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RESEARCH ARTICLE

Interest of seroprevalence surveys for the epidemiological surveillance of the SARS-CoV-2 pandemic in African populations: Insights from the ARIACOV project in Benin

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Abstract

Background: Many SARS-CoV-2 seroprevalence surveys since the end of 2020 have disqualified the first misconception that Africa had been spared by the pandemic. Through the analysis of three SARS-CoV-2 seroprevalence surveys carried out in Benin as part of the ARIACOV project, we argue that the integration of epidemiological serosurveillance of the SARS-CoV-2 infection in the national surveillance packages would be of great use to refine the understanding of the COVID-19 pandemic in Africa.

Methods: We carried out three repeated cross-sectional surveys in Benin: two in Cotonou, the economic capital in March and May 2021, and one in Natitingou, a semi-rural city in the north of the country in August 2021. Total and weighted-by-age-group seroprevalences were estimated and the risk factors for SARS-CoV-2 infection were assessed by multivariate logistic regression.

Results: In Cotonou, a slight increase in overall age-standardised SARS-CoV-2 seroprevalence from 29.77% (95% CI: 23.12%–37.41%) at the first survey to 34.86% (95% CI: 31.57%–38.30%) at the second survey was observed. In Natitingou, the globally adjusted seroprevalence was 33.34% (95% CI: 27.75%–39.44%). A trend of high risk for SARS-CoV 2 seropositivity was observed in adults over 40 versus the young (less than 18 years old) during the first survey in Cotonou but no longer in the second survey.

Conclusions: Our results show that, however, rapid organisation of preventive measures aimed at breaking the chains of transmission, they were ultimately unable to prevent a wide spread of the virus in the population. Routine serological surveillance on strategic sentinel sites and/or populations could constitute a cost-effective compromise to better anticipate the onset of new waves and define public health strategies.

KEYWORDS
epidemiological, SARS-CoV-2, seroprevalence, surveillance

INTRODUCTION

In Benin, the first case of SARS-CoV-2 infection was detected on March, 16th 2020 [1]. From the very first months of the COVID-19 pandemic, most African countries implemented public health measures [2, 3] to counter the spread of the virus, in particular, the definition of sanitary cords around a geographical area to contain the spread of the virus, the closure of borders and sometimes the containment of populations. These decisions were accompanied by sensitization campaigns that recommended the wearing of masks, social distancing measures, and hand washing [4].

Sustainable Development Goal: Good Health and Well-being.

Improvements in the capacities of national laboratories in terms of SARS-CoV-2 infection screening by Polymerase Chain Reaction (PCR) [5] were also encouraged. The objective of this strategy was to interrupt the chains of transmission as quickly as possible, knowing that the capacity to handle severe cases in hospitals was limited in most countries [6]. Nevertheless, the number of tests carried out in Africa remained low compared to the rest of the world [7, 8] and most often they were carried out in capitals or large cities, making it difficult to assess the true burden and distribution of the disease in all countries. Severe forms of the disease were less common than in other parts of the world [9–11] and the proportion of asymptomatic or paucisymptomatic cases among infected people was found to be very high [12–15]. The small number of detected cases and reported deaths in Africa [16–23] could suggest that the continent had been spared from the pandemic. However, many SARS-CoV-2 seroprevalence surveys (following the WHO unity protocol [24]) published since the end of 2020 from different African countries, have now definitively disqualified this misconception [25]. The ARIACOV project (Support for the African Response against COVID-19) which carried out SARS-CoV-2 seroprevalence surveys in six West and Central African countries (Benin, Ghana, Cameroon, Democratic Republic of Congo, Guinea and Senegal) together with other initiatives helped to demonstrate that the virus has circulated widely on the continent [5, 25–27]. All these results show that as highly useful as it is to understand the general dynamics of the COVID-19 pandemic, routine epidemiological surveillance in Africa exclusively based on the detection of infected cases by PCR (or antigenic RDT more recently) has proved unable to account for the true extent of the pandemic in the African context (limited screening capacity, proportion of asymptomatic cases, limited number of tests and urban concentration of testing). Through the analysis of three SARS-CoV-2 seroprevalence surveys carried out in Benin as part of the ARIACOV project, we argue that the integration of epidemiological serosurveillance based on the detection of antibodies against SARS-CoV-2 in the national surveillance package would improve the understanding of the distribution and burden of the epidemic as well as, the identification of the groups most at risk of infection in African populations.

