

Seroprevalence of anti-SARS-CoV-2 antibodies in Senegal: a national population-based cross-sectional survey, between October and November 2020

Cheikh Talla, Cheikh Loucoubar, Jerlie Loko Roka, Mamadou A. Barry, Seynabou Ndiaye, Maryam Diarra, Oumar Faye, Moussa Dia, Mamadou Diop, Oumar Ndiaye, et al.

▶ To cite this version:

Cheikh Talla, Cheikh Loucoubar, Jerlie Loko Roka, Mamadou A. Barry, Seynabou Ndiaye, et al.. Seroprevalence of anti-SARS-CoV-2 antibodies in Senegal: a national population-based cross-sectional survey, between October and November 2020. 2022. pasteur-03752584v1

HAL Id: pasteur-03752584 https://pasteur.hal.science/pasteur-03752584v1

Preprint submitted on 2 Jan 2022 (v1), last revised 17 Aug 2022 (v2)

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Seroprevalence of anti-SARS-CoV-2 antibodies in Senegal: a national population-based cross-sectional survey, between October and November 2020

Authors: Cheikh Talla¹, Cheikh Loucoubar¹, Jerlie Loko Roka², Mamadou A. Barry¹, Seynabou Ndiaye¹, Maryam Diarra¹, Oumar Faye³, Moussa Dia³, Mamadou Diop¹, Oumar Ndiaye³, Adama Tall¹, Rokhaya Faye⁴, Adji Astou Mbow⁴, Babacar Diouf⁴, Jean Pierre Diallo⁵, Mamadou Ndiaye⁵, Tom Woudenberg⁶, Michael White⁶, Ting, Jim², Cheikh Tidiane Diagne³, Omer Pasi², Boly Diop⁵, Amadou A. Sall³, Inès Vigan-Womas⁴, Ousmane Faye³

Affiliations of authors:

¹Institut Pasteur de Dakar, Epidemiology, Clinical Research and Data Sciences Department, Dakar, Senegal

²Centers for disease control and prevention (CDC), Atlanta, Georgia, USA

³Institut Pasteur de Dakar, Virology Department, Dakar, Senegal

⁴Institut Pasteur de Dakar, Immunophysiopathology and Infectious Diseases Department, Dakar, Senegal

⁵Ministry of Health and Social Action, Surveillance Division, Prevention Department, Dakar, Senegal

⁶Institut Pasteur, Malaria: Parasites and Hosts Unit, Department of Parasites and Insect Vectors, Paris, France.

Summary

Background: Senegal reported the first COVID-19 case on March 2, 2020. A nationwide cross-sectional epidemiological survey was conducted to capture the true extent of COVID-19 exposure

Methods: Multi-stage random cluster sampling of households was carried out between October 24 and November 26, 2020, at the end of the first wave of COVID-19 transmission. Anti-SARS-CoV-2 antibodies (IgG and/or IgM) were screened using three distinct ELISA assays. Adjusted prevalence for the survey design were calculated for each test separately, and thereafter combined. Crude, adjusted prevalence based on tests performances and weighted prevalence by sex-age strata were estimated to assess the seroprevalence.

Findings: Of the 1,463 participants included in this study, $58 \cdot 8\%$ were women and the mean age of participants was $29 \cdot 2$ years (range $0 \cdot 25 - 82 \cdot 0$). The national seroprevalence was estimated at $28 \cdot 4\%$ (95% CI: $26 \cdot 1 - 30 \cdot 8$). There was substantial regional variability. Four regions recorded the highest seroprevalence: Ziguinchor ($56 \cdot 7\%$), Sedhiou ($48 \cdot 0\%$), Dakar ($44 \cdot 0\%$) and Kaolack ($32 \cdot 7\%$) whereas, Louga ($11 \cdot 1\%$) and Matam ($11 \cdot 2\%$), located in the Center-North, were less impacted in our analysis. All age groups were impacted and the prevalence of SARS-CoV-2 was comparable in symptomatic and asymptomatic groups. We

estimated 4,744,392 SARS-CoV-2 (95% CI: 4,360,164 – 5,145,327) potential infected in Senegal compared to 16,089 COVID-19 RT-PCR laboratory-confirmed cases reported at the time of the survey.

Interpretation: These results provide an estimate of SARS-CoV-2 virus dissemination in the Senegalese population. Preventive and control measures need to be reinforced in the country and especially in the south border regions.

Funding: US Centers for Disease Control and Prevention.

