High SARS-CoV-2 seroprevalence in health care workers but relatively low numbers of deaths in urban Malawi [version 2; peer review: 2 approved]

Marah G. Chibwana\textsuperscript{1}\textsuperscript{*}, Khuzwayo C. Jere\textsuperscript{1,2}\textsuperscript{*}, Raphael Kamng'ona\textsuperscript{1}, Jonathan Mandolo\textsuperscript{1}, Vincent Katunga-Phiri\textsuperscript{1}, Dumizulu Tembo\textsuperscript{1}, Ndaona Mitole\textsuperscript{1}, Samantha Musasa\textsuperscript{3}, Simon Sichone\textsuperscript{1}, Agness Lakudzala\textsuperscript{1}, Lusako Sibale\textsuperscript{1}, Prisca Matambo\textsuperscript{1}, Innocent Kadwala\textsuperscript{1}, Rachel L. Byrne\textsuperscript{4}, Alice Mbewe\textsuperscript{1}, Marc Y. R. Henrion\textsuperscript{1,4}, Ben Morton\textsuperscript{1,4}, Chimota Phiri\textsuperscript{5}, Jane Mallewa\textsuperscript{3}, Henry C. Mwandumba\textsuperscript{1,4}, Emily R. Adams\textsuperscript{4}, Stephen B. Gordon\textsuperscript{1,4}\textsuperscript{*}, Kondwani C. Jambo\textsuperscript{1,4}\textsuperscript{*}

\textsuperscript{1}Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Blantyre, Malawi
\textsuperscript{2}Centre for Global Vaccine Research, Institute of Infection, Veterinary Ecological Sciences, University of Liverpool, Liverpool, UK
\textsuperscript{3}Department of Medicine, College of Medicine, University of Malawi, Blantyre, Malawi
\textsuperscript{4}Liverpool School of Tropical Medicine, Liverpool, L3 5QA, UK
\textsuperscript{5}Ministry of Health, Queen Elizabeth Central Hospital, Blantyre, Malawi

* Equal contributors

Abstract

\textbf{Background:} In low-income countries, like Malawi, important public health measures including social distancing or a lockdown have been challenging to implement owing to socioeconomic constraints, leading to predictions that the COVID-19 pandemic would progress rapidly. However, due to limited capacity to test for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, there are no reliable estimates of the true burden of infection and death. We, therefore, conducted a SARS-CoV-2 serosurvey amongst health care workers (HCWs) in Blantyre city to estimate the cumulative incidence of SARS-CoV-2 infection in urban Malawi.

\textbf{Methods:} We recruited 500 otherwise asymptomatic HCWs from Blantyre City (Malawi) from 22\textsuperscript{nd} May 2020 to 19\textsuperscript{th} June 2020 and serum samples were collected from all participants. A commercial ELISA was used to measure SARS-CoV-2 IgG antibodies in serum.

\textbf{Results:} A total of 84 participants tested positive for SARS-CoV-2 antibodies. The HCWs with positive SARS-CoV-2 antibody results came from different parts of the city. The adjusted seroprevalence of SARS-
CoV-2 antibodies was 12.3% [CI 8.2 - 16.5]. Using age-stratified infection fatality estimates reported from elsewhere, we found that at the observed adjusted seroprevalence, the number of predicted deaths was eight times the number of reported deaths. **Conclusions:** The high seroprevalence of SARS-CoV-2 antibodies among HCWs and the discrepancy in the predicted versus reported deaths suggests that there was early exposure but slow progression of COVID-19 epidemic in urban Malawi. This highlights the urgent need for development of locally parameterised mathematical models to more accurately predict the trajectory of the epidemic in sub-Saharan Africa for better evidence-based policy decisions and public health response planning.