METHODS

Study area

The first two surveys of the study were conducted in Cotonou, the economic capital of Benin with the port and the airport, with an area of 79 km^2 and an estimated population of 840,313 in 2020 [28]. To assess the circulation of the SARS-CoV-2 virus in a city where epidemic activity was assumed to be low according to the national surveillance, the third survey was carried out at Natitingou, a middle-size city located more than 500 kilometres in the north of Benin. Natitingou has an area of 3045 km^2 with an estimated population of 128,511 in 2020 [28].

Study design and participants

We conducted three cross-sectional SARS-CoV-2 seroprevalence studies in households. The first and the second studies took place in Cotonou, from March 3 to 15, 2021 (during the decline of the second wave), and May 27 to June 8, 2021 (between the second and the first wave), respectively. The third study took place in Natitingou from August 20 to 26, 2021 (during the start of the third wave). These studies were carried out as part of the ARIACOV project and used the WHO Unity population-based age-stratified seroepidemiological investigation protocol for COVID-19 infection version 2.0 [24].

Two-stage cluster sampling was employed in each study of Cotonou. At the first stage, 50 neighbourhoods among 143 were randomly drawn with a probability proportional to their population size, and at the second stage, 12 households were randomly selected per neighbourhood from a sampling step. People over 40 years represent only 17.3% of the population in Benin. In order to balance the age groups in the study sample to be able to compare the two surveys by age groups, in half of the households all the residents were invited to participate in the study, and in the remaining 50%, only the residents aged 40 years and older were invited to participate.

In Natitingou, a two-stage cluster sampling was also carried out, where 30 neighbourhoods among 77 were randomly drawn with a probability proportional to their size, and then 5 households were randomly selected in each neighbourhood by the data collector based on the sampling step. In Natitingou, all the residents of any household were invited to participate in the study.

In each study, the eligible participants were requested to answer a questionnaire on smartphones by the field team. Data collected included general demographic information, socio-economic (occupation, individual family home and common yard) data, as well as experience of COVID-19 confirmed disease or related symptoms in the past 3 months and/or hospitalisation (defined asymptomatic if no symptom). The participants were requested to provide a blood sample for SARS-CoV-2 serological analysis in the laboratory.

Ethical considerations

Ethics approval (N°131/MS/DRFMT/CNERS/SA) was obtained respectively from the Comité d'Ethique pour la Recherche en Santé and the Conseil National de la Statistique. All residents (adults and children) were informed about the study objectives and procedures. Adults provided written consent to participate in the study and to be tested for SARS-CoV-2 serology prior to starting the interview. Written parental consent and children assent were obtained prior to enrollment of participants aged from 10 to 17 years. For participants under 10 years old, only written parental consent was required. At the end of the study, each participant was informed about their serology status.

Antibody detection to SARS-CoV-2/COVID-19

Venous blood samples (2-5 mL) collected in Ethylenediaminetetraacetic acid tubes from participants were carefully transported to the laboratory of the Clinical Research Institute of Benin. After centrifugation and inactivation at 56°C for 45 min, plasma samples were used for SARS-CoV-2 serological analyses. In this study, the Luminex-based assay was used to simultaneously detect IgG antibodies to two viral antigens, that is, recombinant nucleocapsid and spike proteins of SARS-CoV-2, as previously described [29]. This dual-target strategy for SARS-CoV-2 IgG antibodies detection has a high sensitivity (100%) and specificity (99.7%) that was previously validated on a panel of more than 1000 samples from Africa (including 160 samples from Benin) before COVID-19 [27, 29]. Results were expressed as the median fluorescence intensity (MFI) for 100 beads. Cutoff values were also previously determined using pre-COVID-19 samples and those from COVID-19-positive and hospitalised patients [29]. An experiment was validated when [1] the MIF value of the blank is less than 50 MFI regardless of the antigen and [2] the MFI value of the negative control is less than 100. A sample is considered positive for anti-SARS-CoV-2 IgG when positive both for anti-SP IgG and anti-NP IgG and is declared negative when negative for this both antigens.

