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Serologic response to SARS-CoV-2 in an African population

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ABSTRACT

Official case counts suggest Africa has not seen the expected burden of COVID-19 as predicted by international health agencies, and the proportion of asymptomatic patients, disease severity, and mortality burden differ significantly in Africa from what has been observed elsewhere. Testing for SARS-CoV-2 was extremely limited early in the pandemic and likely led to under-reporting of cases leaving important gaps in our understanding of transmission and disease characteristics in the African context. SARS-CoV-2 antibody prevalence and serologic response data could help quantify the burden of COVID-19 disease in Africa to address this knowledge gap and guide future outbreak response, adapted to the local context. However, such data are widely lacking in Africa. We conducted a cross-sectional seroprevalence survey among 1,192 individuals seeking COVID-19 screening and testing in central Cameroon using the Innovita antibody-based rapid diagnostic. Overall immunoglobulin prevalence was 32%, IgM prevalence was 20%, and IgG prevalence was 24%. IgM positivity gradually increased, peaking around symptom day 20. IgG positivity was similar, gradually increasing over the first 10 days of symptoms, then increasing rapidly to 30 days and beyond. These findings highlight the importance of diagnostic testing and asymptomatic SARS-CoV-2 transmission in Cameroon, which likely resulted in artificially low case counts. Rapid antibody tests are a useful diagnostic modality for seroprevalence

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surveys and infection diagnosis starting 5-7 days after symptom onset. These results represent the first step towards better understanding the SARS-CoV-2 immunological response in African populations.

Research in context

Evidence before this study

We searched PubMed, MEDLINE and Google Scholar on the March 20, 2021 using the search terms "novel coronavirus" or "2019nCOV" or "SARS-CoV-2" and "seroprevalence" for studies done in Africa with no restrictions on date or language. We found very little literature (23 papers) on the subject and the available evidence was focused on selected populations like healthcare workers or blood donors.

Added value of this study

This study elucidates the epidemiological patterns of COVID-19 immunity in a small population-based cohort in Cameroon, showing immunoglobulin prevalence and duration over time. This is among the first evidence on SARS-CoV-2 population-level immunity in a sub-Saharan African population.

Implications of all available evidence

This is an early seroprevalence survey in a representative sample of the African population, including both symptomatic and asymptomatic people, and represents a forecast of what can be obtained in a future large-scale population-based seroprevalence studies in sub-Saharan Africa.

Introduction

The world is currently striving to understand, control, and limit morbidity from the novel coronavirus disease (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. A fundamental challenge in achieving COVID-19 control is understanding transmission dynamics and regional disease prevalence to implement appropriate distancing measures and plan vaccination strategies. Though expanded testing has contributed to a better understanding of disease prevalence in China and elsewhere, low-resource settings including Cameroon and much of sub-Saharan Africa have not accessed widespread real-time SARS-CoV-2 testing, limiting the ability to detect transmission hotspots, predict further transmission, and identify the highest-impact settings for early interventions. Furthermore, knowing what proportion of the population has been infected with the virus is an important step towards understanding whether herd immunity sufficient population-level immunity to prevent onward transmission of the disease is a realistic goal [2–5]. In Cameroon, as in much of sub-Saharan Africa (sSA), SARS-CoV-2 molecular diagnostic testing capacity has been limited, making it difficult to estimate the impact, kinetics, and timing of COVID-19 outbreaks on the African continent.

The first case of COVID-19 in Cameroon was diagnosed on 6 March 2020. As of 31st of March 2021, Cameroon had recorded 57,337 confirmed cases, 851 deaths (case fatality rate 1.5%) and 51,769 recovered (90.3%)[6]. This represents a per-capita case rate of 84.1/100,000, comparable to rates observed in Senegal (95.7/100,000) and Côte d'Ivoire (79.3/100,000). Due to limited testing availability in Cameroon at the beginning of the epidemic, the countrywide response relied on case-based diagnosis and syndromic surveillance. These strategies encouraged people with a COVID-19-like illness or possible exposure to report immediately to healthcare providers or, where feasible, get tested. However, the asymptomatic and minimally symptomatic infection has been reported in up to 45% of infected people [7], and this symptom-driven testing strategy likely underestimated early spread of SARS-CoV-2 in the Cameroonian population due to under-reporting of asymptomatic cases, combined with limited access to diagnostic testing.