**Keywords**
SARS-CoV-2, COVID-19, Malawi, Seroprevalence, IgG
Corresponding author: Kondwani C. Jambo (kondwani.jambo@lstmed.ac.uk)

Author roles: Chibwana MG: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing; Jere KC: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing; Kamng’ona R: Investigation, Methodology, Project Administration, Writing – Original Draft Preparation, Writing – Review & Editing; Mandolo J: Formal Analysis, Investigation, Methodology, Writing – Original Draft Preparation, Writing – Review & Editing; Tembo D: Investigation, Methodology, Supervision, Validation, Writing – Original Draft Preparation, Writing – Review & Editing; Mitole N: Investigation, Project Administration, Writing – Original Draft Preparation, Writing – Review & Editing; Musasa S: Investigation, Methodology, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing; Sibale L: Investigation, Writing – Original Draft Preparation, Writing – Review & Editing; Matambo P: Investigation, Writing – Original Draft Preparation, Writing – Review & Editing; Kadwala I: Investigation, Writing – Original Draft Preparation, Writing – Review & Editing; Byrne RL: Resources, Writing – Original Draft Preparation, Writing – Review & Editing; Phiri C: Conceptualization, Writing – Original Draft Preparation, Writing – Review & Editing; Mallewa J: Conceptualization, Writing – Original Draft Preparation, Writing – Review & Editing; Gordon SB: Conceptualization, Funding Acquisition, Investigation, Methodology, Writing – Original Draft Preparation, Writing – Review & Editing; Adams ER: Methodology, Resources, Writing – Original Draft Preparation, Writing – Review & Editing; Jambo KC: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Supervision, Validation, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: E.R.A. and R.L.B. worked with Mologic (UK) to independently validate the SARS-CoV-2 ELISA at the Liverpool School of Tropical Medicine (LSTM). All other authors report no potential conflicts.

Grant information: This work was funded by the Wellcome Trust through a Wellcome Training Fellowship [201945; to K.C.J]; the Wellcome Trust/DFID Joint Initiative for Research in Epidemic Preparedness and Response [220764; to E.R.A]; and the Malawi-Liverpool-Wellcome Trust Clinical Research Programme [MLW] was supported by a strategic award from the Wellcome Trust awarded to S.B.G. K.C.J was also supported by the NIHR and MRC through grant numbers 3477609 and MR/T008822/1, respectively. M.G.C. is supported by NIH through grant number U01AI131348. E.R.A is also supported by the NIH HPRU [200907] in Emerging and Zoonotic Infection. Disclaimer: The funders had no role in the study design, data collection and interpretation, or the decision to submit the work for publication. Mologic (UK) was provided the opportunity to review a preliminary version of this manuscript for factual accuracy but the authors are solely responsible for final content and interpretation. The authors received no financial support or other form of compensation related to the development of the manuscript. The findings and conclusions in this report are those of the authors. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2020 Chibwana MG et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Chibwana MG, Jere KC, Kamng’ona R et al. High SARS-CoV-2 seroprevalence in health care workers but relatively low numbers of deaths in urban Malawi [version 2; peer review: 2 approved] Wellcome Open Research 2020, 5:199 https://doi.org/10.12688/wellcomeopenres.16188.2

Introduction
Coronavirus disease 2019 (COVID-19) has had a dramatic impact worldwide, with high mortality in Asia, Europe and the Americas\(^1\). Africa reported its first COVID-19 case on 14\(^{th}\) February 2020\(^2\). Due to poor socio-economic conditions, high HIV prevalence, an increase in non-communicable diseases and challenged health system infrastructure, it was predicted that the African pandemic would progress rapidly. As of 16\(^{th}\) July 2020, however, the number of COVID-19 cases was 665,522 and deaths 14,434\(^3\), much lower than predicted by mathematical models\(^4\).

In low-income countries, like Malawi, important public health measures like social distancing or a lockdown are difficult to implement owing to socioeconomic constraints. Furthermore, the limited capacity to test for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection impedes effective public health response planning. Initial testing in Malawi focused on case identification in patients with COVID-19-like symptoms, contacts of index patients and inbound travellers. The first COVID-19 case in Malawi was reported on 2\(^{nd}\) April and as of 16\(^{th}\) July 2020 there were 2716 cases with only 51 deaths reported\(^5\). Given the sampling strategy, the true burden is certainly much greater than the reported cases, but there are no reliable estimates of the true burden of infection and death. Up to now, health services have reported only small number of cases and have not been overwhelmed as predicted\(^6\).

