Seroprevalence of SARS-CoV-2 in 10 Regional Capitals of Cameroon, October-December 2020

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Abstract

Cameroon was among the most affected African countries during the first wave of the COVID-19 pandemic; however, the true prevalence of SARS-CoV-2 remains unknown. From October-December 2020 we conducted a cross-sectional, age-stratified SARS-CoV-2 seroepidemiological survey at 30 purposively selected community-based sites across Cameroon's 10 regional capitals, sampling 10,000 individuals aged 5 years or older. We employed a parallel SARS-CoV-2 antibody testing algorithm (WANTAI ELISA and Abbot Architect) to improve both the positive predictive value and negative predictive value of seroprevalence. The overall weighted and adjusted seroprevalence of SARS-CoV-2 antibodies across the 10 urban capitals of Cameroon was 10.5% (95% CI: 9.1%-12.0%) among participants aged [?]5 years. Of the 9332 participants, 730 males (13.1%, 95% CI: 11.5%-14.9%) had SARS-CoV-2 antibodies compared to 293 females (8.0%, 95% CI: 6.8%—9.3%). Among those who reported a comorbidity at the time of testing, 15.8% (95% CI: 12.8%-19.4%) were seropositive. We estimated that over 2 million SARS-CoV-2 infections occurred in the 10 regional capitals of Cameroon between October and December 2020, compared to 21,160 cases officially reported at that time translating to one laboratory-confirmed case was reported for every 110 SARS-CoV-2 infections across the 10 urban capitals. This study's findings point to extensive and under-reported circulation of SARS-CoV-2 in Cameroon– an almost 100-fold more cases compared to the number of cases reported to the World Health Organization. This finding highlights the importance of conducting serosurveys, especially in settings where access to testing may be limited and to repeat such surveys as part of pandemic tracking.

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Disclaimer

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Abstract

Background

Cameroon was among the most affected African countries during the first wave of the COVID-19 pandemic; however, the true prevalence of SARS-CoV-2 remains unknown.

MethodsFrom October-December 2020 we conducted a cross-sectional, age-stratified SARS-CoV-2 seroepidemiological survey at 30 purposively selected community-based sites across Cameroon's 10 regional capitals, sampling 10,000 individuals aged 5 years or older. We employed a parallel SARS-CoV-2 antibody testing algorithm (WANTAI ELISA and Abbot Architect) to improve both the positive predictive value and negative predictive value of seroprevalence.

ResultsThe overall weighted and adjusted seroprevalence of SARS-CoV-2 antibodies across the 10 urban capitals of Cameroon was 10.5% (95% CI: 9.1%-12.0%) among participants aged [?]5 years. Of the 9332 participants, 730 males (13.1%, 95% CI: 11.5%-14.9%) had SARS-CoV-2 antibodies compared to 293 females (8.0%, 95% CI: 6.8%—9.3%). Among those who reported a comorbidity at the time of testing, 15.8% (95% CI: 12.8%-19.4%) were seropositive. We estimated that over 2 million SARS-CoV-2 infections occurred in the 10 regional capitals of Cameroon between October and December 2020, compared to 21,160 cases officially reported at that time translating to one laboratory-confirmed case was reported for every 110 SARS-CoV-2 infections across the 10 urban capitals.

ConclusionThis study's findings point to extensive and under-reported circulation of SARS-CoV-2 in Cameroon– an almost 100-fold more cases compared to the number of cases reported to the World Health Organization. This finding highlights the importance of conducting serosurveys, especially in settings where access to testing may be limited and to repeat such surveys as part of pandemic tracking.