Keywords: SARS-CoV-2, Serology, ELISA, IgG and IgM, seroprevalence, population-based survey, Senegal

Research in context

Evidence before this study

Coronavirus disease 2019 (COVID-19), first identified in December 2019 in Wuhan, China, is an acute respiratory infectious disease caused by a newly discovered coronavirus named SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). Globalization, urbanization and environmental changes have greatly accelerated this pandemic emergence and spread, generating an unprecedented global pandemic. Almost all countries and territories around the world (with the exception of 22 island states/territories, including Greenland, Tuvalu, Tonga, Tokelau or Saint Helena) are affected. By June 6, 2021, SARS-CoV-2 has infected more than 172 million people worldwide and claimed the lives of more than 3·7 million people, with the European and the America Regions being the most affected and accounting for nearly 80% of all the cases and deaths. The Africa region has reported around 3·5 million cases and 87,731 deaths since the first case of COVID-19 declared on February 15, 2020, in Egypt, with only 2% of reported cases and 3% of recorded death making the Africa region surprisingly less impacted than the rest of the world. Although low testing rates impacting the true extent of COVID-19 prevalence in Africa, there are no tangible indications that a large number of COVID-19 cases and deaths have been missed.

The first case in Senegal was declared on March 2, 2020. Despite an early implementation of prevention and control measures, the virus has gradually spread in the population. As of June 6, 2021, 41,581 confirmed cases and 1,144 deaths have been reported. Senegal's control strategy is largely based on a molecular testing system. As COVID-19 vaccines are being slowly implemented in Africa and in Senegal, it becomes crucial to better evaluate the actual level of the population exposed to the SARS-CoV-2 virus through seroprevalence studies.

Added value of this study

In Africa, several seroprevalence surveys have already been conducted in specific populations groups or targeted areas. To our knowledge, this is the first cross-sectional population-based study conducted in all the administrative regions in an African country. Our results show that the national surveillance system, mostly based on passive detection of symptomatic cases of COVID-19, only captured a tiny part of the virus transmission within the population. With an

estimated national seroprevalence of $28 \cdot 4\%$, this study describes a significant disparity between regions. Alongside the expected high seroprevalence in regions such as Dakar (44%, 11,016 confirmed cases) or Thiès ($24 \cdot 3\%$, 1,974 confirmed cases), our data showed a strong and silent circulation of the virus in regions such as Ziguinchor ($56 \cdot 7\%$), Sédiou ($48 \cdot 0\%$), Kaolack ($33 \cdot 1\%$) or Kaffrine ($26 \cdot 9\%$). This latter region had recorded only 20 COVID-19 confirmed cases during the survey period. All age groups were impacted and the prevalence of SARS-CoV-2 seropositive people was similar between symptomatic ($27 \cdot 8\%$) and asymptomatic ($28 \cdot 1\%$) people.

Implication of all the available evidence

This first African nationwide seroepidemiological survey reveals that even if the COVID-19 outcomes have been less severe among African populations, the spread of SARS-CoV-2 among the population was, to date, heterogeneous, extended and silent. Therefore, African countries must not relax their prevention and surveillance efforts (including repeated cross-sectional serological surveys) as the emergence of several variants, that transmit more easily, thus contribute to a quicker spread, may cause more severe pathology, specifically among the young population leading to the congestion of fragile healthcare systems.

3

Introduction

Classified as a global pandemic on 11 March 2020, COVID-19 is present in almost all the countries and territories of the world with more than 158 million confirmed cases, and 3.2 million deaths reported by May 10, 2021.^{1,2} While European and Americas Regions continue to account for nearly 80% of all the cases and deaths, Africa region is surprisingly less impacted with only 2% (3,320,786) of reported cases and 3% (83,650) of recorded death. Among African countries, South Africa is the most affected (with > 1.5 million confirmed cases and 54,700 deaths), followed by Ethiopia and Nigeria.^{3,4}

The worldwide reported cases, based on molecular diagnostics (real-time reverse transcription polymerase chain reaction, qRT-PCR assays) of nasopharyngeal or oropharyngeal swabs are mainly performed on symptomatic carriers or their contacts which represent only the tip of the COVID-19 transmission iceberg. Indeed, the number of laboratory-confirmed cases does not fully reflect the true extent of infection. Several epidemiological studies have estimated that the proportion of SARS-CoV-2 infected people who are asymptomatic or paucisymptomatic was between 20% to 50%.^{5–7} In Africa, COVID-19 statistics are largely underestimated, in part because of the limited laboratory testing capacity, changing health authority strategies, but also because of the stigmatization associated with this new pathology and fear of attending health facilities. Consequently, studies measuring the prevalence of antibodies directed against SARS-CoV-2 proteins are important to strengthen pandemic surveillance and to timely guide public health strategies. This is needed especially in the context of emerging new variants of concern, the complexity of COVID-19 clinical presentations, and the slow rollout of an effective vaccine in Africa.