Statistical analysis

The statistical analysis was performed using STATA 14 (StataCorp, College Station, TX, USA). We performed descriptive analyses of household and participant characteristics in the study sample. Categorical variables are reported as frequencies and percentages. Continuous variables are summarised using means and standard deviations.

In Cotonou, the weighted estimations of the SARS-CoV-2 seroprevalence (and 95% confidence intervals) were performed in the 2 surveys by age-standardising the crude seroprevalence using available national census data [28]. We estimated the overall SARS-CoV-2 seroprevalence and the seroprevalence stratified by sex and age groups in each of the 3 surveys. A weighted multivariable logistical regression was performed with a stepwise selection procedure to determine the factors associated with positive serology anti-SARS-COV-2. To adjust all models, we kept variables related to gender, age group, the presence or absence of symptom, household population size and type of house. The adjusted odds ratios were presented with 95% confidence intervals.

RESULTS

Study population

In Cotonou, during the first and second surveys, 617 and 606 households were visited respectively, with a total of 1596 and 1842 eligible individuals respectively (Table 1). Among them, 1416 (89%) and 1465 (80%) individuals were present and agreed to participate in the first and second surveys, respectively, and finally, 1405 and 1460 compliant blood samples with epidemiological data were collected, respectively. The average size of the households surveyed was 3.26 ± 1.98 for the first survey and 3.97 ± 1.81 for the second survey. In Natitingou, 155 households were visited for a total of 815 eligible individuals; 742 (91%) individuals were present and agreed to participate in the study, of whom 735 provided a compliant blood sample. The average size of the households surveyed was 6.06 ± 2.16. The general characteristics of the households and participants are described in Table 1.

SARS-CoV-2 seroprevalence in Cotonou and Natitingou

In Cotonou, a slight increase in overall age-standardised SARS-CoV-2 seroprevalence from 29.77% (95% CI: 23.12%–37.41%) at the first survey to 34.86% (95% CI: 31.57%–38.30%) at the second survey was observed (Table 2). In Natitingou, the globally adjusted seroprevalence was 33.34% (95% CI: 27.75%–39.44%). The seroprevalence stratified by sex is presented in Table 2, and the proportion of symptomatic subjects according to the SARS-COV-2 serology is shown in Figure 1. The first survey in Cotonou showed a lower seroprevalence among the youngest age groups (less than 40 years) compared to 40 years and above (Figure 2), but this trend was no longer observed in the second survey. In Natitingou, a higher seroprevalence was observed among people aged over 60 years but the sample size ($N = 47$) in this age group was quite low (Figure 3).

SARS-CoV-2 risk factors in Cotonou and Natitingou

The multivariate logistic regression (Table 3) presents the covariates for which a clear association with a SARS-CoV-2 seropositivity was found in at least one of the surveys. The three surveys showed an odds ratio (OR) greater than 1 in women (higher risk of positive serology, with a significant association only for the second Cotonou survey, OR = 1.30, 95% CI: [1.03–1.65]). A consistent trend towards a higher risk in larger households or in households living in common yards (vs. more isolated structures) was observed during the first survey in Cotonou, but this was not found in the other surveys. Adults over 40 seemed to be more at risk than those under 18 years old (OR = 1.61, 95% CI: [0.68–3.84]) during the first survey in Cotonou, while in the second survey, the

TABLE 1 Characteristics of households and participants surveyed in Cotonou and Natitingou.