To help contain the spread of COVID-19 in sub-Saharan Africa, in April 2020 the Africa Centres for Disease Control and Prevention (Africa CDC) and the African Union (AU) launched the Trace, Test & Track strategy coined the "CDC-T3" [8] which Cameroon adopted in June 2020. Despite implementing this expanded contact-tracing and testing strategy, the scope of the pandemic and population seroprevalence in Cameroon was unknown, as in other African settings. While a plethora of serological surveys have been reported or are underway elsewhere [9–19], there is a paucity of population-level seroprevalence data from Southern countries. Many published seroprevalence surveys used samples obtained from specific populations (blood donors or health professionals) and may not reflect population-level exposure [9,14,17]. Additionally, some studies fail to report the characteristics of the tests used [9], undermining the reliability of seroprevalence estimates.

Although the kinetics of anti-SARS-CoV-2 antibodies are still being studied, it is clear that most infected people develop antibodies against the SARS-CoV-2 nucleoprotein and spike protein receptor receptor-binding domain (RBD) within three weeks of symptom onset [20], which remain detectable for several months after infection [20,21]. However, data on

seroprevalence and duration of antibody response in African populations are lacking. Serologic testing for SARS-CoV-2 antibodies can be a useful complement to viral testing to shed light on SARS-CoV-2 transmission dynamics that occurred before widespread viral testing was available [22]. Population-level seroprevalence data can help define regional transmission dynamics towards achieving CDC-T3 [21]. Together with expanded testing for viral nucleic acids, serologic evidence of infection can inform epidemic response and guide vaccine deployment.

To address the knowledge gap on COVID-19 serologic response in an African population, estimate population-level SARS-CoV-2 seroprevalence in sub-Saharan Africa, and provide insight into disease burden in this low-resource region, we carried out a large prevalence study in the Centre Region of Cameroon, the epicenter of the countrywide epidemic.

Material and methods

Study design

We performed a secondary analysis of data acquired from a SARS-CoV-2 diagnostic accuracy study in COVID-19 testing sites across the Centre Region of Cameroon between June and August 2020. Two sites were dedicated exclusively to screening asymptomatic individuals, while the remaining six provided asymptomatic screening in addition to testing and treating symptomatic individuals. Individuals aged 21 years or older who presented to any of the sites for voluntary screening (hereafter, non-hospitalized) and symptomatic inpatients admitted to one of the six treatment centers for the management of COVID-19 (hereafter, hospitalized) were included. After obtaining informed consent, peripheral blood venipuncture samples were collected from participants into EDTA tubes and red-top tubes to create serum, and nasopharyngeal swab samples were collected, transported in virologic media, and stored at the National Laboratory of Public Health at -20° C. A standardized questionnaire was administered to collect demographic and clinical data. At the initial visit (Visit 1), participants were invited return for up to two follow-up visits. Visit 2 was planned for seven days after Visit 1 and Visit 3 was planned for 14 days after Visit 1. Participants were invited for follow-up visits regardless of their clinical status and severity of illness.

Procedures

The IgM and IgG rapid test (Innovita [Tangshan] Biological Technology Co., Ltd., Beijing, China; Lot: 20200406, Exp: 28/10/2020) was used to determine presence or absence of antibodies against SARS-CoV-2 in serum according to the manufacturer's recommendations. The National Public Health Laboratory of Cameroon conducted all testing, both on-site at each of the eight testing centers, and also at their laboratory, for retrospective testing of samples. Results of tests performed on-site were made available to clinicians and participants for clinical use 10-15 minutes after sample collection. All participants were also tested for SARS-CoV-2 using Abbot RT-PCR (Abbott, Abbott Park, USA)(3) and Biomerieux ELISA (Biomerieux, Marcy l'Etoile, France)(4), performed at the National Public Health Laboratory on nasopharyngeal swab samples. At follow-up visits, participants underwent repeated testing.