Key Words: SARS-CoV-2, Serosurvey, Cameroon, COVID-19

Introduction

Coronavirus disease (COVID-19), caused by SARS-CoV-2, was first reported in China at the end of 2019. The highly contagious virus quickly reached pandemic proportions, and by the end of 2020, over 90 million COVID-19 cases had been reported in 218 countries.[1]

Cameroon had its first registered SARS-CoV-2 positive test in early March 2020.[2,3] Cases started to rise rapidly country-wide, and by March 17, 2020, Cameroon was under a strict public health alert; wearing of

masks became mandatory in all public areas and education and awareness campaigns were implemented. Cameroon had the highest absolute number of cases in the central Africa sub-region in 2020, with almost 30,000 reported cases and nearly 500 deaths[4]. However, the true number of SARS-CoV-2 infections in Cameroon is unknown because of both the likely large number of people with asymptomatic or mildly symptomatic infections, as well as the lack of widespread testing.[5] Thus, determining the extent of community spread and establishing baseline seroprevalence data are needed to better understand population immunity levels and the impact of public health interventions, such as vaccinations, on future waves of transmission.

The purpose of this study was to estimate and describe the prevalence of SARS-CoV-2 antibodies among individuals aged > 5 years in the 10 regional capitals of Cameroon. In addition, we aimed to determine factors associated with seropositivity. Finally, we gauge knowledge and attitudes towards COVID-19. To the best of our knowledge, this was the first national SARS-CoV-2 serosurvey in Cameroon.

Methods

Survey Design and Population

We designed a cross-sectional, age-stratified SARS-CoV-2 seroepidemiological survey in community-based sites across 10 regional capitals of Cameroon, in line with the World Health Organization (WHO) UNITY studies framework.[13,] This survey took place from October to December 2020 at 30 purposively selected community-based sites (three in each regional capital), including marketplaces, bus stops, and busy commercial intersections. High traffic venues were chosen because they most likely had a broad representation of age groups, sexes, and socioeconomic statuses. The sites were selected in conjunction with local stakeholders [Cameroon Ministry of Health, U.S. Centers for Disease Control and Prevention (CDC) in Cameroon]. For a more representative sample of the regional population, we set regional quotas for three broad age groups: 5-19 years, 20-49 years, and 50+ years (Appendix Table 1). The quotas were proportional to the regional population based on census data (2020) for each of these age groups.[14]

Children < 5 years were excluded due to the difficulty in obtaining blood from young children, especially in such public settings. At each site, survey staff used banners and other promotional materials and loudspeakers to announce the survey and attract attention to the on-site survey tent. If participants were traveling in groups, only one adult and one child (5 years and above) per household were eligible to participate based on self-reported relationships. Data were collected at each site for approximately three weeks, until quotas were met.

Prior to participation in the survey, written informed consent was obtained from adults (aged [?]21 years as per Cameroon regulations) and emancipated minors and from parents for participants aged [?]20 years. Written informed assent was also obtained from participants aged 10-20 years. The survey was approved by the Cameroon National Research Ethics Committee and Columbia University reviewed and designated it as public health surveillance. The study was reviewed in accordance with the U.S. CDC human research protection procedures and was determined as research. However the CDC investigators did not interact with any individuals or have access to identifiable data or specimens for research purposes.

Procedures

Data were collected from October 19 to December 17, 2020. A questionnaire was first administered to participants (or their parent/guardian for those ages 5-14 years) using SurveyCTO on tablet. In this survey, which was adapted from the UNITY survey template, researchers collected information about sociodemographic factors; past COVID-19 symptoms; potential COVID-19 exposures [known contact with a laboratory-confirmed case, travel (domestic or international), and health facility use]; co-morbid conditions and other risk factors; history of health seeking behavior; and knowledge, attitudes, and beliefs about COVID-19. To asses past COVID-19-like illnesses, participants were asked whether, since the beginning of the COVID-19 pandemic in January 2020 (before the first reported case in Cameroon), they had experienced any of the following symptoms per case definition used in Cameroon: dry cough, shortness of breath, tiredness, fever, muscle pain, diarrhea, headache, new loss of smell/taste, sore throat, nausea/vomiting.

After completing the questionnaire, 5 mL of venous blood were collected and placed in a cooler box with ice packs. After providing consent, participants who decided they did not want to complete the questionnaire were still allowed to provide their blood specimens. At the end of each day, samples were transported to a local laboratory where, using the plasma preparation tubes (PPTs), they were centrifuged to separate the plasma and then immediately frozen at -20degC. The frozen PPTs were transported under cold chain to the National Public Health Laboratory (LNSP) at the end of each week. At LNSP, the separated plasma in each PPT was pipetted into two 1 mL cryotubes (one cryotube for each assay) and frozen at minus -80degC until testing was performed.

