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Nationwide retrospective mortality and seroprevalence of SARS-CoV-2 antibodies in Cameroon.

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Abstract: (197 words)

Background: Although the first year of the COVID-19 pandemic in Africa did not produce the expected catastrophe, the true impact of COVID-19 in the Cameroonian population was unclear. We therefore assessed the seroprevalence of anti-SARS-CoV-2 antibodies and retrospective mortality in a representative sample of the general population in the 10 administrative regions of Cameroon more than one year after the first confirmed cases of COVID-19 in these regions. we used the SIRS model in our study which allow recovered individuals return to a susceptible state. We aimed to assess the extent of SARS-COV-2 infection and to detect potential increases in the crude mortality rate (CMR) during the SARS-COV-2 pandemic phase.

Methods: We assessed retrospective mortality and seroprevalence of anti-SARS-CoV-2 antibodies in the 10 capital cities of Cameroon using representative samples of the general population. The study included nested anti-SARS-CoV-2 antibody prevalence surveys and retrospective mortality surveys and was conducted between 27 July 2021 and 31 August 2021. To further analyse crude mortality rates by age group and COVID wave, pre-pandemic and pandemic periods were stratified. Both laboratory-based assays (ELFA) and rapid diagnostic tests (RDT) were used to measure anti-SARS-CoV-2 seroprevalence.

Results: The crude mortality rate (CMR) increased from 0.06 deaths per 10 000 persons per day (pre-pandemic) to 0.17 deaths per 10 000 persons per day (pandemic). The increase in CMR was more pronounced in people aged 20-35 years (pre-pandemic 0.02 deaths per 10 000 persons per day; pandemic 0.06 deaths per 10 000 persons per day). The estimated seroprevalence among unvaccinated persons was 9.5% (RDT) and 15.4% (laboratory-based).

Conclusion: The seroprevalence results showed that cases were significantly underdetected by the national surveillance systems.

Keywords: Mortality, Seroprevalence, SARS-CoV-2, Cameroon

Introduction.

The first case of SARS-CoV-2 (COVID-19) infection was recorded in Cameroon on March 6, 2020 (1), three months after the first case was detected in Wuhan, China in December 2019. From that moment, measures to reinforce the surveillance have been put in place by the government. In a short period of time, an increasing number of COVID-19 positive cases have been reported in other regions of the country, from the Centre and Littoral regions were the first cases were detected in their capitals (Yaoundé and Douala) to the 8 other regions of the country, going from the south to the northern parts of the country (2). As from March 18, 2020, measures have been taken by the government of Cameroon to control the epidemic, such as the closure of Cameroon's borders, the closure of all public and private schools, the restriction of gatherings and inter-city travel, the establishment of a curfew from 6 p.m. and encouraging people to respect barrier measures recommended by the World Health Organization (3). Cameroon handled the COVID-19 pandemic with three objectives 1) reduce viral transmission in the community, 2) limit the number of deaths, and 3) lessen the socioeconomic impact of COVID-19. Therefore, the country implemented contextualized mitigation measures, a bold testing strategy incorporating rapid diagnostic tests, treatment of patients with COVID-19 exclusively in specialized treatment centers, re-opening schools during the peak of the pandemic, and integrating mental health care into the national response (4).

Following the first confirmed case on March 6th, 2020 (Figure 1), Cameroun has registered 80,953 COVID-19 cases with a fatality rate of 1.6% on the 31 August 2021 (5). The second wave of the COVID-19 epidemic from December 2021 to May 2022 was more important with a peak of 8,681 cases and 133 deaths (March 2021), than the first wave from April to August 2020 with a peak of 2,170 confirmed cases and 61 deaths (June 2020) (6). Confirmed COVID-19 cases in Cameroon were relatively young (median age of approximately 40 years); and around 60% of cases were male (7).