	Cotonou		
	Survey 1 *n* (%)	Survey 2 *n* (%)	Natitingou *n* (%)
Households characteristics	*N* = 617	*N* = 606	*N* = 155
Size of households			
1–3	383 (62.07)	242 (39.93)	28 (18.06)
4–5	163 (26.42)	256 (42.24)	58 (37.42)
>5	71 (11.51)	108 (17.82)	69 (44.52)
Type of dwelling			
Common yard	508 (82.33)	499 (82.34)	145 (93.55)
Building	15 (2.43)	5 (0.83)	0
Private residence	94 (15.24)	102 (16.83)	10 (6.45)
Presence of hands washing facility			
Yes	124 (20.1)	64 (10.56)	10 (6.45)
No	493 (79.9)	542 (89.44)	145 (93.55)
Participants characteristics	*N* = 1405	*N* = 1460	*N* = 735
Gender			
Male	606 (43.13)	657 (45.0)	290 (39.46)
Female	799 (56.87)	803 (55.0)	445 (60.54)
Age group (years)			
<18	199 (14.16)	379 (25.96)	309 (42.04)
18–39	466 (33.17)	428 (29.32)	259 (35.24)
40–59	487 (34.66)	438 (30.0)	120 (16.33)
≥60	253 (18.01)	215 (14.73)	47 (6.39)
School level			
No one	267 (19.0)	233 (15.27)	149 (20.27)
Primary	527 (37.51)	645 (44.18)	386 (52.52)
Secondary 1	296 (21.07)	317 (21.71)	142 (19.32)
Secondary 2	190 (13.52)	198 (14.0)	38 (5.17)
University	125 (8.9)	77 (5.27)	20 (2.72)
Occupation			
Salaried worker	99 (7.05)	75 (5.14)	25 (3.4)
Trader/Entrepreneur	471 (33.52)	435 (29.79)	156 (21.22)
Artisan	286 (20.32)	209 (14.32)	98 (13.33)
Home-maker/unemployed/retired	308 (21.92)	337 (23.08)	191 (25.99)
Student	241 (17.15)	404 (27.67)	265 (36.05)
Marital status			
Single	243 (20.15)	210 (19.43)	108 (25.35)
Married	650 (53.9)	518 (46.92)	225 (52.82)
Living in union	176 (14.59)	169 (15.63)	42 (9.86)
Divorced/separated	39 (3.23)	92 (8.51)	29 (6.81)
Widower	98 (8.13)	92 (8.51)	22 (5.16)
Tested for COVID-19			
Yes	65 (4.63)	36 (2.47)	12 (1.63)
No	1340 (95.37)	1424 (97.53)	723 (98.37)

OR = 1.22, 95% CI: [0.72–2.08] in the different age groups were more homogeneous. In Natitingou, a clear increased risk with age was found, with an OR of 2.67 for people aged 60 and over (95% CI: [1.21–5.89]). No association was found between symptomatic status during the last 3 months and SARS-CoV-2 positive serology in surveys in Cotonou.

TABLE 2 Adjusted seroprevalence of SARS-CoV-2 in Cotonou and Natitingou.

	Cotonou		
	Survey 1	**Survey 2**	**Natitingou**
Adjusted seroprevalence—total population	29.77% (23.12–37.41)	34.86% (31.57–38.30)	33.34% (27.75–39.44)
Adjusted seroprevalence—females	30.72% (22.49–40.38)	38.08% (33.53–42.87)	34.93% (29.24–41.10)
Adjusted seroprevalence—males	28.64% (22.05–36.28)	31.50% (27.63–35.65)	30.99% (22.96–40.36)

Note: Seroprevalence was adjusted with age and gender.