Given the expected low seroprevalence (<10%) of SARS-CoV-2 antibodies in the Cameroon population at that time a parallel testing algorithm was used to improve both the positive predictive value (PPV) and negative predictive value (NPV). Two enzyme-linked immunosorbent assays (ELISA) tests available at the time (2020) were selected based on several factors: (1) sensitivity and specificity of available tests [minimum 95% sensitivity and 90% specificity, in line with United States Food and Drug Administration Emergency Use Authorizations (US FDA EUA) validation requirements], (2) the best overall PPV and NPV of the testing algorithm using the FDA/CDC calculator on available tests on the market at that time point, (3) availability of plasma as the specimen type for testing, (4) authorization for use by US FDA EUA, WHO emergency use listing, CDC, or other internationally recognized bodies, and (5) test availability for purchase and shipment within a reasonable timeframe to complete this survey. The tests chosen were the Abbott Architect SARS-CoV-2 IgG (Abbott Diagnostics, Illinois, United States; 100% sensitivity and 99.6% specificity based on FDA report) and the WANTAI SARS-CoV-2 Ab ELISA (Beijing, China; 94.5% sensitivity and 100% specificity according to manufacturer's report). The WANTAI assay detects total antibody to the receptor binding domain (RBD) of the SARS-CoV-2 spike protein, including IgM, IgA, and IgG antibodies, while the Abbott assay only detects IgG antibodies against the nucleocapsid (N) protein. When used in combination, this testing algorithm theoretically should produce an overall PPV of ˜100% based on a prevalence from 1-10% according to the FDA PPV calculator.[15]

WANTAI ELISA testing was performed at the LNSP while the Abbott Architect assay was performed at the Blood Bank of Yaounde Central Hospital because of the availability of appropriate equipment. Each assay and test interpretation was performed according to the manufacturer's instructions and included appropriate quality controls to validate each run.[16,17] After the testing was completed in the laboratory, remnant plasma specimens were returned to the -80degC freezers for long-term storage.

A call center with set operational hours was established to return the results, which was listed on the consent forms provided to the participants. Survey participants would call and obtain their test results using a random identification number assigned at enrollment.

Statistical analysis

For the analysis, only those who tested positive on both WANTAI and Abbott assays were classified as SARS-CoV-2 antibody positive (Figure 1). Those who tested negative on only one or both assays were classified as negative.

Sampling weights were calculated based on selection probabilities at each site. Non-response weights (for questionnaire and each laboratory test) were calculated at the individual level. Weights were calibrated to regional population estimates by age and sex, and then normalized to the total sample size.[14] Weighting was carried out in R using the glm, anesrake, and survey packages.

Seroprevalence estimation was done in SAS using the SURVEYFREQ procedure and the calibrated survey weights. Taylor series variance estimation was used to account for the clustered sample design, treating each survey site as a separate cluster. Unweighted counts and proportions were also computed in SAS. The χ^2 test was used to assess the differences in prevalence across demographic characteristics.

We conducted unadjusted and multivariable logistic regression, incorporating survey weights, to assess the associations between demographic and behavioral characteristics with seropositivity. The analysis included

participants with complete age and sex data. Final multivariable models included variables with $p<0.05$ and variables (sex, age, residence, region, number of household members, comorbid conditions, and history of COVID-19, travel) chosen *a priori* based on their likely association with the risk of SARS-CoV-2 past infection.

Regional-level seroprevalence estimates were applied to 2020 region-specific population projections from the Cameroon Statistics Agency to estimate the total number of SARS-CoV-2 infections in each region that had occurred in the survey period (October-December 2020). These numbers were compared with the total number of reported cases in the country at the end of the study (December 2020) to estimate the ratio of reported cases to total SARS-CoV-2 infections.

Agreement in test results between the WANTAI and Abbott assays was also evaluated using kappa statistics.

