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SARS-CoV-2 prevalence and immunity: a hospital-based study from Malawi

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ABSTRACT

Background: COVID-19 transmission and disease dynamics in sub-Saharan Africa are not well understood. Our study aims to provide insight into COVID-19 epidemiology in Malawi by estimating SARS-CoV-2 prevalence and immunity after SARS-CoV-2 infection in a hospital-based setting.

Methods: We conducted a hospital-based, convenience sampling, cross-sectional survey for SARS-CoV-2 in Lilongwe, Malawi. Participants answered a questionnaire and were tested for SARS-CoV-2 by enzyme-linked immunosorbent assay and real-time reverse-transcription polymerase chain reaction (RT-PCR). A surrogate virus neutralization test (sVNT) was performed in seropositive samples to estimate immunity. Poisson regression was used to assess SARS-CoV-2 point prevalence association with demographic and behavioral variables.

Findings: The study included 930 participants. We found a combined point prevalence of 10.1%. Separately analyzed, RT-PCR positivity was 2.0%, and seropositivity was 9.3%. Of tested seropositive samples, 90.1% were sVNT positive. We found a high rate (45.7%) of asymptomatic SARS-CoV-2 infection. SARS-CoV-2 point prevalence was significantly associated with being a healthcare worker.

Interpretation: Our study suggests that official data underestimate COVID-19 transmission. Using sVNTs to estimate immunity in Malawi is feasible and revealed considerable post-infection immunity in our cohort. Subclinical infection and transmission are probably a game-changer in surveillance, mitigation and vaccination strategies.

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Research in context

Evidence before this study

SARS-CoV-2 burden of disease in sub-Saharan Africa (sSA) is still not well understood. Serological surveillance has been implemented to estimate the true SARS-CoV-2 prevalence, however, predominantly in high-income countries. Currently, there is an increasing but still small number of reported estimates of SARS-CoV-2 prevalence regarding sSA countries in the literature. We searched the PubMed database until July 29th, 2021, for the following terms: "COVID-19" or "SARS-CoV-2" and "Africa" and "prevalence" or "seroepidemiology". In a recent meta-analysis from Chisale et al., 23 studies from 16 countries were included. In the published studies, a wide range of estimated prevalence (0%-63%) reflects actual differences in regional prevalence and differences in selection criteria. There is only one serosurvey from Malawi that was conducted in 500 HCWs in Blantyre.

Regarding further waves of the COVID-19 pandemic and the worldwide initiation of vaccination campaigns, not only disease prevalence but also acquired, valid immunity after in-

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fection is of utmost interest for public health decisions. Recently, a surrogate virus neutralization test has been established to detect neutralizing antibodies as markers of immunity post-infection or post-vaccination without using live viruses. We searched the PubMed database until July 29th for the following terms: "COVID-19" or "SARS-CoV-2" and "Africa" and "immunity" and "surrogate virus neutralization assay". Although especially useful in settings with limited laboratory infrastructure, it has only been evaluated in one study in sub-Saharan Africa.

Added value of this study

To our knowledge, this is the first large-scale SARS-CoV-2 serosurvey in sSA that also included PCR testing to complement overall prevalence with recent SARS-CoV-2 infections and an sVNT assay to investigate for immunity. Our study adds to the growing evidence that official data might underestimate the true magnitude of COVID-19 transmission in sSA. We also found a high rate (45•7%) of SARS-CoV-2 positive participants that did not report any symptoms related to COVID-19 in the last six months.

Performance of sVNT to estimate immunity is feasible in Malawi and revealed a considerable rate of post-infection immunity in our cohort. Of those that were tested seropositive, 90•1% displayed neutralizing antibodies. This is a considerable and higher than expected rate of seemingly protected individuals at the early stage of the epidemic.

Implications of all available evidence

SARS-CoV-2 prevalence and immunity are vastly underestimated by official surveillance. Serial population-based serosurveys, preferably in combination with excess mortality studies, need to be implemented into the national testing strategy to respond to the pandemic's continuously changing dynamics. Asymptomatic and subclinical infection and transmission seem to be high. It would be most helpful to now identify factors associated with SARS-CoV-2 infections, severity of infection and immunity in sub-Saharan Africa. A targeted risk group approach in prevention that includes vaccination and adequate treatment options for the affected might be more effective than undirected lockdown and distancing measures to reduce socio-economic impact and maintain health likewise.

Introduction

Sub-Saharan Africa countries were expected to be highly vulnerable to COVID-19 due to fragile healthcare systems and potentially vulnerable populations (Gilbert et al., 2020). However, according to official surveillance data, prevalence and mortality continue to be comparably low. To date, the USA has an approximately 25-fold higher number of registered cases and deaths than Malawi, raising questions about the actual prevalence of the disease and potential pre-existing immunity in Malawi https://covid19.who.int/ WHO Coronavirus (COVID-19) Dashboard 2021.

Serological surveillance has been implemented to estimate true SARS-CoV-2 prevalence; however, this is predominantly in high-income countries (Bobrovitz et al., 2021). Currently, there are increasing but still small reported estimates of SARS-CoV-2 prevalence in African countries. In a recent meta-analysis from Chisale et al. of 23 studies from 16 countries, a wide range of reported estimated prevalence (0%–63%) reflected actual differences in regional prevalence and differences in selection criteria (Chisale et al., 2021). In general, SARS-CoV-2 exposure is reported to be more extensive than indicated by case-based surveillance, suggesting that the total number of cases is greatly underestimated.