Health professionals at each site administered a standardized questionnaire to participants to collect information on age, sex, history of symptoms compatible with COVID-19 (fever, chills, severe tiredness, sore throat, cough, shortness of breath, headache, anosmia or ageusia, and nausea, vomiting, or diarrhoea), time of onset of symptoms, education level, profession, religion, chronic comorbidities, and tobacco use.

Outcomes and statical analysis

We described categorical variables in terms of absolute and relative frequencies. We summarized quantitative variables, using the median and the interquartile range. We estimated immunoglobulin prevalence as the proportion of participants with a rapid antibody test result positive for IgM, IgG, or both. We adjusted immunoglobulin prevalence for age and sex using direct standardization and compared the prevalence to the World Bank 2018 estimated Cameroon population stratified into 13 age categories (Perspective Monde, 2020). We then repeated the analyses separately for each test result combination: IgM-positive only, IgG-positive only, IgM- and IgG-positive, and IgM- or IgG-positive. For each analysis, we calculated the crude and adjusted prevalence and corresponding 95% confidence intervals. Antibody and PCR test positivity rates over time (days since symptom onset) were estimated using a generalized additive model (GAM) with a random effect for each study participant to take repeated sampling into account using R software (V4.0, R Core Team). IgM and IgG positive rates were further estimated over time in PCR confirmed individuals, i.e. individuals that were tested positive by PCR at any sampling moment.

Ethical considerations

The study protocol was approved by the Cameroon National Ethics Committee (Reference number: 2020/05/1220CE/CNERSH/SP). Written informed consent was obtained from all participants.