Role of the funding source

The funder of the study was involved in the study design, data analysis, data interpretation, and writing of the manuscript.

Results

Overall, 10,386 individuals were enrolled in the survey. Of these, 9,180 (88.7%) completed an interview, 10,071 (97.0%) completed a blood draw, and 9,686 (93.3% of 10,071) had results from both antibody assays. In total of 9,332 (89.9% of 10,386) participants who had valid results from both assays as well as valid age and sex data were included in the analysis (Appendix Figure 1). From both assays, 4,738 (50.8%) participants tested negative, 1,023 (10.9%) tested positive, and 3,571 had discordant results resulting in a concordance of 61.7% and a kappa of 0.19 (Appendix Table 2). The WANTAI assay produced more positive results than the Abbott assay (4,280 (45.8%) vs. 1,337 (14.3%)).

Approximately half of the participants were female (51.2%, or 3,548) and were age 15-25 years (48.8% or 4,529). All participants who enrolled were Cameroonians, and the majority had completed secondary education (43.1%) (Table 1). Only 9.2% of participants reported co-morbid medical conditions (Table 1). Almost half of the participants age [?]15 years (43.7% or 3,351 of 8,399) reported being unemployed. The majority of those who were employed, worked in the informal trade sector (17.0%) (Table 1).

The overall weighted and adjusted seroprevalence of SARS-CoV-2 among those aged 5+ years across the 10 regional capitals of Cameroon was 10.5% (95% confidence interval [CI]: 9.1%-12.0%) among participants [?]5 years (Table 2). The seroprevalence was higher among males than it was among females [13.1% (95% CI: 11.5%-14.9%) vs. 8.0% (95% CI: 6.8%—9.3%); $p<0.0001$). Seroprevalence also differed by location ($p=0.0062$), ranging from 7.5% (95% CI: 5.9%-9.5%) in the East region to 12.4% in the Far North (95% CI: 10.4%-14.8%) and North West (95% CI: 10.5-14.7) regions. Across age groups, seroprevalence ranged from 7.9% (95% CI: 5.9%-10.7%) among participants 5-14 years, to 19.2% (95% CI: 14.7%-24.8%) among those aged 60 years and above. Among those who reported a comorbidity at the time of testing, 15.8% (95% CI: 12.8%-19.4%) were seropositive. Further, among participants who were diabetic at the time of testing, 24.2% (95% CI: 15-4%-36.0%) tested positive for SARS-CoV-2 antibodies. Among participants who had reported recent travel, 15.9% (95% CI:11.6%-21.5%) of those who had reported recent international travel were seropositive and 11.9% (95% CI:10.6%-13.4%) of participants who had reported recent domestic travel were seropositive (Table 2).

In the multivariable analysis females had a significantly lower seroprevalence than males (adjusted odds ratio [aOR]: 0.61, 95% CI: 0.51-0.74). Further, participants residing in the North (OR: 0.54 [95% CI: 0.30-0.99], Adamawa (aOR: 0.62 [95% CI: 0.41-0.94]), the East (aOR: 0.57 [95% CI: 0.40-0.82]), and Littoral (aOR: 0.60 [95% CI: 0.40-0.88]) regions had a lower seroprevalence compared to those in the South West (Table 2). The odds of having tested positive for this virus were also significantly higher across older age groups (as compared to children ages 5-14 years); (aOR: 1.15 [95% CI: 0.12-10.76), for those ages 15-25 years and aOR: 1.72 [95% CI: 0.21-14.25) for persons aged 26-35 years). Seroprevalence was higher among participants who reported having ever tested SARS CoV-2 positive (aOR: 3.61 [95% CI:2.01-6.47]), and among participants

who traveled internationally in the past year compared to those who did not travel at all within the past year (aOR: 1.56 [95% CI: 1.10-2.22]). In multivariable analyses, seroprevalence did not differ by residential setting (urban vs. rural), history of comorbidities, or household size.