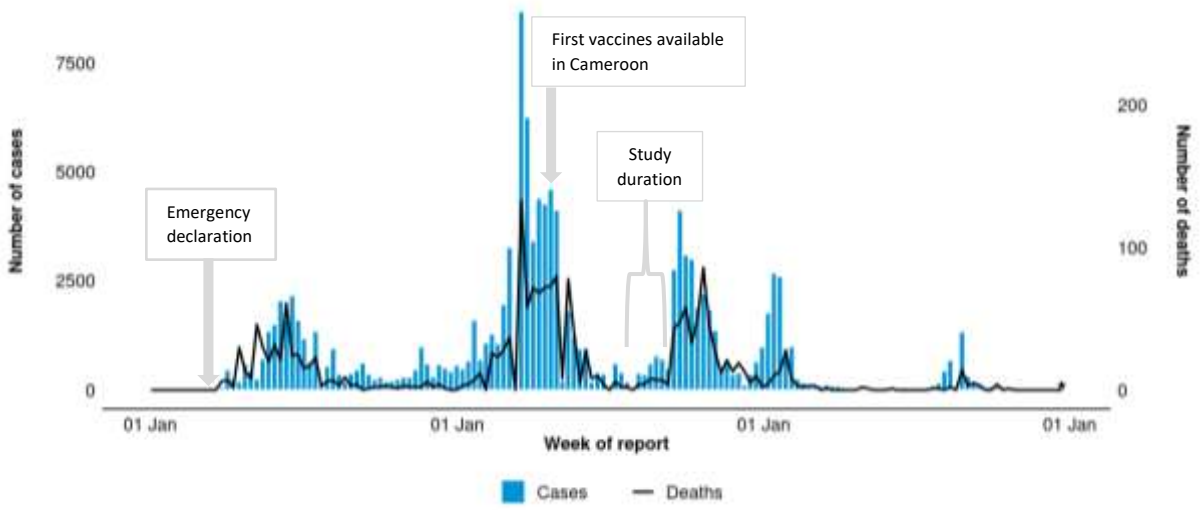

Figure 1. *Number of confirmed COVID cases and deaths by week (January 1st, 2020 – January 1st, 2023), Cameroon* (8)

Many key epidemiological characteristics of COVID-19, such as how the ability of spread or the severity of illness varies by context are still not well understood. Several countries have conducted seroprevalence studies, and demonstrated that the number of infected individuals equate to several times the number of reported cases in most locations, which can be explained by the large number of asymptomatic cases and limitations of deployed surveillance/testing strategies (9–11). The differences in observed mortality across Africa compared to that observed in Asia, the Americas and Europe are even less understood; potential explanations include the younger population structure, the epidemiology of comorbidities and a more limited capacity for diagnosis (12).

Although the first year of the COVID-19 pandemic in Africa did not produce the expected catastrophe, the true impact of COVID-19 in the Cameroonian population was unclear because of massive underreporting of COVID-19 cases and deaths due to the observed decline in hospital attendance over time, continued misinformation on social networks, and irregular reporting of cases from June 2020 (13). We therefore evaluated the seroprevalence of anti-SARS-CoV-2 antibodies and retrospective mortality in a representative sample of the general population in the 10 administrative regions of Cameroon more than one year after the first confirmed cases of COVID-19 in these regions. Our study relies on the SIRS models. While the SIR model assumes people carry lifelong immunity to a disease upon recovery which is the case for Ebola virus disease; for COVID-19, an individual's immunity may wane over time. According to our previous study (14), it takes an average of three months for the immunity to wane. Therefore, we used the SIRS model in our study which allow recovered individuals return to a susceptible state. Patients who tested positive (ELFA and RDT) during our seroprevalence survey are considered to have been exposed within a period of three months. Given the possible

interactions as described by other authors (15) between SARS-CoV-2 infection and other factors such as underlying diseases, socio-economic factors and health policies, justifying the spread of SARS-CoV-2 between people in different ways and for different reasons, our study is part of a syndemic framework. We aimed to assess the extent of SARS-COV-2 infection and to detect potential increases in the crude mortality rate (CMR) during the SARS-COV-2 pandemic phase.

Methods

We conducted a cross-sectional household-based survey in the ten regional capitals in Cameroon from 27 July, 2021 to 31 August, 2021. The study included a retrospective mortality survey and a nested SARS-CoV-2 seroprevalence study. Based on national COVID-19 surveillance data, the 10 regions of Cameroon were divided into two strata at the time of study protocol writing (April 2021): a high transmission stratum (≥ 0.5 cases per 1,000 individuals), including the Centre, Littoral, Northwest, West, East and South regions; and a low transmission stratum (< 0.5 cases per 1,000 individuals), including the Adamawa, Southwest, North and Far North regions. In summary, 198 clusters were first randomly selected on the basis of spatial sampling, and then 5 randomly selected households per cluster were included in the mortality survey. For the seroprevalence survey, the first 10 households per cluster were included in the high transmission regions and the first 5 households per cluster in the low transmission regions. A detailed description of the study design and sampling methodology is provided in the supplementary text (S1). The mortality questionnaire consisted of sections on housing characteristics, demographic data and information on deaths (including date of death) that occurred during the recall period. For the seroprevalence survey, individual questionnaires were administered to each household member or their parent/guardian for children, collecting information on sociodemographics, medical history, potential SARS-CoV-2 exposures, history of possible COVID-19-related symptoms since 2020, and COVID-19 vaccination status. The questionnaires are provided in S2 text.