Data on disease prevalence and acquired immunity after infection is important for public health decisions in future waves of the COVID-19 pandemic and worldwide initiation of vaccination campaigns (Salyer et al., 2021). Recently, a surrogate virus neutralization test has been established as an alternative to the gold standard conventional virus neutralization test, to detect neutralizing antibodies without using live viruses (Tan et al., 2020). It is a high-throughput serological test that mimics virus-host interaction in an enzyme-linked immunosorbent assay (ELISA) plate, especially useful in settings with limited laboratory infrastructure (Marien et al., 2021).

Malawi is a low-income country in south-eastern Africa with a population of approximately 17.6 million people. It is characterized by a predominantly rural (84%) and young (median age 17 years) population http://www.nsomalawi.mw (Malawi National Statistical Office 2021). Multidimensional poverty, other pre-existing health priorities such as malaria, malnutrition, tuberculosis and HIV, and deficiencies in the health infrastructure (Sonenthal et al., 2020) are considered key contextual contributors to COVID-19 severity and mortality. One estimate projected 16 million infections and 50 000 COVID-19 deaths over one year in an unmitigated scenario in Malawi (Green et al., 2020).

On 20 March 2020, COVID-19 was declared a national disaster in Malawi, and a multisectoral approach to mitigate the spread of the disease in consideration of competing health priorities and the socioeconomic context was initiated (Mzumara et al., 2021; The Republic of Malawi 2021). However, political mistrust and gatherings hampered mitigation strategies during anti-government demonstrations and mass political campaigns (Mzumara et al., 2021, Nyasulu et al., 2021). Community belief of unjustified national regulations and a perception of a low risk of COVID-19 negatively affected adherence to prevention measures (Banda et al., 2021). In addition, an already challenged health sector with limited financial and personal resources was further challenged by a strike of health providers demanding the provision of adequate personal protective equipment and staffing levels (Nyasulu et al., 2021). In 2020, the recorded incidence of SARS-CoV-2 infections peaked at the end of July and slowly decreased until the start of September. As of 24 August 2020, Malawi had cumulatively recorded 5419 cases, including 169 deaths, contrasting to predicted higher numbers https://www.malawipublichealth.org/ (Public Health Institute of Malawi 2021). Our study aimed to provide insight into the epidemiology of COVID-19 in Malawi by estimating SARS-CoV-2 prevalence and immunity after SARS-CoV-2 exposure in a convenience sample from healthcare workers (HCWs) and non-HCWs in a hospital-based setting.

Methods

Study design and study population

Between 24 August and 3 September 2020, we conducted a hospital-based, cross-sectional survey for SARS-CoV-2 at Kamuzu Central Hospital in Lilongwe, Central Region, Malawi. Kamuzu Central Hospital is a tertiary level hospital that provides referral services for 5 district hospitals. The population in the direct community numbers 1.6 million, but the hospital also serves the population across the wider region.

The study population included participants working or presenting at the facility. HCWs were defined as healthcare professionals, allied health workers and administration; non-HCWs were defined as patients, guardians and relatives. Participants were selected through convenience sampling, aimed at an equal distribution of HCWs and non-HCWs. Participants under 18 years and those who presented with contraindications to venipuncture or who were unwilling or unable to consent were excluded. Written informed consent (in Chichewa or English) was obtained from all

participants. Witnesses assisted participants who were not able to read in English or Chichewa.

Data collection

The collection of serum samples and nasopharyngeal swabs was carried out following a standard protocol approved by the College of Medicine Research and Ethics Committee Malawi. Participants answered a questionnaire that included sociodemographic information, possible exposure with SARS-CoV-2, medical and travel history. Anonymized data entry was performed using an electronic case report form on a tablet. Serum samples were collected by venipuncture and were tested for SARS-CoV-2 antibodies by ELISA. Participants were also offered testing for acute SARS-CoV-2 infection by nucleic acid testing.

Detection of SARS-CoV-2 antibodies

Serum samples were tested in a single replicate for the presence of SARS-CoV-2-IgG antibodies using a commercially available ELISA from Euroimmun (Lübeck, Germany). Assays were performed according to the manufacturer's guidelines. The microplate wells are coated with recombinant S1 structural protein, and the assay detects SARS-CoV-2-IgG antibodies against the viral spike protein. Results were evaluated semi-quantitatively by calculating a ratio of the extinction of the control or patient sample over the extinction of the calibrator. According to the manufacturer's guidelines, the ratio was interpreted as following <0.8 negative, ≥0.8 ≤1.1 borderline, >1.1 positive. Borderline results were re-run in a single replicate and considered positive, negative or borderline accordingly. Validation of the Euroimmun test kit in the Malawian setting was performed in 81 respective negative sera collected before July 2019.