Based on the seroprevalence results from the study, we estimated that over 2 million persons were seropositive for SARS-CoV-2 (cumulatively) in the 10 regional capitals of Cameroon during October to December 2020, as compared to 21,160 cases officially reported at that time (Table 3).[18] We estimate that one laboratory-confirmed case was reported for every 110 SARS-CoV-2 infections across the 10 regional capitals. The highest number of SARS-CoV-2 infections was estimated to be in Center region with an estimated 475,000 infections, and the lowest number in Adamawa with an estimated 64,700 infections.

Discussion

Overall, we found that an estimated 10.5% of Cameroonian survey participants aged [?]5 years were SARS-CoV-2 seropositive at the end of 2020. SARS-CoV-2 seroprevalence ranged widely by region (7.5% to 12.4%) and seroprevalence was higher among males, persons older than 25 years of age, those who reported ever having tested positive for SARS-CoV-2 and among those who had reported having a recently traveled. However, seroprevalence did not differ by, residential setting (urban vs. rural), having a comorbid condition, or household size.[19] Another seroprevalence survey conducted in Cameroon that sampled households in Yaoundé, Cameroon that was conducted around the same time as this study found a seroprevalence of 29.2% (95% CI 24·3–34·1).[20] This was more than 2.5 times higher than what we found for the seroprevalence (11.8%) in the Center region where Yaoundé is located. Like our survey, this survey also found that seroprevalence was higher among males. However, unlike our survey, they found a higher seroprevalence among participants living in larger households.

Our finding of 10.5% seroprevalence translates to an estimated 2,347,500 total infections across Cameroon at the time of the survey. This is over 100 times higher than the cumulative number of cases reported as of December 30, 2020.[18] Currently, there are 124,392 all time confirmed cases and 1,965 deaths reported in Cameroon due to SARs-CoV-2[40]. A meta-analysis that used data from seroprevalence studies conducted in 2020 found an overall seroprevalence of 19.5% in Sub-Saharan Africa which varied widely and was significantly higher than that in high-income countries.[21] The authors also found that seroprevalence estimates were a median 18.1 times higher than the cumulative incidence of reported cases overall and 600 times higher in Sub-Saharan Africa. Another meta-analysis which used data published through December 2021 from Africa only (including data from our survey) also found a very high ratio (97:1) of seroprevalence to cumulative incidence that remained fairly constant over time. Several reasons have been suggested for the seemingly low number of cases and even lower mortality rate and the misalignment between seroprevalence findings and reported cases in Africa. These factors include lack of access to health services, including SARS-CoV-2 testing; limited public health surveillance capacity and infrastructure, including shortages of SARS-CoV-2 real-time (RT)-PCR test kits and other laboratory supplies; ; swift and wide-reaching public health measures established by many countries; and the overall lower age of its population.[23-24] Further, in 2020, the stigma associated with COVID-19, along with misinformation and disinformation in the community likely resulted in testing avoidance which exacerbated the undercounting of cases.[25]

As SARS-CoV-2 can spread asymptomatically, the official reported number of cases to health reporting systems globally did not include all the possible infections. This underscored the need for seroprevalence surveys that could present a full picture of the disease burden in a population by measuring SARS-CoV-2 antibodies in sampled blood specimens to detect previous infection, regardless of the presence or absence of symptoms. In countries where testing numbers were low, both because of low demand and testing supply shortages, the need for serosurveys was critical to understanding the epidemic both on a national and sub-national level, which was the case for Cameroon. Thus, we designed the first SARS-CoV-2 seroprevalence survey that included all 10 regions of Cameroon comprising over 10,000 adults and children aged [?]5 years. In each city, individuals were recruited from multiple sites to increase the diversity of participants. The survey also demonstrated the feasibility of performing a community-based serological survey in large African regional centers.