Participants of the seroprevalence survey were requested to provide a blood sample, in the form of dried blood spots using finger pricks. Serological testing was done using either Wondfo (16,17), Encode (18), Hightop (17) or Right sign (19) rapid test kits. Dry blood spots (DBS) were also collected from the same finger prick, transported to the central laboratory, and were analyzed for anti-SARS-CoV-2 IgG-2 antibodies using the Enzyme Linked fluorescent assay (ELFA) technique from Biomerieux according to manufacturer's instructions(20). Comparative Performance of Serological test assays performed are provided in the supplementary material (Supplementary table 1)

Crude mortality rates (CMR, expressed as deaths/10,000 people/day) with 95% confidence intervals (95%CI) were calculated taking into account the study design using the survey package in R. The recall period was calculated individually for each household member as the number of days between the beginning of the recall period, the date of arrival in the household, or the date of birth, and either the end of the recall period, the date of death, the date when the household member left the household, or the date of the survey. The analysis was stratified between pre-pandemic and pandemic periods. The pre-pandemic period was defined as 1 January 2019 – 29 February 2020 and pandemic period as 1 March 2020 – date of survey. We excluded from the analysis deaths with missing date of death and those with inconsistent birth and death dates. We estimated rate ratios with 95%CIs comparing mortality rates in each time period to the pre-pandemic period and tested for statistical differences using a Poisson generalized linear model (GLM) approach in the survey package in R.

To estimate seroprevalence, a positive RDT result was defined as positive IgM, positive IgG or positive IgM and IgG. A positive ELFA result was defined using a test cut-off value of 0.14 as described previously using manufacturer-specified cut-off value (20). Seroprevalence and 95%CIs were estimated using the survey package in R, weighting for demographic differences between the survey sample and the general population and adjusting for the design effect. To compare seroprevalence among sub-groups (sex, age-groups, strata) we estimated odds ratios (OR) and 95%CIs and tested for statistical differences taking into account the study design and weightings using the survey package in R.

Ethics statement

The study protocol was approved by the MSF Ethics Review Board (ERB) (ID: 2089e) and the national ERB of Cameroon (CNERSH N°: 2021/07/1371/CE/CNERSH/SP) and received administrative authorization from the Ministry of Public Health of Cameroon.

Results.

Study population

The mortality and seroprevalence surveys took place in the capital cities of all 10 regions in Cameroon and were implemented from July 27 to August 31, 2021 at the end of the second SARS-CoV-2 wave (Figure 1). The mortality survey included a total of 22314 household members from 6155 households, with a median age of 34 years (interquartile range [IQR] 12-37 years); 53.2% of participants were female. The serosurvey included 5739 individuals from 3992 households with a median age of 30 years (IQR 22-43 years) and 54.8% of participants were female. Matched ELFA results were obtained from 5583 individuals.