Surrogate virus neutralization test

Subsequently, a surrogate virus neutralization test (sVNT, GenScript Biotech Corporation, New Jersey, USA) was performed in seropositive samples. Assays were performed according to the manufacturer's guidelines. This assay detects circulating neutralizing antibodies against SARS-CoV-2 that specifically inhibit the interaction between the receptor-binding domain of the viral spike glycoprotein and the angiotensin-converting enzyme 2 cell surface, without the use of live SARS-CoV-2. Results are evaluated semi-quantitatively by calculating the percentage inhibition ([1- average optical density of sample/average optical density of negative control] x 100%). According to the manufacturer's information, a sample with an inhibition ≥20% was considered positive and <20% negative. Validation of the sVNT test kit in the Malawian setting was performed in 44 respective negative sera collected before July 2019.

Laboratory nucleic acid test

Nasopharyngeal swabs were collected whenever additional informed consent was given to determine SARS-CoV-2 prevalence by real-time reverse-transcription polymerase chain reaction (RT-PCR). Viral RNA was extracted manually using QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany), and RT-PCR assays were performed according to the manufacturer's instructions. The LightMix SarbecoV E-Gene PCR assay (TIB Molbiol, Berlin, Germany), targeting the E gene, was used for primary screening. The amplification curve's cycle threshold (Ct) value was defined as positive if Ct <36 and negative if Ct ≥36. All positive results were confirmed using a LightMix Modular Wuhan COV RdRP-gene (TIB Molbiol, Berlin, Germany), targeting the RNA-dependent RNA polymerase (RdRP) gene. The Ct value of the amplification curve was defined as positive if Ct <39 and negative if Ct ≥39. Due to technical reasons resulting in invalid test results, RT-PCR was alternatively performed using the Abbott RealTime SARS-CoV-2 Amplification Reagent Kit on the Abbott m20000rt (Abbott Diagnostics Scarborough, USA) in 105 samples. The panel targets the SARS-CoV-2 RdRP and N genes of the SARS-CoV-2 genome. The Abbott m2000rt instrument automatically reports the results and interpretations on the Abbott m2000rt workstation as positive or negative. Results were considered positive if either confirmed as positive using a LightMix Modular Wuhan COV RdRP-gene (TIB Molbiol, Berlin, Germany) or if reported positive using Abbott RealTime SARS-CoV-2 Amplification Reagent Kit on the Abbott m20000rt.

Statistical analysis

All statistical analyses were performed using STATA (v17, Stata Corp, LP, TX, USA) software. A descriptive analysis was performed to investigate participants' characteristics. Prevalence and 95% CI estimates were calculated for RT-PCR and ELISA separately. The point prevalence of SARS-CoV-2 and 95% CIs were estimated as a combined prevalence by dividing the number of individuals who had a positive result in the ELISA and/or a positive result in the RT-PCR over the number of all participants. To explore differences in SARS-CoV-2 point prevalence according to demographic and behavioral variables, we performed a univariable and multivariable Poisson regression model and calculated prevalence ratios (PR). We applied a multivariable regression model to estimate the adjusted PR, including all variables of interest associated with SARS-CoV-2 point prevalence at a *P*-value <0.20 or clinically appropriate (e.g., sex and age group) for the analysis. We also performed a multicollinearity test for independent variables in the multivariable model to assess any intercorrelations between them. A robust standard error for the parameter estimates was applied to control for mild violation of underlying assumptions (Tamhane et al., 2016). All performed tests were two-tailed. Statistical significance was set at *P*-value <0.05.

Results

A total of 962 participants attended the study. Of those, 32 were excluded from the analysis because of incomplete data and/or withdrawal of consent. Of the 930 included participants, 914 provided a specimen for ELISA and 734 provided a specimen for the RT-PCR. A total of 927 participants had available results on ELISA or/and RT-PCR. For the subsequent sVNT assay, 4 seropositive samples had to be excluded because of insufficient remnant serum. Due to dissociative labeling, 75 laboratory specimens could not be assigned to a participant and were excluded (Figure 1).

Of the 930 participants included for data analysis, 48.3% were HCWs and 51.7% non-HCWs. Of the HCWs, 75.1% were direct clinicians (such as doctors, nurses), 22.0% were direct non-clinical staff (such as guards, housekeeping and others), 0.9% were indirect clinicians (such as staff working at the pharmacy or laboratory), and 2.0% were indirect non-clinical staff (such as administration). Overall, 599 participants were female (64.4%). The majority of the participants were aged 18–29 years (33.3%) or 30–39 years (33.2%). In addition, the majority of the participants resided in Lilongwe (89.1%) and were of Malawian nationality (99.0%). Further participants' demographics are summarized in Table 1.

When combining the prevalence of SARS-CoV-2 seropositivity and the RT-PCR positive cases, there was a combined point prevalence of 10.1% (95% CI 8.4–12.3). Separately analyzed, the RT-PCR positivity was 2.0% (95% CI 1.2–3.4) and the seropositivity 9.3% (CI 95% 7.6–11.4) (Table 2). Of those tested positive for SARS-CoV-2 antibodies or/and RT-PCR, 45.7% did not report any symptoms in the

Table 1
Participant demographics, n (%) or median (range)