Most available serological studies have been conducted in European or American countries.^{8,9} In Africa, few seroprevalence surveys were conducted in specific populations group including blood donors, health care workers and COVID-19 exposed populations or general population in some targeted districts/states.^{10–17} However, to the best of our knowledge, to date, no studies have performed population-based studies at a country level in Africa.

Since the first COVID-19 confirmed case reported in Senegal on March 02, 2020, the country has implemented a range of lockdown measures aimed at spatial and physical distancing.¹⁸ However, the virus gradually spread to all regions with a record of 41,713 COVID-19 confirmed cases and up to 1,148 deaths on June 7, 2021.¹⁹

Senegal has faced the first wave of COVID-19 between March and September, 2020 with a peak of transmission recorded in June-July. Surveillance associated with COVID-19 has mostly focused on symptomatic patients and their close contacts. To better understand the epidemiological characteristics of the COVID-19 outbreak in Senegal, a population-based, seroepidemiological survey was conducted in all regions with an aim to i) assess the level of population exposure to SARS-CoV-2, and ii) guide public health interventions. In this study,

we also compared the seroprevalence results with the reported COVID-19 confirmed-case rates within the same population at the same period.

Methods

Study design and sampling

Senegal is located in West Africa, and bordered by Mauritania, Mali, Guinea, and Guinea-Bissau and surrounds The Gambia. Senegal has around 16.7 million inhabitants, a quarter of whom live in the Dakar region (0.3% of the territory). The country is administratively divided into 14 regions.

To evaluate the seroprevalence of anti-SARS-CoV-2 antibodies, a cross-sectional seroepidemiological survey at the national level was carried out in all regions of Senegal at the end of the first COVID-19 transmission wave (from 25 October to 26 November 2020, Figure 2). A multi-stage cluster sampling was applied to randomly select 70 clusters, provided by the National Agency for Statistics and Demography (ANSD). In the first stage, areas were selected across Senegal by identifying the number of clusters by region according to the population size and using sampling proportional to the probability by size (PPS). In the second step, systematic random sampling to select 10 households in each selected area chosen was performed. Finally, for each selected household, a simple random sampling was done to systematically enroll two people over 5 years old and one child under 5 years old. The geographical localizations of the investigated clusters are shown in Figure S1. The study was designed following the World Health Organization (WHO) protocol for COVID-19 population-based seroepidemiological studies.²⁰

All field workers were trained in protocol procedures, filling up questionnaires, and sample collection procedures under COVID-19 biosafety guidelines.

Ethical considerations

All randomly selected individuals who fulfilled study inclusion criteria were invited to participate. All participants have consented to participate in the study. For people younger than 18 years, a legal representative provided informed consent. The study was approved by the Senegalese National Ethics Committee for Research in Health (reference number N°0176/MSAS/DPRS/CNERS, 10 October 2020).

Sample size calculation

The sample size needed to estimate prevalence in all regions was assessed to be 1215 individuals (with a minimum sample size of 1395 and a maximum sample size of 2100) based on a design effect of 3 to account for intra-household clustering of cases with an absolute error of \pm 0%, a power of 90% and a seroprevalence rate of 50%. The sample size calculation takes into account 10% of non-response rate and 5% of non-valid sample.

Blood sampling and data collection

For each participant, whole blood sample was collected into a dry vacutainer tube by standard venipuncture. Blood samples were immediately stored at 4°C and quickly directed to Institut

Pasteur de Dakar (IPD) within 24-72h, according to the routing conditions required for potentially infectious samples. Tracking procedures were established to ensure temperature-controlled delivery to IPD. After a centrifugation step, sera were collected, aliquoted and stored at -20° C until use. Sociodemographic information such as age, sex, region, place of residence, occupation, education level, contact with COVID-19 confirmed cases and history of symptoms during the 6 months prior to the survey, were also collected.

COVID-19 serological assays (ELISA)

The antibodies specifically directed against SARS-CoV-2 proteins were detected by Enzymelinked Immunosorbent Assay (ELISA). In this study, three CE-marked, indirect semiquantitative commercially available ELISA kits were used. The Omega Diagnostics COVID-19 IgG ELISA Kit (Mologic Ltd. and Omega Diagnostics Ltd, Cambridgeshire, UK, Ref. ODL150/10) that detect IgG directed against the nucleocapsid (NP) and the Spike (subunit S2) proteins.²¹ The ID Screen® SARS-CoV-2-N IgG Indirect ELISA (IDVet, Innovative Diagnostic, France, Ref: SARSCOV2S) intended to detect IgG directed against only the SARS-CoV-2 NP.²² The WANTAI SARS-CoV-2 Ab ELISA (Beijing Wantai Biological Pharmacy Enterprise, Beijing, China; Ref WS-1096) recommended by WHO for seroepidemiological studies and that detects total antibodies (including IgM and IgG) binding SARS-CoV-2 spike protein receptor-binding domain (S1/RBD).²³ The main characteristics of each immunoassay are depicted in Table S8. Sera were analyzed in duplicates according to the recommendations of the suppliers.