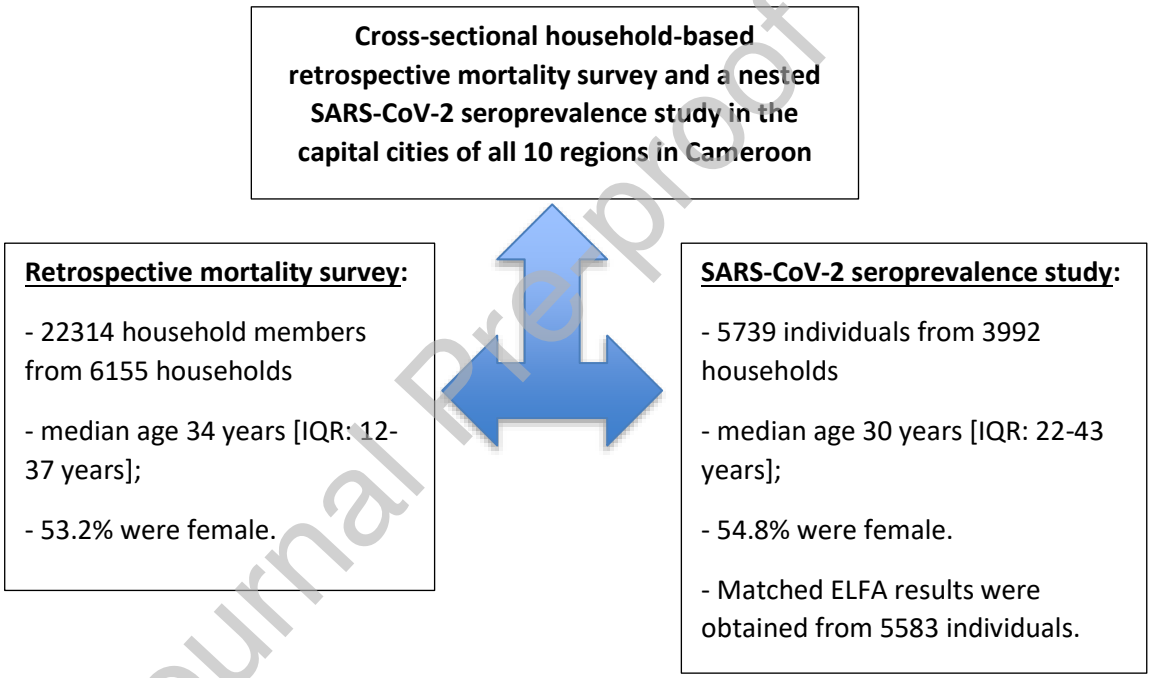

Figure 2. *Breakdown of collected data*

We did not include 55 households in the mortality survey even though they were visited, of which 35 household heads refused to participate and 20 household heads were absent.

Doubling Mortality during the first two waves of the COVID-19 pandemic in Cameroon

During the entire recall period (January 1, 2019 to August 31, 2021), a total of 233 deaths were reported including 56 deaths during the pre-pandemic and 177 deaths during the pandemic period (Figure 3). Further 27 deaths had no valid date of death and had to be excluded from the analysis. The median age of reported deaths was 56 years (IQR 32-75) and 54.9% were male. The main causes of death were chronic diseases (cardiopathy/high blood pressure/cancer/diabetes) (44/233,18.9%),

fever/malaria (26/233,11.2%), and accident/trauma (18/233, 7.7%). COVID-19 was the reported cause for 17/233 deaths (7.1%).

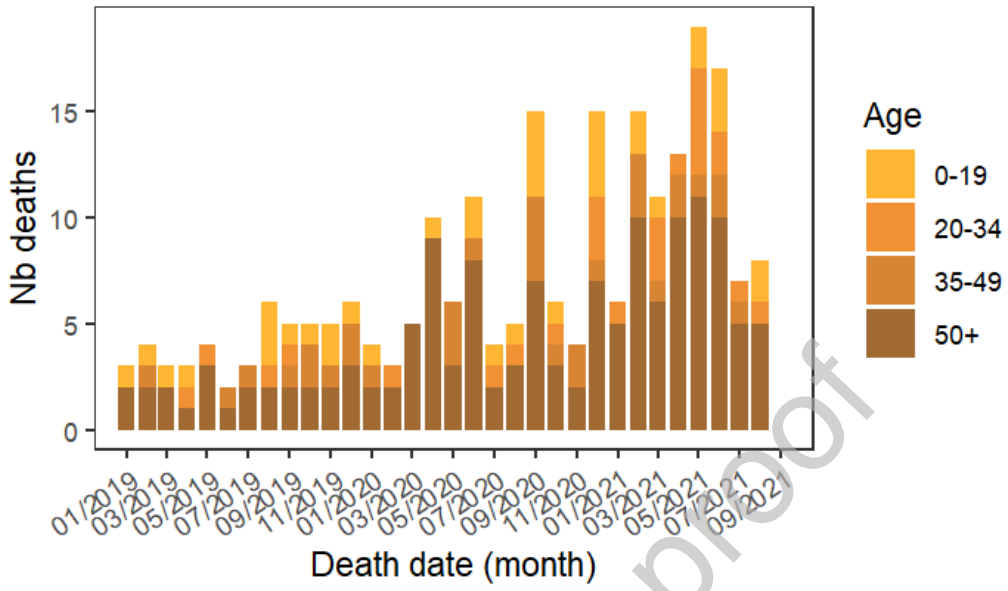

Figure 3. Number of deaths by month and age-group.