The unrestricted nature of the COVID-19 epidemic in Malawi provides an opportunity to compare its trajectory in a low-income setting with what has been reported in high income settings. It has been shown that the rate of asymptomatic SARS-CoV-2 infection among health care workers (HCWs) reflects general community transmission rather than in-hospital exposure\(^7\). We, therefore, conducted a SARS-CoV-2 serosurvey amongst HCWs in Blantyre city to estimate the cumulative incidence of SARS-CoV-2 infection in urban Malawi.

Methods
Ethical statement
Ethical approval was provided by the College of Medicine Research and Ethics Committee (COMREC, Malawi) (P.05/20/3045) and Liverpool School of Tropical Medicine (LSTM, UK) (20-043). All participants gave informed written consent for participation in the study, publication of the data and storage/usage of collected samples.

Study setting and participants
Participant recruitment was done at the Malawi-Liverpool-Wellcome Trust Clinical Research programme (MLW) in Blantyre, Malawi, as part of an ongoing longitudinal study that seeks to investigate markers of SARS-CoV-2 exposure and immunological protection in Malawian adults. The study site is within the compound of the Queen Elizabeth Central Hospital (QECH), the largest tertiary teaching hospital in Malawi. It is easily accessible from most parts of the city. Participants were HCWs from Blantyre City, both clinical and non-clinical. We used a convenience sampling approach, whereby the study was advertised electronically and by word of mouth. The sample size was calculated based on the study primary objective, which was to compare the SARS CoV-2 neutralising antibody titers in recovered COVID-19 patients compared to SARS CoV-2 antibody positive (asymptomatic/mild) individuals. Inclusion criteria for the study included being an HCW resident in Blantyre, aged between 18 and 65 years old, and otherwise asymptomatic. The exclusion criterion was withholding consent.

Electronic case report forms (eCRFs) were used to collect demographic data including age, gender, place of residence, common mode of transportation, occupation and involvement in COVID-19 work. The samples used for this manuscript are from the baseline recruitment arm of the asymptomatic group in the main study.

Sample collection, processing and experimental setup
Peripheral blood (10ml), 7ml in Sodium Heparin tubes and 3ml in serum separation tubes (SST) (All BD Biosciences), was collected from all study participants using venesection by the study clinical team at the study site (MLW) between 22\(^{nd}\) May 2020 and 19\(^{th}\) June 2020. Serum was collected from the SSTs by centrifugation at 500g for 8 mins and stored at -80°C. To measure SARS-CoV-2 antibodies, we used a commercial enzyme linked-imunosorbent assay (ELISA) targeting Spike (S2) and Nucleoprotein (N) from SARS-CoV-2 (Omega diagnostics, UK; ODL 150/10; Lot #103183). The assay was performed as per the manufacturer’s instructions. In brief, participant serum was diluted (1:200) in sample diluent (150M M Tris-buffered saline, pH 7.2 with antimicrobial agent). The diluted samples, diluent alone (negative control), manufacturer’s cutoff control and positive control were added at 100μl per well. The plate was incubated at room temperature for 30 mins. After incubation, the plate was washed three times with wash buffer (100mM Tris-buffered saline with detergent, pH 7.2) using a plate washer (Asys Atlantis, Biochrom Ltd, UK). Anti-human IgG conjugated to horseradish peroxidase was then added to each well at 100μl and incubated for 30 minutes at room temperature. After incubation, the plate was washed four times with wash buffer, and 100μl of TMB (3,3’,5,5’-Tetramethylbenzidine) Substrate (aqueous solution of TMB and hydrogen peroxide) was added. The plate was incubated for 10 minutes at room temperature, before addition of 100μl of Stop Solution (0.25M sulphuric acid). The optical density (OD) of each well was read at 450nm in a microplate reader (BioTek ELx808, UK) within 10 minutes. The assay interpretation was as follows; positive result (OD 0.6), indeterminate result (OD 0.55 to < 0.6) and negative (OD < 0.55) This assay has undergone rigorous independent validation at the Liverpool School of Tropical Medicine (UK) and St George’s University of London (UK)\(^8\).
Statistical analysis
Graphical presentation was performed using GraphPad Prism 8 (GraphPad Software, USA). To integrate uncertainty arising from test sensitivity and specificity, we used a method published by Reiczigel et al., 2010. The geospatial data was plotted in R (v4.0.0) using ggmap (v3.0.0) and ggplot2 (v3.3.1).