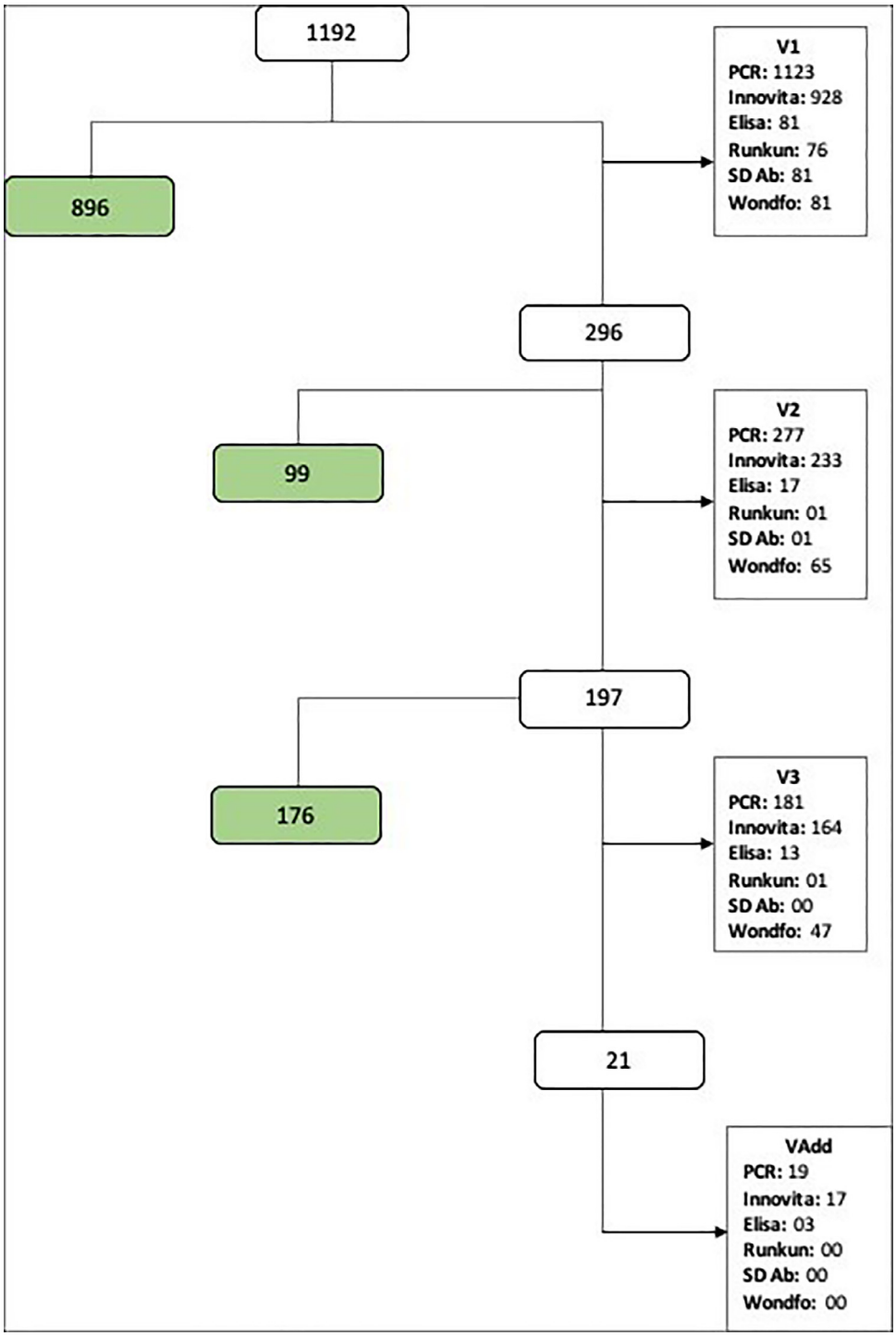

Fig.1. Flow chart for study visits and inclusions.

Results

Participant demographics

We had a total of 1192 study visits. Overall, 347 (29.11%) participants tested positive for SARS-CoV-2 by RT-PCR. 999 (83.8%) were tested using the combined IgM/IgG antibody test (Fig. 1, Table 1). The median age for participants testing

Table 1
Demographic and clinical factors associated with positive rapid antibody test results.

	No antibodies detectedn (%)	IgMpositiven (%)	IgG positiven (%)	IgG and IgM positiven (%)	P-value
	426	72	112	117	
Age (median [IQR])	36.0 [29.0, 46.0]	36.0 [30.0, 47.0]	37.0 [31.0, 45.0]	41.0 [33.8, 51.3]	0.001
Male gender	220 (52.1)	50 (69.4)	61 (54.5)	72 (62.1)	0.02
Hospitalized	27 (6.3)	20 (27.8)	14 (12.5)	20 (17.1)	<0.001
Level of education completed					
None	4 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	
Primary	16 (5.5)	2 (4.0)	11 (13.4)	10 (11.9)	
Secondary	78 (26.9)	14 (28.0)	18 (22.0)	30 (35.7)	
Superior	192 (66.2)	34 (68.0)	53 (64.6)	44 (52.4)	
Profession					0.004
Health worker	27 (8.6)	3 (5.3)	2 (2.3)	2 (2.1)	
Retired	16 (5.1)	3 (5.3)	5 (5.7)	5 (5.3)	
Formal sector	11 (3.5)	1 (1.8)	7 (8.0)	2 (2.1)	
Informal sector	24 (7.6)	9 (15.8)	6 (6.9)	10 (10.6)	
Private sector	123 (39.0)	18 (31.6)	32 (36.8)	29 (30.9)	
Public sector (civil servant)	55 (17.5)	18 (31.6)	15 (17.2)	33 (35.1)	
Health sector	59 (18.7)	5 (8.8)	20 (23.0)	13 (13.8)	
Comorbid disease (Yes)	46 (10.9)	16 (22.5)	18 (16.2)	24 (21.2)	0.006
Symptomatic (Yes) (at time of testing)	187 (44.8)	44 (61.1)	44 (39.6)	60 (51.7)	0.02
Tobacco use					0.38
Unknown	0 (0.0)	0 (0.0)	1 (0.9)	0 (0.0)	
No	419 (99.5)	70 (98.6)	109 (98.2)	112 (99.1)	
Yes	2 (0.5)	1 (1.4)	1 (0.9)	1 (0.9)	