The diagnostic performance (sensitivity and specificity) of each ELISA assay was re-evaluated with a panel of 40 archived, well-documented serum or plasma samples, and data obtained were used to adjust the seroprevalence (details in Supplementary Methods). According to the performance obtained, to limit the rate of false IgG positive results, after an evaluation of IgG directed against virus proteins using the OMEGA assay, all positive and doubtful samples were validated on the IDVet kit. In parallel, the WANTAI kit was used to capture the whole Ab (including IgG and IgM) reactivity to SARS-CoV-2. The flowchart used to analyze the serum samples is described in Figure S1.

Plasmodium falciparum antigen preparation and ELISA assay

Crude schizont antigens from *P. falciparum* 0703 field-adapted strain were prepared from *in vitro* continuous culture on O⁺ erythrocytes in RPMI medium containing 0.5% Albumax. Schizonts stage parasites were harvested and lysed in three volumes of sterile distilled water and stored into aliquotes in liquid nitrogen. The ELISA assay was performed as previously described by Diop and Colleagues and detailed in Supplementary Methods.²⁴ Finite mixture models, assuming two underlying distributions of "negative" (unexposed) and "positive" (exposed) individuals, were created from log-transformed MFI values to determine seropositivity cut-off. Finite mixture models were fit with the flexmix package in R version 3.5.1 (Comprehensive R Archive Network, Vienna, Austria).

Statistical analysis

Crude and standardized seroprevalence was calculated with 95% Confidence Interval (CI). Confidence intervals (95% CI) for seroprevalence were estimated using Clopper-Pearson method. We assessed weighted prevalence estimates using 2020 population data from ANSD and direct standardization on the observed seroprevalence and population weights by age-sex strata. For combined seroprevalence results, samples were counted as positive if they were positive with one of the two tests and negative if they were negative with both tests. The performance of combined tests is calculated using the performance of both tests.²⁵ We assumed statistical significance at p-value <0.05. All statistical analyses were done with R version 4.0.4. The spatial distribution of seroprevalence was mapped using mapplots R package.

Role of the funding source

The study's funder was involved in study design, data collection, data analysis, data interpretation, writing of the manuscript, or the decision to submit it for publication.

Results

Population characteristics

All the Senegalese administrative regions were investigated. Among the 70 clusters initially targeted, only 66 were included in the study. One cluster in Sédhiou, in the south of the country, did not agree to participate in the study, and blood samples from 3 remote clusters in the Diourbel's region, were not delivered to conform to the laboratory requirements. Of the 700 households randomly selected, 628 (89.7%) for which heads of households consented to participate were included in the study. Hemolytic and underfilled blood samples were excluded from the serological analysis. Overall, 1632 people were enrolled and among them, 1463 (89.4%) participants with complete socio-demographic data and conform blood sample were included in the serological analysis (Figure 1).

This cross-sectional household survey was initiated just after the first wave (March to October 2020) of COVID-19 transmission (Figure 2). The mean age was $29 \cdot 2$ years, ranging from $0 \cdot 20$ and $84 \cdot 8$ years old. Females were significantly more represented ($58 \cdot 8\%$) than males (p <0.001). During the survey, a high number of people [n= 958 (67%)] declared to have had a history of symptoms compatible with COVID-19 during the 2-3 months prior to the survey. Fever ($73 \cdot 8\%$), headaches ($32 \cdot 4\%$), and cough ($30 \cdot 0\%$) were the most evocated COVID-19 related symptoms (Table 1). Anosmia and/or ageusia was only reported by $2 \cdot 8\%$ of the participants.

Seroprevalence of anti-SARS-CoV-2 antibody

The prevalence data were weighted according to the age and sex distribution of the general population and adjusted with each immunoassay's performance. According to our serological analysis strategy, seroprevalence data were given first using the results obtained with OMEGA/IDVet ELISA (Table S3 and Table S4) and WANTAI ELISA (Table S5 and Table S6) separately and subsequently after a combination of both results (Table 2 and Table 3). At

the country level, the whole seroprevalence was estimated at $22 \cdot 5\%$ (95% CI: 20.4–24.7) with OMEGA/IDVet ELISA targeting IgG directed against SARS-CoV-2 proteins and at 28.1% (95% CI: 25.8–30.5) with the WANTAI ELISA measuring both IgG and IgM against the SARS-CoV-2 S1/RBD proteins. Combining the two approaches, OMEGA/IDVet and WANTAI ELISA results, the global seroprevalence was estimated at 28.4% (95% CI: 26.1–30.8). SARS-CoV-2 combined seroprevalence data were then presented below (Figure S2).