Results
Demographics of study participants
We recruited 500 asymptomatic HCWs with a median age of 31 (range 20–64 years). The average household size for the participants was four [confidence interval (CI) 3–5]. Of the 500, 331 were clinical HCWs and 169 non-clinical HCWs (Table 1). The clinical HCWs included nurses, medical doctors and clinical officers, while the non-clinical HCWs included clerical/administration, field workers and laboratory scientists. The primary workstation for the HCWs included primary healthcare facilities (35/500), secondary healthcare facilities (291/500), and clinical research facilities (174/500). The majority of the participants were nurses (57%), 41% of all participants were involved in clinical work related to COVID-19 and 73% of the total participants used public transport or walking as their main means of transport. The main characteristics of the participants are summarised in Table 1.

Seroprevalence of SARS CoV-2 antibodies and geospatial location of the antibody positive individuals
84 participants tested positive for SARS-CoV-2 antibodies (Figure 1). After adjusting for test sensitivity (238/270, 88.1% with 95% confidence interval [83.7%, 91.8%]) and the specificity (82/85, 93.2% with 95% CI [85.7%, 97.5%]), the overall seroprevalence of SARS-CoV-2 antibodies was 12.3% [CI 8.2 – 16.5], assuming that the sensitivity and specificity are known exactly. However, propagation of uncertainty into the final adjusted seroprevalence estimate resulted in wider confidence intervals [3.9%, 19.0%] (See details in Supplementary Material). Together, this suggests that local transmission was high and that SARS-CoV-2 may have been circulating for some time in Blantyre City.

To estimate the potential geographical spread of SARS-CoV-2, we plotted the geographical coordinates of place of residence for the individuals with a positive antibody result on the map of Blantyre City. We found that the HCWs with a positive SARS-CoV-2 antibody result came from different parts of the city (Figure 2). This suggests that SARS-CoV-2 local transmission was likely widespread across the city.

Crude projections of mortality based on seroprevalence estimates
Using estimates of infection fatality rates from Verity et al. and the Malawi population census, we estimated the number of deaths that could have occurred at the observed seroprevalence of SARS-CoV-2 antibodies (Table 2). We adjusted the population estimates by inflating them to take into account population annual population growth rate of 2% from 2018 to 2020. We assumed that there was a uniform risk of infection at all age groups and that the age-stratified population seroprevalence in Malawi was similar to that observed amongst asymptomatic HCWs.

The crude estimates suggest that there should have been at least 138 deaths by 19th June 2020. However, four weeks following the serosurvey, only 17 COVID-confirmed deaths in Blantyre have been reported by the Public Health Institute of Malawi, which is approximately eight times below the predicted deaths. When the seroprevalence is extrapolated to the entire Malawi, it predicts approximately 5,295 COVID-19 deaths, but only 51 deaths have been reported as of 16th July 2020. These crude estimates highlight a discrepancy between the predicted deaths using infection fatality rates from elsewhere and the actual number of reported COVID-19 deaths in Malawi.

Conclusions
To our knowledge, this seroprevalence study is the first to report estimates of SARS-CoV-2 exposure among HCWs in an African urban low-income setting. It provides insights into the potentially unique trajectory of the COVID-19 epidemic in Malawi.

Table 1. Study participant demographics.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number of participants</th>
<th>Proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>236</td>
<td>47%</td>
</tr>
<tr>
<td>Female</td>
<td>264</td>
<td>53%</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20–29</td>
<td>209</td>
<td>42%</td>
</tr>
<tr>
<td>30–39</td>
<td>170</td>
<td>34%</td>
</tr>
<tr>
<td>40–49</td>
<td>86</td>
<td>17%</td>
</tr>
<tr>
<td>50–59</td>
<td>28</td>
<td>6%</td>
</tr>
<tr>
<td>60+</td>
<td>7</td>
<td>1%</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical HCW</td>
<td>331</td>
<td>66%</td>
</tr>
<tr>
<td>Non-clinical HCW</td>
<td>169</td>
<td>34%</td>
</tr>
<tr>
<td>COVID-19 work</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical</td>
<td>205</td>
<td>41%</td>
</tr>
<tr>
<td>Non-clinical</td>
<td>43</td>
<td>9%</td>
</tr>
<tr>
<td>None</td>
<td>252</td>
<td>50%</td>
</tr>
<tr>
<td>Transport</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Public + walking</td>
<td>363</td>
<td>73%</td>
</tr>
<tr>
<td>Private</td>
<td>137</td>
<td>27%</td>
</tr>
</tbody>
</table>