The prevalence estimates from this study were based on two assays: WANTAI SARS-CoV-2 Ab ELISA and Abbott Architect SARS-CoV-2 IgG. The WANTAI assay, which was made available by the WHO and was not independently evaluated at the time of the survey while the Abbott assay was authorized for use by the U.S. FDA after independently qualifying the assay with 100% sensitivity and 99.6% specificity.[26] In 2020, many antibody test kits entered the market with variable, and in some cases unreliable, test performance characteristics (sensitivity, specificity, PPV and NPV).[27] To overcome some of these test kit performance issues, we employed a parallel two-test algorithm which increased the overall PPV for more accurate seroprevalence estimates. This approach differed substantially from that used by the majority of serosurveys conducted in the early days of the pandemic whereby a single test was employed to estimate prevalence, leading to uneven surveillance data quality.[7,28] Some of these antibody tests included lateral flow immunoassays that had poorer performance than ELISA assays.[29]

In our parallel two-test algorithm, we found poor concordance between the WANTAI and Abbott assays (Kappa value = 0.19), with the WANTAI assay producing a very high positivity rate of 45.9% compared to that of the Abbott assay, which was 14.3%. Similar to our findings, other studies have noted high positivity rates with the WANTAI assay compared to other ELISA assays.[30-32] One possible explanation for this discrepancy is that the WANTAI assay detects total antibodies to the receptor binding domain (RBD) of the SARS-CoV-2 spike protein while the Abbott assay only detects IgG antibodies against the N protein. Assays that detect total antibodies have been shown to be more sensitive than those that detect either IgM or IgG and detect antibody earlier in infections (<21 days post-symptom onset).[33-35] Another possible contribution to the high positivity is the lower specificity due to cross-reactivity to other circulating antibodies resulting from past infections from other pathogens. This has been noted in other evaluations, including WHO's own evaluation,[36] resulting in false-positives and higher overall prevalence estimates.[37] Conversely, the lower positivity rate from the Abbott assay may be due to the lower sensitivity associated with only detecting IgG antibodies and timing of testing from days post infection (<21 days). Given the shortcomings of both assays, as well as the unknown prevalence of COVID-19 in Cameroon at the time of the survey, a parallel testing algorithm was used to reduce false positives and improve the overall PPV of the SARS-CoV-2 prevalence estimates for this survey.

This survey had several limitations. First, the use of convenience sampling from the regional capitals may indicate that the results are not representative of the entire population of Cameroon. However, the use of age stratified targets and post-stratification with the participant weights was used to help reduce the effects of this selection bias. Second, bias may have been introduced because of experiences with COVID-19, for example, people may have been more willing to participate if they or someone they know had been affected by COVID-19, or they may have been less willing, if they felt like they had already contracted COVID-19 and were not interested in finding out their antibody statuses. Third, the assays could have missed individuals who were still in the early stages of seroconversion. Our survey also has several strengths. It was the first SARS-CoV-2 seroprevalence survey that included all 10 regions of Cameroon and included over 10,000 adults and children aged [?]5 years. In each city, individuals were recruited from multiple sites to increase the diversity of participants. The survey also demonstrated the feasibility of performing a community-based serological survey in large African urban centers. Further, we used two antibody tests to increase both PPV and NPV.

We conducted our survey in late 2020 before Cameroon experienced its second largest SARS-CoV-2 wave and two subsequent waves that likely increased seroprevalence,[18] especially given that only 3% of the population had been vaccinated by the end of that year.[38] Repeated seroprevalence surveys after each large infection wave would have been useful to understand how readily the virus spreads in this population, and also the impact of vaccines on the spread of the virus and any associated mortality.

Conclusion

Approximately one in ten individuals in the regional capitals of Cameroon had been infected with SARS-CoV-2 by December 2020, indicating extensive and under-reported circulation of SARS-CoV-2 in the country, with almost 100-fold more cases across Cameroon as compared to the number of cases reported to the WHO.

This finding highlights the importance of conducting seroprevalence surveys especially in places where access to testing may be limited and repeating such surveys as part of tracking the trajectory of the pandemic.

Seroprevalence surveys, if conducted using accurate testing algorithms, can capture a fuller burden of infection in a population, allowing public health officials to decide on an effective pandemic response and identify subgroups at higher risk for infection. With the availability of home-testing and more mild disease, reported numbers of infections will become less reliable and national seroprevalence surveys will become more important to understand disease burden and geographical impacts on a population.

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