The overall CMR more than doubled during the pandemic period with 0.17 deaths per 10 000 persons per day (95%CI 0.14 – 0.20) compared to the pre-pandemic period with 0.06 deaths per 10 000 persons per day (95%CI 0.05 – 0.09) (Rate ratio [RR] 2.63 [95%CI 1.89-3.66]; p<0.001) (Figure 4A). The relative CMR increase was more pronounced among 20-35 year-olds (pre-pandemic 0.02 deaths per 10 000 persons per day [95%CI 0.01-0.06]; pandemic 0.06 deaths per 10 000 persons per day [95%CI 0.04-0.11], RR 3.1 [95%CI 1.1-8.5], p=0.033) and 50+ year-olds (pre-pandemic 0.26 deaths per 10 0000 persons per day [95%CI 0.17-0.39]; pandemic 0.76 deaths per 10 000 persons per day [95%CI 0.61-0.95], RR 3.0 [95%CI 1.9-4.7], p<0.001) (Figure 4B, Supplementary table 2).

The CMR increased both in the high (RR 3.2 [95%CI 2.0-5.2], p<0.001) and the low transmission stratum (RR 2.0 [95%CI 1.3-3.1], p=0.002), with increases being more pronounced during the second wave (Figure 4A, Supplementary table 3).

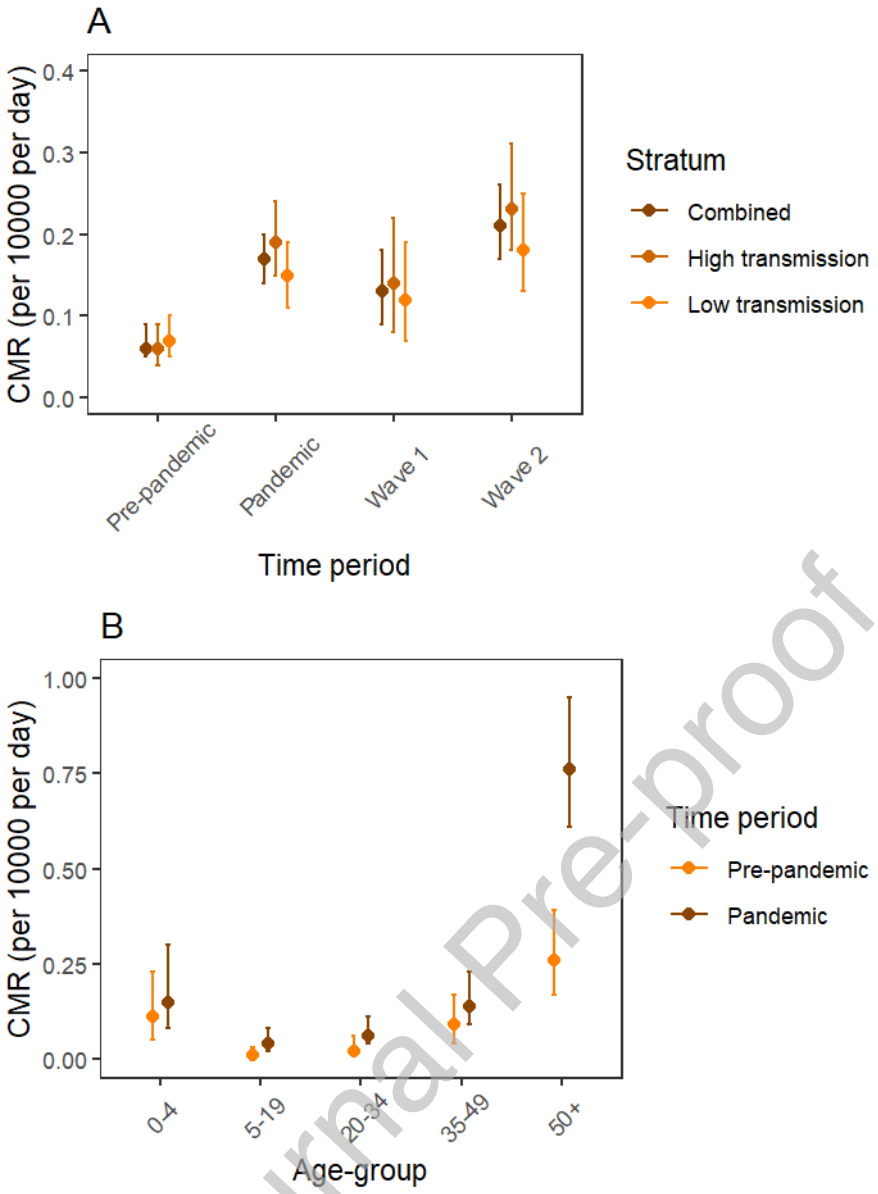

Figure 4. (A) CMR (deaths per 10000 persons per day) by time period, overall and by high/low transmission stratum. (B) Pre-pandemic and pandemic CMR by age-group.