positive for IgM only was 36.0 (interquartile range [IQR] 30.0, 47.0), IgG only was 37.0 (IQR 31.0, 45.0), and for both IgM and IgG was 41.0 (IQR 33.8, 51.3) (P=0.001, Table 1). Hospitalized participants were more likely to test positive for at least one antibody than participants presenting for screening (P<0.001). Male sex was significantly associated with test positivity for at least one immunoglobulin type (P=0.001). Among hospitalized participants, 27.8% tested positive for IgM only, 12.5% tested positive for IgG only, and 17.1% tested positive for both, compared to 5.6%, 12.4%, and 10.7% of non-hospitalized participants presenting for screening, respectively (Table 1). Those tested within 0-7 days of symptom onset were more likely to test negative for antibodies than those within 8-14 days after symptom onset (IgM) and 15 days after symptom onset (IgG, P<0.001). Being a healthcare worker was significantly associated with positive antibody results (P=0.004), with the highest positivity rates in that group, and the lowest rates in retired participants.

RT-PCR and immunoglobulin positivity over time

The proportion of samples testing positive by each method changed over time since symptom onset. The proportion positive by RT-PCR was 50.6% (95% CI 39.4-61.8) on the day of symptom onset and dropped to 43.2% (95%CI 35.7-51.0) 14 days after symptom onset (Fig. 2). For IgM, the proportion positive increased from 17.5% (95%CI 8.4-33.0) on the day of symptom onset to 61.3% (95%CI 49.1-72.2) 18 days after start of symptoms and decreased afterwards. For IgG, the proportion positive increased from 13.5% (95%CI 8.7-20.4) on the day of symptom onset to 60.4% (95%CI 51.7-68.4) after 30 days. Considering only individuals that tested positive by PCR at any moment of sampling, the proportion positive by IgM was highest 20 days after symptom onset with 55.5% (95%CI 46.7-64.0); while it continuously increased for IgG to 62.7% (95%CI 52.2-72.2) 30 days after symptom onset (Fig. 2). IgM positivity was low at the start of illness, gradually increasing to a peak around 20 days after symptom onset, and then slowly declining. The trend for IgG positivity was similar, gradually increasing over the first 10 days of illness followed by a more rapid increase to 30 days and beyond (Fig. 3).

Immunoglobulin prevalence

The overall immunoglobulin prevalence in our study was 32.0% (95% CI 27.0 – 35.0%) with 34.0% (95% CI 29.0 – 39.0%) prevalence in men and 28.0% (95% CI 23.0 – 34.0%) prevalence in women (Table 2). IgM prevalence was 20.0% (95% CI 16.0 – 22.0%); 23.0% (95% CI 19.0 – 27.0%) in men and 16.0% (95% CI 12.0 – 20.0%) in women. IgG prevalence was 24.0% (95% CI 20.0 – 27.0%) overall, 25.0% (95% CI 21.0 – 29.0%) in men and 23.0% (95% CI 18.0% - 28.0%) in women (Table 2). Overall, 12.0% (95% CI 9.0 – 14.0%) of participants tested positive for both IgM and IgG, with 13.0% (95% CI 10.0 – 16.0%) of men and 11.0% (95% CI 8.0 – 14.0%) of women testing positive for both immunoglobulins. Immunoglobulin positivity in hospitalized patients was 27.8% for IgM, 12.5% for IgG and 17.1% for both IgM and IgG, and for non-hospitalized patients was 6.5%, 12.1% and 10.5% respectively (Table 2).