We observe a high seroprevalence of SARS-CoV-2 antibodies amongst HCWs. It has been reported elsewhere that HCWs accounted for a high proportion of cases early in the SARS-CoV-2 outbreak when transmission was increasing sharply and personal protective equipment (PPE) provision was patchy. Our data could suggest that Malawi is relatively early in the epidemic and that COVID-19 cases are likely to continue to rise sharply in the coming weeks, but the serology also suggests that large numbers of cases must be either asymptomatic or only show mild disease.

The discrepancy between the predicted compared to reported mortality at the observed seroprevalence estimate may also suggest that there are large numbers of underreported or misclassified deaths in Malawi. However, even in countries like South Africa with relatively abundant testing capacity and strong health systems, there is relatively low mortality with a case fatality rate of 1.5%. This may imply that the impact of SARS-CoV-2 in Africa is potentially much less severe or is following a different trajectory than that experienced in China, Americas and Europe, where case fatality rates were commonly above five percent. This warrants further investigation.

However, the reasons behind the discrepancy in the COVID-19 pandemic trajectory between SSA and elsewhere might include population demography, climate and prior cross-reactive immunity. In Malawi, for example, the population is younger than in many other regions.
Figure 2. Map of Blantyre showing geospatial distribution of seropositive results. We collected geocoordinate data for the place of residence of all study participants at recruitment. The geocoordinates were combined with the ELISA assay results and plotted on the map of Blantyre using R. Black dot, seronegative; Orange dot, indeterminate; Red dot, seropositive.

Table 2. Crude estimates of predicted mortality at the observed seroprevalence.

<table>
<thead>
<tr>
<th>Age</th>
<th>Population* (Blantyre)</th>
<th>Population* (Malawi)</th>
<th>Infection fatality rate †</th>
<th>Predicted Number of Infections</th>
<th>Predicted Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Blantyre</td>
<td>Malawi</td>
</tr>
<tr>
<td>0–9 yrs</td>
<td>207,002</td>
<td>5,394,769</td>
<td>0.00%</td>
<td>24,840</td>
<td>663,557</td>
</tr>
<tr>
<td>10–19 yrs</td>
<td>199,915</td>
<td>4,753,846</td>
<td>0.01%</td>
<td>23,990</td>
<td>584,723</td>
</tr>
<tr>
<td>20–29 yrs</td>
<td>176,360</td>
<td>2,997,379</td>
<td>0.03%</td>
<td>21,163</td>
<td>368,678</td>
</tr>
<tr>
<td>30–39 yrs</td>
<td>130,362</td>
<td>2,160,103</td>
<td>0.08%</td>
<td>15,643</td>
<td>265,693</td>
</tr>
<tr>
<td>40–49 yrs</td>
<td>67,618</td>
<td>1,316,593</td>
<td>0.16%</td>
<td>8,114</td>
<td>161,941</td>
</tr>
<tr>
<td>50–59 yrs</td>
<td>28,397</td>
<td>722,800</td>
<td>0.60%</td>
<td>3,408</td>
<td>88,904</td>
</tr>
<tr>
<td>60–69 yrs</td>
<td>15,225</td>
<td>494,678</td>
<td>1.93%</td>
<td>1,827</td>
<td>60,845</td>
</tr>
<tr>
<td>70–79 yrs</td>
<td>5,715</td>
<td>280,394</td>
<td>4.28%</td>
<td>686</td>
<td>34,488</td>
</tr>
<tr>
<td>80+ yrs</td>
<td>2,001</td>
<td>152,762</td>
<td>7.80%</td>
<td>240</td>
<td>18,790</td>
</tr>
<tr>
<td>Total</td>
<td>832,595</td>
<td>18,273,324</td>
<td></td>
<td>99,911</td>
<td>2,247,619</td>
</tr>
</tbody>
</table>

*Source: 2018 Population and Housing Census. † Estimates derived from Verity, R. et al. (2020). The total number of reported COVID-19 deaths on 16th July in Blantyre was 17 and in Malawi was 51.
(median age 17 years old)\(^2\), and the elderly who mostly experience worse outcomes in other settings\(^3\), are 5.1% of the population\(^4\), largely residing in rural areas. If the prevalence of SARS-CoV-2 is very low in rural areas, this may explain the low number of deaths, and would strengthen the call to shield the elderly\(^5\).