Highest Seroprevalence of SARS-CoV-2 antibodies among elders

In total, 5739 individuals from 3992 households participated in the seroprevalence survey based on RDT and 5583 individuals based on ELFA. Two-hundred-thirty-three households did not participate in the seroprevalence survey (239 household heads refused and 3 household heads were absent). Furthermore, within participating households, 4177 individuals or guardians refused to have a RDT done, or a blood sample taken. The median age of the participants was 30 years (IQR 22-43). COVID-19 vaccination was reported among 312/5739 (5.4%) survey participants (median age 42.5 years, IQR 31-55 years), of which 138 tested positive by RDT (44.2%) and 149 tested positive by ELFA (47.8%).

Among the 5314 non-vaccinated participants, 593 had a positive RDT result and the weighted seroprevalence was 9.5% (95%CI 8.4-10.7). ELFA seroprevalence was 1.6 times higher than based on RDT, with 786/5163 non-vaccinated participants testing positive and a weighted seroprevalence of 15.4% (95%CI 14.0-16.9). A detailed comparison of RDT and ELFA results is provided in the Supplementary material (Supplementary table 4).

We observed significant differences in seroprevalence by age-group of non-vaccinated individuals, with highest seroprevalence among 50+ year-olds (RDT: 13.5% [95%CI 10.9-16.3]; ELFA: 20.1% [95%CI 14.5-26.5]) and lowest among under 20 year-olds (RDT: 7.9% [95%CI 5.8-10.4]; ELFA: 12.1% [95%CI 9.7-14.9]; p-value RDT= 0.002, p-value ELFA= 0.008) (Figure 5).

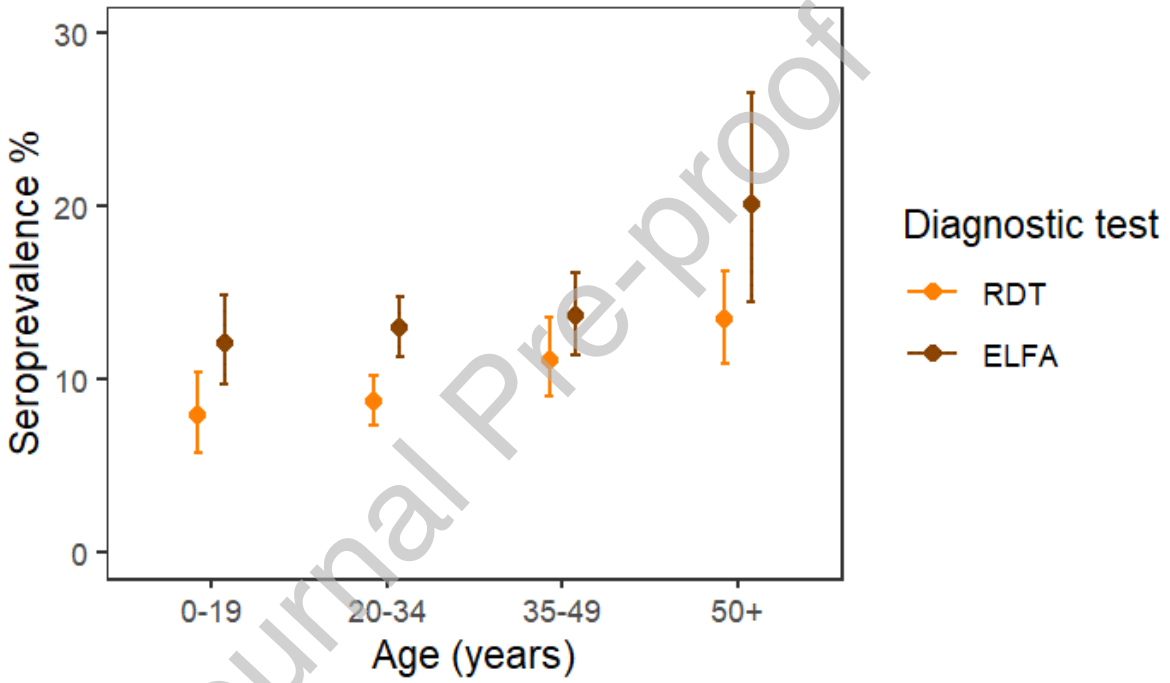

Figure 5. RDT and ELFA based seroprevalence by age-group.