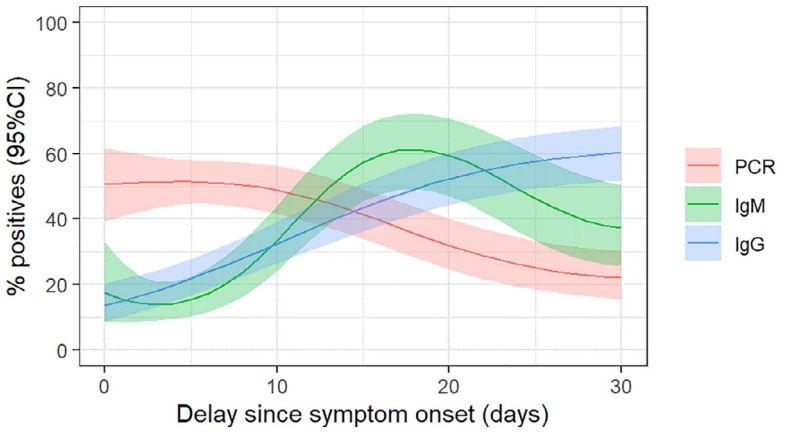

Fig. 2. Comparison of SARS-CoV-2 serologic and PCR test positivity by days since symptom onset.

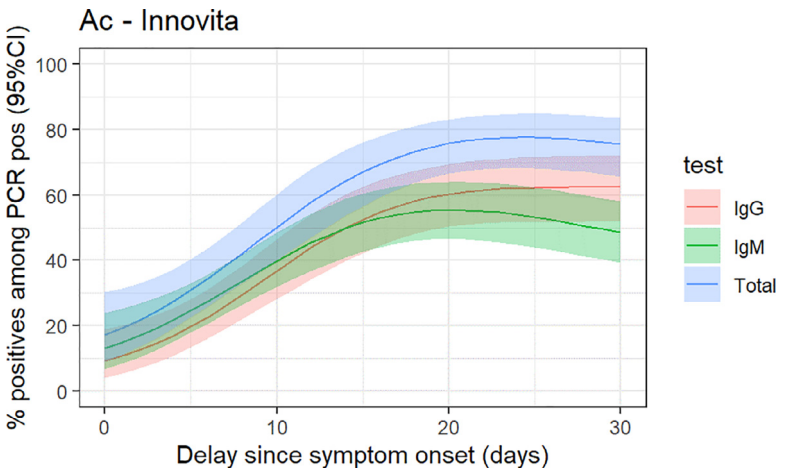

Fig. 3. IgM and IgG antibody positivity by days since symptom onset.

Table 2
Immunoglobulin prevalence stratified by specific immune globulin and participant gender.

	Overall proportion (95% CI)	Men proportion (95% CI)	Women proportion (95% CI)
IgM, IgG or both	32 ([27]-35)	34 (29-39)	28 ([23]-34)
IgM	20 [16-22]	23 [19-27]	16 [12-20]
IgG	24 [20-27]	25 ([21]-29)	23 [18-28]
IgM/IgG	12 [9-14]	13 [10-16]	11 [8-14]

Discussion

Here we present one of the first reports of serologic response data for people living in central Africa who were hospitalized or seeking COVID-19 screening. These results help to fill the knowledge gap concerning the magnitude of the COVID-19 epidemic in Cameroon and may be generalizable to similar sites across sSA.

