age groups (appendix 2 p 11).

363 individuals who were positive for pan-immunoglobulins from 270 families attended the first follow-up visit and 454 individuals from 343 families attended the second follow-up visit. At the second follow-up visit, more than 90% of individuals were still positive for IgG, although the proportion who were positive for IgA and IgM decreased over time (table 2). All individuals in the matched negative families were still negative for anti-SARS-CoV-2 antibodies at the follow-up visits. These findings emphasise that there was no sustained exposure to SARS-CoV-2 in Wuhan, China. 212 (40%) of individuals who were positive for pan-immunoglobulins against SARS-CoV-2 had neutralising antibodies at the baseline visit, a proportion that remained stable over the two follow-up visits (table 2). Although the proportion of individuals who were positive for IgG were comparable among the confirmed cases and the symptomatic and asymptomatic individuals (table 2), the proportion of participants who were positive for neutralising antibodies was higher in confirmed cases and symptomatic individuals than in asymptomatic individuals (table 2). Generally, the titres of pan-immunoglobulins, IgG, and IgA continually decreased significantly across the study period (all p≤0·01; appendix 2 p 12). IgM titres also decreased over time and were significantly lower in the samples taken during the first follow-up than in those taken at baseline and no significant difference was seen between samples taken at the first follow-up and second follow-up visits (appendix 2 p 12).

Of individuals who were positive for pan-immunoglobulins against SARS-CoV-2 at baseline, 335 attended both follow-up visits and provided three serum samples in total, while others were lost to follow-up or did not attend all visits. Between the asymptomatic and symptomatic individuals and confirmed cases, no difference was seen in the proportion who were positive for neutralising antibodies between the baseline and the two follow-up visits (table 3). The proportion of patients who were positive for IgM, IgA, and IgG decreased in all three subgroups from baseline to the second follow-up visit (appendix 2 p 13).

Among the 335 participants who were positive for pan-immunoglobulins and who had three consecutive

of 532 self-reported no COVID-19-related symptoms, such that the adjusted seroprevalence among individuals with asymptomatic infection was 5·99% (95% CI 5·50–6·48), which was lower than that among individuals with symptoms (26·13% [21·95–30·31]). Participants who had visited hospital in the past 5 months, had a known contact with someone with a confirmed COVID-19 diagnosis, and people with respiratory symptoms in the past 5 months had a significantly higher seroprevalence than those without these risk factors (table 1).

The 532 positive individuals were from 391 families, which were defined as positive families. To assess whether family-size-related antibody-positive clusters existed, we compared the number of family members in positive families and negative families. Among the 3556 families in the baseline population, 1984 (55·8%) had two or three members. We found that 209 (10·5%) of 1984 families with two or three members were positive for antibodies, and that 118 (15·6%) of 756 families with four or more members were positive for antibodies, indicating that families with more members have an increased likelihood of contracting

serum samples, neutralising antibody titres did not significantly decreased over the study period (median 1/5·6 [IQR 1/2·0 to 1/14·0] at baseline *vs* 1/5·6 [1/4·0 to 1/11·2] at first follow-up [p=1·0], and 1/6·3 [1/2·0 to 1/12·6] at second follow-up [p=0·29]; figure 4A). IgG levels in confirmed cases and symptomatic individuals were higher than those in asymptomatic individuals at baseline, whereas the titres were similar between the symptom subgroups at the second follow-up visit (figure 4B). Moreover, neutralising antibody titres were lower in asymptomatic individuals than in confirmed cases and symptomatic individuals across all three visits (figure 4C). Diverse antibody kinetics were found in the study participants. Among the 335 individuals who had three consecutive plasma samples, we found that IgG antibody concentrations were relatively stable in 98 individuals over the study period, continuously increasing in 65 individuals, and continuously decreasing in 114 (appendix 2 p 14). We found no association between sustained concentrations of neutralising antibodies and the sex or age of participants, although we did find an association with whether they were symptomatic or not (appendix 2 p 15).

Discussion

We found that the adjusted seroprevalence of pan-immunoglobulins against SARS-CoV-2 in Wuhan in April, 2020, was 6·92% (95% CI 6·41–7·43), and that more than 80% of people infected with SARS-CoV-2 were asymptomatic during the first wave of the pandemic. Among those with available data, the proportions of participants who were positive for IgG and neutralising antibodies and the concentrations of neutralising antibodies were relatively stable for at least 9 months across the study period, regardless of whether the individuals were symptomatic or not. Individuals who were positive for antibodies against SARS-CoV-2 tended to be clustered in family groups.