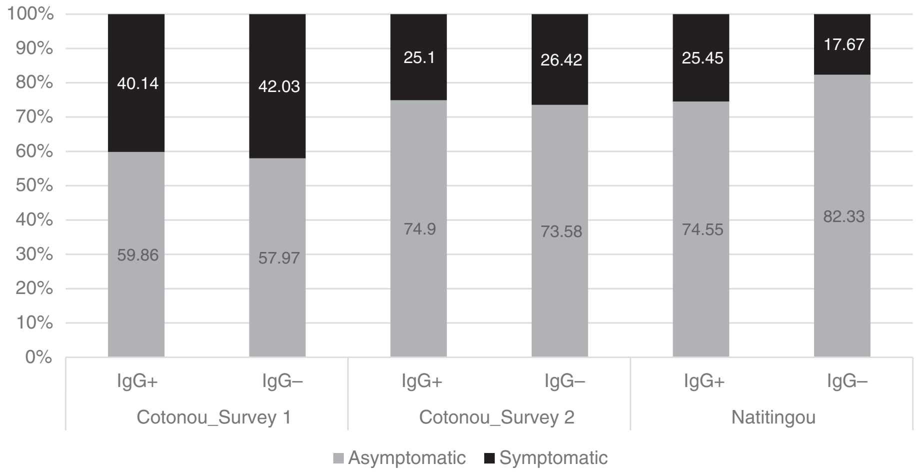

FIGURE 1 Proportion of asymptomatic and symptomatic by IgG results.

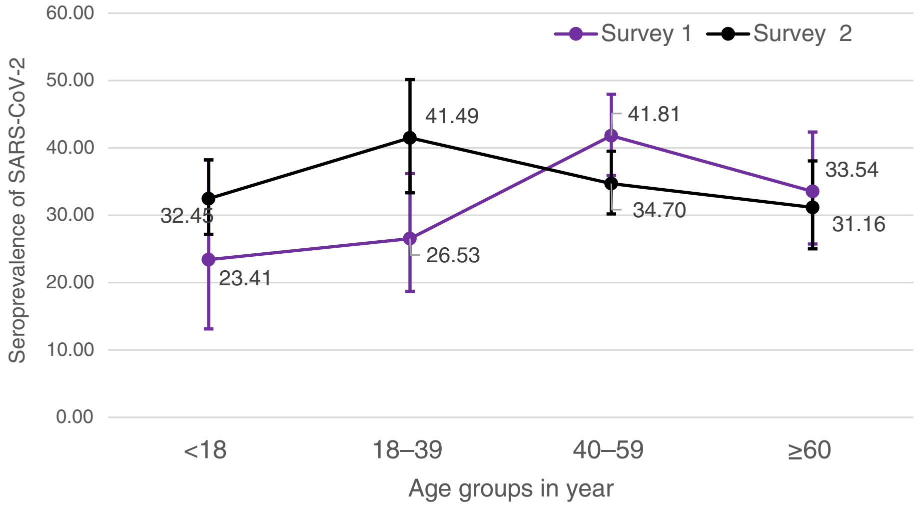

FIGURE 2 Seroprevalence of SARS-CoV-2 according to age groups in Cotonou in the first and second surveys (March and May 2021).

In Natitingou, an increased risk OR in symptomatic people was observed (OR = 1.33), although this association was not very clear (95% CI: [0.81–2.17]).

DISCUSSION

We presented three cross-sectional seroprevalence studies in Benin, two carried out in Cotonou in March and May–June 2021, and the third in Natitingou (a medium-sized city in the north of Benin) in August 2021. The main result was that the seroprevalence in the three surveys turned out to be much higher than shown by virological surveillance based on PCR tests. For example, based on the available national demographic data [28], the estimated number of people have been in contact with the virus from the first survey in Cotonou amounts to 250,161 (95% CI: [194,289; 314,361]) for an estimated population of around 840,000 inhabitants

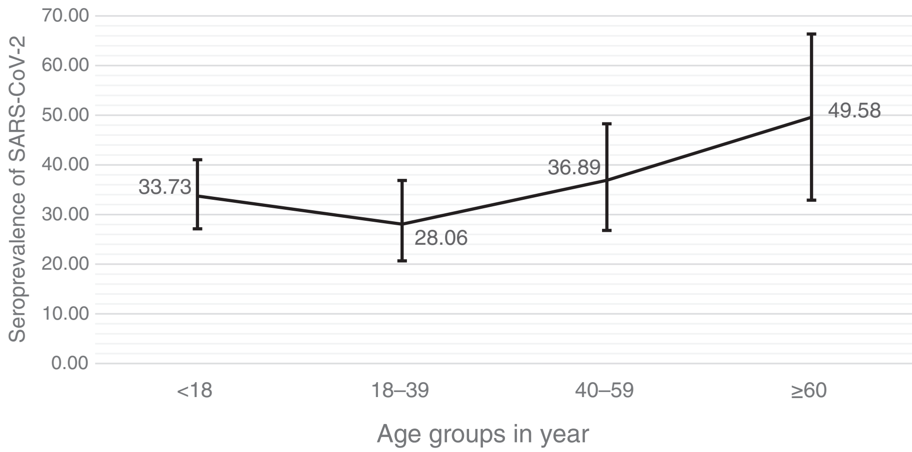

FIGURE 3 Seroprevalence of SARS-CoV-2 according to age groups in Natitingou (August 2021).