	All participants Total = 930	HCW Total = 449	Non-HCW Total = 481
Sex			
Female	599 (64.4%)	283 (63.0%)	316 (65.7%)
Male	331 (35.6%)	166 (37.0%)	165 (34.3%)
Age (years)			
Median (IQR)	35 (27–43)	33 (27–41)	35 (27–45)
Age group			
18-29	310 (33.3%)	154 (34.3%)	156 (32.4%)
30-39	309 (33.2%)	161 (35.9%)	148 (30.8%)
40-49	178 (19.1%)	92 (20.5%)	86 (17.9%)
50-59	91 (9.8%)	34 (7.6%)	57 (11.9%)
>=60	42 (4.5%)	8 (1.8%)	34 (7.1%)
District			
Lilongwe	829 (89.1%)	449 (100%)	380 (79%)
Other	100 (10.8%)	..	100 (20.8%)
Unknown	1 (0.1%)	..	1 (0.2%)
Household number			
Median (IQR)	4 (3–6)	4 (3–6)	5 (3–6)
Nationality			
Malawian	921 (99.0%)	440 (98.0%)	481 (100%)
Other	9 (1.0%)	9 (2.0%)	0
Educational attainment			
None	74 (8.0%)	6 (1.3%)	68 (14.1%)
Primary	256 (27.5%)	34 (7.6%)	222 (46.2%)
Secondary	289 (31.1%)	130 (29.0%)	159 (33.1%)
Higher	310 (33.3%)	278 (61.9%)	32 (6.7%)
Unknown	1 (0.1%)	1 (0.2%)	-
Pregnancy (only female, n=599)			
Yes	20 (3.3%)	11 (3.9%)	9 (2.9%)
No	572 (95.5%)	269 (95.1%)	303 (95.9%)
Unknown/Missing	7 (1.2%)	3 (1.1%)	4 (1.3%)
Chronic precondition			
Yes	209 (22.5%)	66 (14.7%)	143 (29.7%)
No	721 (77.5%)	383 (85.3%)	338 (70.3%)
Cancer			
Yes	33 (3.6%)	..	33 (6.9%)
No	896 (96.3%)	449 (100%)	447 (92.9%)
Unknown	1 (0.1%)	..	1 (0.2%)
Diabetes			
Yes	22 (2.4%)	8 (1.8%)	14 (2.9%)
No	904 (97.2%)	440 (98.0%)	464 (96.5%)
Unknown	4 (0.4%)	1 (0.2%)	3 (0.6%)
HIV			
Yes	112 (12.0%)	26 (5.8%)	86 (17.9%)
No	808 (86.9%)	417 (92.9%)	391 (81.3%)
Unknown	10 (1.1%)	6 (1.3%)	4 (0.8%)
Heart disease			
Yes	32 (3.4%)	18 (4.0%)	14 (2.9%)
No	897 (96.5%)	430 (95.8%)	467 (97.1%)
Unknown	1 (1.0%)	1 (0.2%)	..
Sickle cell disease			
Yes	1 (0.1%)	..	1 (0.2%)
No	926 (99.6%)	448 (99.8%)	478 (99.4%)
Unknown	3 (0.3%)	1 (0.2%)	2 (0.4%)
Asthma			
Yes	34 (3.7%)	21 (4.7%)	13 (2.7%)
No	896 (96.3%)	428 (95.3%)	468 (97.3%)
Tested for Malaria			
Yes	240 (25.8%)	119 (26.5%)	121 (25.2%)
No	688 (74%)	329 (73.3%)	359 (74.6%)
Unknown/Missing	2 (0.2%)	1 (0.2%)	1 (0.2%)
Positive Malaria test (n=240)			
Yes	75 (31.3%)	22 (18.5%)	53 (43.8%)
No	165 (68.8%)	97 (81.5%)	68 (65.2%)
Symptoms of COVID-19 in the previous 6 months			
Yes	341 (36.7%)	203 (45.2%)	138 (28.7%)
No	582 (62.6%)	224 (54.3%)	338 (70.3%)
Unknown/Missing	7 (0.8%)	2 (0.5%)	5 (1.0%)
Fever	126 (13.6%)	61 (13.6%)	65 (13.5%)
Cough	202 (21.7%)	132 (29.4%)	70 (14.6%)
Shortness of Breath	29 (3.1%)	16 (3.6%)	13 (2.7%)
Runny nose	186 (20.0%)	119 (26.5%)	67 (13.9%)
Sore throat	59 (6.3%)	54 (12.0%)	5 (1.0%)
Hospitalized	16 (1.7%)	3 (0.7%)	13 (2.7%)

(*continued on next page*)

Table 1 (*continued*)

	All participants Total = 930	HCW Total = 449	Non-HCW Total = 481
Travel history last 6 months			
Yes	15 (1.6%)	10 (2.2%)	5 (1.0%)
No	915 (98.4%)	439 (97.8%)	476 (99.0%)
Contact to COVID-19 case inside the hospital			
Yes	157 (16.9%)	147 (32.7%)	10 (2.1%)
No	709 (76.2%)	266 (59.2%)	443 (92.1%)
Unknown/Missing	64 (6.9%)	36 (8.0%)	28 (5.8%)
Contact to COVID-19 case outside the hospital			
Yes	47 (5.0%)	35 (7.8%)	12 (2.5%)
No	820 (88.2%)	369 (82.2%)	451 (93.8%)
Unknown/Missing	63 (6.8%)	45 (10.0%)	18 (3.8%)

HCW=healthcare worker

Table 2
SARS-CoV-2 prevalence in healthcare workers and non-healthcare workers by enzyme-linked immunosorbent assay, reverse-transcription polymerase chain reaction and combined

	Frequency, n	Prevalence, % (CI 95%)
SARS-CoV-2 seropositivity (n=914)	85	9.3 (7.6–11.4)
SARS-CoV-2 PCR (n=734)	15	2.0 (1.2–3.4)
Combined (n=927)	94	10.1 (8.4–12.3)

last 6 months before data collection. None of the seropositive participants were hospitalized in the last 6 months. Of the 81 tested seropositive samples, 90.1% (95% CI 81.2%-95.1%) tested positive in the surrogate virus neutralization assay.