At the demographic level, SARS-CoV-2 transmission occurred in all age groups investigated, with the 15-30 years old group showing the highest seroprevalence at $37 \cdot 3\%$ (95% CI: $32 \cdot 2 - 42 \cdot 8$) (Table 2). The lowest weighted prevalence of $16 \cdot 1\%$ (95 CI: $12 \cdot 2 - 21 \cdot 1$) was observed in children under 5 years old. Adjusted prevalence by sex was similar for women and men, $28 \cdot 0\%$ (95% CI: $25 \cdot 0 - 31 \cdot 2$) and $26 \cdot 9\%$ (95% CI: $23 \cdot 3 - 30 \cdot 6$), respectively (Table 2).

Regarding the SARS-CoV-2 seroprevalence at a regional level, substantial variability was observed (Table 3 and Figure 3). The regions of Dakar expectedly recorded a high seroprevalence with $44\cdot0\%$ (95% CI: $38\cdot3-49\cdot9$) of people harboring Abs against SARS-CoV-2. Interestingly, two south border regions displayed the highest seroprevalence: Ziguinchor with 56.7% (95% CI: $41\cdot3-70\cdot0$) followed by Sedhiou with $48\cdot0\%$ (95% CI: $34\cdot0-61\cdot0$). The two regions located on the Center North of the Senegal, Louga and Matam, presented the lowest COVID-19 prevalence of $11\cdot1\%$ (95% CI: $6\cdot0-18\cdot1$) and 11.2 (95% CI: $3\cdot7-24\cdot1$) respectively. Of note, in Kaffrine, only 20 qRT-PCR confirmed cases were recorded during the survey period, while the seroprevalence was up to $26\cdot9\%$, (95% CI: $17\cdot3-37\cdot7$), which was similar to that found at Thiès ($24\cdot0\%$, 95% CI: $18\cdot7-31\cdot1$), the second most impacted region (after Dakar) with 1,974 COVID-19 qRT-PCR confirmed cases.

Of the 958 (67%) participants that have reported a history of symptoms compatible with COVID-19, $28 \cdot 1\%$ (n = 269) were COVID-19 seropositive. The prevalence of anti-SARS-CoV-2 Abs among participants without documented COVID-19 symptomatology history (n = 464) was comparable ($27 \cdot 8\%$, n = 169). The seroprevalence of antibodies to SARS-CoV-2 among people reporting a history of COVID-19 related symptoms were variable: $27 \cdot 0\%$ (191 of 707) for fever, $31 \cdot 1\%$ (97 of 311) for headache, $24 \cdot 8\%$ (71 of 286) for cough, $29 \cdot 2\%$ (21 of 72) for myalgia and $40 \cdot 7\%$ (11 of 27) among participants with anosmia and/or ageusia. No significant correlation was observed between reported COVID-19 symptoms and the serostatus (Table S7).

Risk factors associated with COVID-19 transmission

Participants aged 15 to 30 years [OR 2·49 (95% CI: 1.57-3·98, p<0.001)] and 45 to 60 years [OR 1·79 (95% CI: 1·12-2·89, p=0·016)] were around two times more infected than those under 0 to 5 years old children. No association were observed between SARS-CoV-2 seroprevalence and sex. The Dakar region was more at risk than all the other regions except for Sedhiou (OR 1·07, p=0·832) and Ziguinchor (OR 1·42, p=0·286) (Table S1 and Figure 4). No association were observed between COVID-19 seropositivity and occupation. Interestingly, a significant association was identified between SARS-CoV-2 seroprevalence and the attendance at Koranic schools (OR 1·54, p=0·030).

Estimation of the national prevalence of COVID-19 in Senegal

Seroprevalence estimate results highlighted a strong discrepancy with the reported prevalence of COVID-19 based on RT-PCR confirmed COVID-19 cases (Table 4). Indeed, our analyses estimate 4,744,392cases of SARS-CoV-2 infections (95% CI: 4,360,164 –5,145,327) potentially occurred while only 16,089 cases were detected in the country during the survey period with important variability between regions. At the national level, the ratio of reported cases to estimated infections was 1:295 and ranged between regions from 1:153 in Dakar to 1:9,804 in Kaffrine.