Seroprevalence estimates further varied substantially by region, between 3.9% and 14.8% based on RDT results and between 4.7% and 32.0% based on ELFA results (Figure 6). Highest seroprevalence (both based on RDT and ELFA results) was observed in South-West and West regions and lowest seroprevalence in Adamawa and Far-North regions. The RDT based seroprevalence was significantly higher in regions contained in the high transmission stratum (11.1 % [95%CI 9.9-12.4]) compared to the low transmission stratum (7.7% [95%CI 5.8-9.9], p=0.011). However, there was no significant difference between high and low transmission stratum according to ELFA results (high transmission 14.4% [95%CI 13.1-15.8]; low transmission 13.1% [95%CI 10.5-16.0], p=0.416) (Table 1).

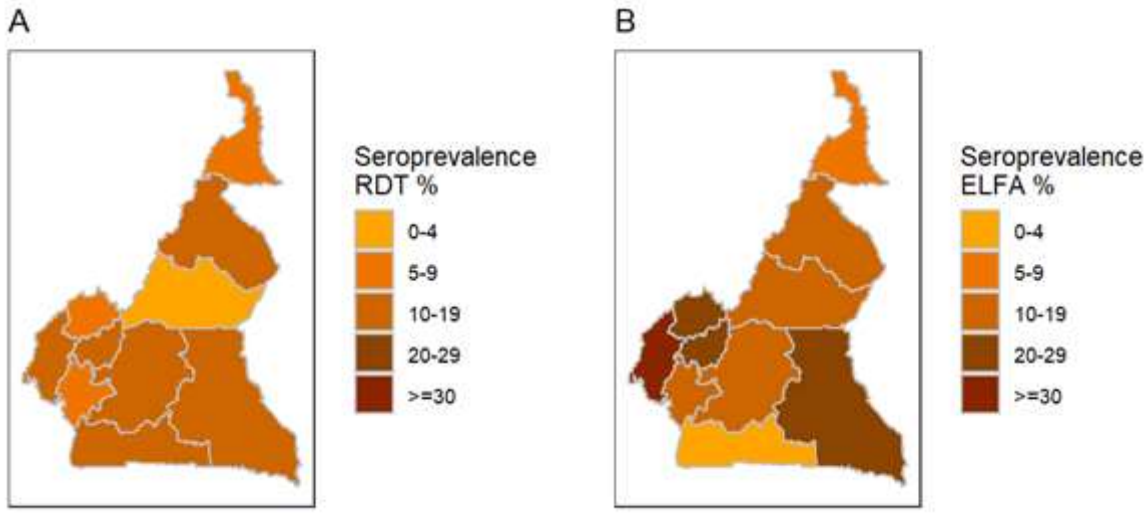

Figure 6. RDT (A) and ELFA (B) based seroprevalence by region.

Table 1. RDT and ELFA based seroprevalence by low/high transmission stratum.

	RDT		**ELFA**	
Stratum	**Seroprevalence (95%CI)**	**OR (95%CI)**	**Seroprevalence (95%CI)**	**OR (95%CI)**
Low transmission	7.7 (5.8-9.9)	**Reference**	13.1 (10.5-16.0)	**Reference**
High transmission	11.1 (9.9-12.4)	1.5 (1.1-2.1)	14.4 (13.1-15.8)	1.1 (0.9-1.5)

Considering only non-vaccinated individuals, 526/3992 (13.2%) of included households had at least one seropositive household member. Among these, 478 households had 1 positive and 48 households 2 or more seropositive individuals. The risk of being seropositive was higher in households with another non-vaccinated seropositive household member (RDT based: OR 5.7; 95%CI 3.7-8.6, p<0.001) compared to households without another non-vaccinated seropositive household member. We did not detect a statistically significant higher risk of a deceased household member among households with one or more seropositive household member (RDT based: OR 1.4; 95%CI 0.7-2.8, p=0.324) (ELFA based estimates are provided in the Supplementary material).

Among 572 non-vaccinated RDT positive individuals, 207 reported at least one COVID-related symptom (weighted estimate 32.0%; 95%CI 27.2-37.1) while among 4488 RDT negative individuals, 116 reported

such symptoms (weighted estimate 22.0%; 95%CI 20.1-24.0). These results are consistent with ELFA based estimates (Supplementary material).

Discussion.

This study represents, to our knowledge, the first COVID-19 combined mortality and seroprevalence survey implemented at national level in an African country. We conducted this survey after the second wave of the pandemic in Cameroon.

We observed an increased mortality rate during the pandemic compared to the pre pandemic period with an overall crude mortality rate (CMR) that more than doubled during the pandemic period compared to the pre-pandemic period. The increase was more pronounced during the second wave. The increased mortality rate was seen in both the high and the low transmission stratum, and among all age groups but with a much higher impact among 50+. The predominance of chronic diseases and infectious diseases among the causes of death in our study is similar to the 43% and 56% respectively found by Echouffo-Tcheugui et al. on burden of chronic non-communicable diseases in Cameroon (21). Our study shows that some of these deaths are directly linked to Covid, however some other may represent collateral effects of the pandemic, such as reduced access to health care, social isolation, or economic impact.