The seroprevalence of SARS-CoV-2 varies at the population level.[8,9,12,19,20] Liu and colleagues[21] completed a cross-sectional study involving healthy adults in Wuhan and found a seropositivity rate for antibodies against SARS-CoV-2 of 3·9%, which is lower than our estimate. The disparity between these two estimates might be attributable to potential sampling bias because the convenient samples in Liu and colleagues' study comprised only adults, with few participants older than 60 years, whose seroprevalence is known to be relatively high.[9] Another study in Wuhan included small numbers of voluntary participants from different settings,[10] and such sampling cannot represent the whole population accurately. To date, most reports indicate similar antibody positivity between the sexes. We found a lower seroprevalence of IgG and neutralising antibodies in male participants than in female participants. We observed differences in seroprevalence between the central and rural areas of Wuhan. Since the outbreak was first reported in JiangHan district, one of the

	IgG	IgA	IgM	Neutralising antibodies
Baseline (n=532)	532 (100%)	84 (15·8%)	69 (13·0%)	212 (39·8%)
Confirmed cases (n=30)	30 (100%)	3 (10·0%)	2 (6·7%)	18 (60·0%)
Symptomatic infection (n=65)	65 (100%)	12 (18·5%)	3 (4·6%)	36 (55·4%)
Asymptomatic infection (n=437)	437 (100%)	69 (15·8%)	63 (14·4%)	158 (36·2%)
p value	NA	0·56	0·051	0·0009
First follow-up (n=363)	354 (97·5%)	36 (9·9%)	14 (3·9%)	162 (44·6%)
Confirmed cases (n=27)	27 (100%)	2 (7·4%)	0	15 (55·6%)
Symptomatic infection (n=56)	56 (100%)	6 (10·7%)	2 (3·6%)	35 (62·5%)
Asymptomatic infection (n=280)	271 (96·8%)	28 (10·0%)	12 (4·3%)	112 (40·0%)
p value	0·25	0·89	0·54	0·0042
Second follow-up (n=454)	413 (91·0%)	16 (3·5%)	7 (1·5%)	187 (41·2%)
Confirmed cases (n=29)	26 (89·7%)	0	0	17 (58·6%)
Symptomatic infection (n=63)	58 (92·1%)	0	1 (1·6%)	38 (60·3%)
Asymptomatic infection (n=362)	329 (90·9%)	18 (5·0%)	6 (1·7%)	132 (36·5%)
p value	0·92	0·092	0·78	0·0026

p values are for the comparison in proportions of patients in each symptom subgroup who were positive for each antibody at each timepoint, calculated using the χ^2 test. For the comparison of proportions of patients who are positive for neutralising antibodies: the p values were 0·84 at baseline, 0·71 at first follow-up and 0·94 at second follow-up for confirmed cases *vs* symptomatic individuals; 0·016 at baseline, 0·17 at first follow-up, and 0·030 at second follow-up for confirmed cases *vs* asymptomatic individuals, and 0·0046 at baseline, 0·0032 at first follow-up, and 0·0006 at second follow-up for symptomatic *vs* asymptomatic individuals.

Table 2: Temporal changes in the proportion of participants who were positive for IgG, IgA, IgM, and neutralising antibodies among those who were positive for antibodies against SARS-CoV-2

	Baseline	First follow-up	Second follow-up	p value
Confirmed (n=27)*	18 (66·7%)	14 (51·9%)	16 (59·3%)	0·54
Symptomatic (n=55)†	35 (63·6%)	35 (63·6%)	35 (63·6%)	1·000
Asymptomatic (n=253)‡	88 (34·8%)	94 (37·2%)	103 (40·7%)	0·38
Total (n=335)	141 (42·1%)	143 (42·7%)	154 (46·0%)	0·55
p value	<0·0001	0·0009	0·003	..

*The diagnosis of COVID-19 was confirmed by quantitative PCR assay and lung CT scan, according to diagnostic guidelines for COVID-19. †Participants self-reported fever of respiratory symptoms, or both. ‡Participants self-reported having no fever or respiratory symptoms.

Table 3: Seroconversion rates of neutralising antibodies in 335 participants who were positive for pan-immunoglobulins against SARS-CoV-2 and who had three consecutive serum samples, by COVID-19 symptom subgroup

central districts, the geographical distribution of infections indicates that routes of transmission were stopped by these measures. We observed the highest seroprevalence in people aged 66 years and older, which is similar to findings in some parts of the USA, such as southern Florida and western Washington;[22] however, this finding is not consistent across all population-level reports.[9]