Relationships between SARS-CoV-2 seropositivity and malaria seropositivity

To explore the impact of malaria seropositivity on immune reactivities to SARS-CoV-2 proteins used on the serological tests, we also analyzed the immune reactivities (IgG) against a crude antigen extract of *Plasmodium falciparum* and SARS-CoV-2 ELISA on 863 randomly selected samples. Overall, 541 participants (62.7%) have IgG against *P. falciparum* antigens. The proportion of individuals seropositive for SARS-CoV-2 -using OMEGA/IDVet ELISA screening- was similar between individuals with and without immune reactivity toward *P. falciparum* antigen, i.e., 60.9% (142 of 233) and 59.7% (203 of 340), respectively. Similarly, SARS-CoV-2 seropositivity was not significantly different between subjects with (26.2%, 142 of 541) or without (28.2% (91 of 322) *P. falciparum* Abs (p-value = 0.833). Comparable results were obtained with the WANTAI COVID-19 total Ig immunoassay (Table S2).

Discussion

Since the beginning of the pandemic, to our knowledge, this is the first COVID-19 crosssectional, sero-epidemiological survey, performed at a national level in Africa. This survey was carried out at the end of the first wave of the COVID-19 epidemic and before the roll-out of COVID-19 vaccines in Senegal.

Several studies, including ours, have reported cross-reactivities on SARS-CoV-2 serological tests usually validated in high-income countries with pre-pandemic samples taken in malaria endemic areas.^{26–28} This cross-reactivity was detected either on SARS-CoV-2 Nucleocapsid (N) or Spike (S) proteins and thus may complicated the use of any of the homemade or already available serological tests. They may be due to the high exposure of African populations to multiple pathogens, including one of the most prevalent parasites, *Plasmodium*. Our results suggest no evidence of cross-reactivity interference between malaria and SARS-CoV-2 ELISA immunoassays using the double screening strategy with OMEGA/IDVet or WANTAI ELISA assays. (Table S2).

The adjusted national prevalence of anti-SARS-CoV-2 IgG/IgM was estimated at $28 \cdot 4\%$ with a great disparity between regions. Despite some areas of transmission like Dakar, Ziguinchor or Sedhiou presenting high seroprevalences (> 40%), the global seroprevalence suggested that a vast majority (around 2/3) of Senegalese people still remained susceptible to SARS-CoV-2 infection. Indeed, we estimate that about 4,744,392 cases of SARS-CoV-2 infections, among the 16.7 million population, potentially occurred. With the emergence of variants of concern

in December 2020, these data may explain the rebound observed following the survey period with a second wave that presented a higher amplitude in terms of the number of cases (41,416 compared to 16,089) and deaths (333 compare to 1,139) compared to the first one as herd immunity was not reached.

The highest prevalence was observed in the Ziguinchor and Sedhiou regions, with seroprevalence of 56.7% (574 confirmed cases) and 48.0% (151 confirmed cases), respectively. These regions are located in the South-Western part of the country in close borders with Guinea-Bissau and Gambia, the neighboring countries. These high seroprevalence data do not mirror the surveillance data recorded at the survey time. Several factors could explain these discrepancies and among them, i) the lack of molecular testing at the beginning of the COVID-19 pandemic in these remote areas (an on-site laboratory testing was put in place only in August 2020), ii) an airport in Ziguinchor (a touristic region) facilitating access of imported cases before the closure of air traffic and iii) difficulty to control inter-regional and cross-border trade. Louga and Matam, with a seroprevalence of around 11% seems to be less impacted by the COVID-19 epidemic. After the Tambacounda region, these rural regions are the two largest regions in the country, with low population densities of around 20-30 inhabitants per km². These socio-demographic characteristics could explain a lower circulation of the virus. Kaffrine, one of the main commercial crossroads of the country, recorded a high seroprevalence (26.9%) regarding the low number of COVID-19 confirmed cases (n=20)registered. High population movement with a lack of on-site laboratory for molecular testing may greatly contribute to this underestimation of COVID-19 burden extend. This underestimation can be explained by the large number of asymptomatic individuals or those with few symptoms that do not require medical consultation.

Other studies showed the same results in Africa approximately over the same period or at the beginning of 2021, i.e Juba, South-Sudan (22.6% in August-September); or our neighboring country, Mali, 54.7% in Dec-Jan 2021, although these studies concern a part of the population or a targeted population ^{16,29}. Altogether, this demonstrated an important circulation of SARS-CoV-2 in Senegal and in general in Africa. This slow SARS-CoV-2 transmission was not mirrored by the data obtained through surveillance system, highlighting the lack to assess the true exposure of COVID-19.