The proportion of all deaths directly linked to COVID-19 was higher (7.1%) than the 1.6% officially reported by the Ministry of Health(22). During the first year of the pandemic people dying of COVID-19 were buried with 48 hours according to the governmental rules (23,24). Therefore, many families were not reporting COVID-19 as the cause of death to carry the burial of their relatives according to their cultural and religious rules. Moreover, some COVID-19 died in the community before reaching hospital especially during the second wave when COVID-19 treatment centers were full with limited oxygen availability.

Among non-vaccinated population, ELFA seroprevalence [15.4% (95%CI 14.0-16.9)] was 1.6 times higher than based on RDT [9.5% (95%CI 8.4-10.7)]. We found two factors correlated with seropositivity: being aged 50 years and over (the lowest seroprevalence was found among under 20-year-olds) and having another case in the household. This highest SARS-CoV-2 infection risk among elderly individuals was also shown by several studies (25–28). However, some studies have shown a variability of the SARS-CoV-2 seroprevalence according to age with a higher seroprevalence among middle ages or

younger (29–31). The high seroprevalence and CMR highlight the importance in the vaccination strategies, of targeting populations identified as vulnerable according to their risk factors.

As expected, the seroprevalence was highest in the region of the high transmission strata including South-West (in the context of an ongoing socio-political crisis since 2016) and West (one of those the most where funeral practices face the care of the Covid-19 dead (32)) regions as compared to those in low transmission strata especially Adamawa and Far-North regions. The results of RDT and ELFA confirmed the high circulation of the SARS-CoV-2 in the region with the highest density of population and the high number of confirmed cases of COVID-19.

A small proportion (5%) of participants in the seroprevalence survey were vaccinated. This can be due to the late start of the vaccination in April 2021 and the vaccine hesitancy described in Cameroon (33) and in Africa (34) (35,36). According to the laboratory-based results, 44.2% (RDT; 138/312) and 47.8% (ELFA; 149/312) of vaccinated participants have antibodies against COVID-19. This low seroprevalence among vaccinated could be explained by the duration of antibodies due to natural infection and due to the vaccine(37).

Limitations:

Our study had several limitations:

Our study had several limitations. Despite their configuration (urban, semi-urban or both), the populations of regional capitals are not representative of the whole population. Capital cities are at high risk of SARS-CoV-2 transmission due to higher population density, activity and movement of people and goods, and better access to health care. A survey in these capitals may overestimate the burden of COVID-19 in the country.

The high number of refusals to participate in the seroprevalence survey at the household or individual level is another limitation of the study, especially if refusals differed from participants in their serological status and other key characteristics.

In this study, different RDTs were used to assess the seroprevalence of anti-SARS-CoV-2 antibodies, and the performance of these tests in terms of sensitivity and specificity may vary (37). However, the use of ELFA for all study participants strengthens the robustness of the results presented in this study. However, we do not have a good understanding of how diagnostic test performance evolves with time since infection.

In some cases, deaths may have been missed or household members may have been lost due to the long recall period, which may have affected the accuracy of the data on deaths, especially those that occurred early in the period.

Despite these limitations, our study provides a good estimate of the extent of SARS-CoV-2 infection in Cameroon and its impact on mortality.

Conclusion

Although SARS-CoV-2 appears to have spread widely in Cameroon, the overall public health impact of COVID-19 was minimal compared with that in Europe, Asia or the Americas. The seroprevalence and mortality results showed an increased risk of infection in the oldest age group, who are also at risk of more severe disease. We also found a significant under-reporting of infections by national surveillance systems. Finally, our findings suggest that strengthened surveillance systems and tailored vaccination campaigns are appropriate to limit the impact of COVID-19 on the population death.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

A minimal dataset underlying the findings of this study is available on request, in accordance with the legal framework set forth by Médecins Sans Frontières (MSF) data sharing policy. MSF is committed to share and disseminate health data from its programs and research in an open, timely, and transparent manner in order to promote health benefits for populations while respecting ethical and legal obligations towards patients, research participants, and their communities. The MSF data sharing policy ensures that data will be available upon request to interested researchers while addressing all security, legal, and ethical concerns. All readers may contact data.sharing@msf.org or Nouha TOUATI (nouha.touati@epicentre.msf.org) to request data.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.