TABLE 3 Multivariate risk factors analysis for SARS-CoV-2 seropositivity.

	Cotonou				Natitingou	
	Survey 1		Survey 2			
	aOR 95% of CI	*p* value	aOR 95% of CI	*p* value	aOR 95% of CI	*p* value
Gender						
Male	Ref		Ref		Ref	
Female	1.21 (0.90–1.62)	0.20	1.30 (1.03–1.65)	0.03	1.30 (0.85–1.99)	0.22
Age group (years)						
<18	Ref		Ref		Ref	
18–39	1.24 (0.6–2.56)	0.55	1.37 (0.85–2.19)	0.19	1.01 (0.50–2.01)	0.99
40–59	1.61 (0.68–3.84)	0.28	1.22 (0.72–2.08)	0.45	1.59 (0.78–3.21)	0.19
≥60	1.18 (0.50–2.83)	0.70	1.19 (0.67–2.11)	0.54	2.67 (1.21–5.89)	0.02
Symptoms[a]						
No	Ref		Ref		Ref	
Yes	1.05 (0.74–1.49)	0.78	0.89 (0.67–1.18)	0.41	1.33 (0.81–2.17)	0.25
Size of household (people)						
1–3	Ref		Ref		Ref	
4–5	1.32 (0.95–1.83)	0.09	1.01 (0.74–1.37)	0.95	0.92 (0.45–1.89)	0.81
>5	1.50 (0.96–2.34)	0.07	0.84 (0.59–1.83)	0.31	1.18 (0.58–2.40)	0.63
Type of living						
Common yard	Ref		Ref		Ref	
Building	0.65 (0.25–1.67)	0.36	0.54 (0.16–1.82)	0.31	–	
Private residence	0.67 (0.41–1.07)	0.09	0.86 (0.63–1.18)	0.34	0.82 (0.37–1.82)	0.61

Note: Ref: Reference category of multivariate logistic regression model.
[a]Presence of at least one symptom related to COVID-19 since the last 3 months.

living in Cotonou whereas just after the first survey in Cotonou, the number of cases diagnosed by PCR nationwide was 6501 (only one case detected for every 40 infections). This ratio is in accordance with numerous seroprevalence surveys carried out in Africa [5, 26, 30, 31]. The seroprevalences we found in our three surveys fit also well with a recent meta-analysis showing a combined seroprevalence of 25% (95% CI [13%–39%]) in West Africa [25], or a SARS-CoV-2 seroprevalence rose from 3% to 65% in Africa [13].

Some studies, for example in Mali or in Zimbabwe, have measured seroprevalences before and after epidemic waves, demonstrating the substantial impact of these waves on seroprevalence estimates [26, 32]. Unlike these studies, the two surveys in Cotonou were both carried out during and after the second wave (end of January to end of March) and were only 2 months apart. This largely explains the smaller increase in seroprevalence between the two surveys (from 29.77% to 34.86%). Interestingly in the second survey in

Cotonou, the prevalence among the age group under 18 years old caught up with the prevalence of the older age groups. This could suggest an impact of the sensitization discourse (particularly oriented towards older age groups), associated with a greater respect for distancing measures between the two surveys by older age groups compared to younger ones. In two studies of repeated cross-sectional surveys in Cameroon and Guinea, seroprevalence had increased for all age groups between the two surveys, but unlike our study, an epidemic wave had occurred between the two surveys [33, 34]. This probably explains the difference in our results, which reflect the persistence of virus circulation even outside of waves, so even when virological PCR monitoring does not seem to suggest any increase in COVID-19 cases incidence.