In our study, the proportion of asymptomatic individuals was much higher than the average proportions of 40–45% that have been reported worldwide.[7] This discrepancy might be due to recall bias, because participants were asked to self-report whether they had symptoms over the past 5 months. However, such recall bias is unlikely to overestimate the incidence of asymptomatic infection to a large extent in our study. After the lockdown of Wuhan, stringent measures were
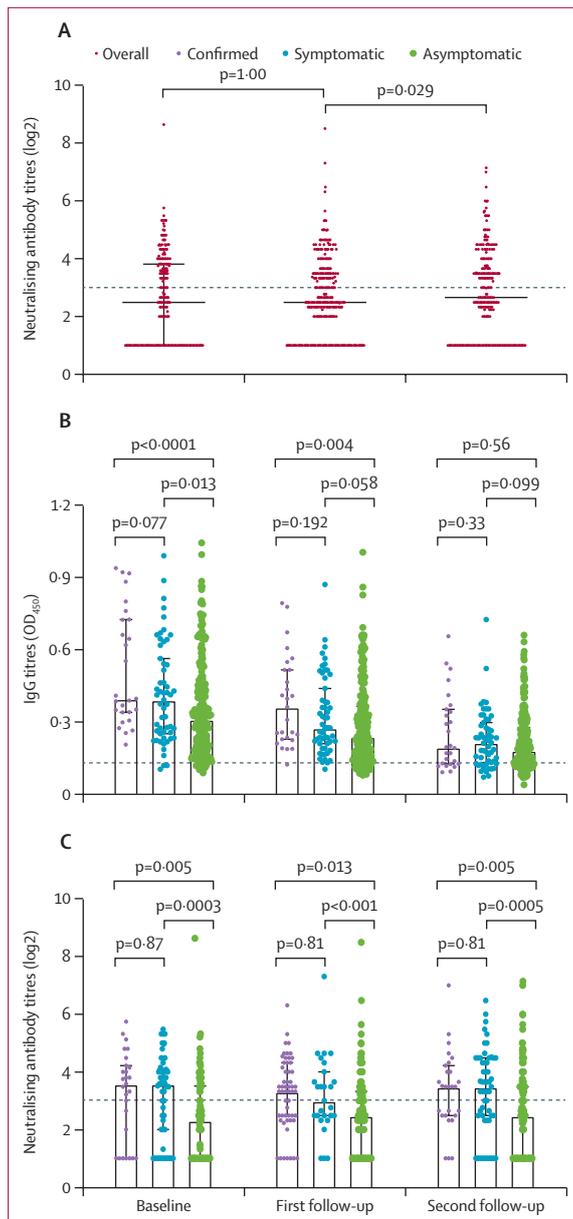
Figure 4: **Longitudinal changes in titres of antibodies against SARS-CoV-2 in 335 participants who had three consecutive serum samples across the study period**

(A) Longitudinal changes in neutralising antibody titres overall.
(B, C) Longitudinal changes in IgG and neutralising antibody titres in individuals with confirmed infection, symptomatic individuals, and asymptomatic individuals. Each datapoint indicates a serum sample and vertical bars denote median with IQRs. The y axis is on a logarithmic scale. p values were calculated using the Kruskal-Wallis test. OD_{450}=optical density at 450 nm.

taken to identify individuals infected with SARS-CoV-2 including testing all individuals with fever or respiratory symptoms, or both; quarantining and isolating close contacts; actively identifying infected individuals via door-to-door investigation and self-report; and treating each individuals with a confirmed infection in hospital. 50 008 confirmed COVID-19 cases had been identified as

of April 8, 2020, and all of them had been admitted to and treated in hospital.[23] Moreover, because residents of Wuhan were vigilant to the need to record their symptoms during the COVID-19 outbreak, the magnitude of recall bias might be reduced. Confirmed COVID-19 cases and symptomatic individuals accounted for 18% of participants who were antibody positive. This study was done after the lockdown was lifted in Wuhan, and most of the patients who had been admitted to hospital for COVID-19 had been discharged, which enabled us to obtain representative samples without obvious selection bias.

Knowing the population-level seroprevalence and kinetics of humoral immunity are crucial for vaccination strategies.[24] However, little is known of the durability of humoral responses against SARS-CoV-2 over a long period. Pan-immunoglobulins against SARS-CoV-2 have been reported to remain stable within 4 months after a PCR-based diagnosis of COVID-19,[12] and titres have been reported to remain relatively stable for 5 months after infection.[13] In our study, we found that the proportion of participants with antibodies against SARS-CoV-2 was sustained for at least 9 months. Importantly, we found that neutralising antibody titres remained stable for at least 9 months.

A previous study showed that the protection induced by seasonal human coronaviruses might last for 1–2 years.[25] Neutralising antibodies against SARS-CoV were detectable 2 years after infection,[26] and IgG and neutralising antibodies against MERS-CoV were detectable up to 15 months after symptom onset.[24] Further investigations on IgG and neutralising antibodies against SARS-CoV-2 should be done to assess the kinetics of waning immunity over longer time periods.