This study has some limitations. First, selection bias is likely due to the convenience sampling approach; however, targeting HCW for regular serosurveys could help predict local transmission outbreaks. Second, this serosurvey focused on an urban population where Malawi has reported the highest concentration of COVID-19 cases. The seroprevalence in the rural population remains unknown, but if high, may prompt other explanations for the African/Malawi situation. Third, current SARS-CoV-2 ELISAs are still undergoing rigorous validation and verification in the African settings, hence seroprevalence estimates could change with new information on the accuracy of the test kits. In addition, not all individuals exhibit SARS-CoV-2 antibodies following COVID-19, hence the seroprevalence would be an underestimate of true exposure.

In conclusion, our findings indicate a major discrepancy between predicted COVID-19 mortality at the observed SARS-CoV-2 seroprevalence in HCWs with reported COVID-19 deaths in urban Malawi. The high seroprevalence estimate implies earlier seroprevalence in HCWs with reported COVID-19 deaths in urban Malawi. The high seroprevalence estimate implies earlier exposure of SARS-CoV-2 than that reported but with slow progression of the COVID-19 epidemic. Development of locally parameterised mathematical models should be prioritised to more accurately predict the trajectory of the epidemic in SSA. This will allow better evidence-based policy decision-making and public health response planning.

**Data availability**

Figshare: High SARS-CoV-2 seroprevalence in Health Care Workers but relatively low numbers of deaths in urban Malawi. https://doi.org/10.6084/m9.figshare.12745214.v3\(^10\)

This project contains the following underlying data:
- PROTECT_FigShare Part 1 31072020.csv
- PROTECT_screening_enrolment_data_dictionary Figshare 31072020.pdf

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

**Acknowledgements**

We acknowledge the PROTECT Study Team at the Malawi-Liverpool-Wellcome Trust Clinical Research Programme, namely Alice Kaililani, Gift Sagawa, Martha Moyo, Orpha Kumwenda, Sharon Nihala, Chisomo Jassi, Neema Nyakuleha and Tayamika Banda.

We thank Dr Joe Fitchett at Mologic (UK) for providing the SARS-CoV-2 ELISA kits and Dr Peter McPherson (MLW/LSTM) for the epidemiological support.
Open Peer Review

Current Peer Review Status: ✔ ✔

Version 2

Reviewer Report 18 December 2020

https://doi.org/10.21956/wellcomeopenres.18194.r41879

© 2020 Burgess S. This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Stephen Burgess
MRC Biostatistics Unit, University of Cambridge, Cambridge, UK

Thank you for making the changes as requested. The updated Table 2 is now much clearer that these are predicted numbers, not observed numbers.

My only reservation is to suggest that the proportions of infections in clinical versus non-clinical HCWs are provided in the article [13.9% (9.8-18.3) vs 9.5% (4.2-15.3)]. While the authors are technically correct that this difference is not statistically significant, this is due to imprecision of the two estimates. The proportion of infections is 46% higher in clinical versus non-clinical HCWs, and could well be lower still in the general population. At the very least, these numbers are of relevance to the interested reader.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Statistics, Epidemiology.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 11 November 2020

https://doi.org/10.21956/wellcomeopenres.17773.r41042

© 2020 Burgess S. This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
This study is a valuable contribution to the literature. However, potential bias in the sampling strategy should be acknowledged. There are several potential sources of bias.