In the univariable Poisson regression model, SARS-CoV-2 point prevalence was significantly associated with being an HCW (PR 1.7; 95% CI 1.1–2.5). The SARS-CoV-2 point prevalence was 1.9 times greater in those who had contact with a COVID-19 case inside the hospital than those who had not (PR 1.9; 95% CI 1.2–2.9). We found a significant association between higher education and SARS-CoV-2 point prevalence (PR 5.3; 95% CI 1.3–21.4) (Figure 2 and Table 3); however, this is because most HCWs have higher education than non-HCWs (62.1% of HCWs reported higher education compared with 6.6% among non-HCWs). In the multicollinearity test, both education attainment and COVID-19 contact inside the hospital showed strong intercorrelations with HCW status, and therefore they were not considered in the multivariable model. In the final model adjusted for sex, age group and symptoms, HCW remained significantly associated with higher SARS-CoV-2-point prevalence (PR 1.6; 95% CI 1.1–2.4; *P*-value 0.026) (Table 4).

Discussion

To our knowledge, this is the first large-scale SARS-CoV-2 serosurvey during the first wave of the coronavirus pandemic in sub-Saharan Africa (Malawi) that included PCR testing to complement overall prevalence for recent SARS-CoV-2 infections plus an sVNT assay to investigate for immunity after SARS-CoV-2 infection. Our study adds to the growing evidence that official data might underestimate the true magnitude of COVID-19 transmission in sub-Saharan Africa. We found a much higher than expected SARS-CoV-2 infection rate with a point prevalence of 10.1% (95% CI 8.4–12.3) that consists of a low rate of acute infections (positive RT-PCR: 2.0%, 95% CI 1.2–3.4) and high seropositivity of 9.3% (95% CI 7.6–11.4); compatible with the fact that our study was conducted after the peak of the first COVID-19 wave in Malawi https://www.malawipublichealth.org/ (Public Health Institute of Malawi 2021).