Given the scale of the pandemic, the Senegalese authorities quickly implemented several measures to limit the spread of the virus, such as social distancing, lockdown of markets, schools, universities, masking in public places and public transport, hand washing, contact tracing and testing, isolation and treatment of COVID-19 positive persons in health centers, etc. It is difficult to assess how much and strictly these measures, were followed by the populations between March and July 2020. However, transmission was not stopped and social living conditions in Africa remain favorable to airborne transmissible viruses. Our study clearly demonstrated this invisible and extended spread of the virus in this population regardless of age, sex or level of education. This is corroborated by other studies showing that the rate of

infection with the SARS-CoV-2 virus is higher than the rate announced by the surveillance systems. ^{29,30} This hidden face of the progression of the virus was enhanced by i) a low number of molecular tests carried out and, ii) a lesser proportion of severe cases of the disease as compared to European and American Regions. The severity of COVID-19 disease may be tempered by one of the demographic features of the African population i.e the youth of the Senegalese population (mean age of 19 years), iii) a more robust non-specific immunity gained by multiple and abundant exposures to pathogens that protect against the severe form of COVID-19 and iv) the absence of self-reporting of mild symptoms compatible COVID-19 during the first wave. Indeed, a high proportion of participants (67.2%) reported to having a history of COVID-19 related symptoms during the 2-3 months prior to the survey (corresponding to the peak of transmission of June-July). However, in the absence of more severe symptoms, none of them made a voluntary laboratory diagnosis of COVID-19. This observation highlights i) the complexity to attribute these non-specific symptoms to COVID-19, ii) the stigmatization around this pathology, and also ii) the fear to attend health facilities or being diagnosed COVID-19 positive.

Women were more represented than men, however, no significant differences were observed on seroprevalence data between the two groups. The most affected age group was 15-30, which is the most active age group followed by 45-60 compared to the under-5 age group. The household contact mode seems to be the main transmission mode as the under-5 age group and the over-60 age group was also impacted. No significant difference in the level of infection by profession was noted. This can be explained by the fact that all occupations were at the same risk level with a significant community-based transmission. Interestingly, a significant association was identified between SARS-CoV-2 seroprevalence and the attendance at Koranic schools [OR 1.54 (95% CI: 1.04-2.28, p=0.030). This could reflect that schools in general (including Koranic schools) are places of favorable transmission due to the regrouping of individuals of all age groups without necessarily a respect for social distancing measures.

The present study is the first population-based representative national survey to estimate the number of SARS-CoV-2 infection in Senegal and in West Africa. This study provides an estimate of the current level of transmission of the disease, points out the impact of (i) COVID-19 community-based transmission and (ii) the cross-regionals or cross-borders transmission increased by population movement. Our data also demonstrate the need to strengthen, in all regions, decentralized laboratories equipped with a capacity for molecular biology analysis of SARS-CoV-2 infections to improve the monitoring. With the emergence of SARS-CoV-2 variants, regular population-based national sero-epidemiological studies will be mandatory to guide prevention, control and vaccination strategy. A larger number of clusters in some regions would provide a better estimate of the prevalence.

Contributors

OusF, CT, AAS, JLR, TJ, OF, CL, BD, MN, CTD, OP and IVW conceived and designed the study. CT, SN, MB, AT, MD, MarD and JLR co-coordinated the survey and data collection. ON, OF, RF, AAM, IVW, BaD, MDia, and CTD performed serological analysis. CT, IVW and MD accessed and verified the underlying data. CT, TD, MarD, TW, MW, ON and IVW analyzed the data. CT, IVW, JLR, and BD wrote the manuscript.

All authors contributed to data interpretation, critically reviewed the first draft and approved the final version of the manuscript, and agreed to be accountable for the work. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

We declare no competing interests.

Data sharing

De-identified participant data used for this analysis can be requested from Institut Pasteur de Dakar. Interested researchers must submit a research proposal for consideration by the study investigators. If approved, the requestor must sign a data use agreement. Additionally, the study protocol is available for request. All data requests should be directed to the corresponding author.

Acknowledgments:

This work was supported by US Centers for Disease Control and Prevention (CDC), the Senegalese Ministry of Health, the Senegalese National Statistics and Demography Agency (ANSD), the WHO Unity program and the COVID-19 Task-force of the International Pasteur Institute Network (IPIN, REPAIR project). ANSD also provided the census information necessary for the random selection of households. Beijing WANTAI SARS-Cov-2 Ab ELISA kits were a generous gift of the WHO Unity program. We express our gratitude to the population of the regions and districts investigated and especially to the children, parents, guardians, who participated in the study. We also thank all the people who facilitated the survey, heads of regions and districts, local administration authorities, local health authorities from the Ministry of Health and community health workers (especially the Badiène-gox). We also thank the survey teams. Additionally, we would like to thank Dr. Thomas Poiret and Dr. Amy K. Bei for their review and feedback on the writing of the manuscript. The findings and conclusions in this article are those of the authors and do not necessarily reflect the position of the Senegalese Ministry of Health or US Centers for Disease Control and Prevention (CDC).

Funding: US Centers for Disease Control and Prevention (CDC).