1. Prevalence of infection amongst healthcare workers (HCW) is likely to be substantially higher than prevalence amongst non-healthcare workers. Therefore the extrapolation of this survey result to the whole of Blantyre city is likely to over-estimate population seroprevalence. A crude test of this would be to estimate seroprevalence separately in clinical and non-clinical HCWs, and according to the strata for "COVID-19 work" in Table 1. I would encourage the authors to present stratified seroprevalence estimates according to these strata. If prevalence is substantially lower in non-clinical HCWs, perhaps the authors should repeat their calculations using the prevalence estimates in this stratum as a supplementary analysis to indicate the level of uncertainty in findings.

2. While I expect point 1 to be the greatest source of bias, the restriction to asymptomatic individuals will also be a source of bias - in this case, this would lead to underestimation of prevalence.

3. By my calculation, the crude seroprevalence is $84/500 = 16.8\%$. But the seroprevalence after adjusting for test sensitivity and specificity is given as 12.3%. What values of specificity and sensitivity were used here? Typically test specificity is very high and sensitivity is lower, meaning that there are more false negatives than false positives. Particularly as sensitivity for asymptomatic individuals is typically lower than manufacturer claimed levels (as manufacturer calibration is typically done using hospitalized cases). If sensitivity is around 50% (as was observed for asymptomatic individuals in Iceland for some antibody tests), what would the corrected seroprevalence be?

4. How many individuals had an intermediate test result?

5. The text states that South Africa had a "case fatality ratio of 1.5", whereas outside of Africa, "case fatality ratios were commonly above five". What do these numbers mean? 1.5 of what?

6. The idea of climate influencing the severity of illness is an important one. Increased ventilation and distancing due to hot climate may lead to lower dosage at the point of viral transmission, which would lead to less severe illness.

7. Table 2: please be more clear which numbers are measured and which numbers are estimates / extrapolation. This should be very clear from a first glance (e.g. in the overall heading and the column heads). A casual glance at this table would lead a reader to believe that there have been 5295 COVID-19 deaths in Malawi.

Overall, it's great to see this in the literature, but I am somewhat uncomfortable about the level of extrapolation here. It would be good if the assumptions were clearly stated in the most prominent parts of the manuscript. For example, after the final line of the results, you could state: "This estimate assumes that age-stratified population seroprevalence in Malawi is similar to that observed amongst asymptomatic HCWs."
Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Statistics, Epidemiology.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 11 Dec 2020

Kondwani Jambo, Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Blantyre, Malawi

Prevalence of infection amongst healthcare workers (HCW) is likely to be substantially higher than prevalence amongst non-healthcare workers. Therefore the extrapolation of this survey result to the whole of Blantyre city is likely to over-estimate population seroprevalence. A crude test of this would be to estimate seroprevalence separately in clinical and non-clinical HCWs, and according to the strata for “COVID-19 work” in Table 1. I would encourage the authors to present stratified seroprevalence estimates according to these strata. If prevalence is substantially lower in non-clinical HCWs, perhaps the authors should repeat their calculations using the prevalence estimates in this stratum as a supplementary analysis to indicate the level of uncertainty in findings.

We agree with the reviewer that the prevalence of SARS-CoV-2 infection is expected to be higher amongst HCW compare to non-HCWs. There is evidence that this is mostly true during the early stages of the pandemic, but as the pandemic matures, the prevalence of SARS-CoV-2 infection among HCW has been shown to reflect general community transmission rather than in hospital exposure [Lancet. 2020; 395(10237): 1608–10]. In this study, the seroprevalence of SARS-CoV-2 antibodies was not significantly different between clinical and non-clinical HCWs [13.90(9.80-
18.30) vs. 9.5(4.2-15.3)]. In our setting, unpublished work from Blantyre City also confirms this observation, showing that the prevalence of PCR-confirmed COVID-19 was similar between HCWs and non-HCWs during the time this study was conducted.

While I expect point 1 to be the greatest source of bias, the restriction to asymptomatic individuals will also be a source of bias - in this case, this would lead to underestimation of prevalence.

We agree with the reviewer that restriction of study to asymptomatic individuals would underestimate the seroprevalence. However, the numbers of symptomatic cases were relatively very low in Malawi, like in most of the sub-Sahara region. Hence, studying asymptomatic provides a good estimate of SARS-CoV-2 exposure.