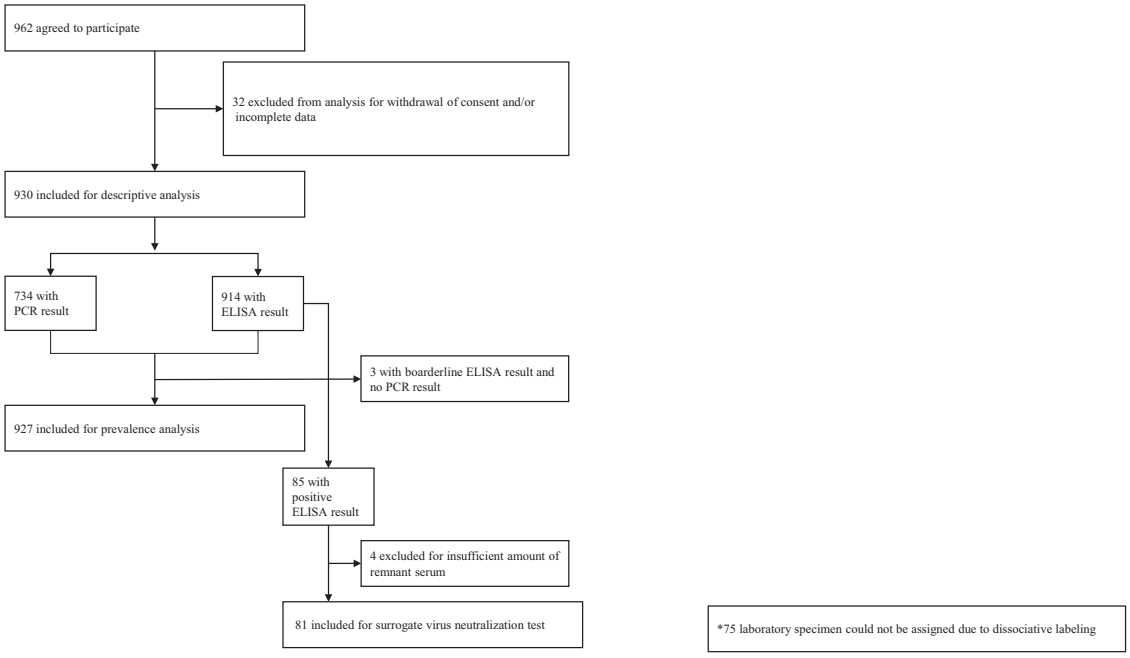

Figure 1. Participants flow chart

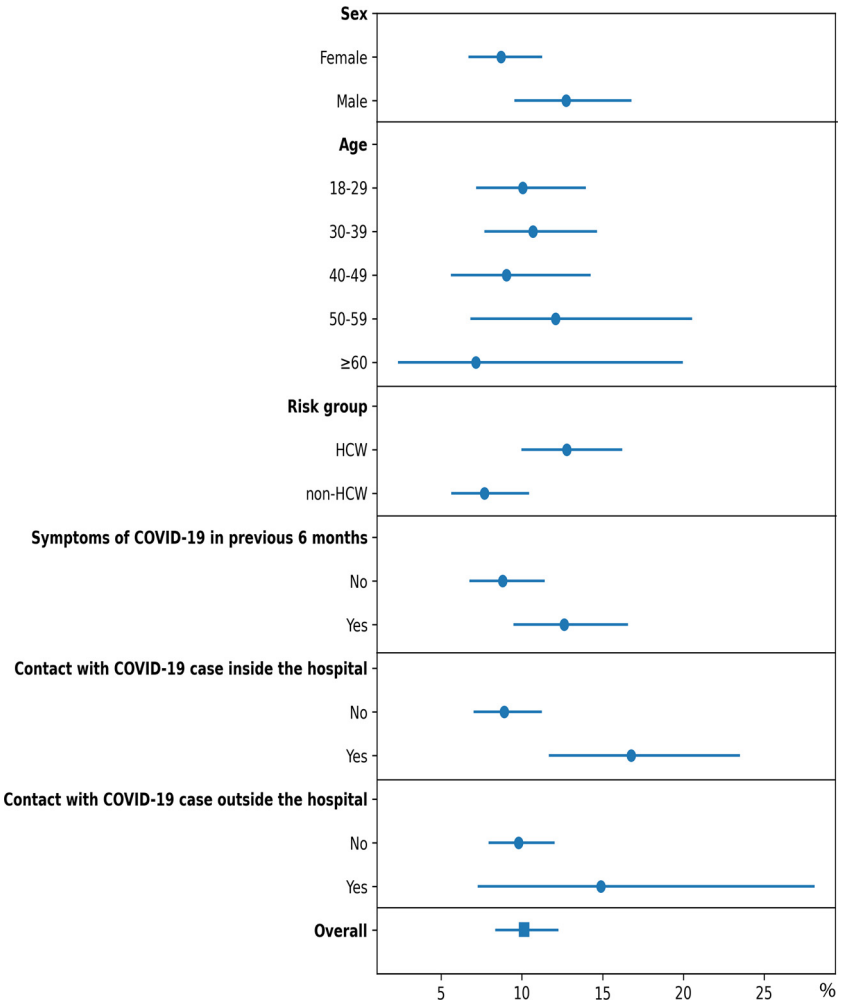

Figure 2. SARS-CoV-2 prevalence with 95% CI

Since we conducted a hospital-based, convenience sampling study, it is not possible to directly compare data from the general population. However, official surveillance data might still serve as an indicator, reporting 5593 confirmed cases of SARS-CoV-2 infection in Malawi and 1290 confirmed cases of SARS-CoV-2 infection in Lilongwe at the end of our study (3 September 2020) https://www.malawipublichealth.org/ (Public Health Institute of Malawi 2021). With a population of approximately 17.6 million in Malawi and 1.6 million in Lilongwe, these official numbers correlate with a SARS-CoV-2 prevalence of 0.03% and 0.08%, respectively.

Due to methodological limitations, SARS-CoV-2 point prevalence might be underestimated in our study. Only 78.9% of the participants consented to the performance of a nasopharyngeal swab. Due to a lower sensitivity of the Euroimmun IgG assay in an earlier phase of infection (Kohmer et al., 2020), acute infections with SARS-CoV-2 might be underestimated. In addition, there is emerging evidence for the waning of antibodies that may lead to undetectable antibody titers, especially after asymptomatic and paucisymptomatic infection (Long et al., 2020). Potential cross-reactivity of serological tests with other circulating pathogens resulting in reduced specificity of serological tests for SARS-CoV-2 has been reported in sub-Saharan Africa (Emmerich et al., 2021). To address this challenge, we validated the Euroimmun assay in a panel of 81 samples collected before the COVID-19 pandemic with no false-positive results observed.

Of those who tested positive for SARS-CoV-2 infection, a high number did not recall any of the queried symptoms associated with COVID-19 (45.7%) in the last 6 months. In addition, none of the SARS-CoV-2 positive participants in our study reported hospitalization in the last 6 months. Recall and selection bias might have led to underestimating the rate of symptomatic SARS-CoV-2 infection in our cohort. High rates of subclinical infections have also been shown in other studies from sub-Saharan Africa. For example, Mulenga et al. reported an even higher rate (76.2%) of asymptomatic SARS-CoV-2 prevalence in a population-based serosurvey in Zambia (Mulenga et al., 2021). We hypothesize that circulation of the virus in the population might have gone on silently and might have appeared much earlier and more than predicted, even before or despite measures put in place. High rates of subclinical infections and silent community transmission have implications when designing surveillance and mitigation strategies. Asymptomatic surveillance needs to be incorporated to understand and prevent widespread community transmission. However, given the current constraints, including limited funding for other concurring health threats, and even with upscaled testing capacities, asymptomatic surveillance does not seem feasible in a low-resource setting like Malawi. Given the costs and collateral damage of preventive measures in this crisis, such as widespread hunger, economic insecurity, disruption of essential medical services and access to education (Haider et al., 2020), a targeted approach might be more resource-efficient in African countries.