The immune response of individuals after natural exposure to SARS-CoV-2, especially the presence of neutralising antibodies in asymptomatic individuals, which accounts for the majority of SARS-CoV-2 infections, is not well understood. Additionally, seroconversion of neutralising antibodies in patients with mild COVID-19 might take longer than in those with severe disease.[13,27] Wajnberg and colleagues[13] reported that 50–100% of patients with confirmed COVID-19 seroconverted to have neutralising antibodies. We observed similar rates of neutralising antibodies in confirmed cases at baseline (18 [60%] of 30, but a relatively low proportion of asymptomatic individuals had neutralising antibodies (158 [36%] of 437). In both Wajnberg and colleagues' study and our study the presence of neutralising antibodies was determined using cultures of the virus,[13] although different methods were used to interpret the neutralising antibodies titres. We believe the disparity in the proportion of seroconverters is more likely to be related to the high proportion of asymptomatic infections in our study, rather than differences in methods.

Regardless of symptoms, we found no significant decrease in neutralising antibody titres over the study period, even though the titres were relatively low. Such

findings were not consistent with a hospital-based study, in which neutralising antibody titres decreased significantly over 3 months.[28] The different sampling intervals at population-level and hospital-based studies might cause such disparities. In another study,[29] most patients with COVID-19 were reported to have low levels of neutralising antibodies in their convalescent serum samples. All these individuals had receptor-binding domain-specific antibodies with potent antiviral activity.[29] Although the definite protective activity of neutralising antibodies remains unclear, understanding the wan of neutralising antibody responses at the population level is crucial for herd immunity.

After acute viral infection, the number of plasma cells peaks at day 6 or 7 and decreases to baseline levels within 2–3 weeks after the onset of disease.[30] However, long-term serum antibody production is maintained by long-lived plasma cells.[31] Thus, sustained production of neutralising antibodies in individuals with asymptomatic and mild infections is probably produced by long-lived plasma cells. Follicular and extrafollicular B cells and their development will also contribute to the kinetics of the antibody responses.[32]

A key strength of this study is that the seroprevalence and kinetic changes in antibodies, including pan-immunoglobulins, IgG, IgA, IgM, and especially neutralising antibodies, against SARS-CoV-2 were assessed in a general population-based study in China for the first time to our knowledge. In a city-wide SARS-CoV-2 nucleic acid screening programme run between May 14 and June 1, 2020, in which 9 899 828 participants were tested in Wuhan, no symptomatic cases and 300 asymptomatic cases were identified.[33] These findings suggested a very low prevalence of SARS-CoV-2 5–8 weeks after the end of city-wide lockdown. Furthermore, we followed up location-matched families who were negative to anti-SARS-CoV-2 antibodies to confirm that no new infections with SARS-CoV-2 occurred in our sampled population. Hence, for our study we were able to estimate the seroprevalence and humoral immune durability of anti-SARS-CoV-2 antibodies without bias caused by duplicate exposure. Thus, our study avoided the interference of repeated exposure that other studies might face, especially in countries that are still having surges in infection rates. A representative sample was randomly selected from all districts in Wuhan using probability-proportionate-to-size sampling. Furthermore, we adjusted the seroprevalence to match the population profile in Wuhan. Importantly, all samples that were positive for pan-immunoglobulins were examined in the presence of neutralising antibodies using live SARS-CoV-2, providing authentic neutralising activity.

Our study also has several limitations. Because most of the individuals with antibodies were not confirmed cases and reported no COVID-19-related symptoms, we cannot determine when they were infected, which restricts our ability to assess when the antibody was produced.

Nevertheless, because very few cases of COVID-19 were reported between the middle of March and April, 2020, in Wuhan,[23] we reasoned that these samples were collected at least 4 weeks after antibody production. Additionally, because the COVID-19-related symptoms were self-reported by the participants, another potential limitation is recall bias, although we believe such bias is unlikely to have resulted in too much overestimation of the incidence of asymptomatic infection in our study.

In summary, we provide regional estimates of the spread of SARS-CoV-2 in Wuhan, China, between April and December, 2020. Even at the epicentre of the pandemic, with more than 50 000 confirmed cases as of April 8, 2020, the estimated seroprevalence remains low, suggesting that vaccinations will be required to promote herd immunity. We found that the proportion of infections that are asymptomatic can be as high as 80%, suggesting that symptoms in many infected individuals might be too mild for them to need medical attention. The proportions of participants who were positive for IgG and neutralising antibodies were stable for at least 9 months after exposure, regardless of whether individuals were asymptomatic, which indicates that passive and active immune strategies could be considered to protect the at-risk population from severe infection or reinfection.

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