By my calculation, the crude seroprevalence is 84/500 = 16.8%. But the seroprevalence after adjusting for test sensitivity and specificity is given as 12.3%. What values of specificity and sensitivity were used here? Typically test specificity is very high and sensitivity is lower, meaning that there are more false negatives than false positives. Particularly as sensitivity for asymptomatic individuals is typically lower than manufacturer claimed levels (as manufacturer calibration is typically done using hospitalized cases). If sensitivity is around 50% (as was observed for asymptomatic individuals in Iceland for some antibody tests), what would the corrected seroprevalence be?

We used the independently validated sensitivity (238/270, 88.1% with 95% confidence interval [83.7%, 91.8%]) and specificity (82/85, 93.2% with 95% CI [85.7%, 97.5%]) to adjust our seroprevalence. The independent validation was done by the Liverpool School of Tropical Medicine and St George's University of London [https://doi.org/10.1101/2020.04.29.20082099]. However, since both the sensitivity and specificity are only estimates, we also propagated that uncertainty through into the final adjusted seroprevalence estimate using https://cran.r-project.org/package=bootComb using our published the R code [details are included in the Supplementary Material]. Applying this to our data, we get the same adjusted seroprevalence estimate (12.3%) but with a larger 95% confidence interval: [3.9%, 19.0%]. However, the conclusions from the main text are not affected by this wider confidence intervals.

How many individuals had an intermediate test result?

There were 16 individuals with an indeterminate result.

The text states that South Africa had a "case fatality ratio of 1.5", whereas outside of Africa, "case fatality ratios were commonly above five". What do these numbers mean? 1.5 of what?

There was a typo, it supposed to be “case fatality rate”. This has been corrected in the manuscript. The case fatality rate, in this case, refers to the percentage of individuals that died due to COVID-19 out of all COVID-19 PCR confirmed cases in those regions.

The idea of climate influencing the severity of illness is an important one. Increased ventilation and distancing due to hot climate may lead to lower dosage at the point of viral transmission, which would lead to less severe illness.
We agree with the reviewer’s suggestion.

Table 2: please be more clear which numbers are measured and which numbers are estimates extrapolation. This should be very clear from a first glance (e.g. in the overall heading and the column heads). A casual glance at this table would lead a reader to believe that there have been 5295 COVID-19 deaths in Malawi.

We have edited the table and added “predicted” to the number of infections and deaths in the table. We have also included a clearer title “Crude estimates of predicted infection and mortality at different infection rates”.

Overall, it's great to see this in the literature, but I am somewhat uncomfortable about the level of extrapolation here. It would be good if the assumptions were clearly stated in the most prominent parts of the manuscript. For example, after the final line of the results, you could state: "This estimate assumes that age-stratified population seroprevalence in Malawi is similar to that observed amongst asymptomatic HCWs."

We have included this phrase as part of the results section.

Competing Interests: No competing interests were disclosed.
authors can mention in the discussion if they agree.

3. Minor comment: Methods section has a phrase "Human IgG conjugated to horseradish peroxidase" which I assume could be referring to Anti-human IgG conjugated to...."

**Is the work clearly and accurately presented and does it cite the current literature?**
Yes

**Is the study design appropriate and is the work technically sound?**
Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**
Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**
Yes

**Are all the source data underlying the results available to ensure full reproducibility?**
Yes

**Are the conclusions drawn adequately supported by the results?**
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Humoral immunity

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 11 Dec 2020

Kondwani Jambo, Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Blantyre, Malawi

Are the authors able to include ELISA data from pre-covid era to determine if there is pre-existing cross-reactivity to the assay in the region? This can be easily done using archived serum/plasma samples.

Yes, we have ongoing work addressing this very question. We did not include it in this manuscript as it was beyond the scope of this work.

While the results are surprising, they could still be understating the cumulative incidence of SARS-CoV-2 if some mild/asymptomatic people failed to develop detectable antibodies. The authors can mention in the discussion if they agree.

Yes, we agree with the reviewer. The is now substantial evidence that some individuals don't
exhibit detectable SARS-CoV-2 antibodies following COVID-19. We have included this in the discussion to acknowledge this potential limitation.

Minor comment: Methods section has a phrase "Human IgG conjugated to horseradish peroxidase" which I assume could be referring to Anti-human IgG conjugated to....".

Yes, this has been corrected.

**Competing Interests:** No competing interests were disclosed.