It would be most helpful to identify factors associated with SARS-CoV-2 infection, severity of infection and immunity in sub-Saharan Africa. Furthermore, identifying and shielding the most

Table 3
Association between SARS-CoV-2 prevalence (combined measure) and demographic, behavioral and other factors (n=927)

	Combined Prevalence, % (95% CI)	Univariable analysis Prevalence ratio (95% CI)	*P*-value
Healthcare worker			
No	7.7 (5.6–10.4)	1	
Yes	12.8 (10.0–16.2)	1.7 (1.1–2.5)	0.012
Sex			
Female	8.7 (6.7–11.3)	1	
Male	12.7 (9.5–16.8)	1.5 (1.0–2.2)	0.053
Age group			
18–29	10.1 (7.2–14.0)	1	
30–39	10.7 (7.7–15.0)	1.1 (0.7–1.7)	0.802
40–49	9.0 (5.6–14.3)	0.9 (0.5–1.6)	0.714
50–59	12.1 (6.8–20.5)	1.2 (0.6–2.3)	0.579
>=60	7.1 (2.3–20.0)	0.7 (0.2–2.2)	0.556
Education attainment			
None	2.7 (0.7–10.2)	1	
Primary	7.4 (4.8–11.4)	2.8 (0.7–11.6)	0.168
Secondary	10.0 (7.1–14.1)	3.7 (0.9–15.3)	0.069
Higher	14.3 (10.8–18.7)	5.3 (1.3–21.4)	0.019
Symptoms of COVID-19 in the previous 6 months			
No	8.8 (6.8–11.4)	1	
Yes	12.6 (9.5–16.6)	1.4 (1.0–2.1)	0.067
Chronic precondition (diabetes, HIV, heart disease and/or asthma)			
No	10.6 (8.6–13.0)	1	
Yes	8.7 (5.5–13.3)	0.8 (0.5–1.34)	0.4
Diabetes			
No	10.1 (8.3–12.3)	1	
Yes	9.1 (2.3–30.0)	0.9 (0.2–3.4)	0.877
HIV			
No	10.1 (8.2–12.3)	1	
Yes	11.7 (7.0–19.2)	1.2 (0.7–2.0)	0.586
Heart Disease			
No	10.3 (8.5–12.5)	1	
Yes	6.3 (1.6–21.9)	0.6 (0.2–2.3)	0.471
Asthma			
No	10.4 (8.6–12.6)	1	
Yes	2.9 (0.4–18.2)	0 .3 (0.0–2.0)	0.202
Tested for Malaria			
No	10.1 (8.0–12.6)	1	
Yes	10.4 (7.1–15.0)	1.0 (0.7–1.6)	0.879
Positive test for malaria (n =240)			
No	11.5 (7.4–17.4)	1	
Yes	8.0 (3.6–16.8)	0.7 (0.3–1.7)	0.417
Contact inside the hospital			
No	8.9 (7.0–11.2)	1	
Yes	16.8 (11.7–23.5)	1.9 (1.2–2.9)	0.003
Unknown	7.8 (3.3–17.5)	0.9 (0.4–2.1)	0.771
Contact outside the hospital			
No	9.8 (7.9–12.0)	1	
Yes	14.9 (7.3–28.1)	1.5 (0.7–3.1)	0.250
Unknown	11.1 (5.4–21.6)	1.1 (0.6–2.4)	0.734

vulnerable groups and providing adequate treatment options for the infected might allow a more targeted approach and be more effective than undirected lockdowns and distancing measures in reducing socioeconomic impact while maintaining health.

Our study found a higher SARS-CoV-2 prevalence in HCWs (12.8%) than non-HCWs (7.7%). Chibwana et al. found a similar rate (12.5%) of SARS-CoV-2 prevalence in 500 HCWs from Blantyre; interestingly, a similar finding was detected at an earlier stage of the epidemic in Malawi (Chibwana et al., 2020). HCWs are generally considered at risk for SARS-CoV-2 infection and transmission, especially in sub-Saharan Africa, due to a shortage of personal protective equipment, training and human resources (Bandyopadhyay et al., 2020, Mukwege et al., 2021, Olayanju et al., 2021). Convenience sampling and the stigma related to COVID-19 infection might have introduced a selection bias in our study, underestimating the true prevalence of SARS-CoV-2 infection in HCWs. Limited caseloads in hospitals in Malawi due to concerns in the population about the health system, strikes and absenteeism of HCWs from work due to fear of infection might have limited in-hospital transmission during the first wave of the pandemic. SARS-CoV-2 infection was also attributable to COVID-19 exposure in the hospital, underlining the need for adequate personal protective equipment and training. However, general (high) transmission in urban Lilongwe, where all HCWs resided, must be considered.