Our overall 32% detectable immunoglobulin prevalence is significantly higher than that reported by Havers and collaborators in the San Francisco metropolitan area (1%), New York City (8%) [23], and a meta-analysis of populations worldwide (6%) [24], but similar to reports from Pakistan (36%) [25], New York State (23%) [26] and an urban neighbourhood near Boston (32%) [27]. Vast differences in prevalence can be explained by differences in population heterogeneity, demographics, urbanity, study design, and timing of sample collection relative to symptom and epidemic onset. We began collecting samples in central Cameroon in June 2020, far into the global pandemic. By that time, SARS-CoV-2 had likely been cir-

culating in the population for months, increasing population seroprevalence over time. In contrast, studies reporting low seroprevalence <10% were generally conducted on samples collected during the early phase of the pandemic and in less urban centers with high population heterogeneity. The population studied here was comprised of 10% hospitalized patients and 90% non-hospitalized participants seeking screening. Although our study population does not reflect a household seroprevalence survey, our overall IgG prevalence result (24%) is similar to that reported by the national COVID-19 response team (17%) among 3,218 people serosurveyed using a mobile testing laboratory in Cameroonian marketplaces (unpublished data 2020). The slightly higher prevalence seen in our study may be explained by the higher number of hospitalized and symptomatic participants included in our studied population, also self-selection occurrence is likely, people with a higher risk of getting the disease may have enrolled massively, increasing the seroprevalence.

To our knowledge, our report is among the first to describe dynamics of SARS-CoV-2-specific IgM and IgG kinetics in an African population. In our study, immunoglobulin positivity was inversely related to antigen and RT-PCR positivity. During the first seven days of symptoms, IgM and IgG antibody positivity was very low, followed by a more rapid rise in IgM than IgG to a peak around day 20 followed by an IgG plateau between symptom days 20-30 and an earlier slow decline in IgM positivity. These trends are in agreement with other studies demonstrating a high proportion of negative antibody tests during the early stages of infection. Therefore, we agree with others that rapid antibody tests alone should not be used to diagnose COVID-19 within the first 7-10 days of symptoms. In symptomatic patients, IgM antibody peaked 20 days after symptom onset, before IgG peaked at 30 days. These results differ from what was observed in China, were seroconversion began earlier, on symptom day five, and IgG peaked before IgM [28]. However, the study in China included only people testing SARS-CoV-2 PCR-positive, while our study was largely comprised of PCR-negative participants. Longer delay to antibody development among individuals who are PCR-negative could partially explain the differences in antibody kinetics between the two populations. Antibody positivity was highest in healthcare workers, likely as a result of occupational exposures, and lowest in retired participants, likely due to lack of contact with persons outside the come. Level of education was not associated to antibody positivity, and may reflect highly inclusive sensitisation programmes on COVID-19 conducted by the Cameroonian Ministry of Public Health and a large proportion of highly educated study participants.

One limitation of our study is that although it was not targeted to a specific demographic group, it is not fully population-based since individuals self-selected for screening or were enrolled after hospitalization with COVID-19 symptoms. In addition, the rapid diagnostic antibody test employed here did not test the neutralizing ability of IgM and IgG, limiting our ability to make inferences about reinfection risk and population immunity. Another limitation of our study is the use of generalized additive modeling to estimate the time to test positivity from symptom onset, though our model estimates were robust.

Major strengths of our study include broad inclusion criteria not limited to a specific population sector, and concomitant antigen and PCR testing for SARS-CoV-2. In addition, our results are among the first describing immunoglobulin prevalence and kinetics among people living in sub-Saharan Africa, which is important to better understand regional transmission dynamics, target vaccine deployment, and monitor the development of possible herd immunity. Current estimates of the herd immunity threshold needed to prevent onward SARS-CoV-2 transmission range from 43% [2,3] to 75% [4,5] depending on regional factors including population demographics and heterogeneity. Our results are likely generalizable to similar settings and populations in sub-Saharan Africa and suggest that herd immunity is not a realistic near-term goal at a country level but can happen in some regions or cities.

Conclusion

This serosurvey is an important first step towards filling the knowledge gap concerning the effects of COVID-19 on African populations and represents a preview of what can be expected and achieved in future population-based seroprevalence studies across sub-Saharan Africa.

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Declaration of Competing Interest

We declare no competing interests.

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