Based on the findings in Cotonou, our hypothesis was that the prevalence of infected people with the SARS-CoV-2 virus was undoubtedly underestimated throughout the country. The goal of the investigation in Natitingou was then to assess the situation in an area where available data suggested very low circulation of the virus, despite the start of the third COVID-19 epidemic wave in Benin (August to October 2021). The estimation of age-group prevalence was not a main objective, which justifies the non-age-group stratified design in Natitingou. Our hypothesis was confirmed, with an estimated overall adjusted prevalence of 33.34%, well above the low PCR positive rate detected by the epidemiological surveillance in the study area (data not shown), and in agreement with the high prevalence observed in semi-rural areas after the second wave in Mali for example [31].

Across the three surveys, the presence of symptoms in the past 3 months has not shown any relationship with positive serology, as observed in several other studies [5, 35–38]. This supports the hypothesis that the vast majority of the infected people have been asymptomatic or paucisymptomatic, even if the information regarding the symptoms may be affected by a recall bias. The proportion of people (even symptomatic) having tested for COVID-19 was very low (less than 5%, data not shown), which shows the limits of surveillance based on PCR tests intended for people presenting spontaneously.

Our study has limitations. In Cotonou, the design was repeated cross-sectional rather than longitudinal surveys. Consequently, the differences between the prevalence by age groups in the two surveys may be because the samples were carried out in different neighbourhoods of Cotonou. Nonetheless, the relatively small increase in prevalence between the two surveys is consistent with the relatively narrow time interval (2 months) with no epidemic wave in between. Another limitation is that in Natitingou the design does not allow to estimate the prevalence by age group as precisely as in Cotonou. However, all age groups seem to be highly affected, which is consistent with what was observed in Cotonou and other studies including the ARIACOV project [26].

All of these results show the essential value of seroprevalence surveys in providing a more accurate view of the spread and the extent of the COVID-19 epidemic than the reported cases from virological PCR-based surveillance only. Virological surveillance seems suitable for describing epidemic waves when they are in progress but turns out to be inadequate neither for capturing a precise quantitative estimate of the extent and spread of the epidemic in populations. It fails also to apprehend clearly the persistence of the virus and its basal dynamics between epidemic waves. Our results show, along with others in Africa, that a more systematic use of serological surveillance to support an overall strategy of routine epidemiological surveillance would certainly be useful in overcoming these shortcomings. The rapid integration of seroprevalence studies into the national packages for monitoring the pandemic in northern countries has been recommended in early 2020 by international health institutions such as the World Health Organisation [39] and the Center of Disease Control and Prevention [40]. It effectively made it possible to better estimate the real extent of the transmission of the virus, the risk factors for infection and the foci of transmission. It has also helped to establish public health measures and control strategies.

In particular, before mass vaccination of populations, knowledge of the true proportion and sub-groups of the population with anti-SARS-COV-2 antibodies is an essential element to better guide the national vaccine strategies. These considerations are particularly important in an African context where many countries are constrained to lighten, sometimes significantly, their epidemiological surveillance systems because of their logistical burden and high cost, especially between epidemic waves when the epidemic situation seems stable. However, as shown here, the virus probably continues to diffuse quietly, contributing to the emergence of new variants and new waves. Our results, associated with many other seroprevalence studies in Africa, have implications in terms of public health. They show in particular that, however rapid the organisation of the response and preventive measures aimed at breaking the chains of transmission, they were ultimately unable to prevent a wide spread of the virus in the population. It is therefore essential to systematically combine these measures with awareness-raising aimed at the groups most at risk (elderly people, people with comorbidities…). They also raise the question of the interest of a generalised vaccination of a population having been mainly infected by the virus with a severity that finally turned out to be less than initially feared [41]. Routine serological surveillance on strategic sentinel sites and/or populations could constitute a cost / effective compromise to better anticipate the onset of new waves and characterise the most vulnerable subgroups of populations. In order to take into account the logistical constraints, the use of an easy-to-use diagnostic tool such as serological RDTs validated with good sensitivity and specificity, well known to health workers as well as to populations, as it is already used for the COVID-19 case detection (antigenic RDTs) and in other pathologies like malaria, could prove interesting.

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