Concerning the emergence of new virus variants and the desire to implement vaccination strategies, there is a pressing need to estimate immunity after SARS-CoV-2 infection. Our study used an sVNT as a surrogate assay test to measure neutralizing antibodies in individuals who tested positive for SARS-CoV-2 IgG. To our knowledge, this is the first study that included a surrogate assay for neutralizing antibodies in a serosurvey in sub-Saharan Africa. We demonstrated that the performance of an sVNT was feasible and with limited laboratory infrastructure. Marien et al. have recently evaluated a surrogate virus neutralization assay in comparison with a conventional neutralization assay in samples from Belgian and Congolese participants. In a sample of 184 Congolese probes, Marien et al. found a good correlation of the results of the sVNT with the cVNT with a sensitivity of 88.4% and a specificity of

Table 4
Association between SARS-CoV-2 prevalence (combined measure) and demographic, behavioral and other factors, adjusted multivariable analysis (n = 854)*

	Multivariable analysis	
	Prevalence ratio	*P*-value
Healthcare worker		
No	1	
Yes	1.6 (1.1–2.4)	0.026
Sex		
Female	1	
Male	1.4 (1.0–2.1)	0.074
Age group		
18–29	1	
30–39	1.1 (0.7–1.7)	0.842
40–49	0.9 (0.5–1.7)	0.826
50/59	1.3 (0.7–2.6)	0.377
>=60	0.9 (0.3–3.0)	0.906
Symptoms in the last 6 months		
No	1	
Yes	1.3 (0.9–2.0)	0.148

* All variables of interest associated with SARS-CoV-2 point prevalence with a *P*-value <0.20 (healthcare worker, sex, education attainment, symptoms, contact to case inside the hospital) or clinically appropriate (sex, age group) for the analysis were considered for a multivariable model. Due to strong intercorrelations between healthcare worker and education (60%) and mild intercorrelations between healthcare worker and COVID-19 contact inside the hospital (30%), we removed these 2 variables from the model.

98.2% (Marien et al., 2021). Regarding potential cross-reactivity of serological assays, we also validated the GenScript sVNT in a panel of 44 zero samples with no false-positive results observed. Of those that tested positive for SARS-CoV-2 IgG, 90.1% displayed neutralizing antibodies. Although this is a snapshot and one might question generalizability, it is a considerable and higher than expected rate of seemingly protected individuals.

High rates of protection after infection and comparably low rates of severe infections question the current public health approach regarding vaccination. Moreover, they have implications for the economics, logistics and effectiveness of a national strategy, moving from a broad horizontal approach to a selective and targeted one, namely the vaccination of high-risk groups (e.g., the elderly, HCWs) only. In this light, the measurement of progress in African countries by vaccine coverage targets—as most recently posted by the WHO regional director for Africa https://www.afro.who.int/news/eight-10-african-countries-miss-crucial-covid-19-vaccination-goal (WHO. Regional Office for Africa 2021).—is not recognizing the immunological situation detected and presented here and is potentially misleading.

The emergence of new variants, possibly more transmissible, more lethal and more easily escaping immunity, underlines the importance of continuous seroepidemiological surveillance of the COVID-19 pandemic. Serial population-based serosurveys, preferably in combination with excess mortality studies, need to be implemented into the national testing strategy to understand viral transmission and disease dynamics and tailor mitigation strategies to the local context.

Conclusion

Our study presents African figures for African estimates—context matters in predictions. Even revised epidemiological estimates proved to be wrong, predicting high morbidity and mortality, using European epidemiological data to calculate Malawi estimates. Conversely, we found a SARS-CoV-2 prevalence and post-infection immunity much higher than expected from official numbers, while there was a high rate of presumably asymptomatic SARS-CoV-2 infections. A targeted approach that includes the identification and shielding of and adequate treatment options for the most vulnerable might be more effective than strict lockdown measures in a setting with high rates of subclinical infections to reduce health and socioeconomic impact. Acceptable levels of immunity in sub-Saharan Africa countries will most likely be reached by natural acquisition, enhanced by vaccination; diametrically opposed to European countries' vaccination enhanced by natural acquisition.

We need up-to-date epidemiological data to foster community-based decisions on COVID-19 mitigation and vaccination measures specific to the country.

Authors and their contributions

AS, BN, OJ and RS were involved in the conceptualization of the study. Other authors contributed the following: data curation (AS, BN, OJ), formal analysis (BM, CM, BK, JB, RS), funding acquisition (AJ, AS), investigation (BB, BM, BN, OJ), methodology (AS, BB, BN, BK, RS), project administration (AS, OJ), resources (AJ, AS, BM), supervision (AS), validation (AS, BB, BK, RS) and visualization (CM, BK, RS). CM prepared the original draft of the manuscript. All co-authors reviewed and edited the manuscript before submission. AS, CM and RS have directly accessed and verified the underlying data reported in the manuscript and AS was responsible for the overall design and execution of the study as well as the final manuscript. All authors meet the ICMJE criteria for authorship. This work has emerged from collaboration and co-authorship with colleagues in the locations where the research was conducted.

Declaration of interest

AS is staff member of GIZ German development who partly funded the study. RS, BB, BK and AJ are staff members of RKI Germany who gave technical and material support. All remaining authors declare no competing interests.

AS had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Data sharing

Deidentified participant data and study protocol with publication at Mendeley Data DOI (10.17632/hz5mfsmpk5.1) will be shared upon request and after review of a research proposal. All data requests should be directed to the corresponding author.

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Ethical statement

Ethical approval was obtained from the College of Medicine Research and Ethics Committee Malawi under the approval number P.07/20/3094. Written informed consent was obtained from all participants.

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