Role of the funding source (At the end of the Methods section)

The study's funder had a role in study design, data collection, data analysis, data interpretation, or writing of the report.

List of Tables and Figures

Table 1. Main characteristics of the study population

Table 2. Seroprevalence of anti-SARS-CoV-2 IgG per age group and sex: combined results obtained with OMEGA/IDVet and Wantai ELISA

Table 3. Seroprevalence of anti-SARS-CoV-2 IgG per regions: combined results obtained with

 OMEGA/IDVet and Wantai ELISA

Figure 1: Flowchart of participants enrolment for the anti-SARS-CoV-2 antibody national seroprevalence study in Senegal

Figure 2: Mapping of clusters investigated and Senegal COVID-19 epidemic curve from 2 March to 31 December 2020 (Data sourced from the Ministry of Health)

Figure 3: Mapping of the seroprevalence data obtained by ELISA (panel A, B and C) and COVID-19 attack rate cases at the time of the household Sero-survey (panel D)

Figure 4: Association between SARS-CoV-2 prevalence and sociodemographic factors

List of Supplementary Tables and Figures

Table S1. Association between sociodemographic factors and SARS-CoV-2 seroprevalence

 (combined measures)

Table S2. COVID-19 and malaria seropositivity

Table S3. Seroprevalence of anti-SARS-CoV-2 IgG per age group and sex: results obtained with OMEGA/IDVet ELISA

 Table S4.
 Seroprevalence of anti-SARS-CoV-2 IgG per regions: results obtained with

 OMEGA/IDVet ELISA
 Image: Same series of the series

Table S5. Seroprevalence of anti-SARS-CoV-2 IgG per age group and sex: results obtained with WANTAI ELISA

 Table S6. Scroprevalence of anti-SARS-CoV-2 IgG/IgM per regions: results obtained with

 Wantai ELISA

 Table S7. Seroprevalence of anti-SARS-CoV-2 Abs according to COVID-19 related symptoms

Table S8. Main characteristics of ELISA immunoassays used in this study

Figure S1: Flowchart of the serological analysis (ELISA)

Figure S2: SARS-CoV-2 seroprevalence for IgG (OMEGA/IDVet ELISA), IgG/IgM (Wantai ELISA) and for the combined measures by regions

References

- 1 (WHO) WHO. COVID-19 Weekly Epidemiological Update.
- DOI:https://www.who.int/emergencies/diseases/novel-coronavirus-2019.
- 2 Johns Hopkins. Coronavirus Resource Center. COVID-19 Dashboard.
- 3 (WHO) WHO. COVID-19 WHO African region. .
- 4 Lukman AF, Rauf RI, Abiodun O, Oludoun O, Ayinde K, Ogundokun RO. COVID-19 prevalence estimation: Four most affected African countries. *Infectious Disease Modelling* 2020; **5**. DOI:10.1016/j.idm.2020.10.002.
- 5 Buitrago-Garcia D, Egli-Gany D, Counotte MJ, *et al.* Occurrence and transmission potential of asymptomatic and presymptomatic SARSCoV-2 infections: A living systematic review and metaanalysis. PLoS Medicine. 2020; **17**. DOI:10.1371/journal.pmed.1003346.
- 6 Al-Qahtani M, AlAli S, AbdulRahman AK, Salman Alsayyad A, Otoom S, Atkin SL. The prevalence of asymptomatic and symptomatic COVID-19 in a cohort of quarantined subjects. *International Journal of Infectious Diseases* 2021; **102**. DOI:10.1016/j.ijid.2020.10.091.
- 7 Syangtan G, Bista S, Dawadi P, *et al.* Asymptomatic SARS-CoV-2 Carriers: A Systematic Review and Meta-Analysis. Frontiers in Public Health. 2021; **8**. DOI:10.3389/fpubh.2020.587374.
- 8 Lai CC, Wang JH, Hsueh PR. Population-based seroprevalence surveys of anti-SARS-CoV-2 antibody: An up-to-date review. International Journal of Infectious Diseases. 2020; 101. DOI:10.1016/j.ijid.2020.10.011.
- 9 Chen X, Chen Z, Azman AS, *et al.* Serological evidence of human infection with SARS-CoV-2: a systematic review and meta-analysis. *The Lancet Global Health* 2021; **9**. DOI:10.1016/S2214-109X(21)00026-7.
- 10 Uyoga S, Adetifa IMO, Karanja HK, *et al.* Seroprevalence of anti-SARS-CoV-2 IgG antibodies in Kenyan blood donors. *Science* 2021; **371**. DOI:10.1126/science.abe1916.
- 11 Etyang AO, Lucinde R, Karanja H, *et al.* Seroprevalence of Antibodies to SARS-CoV-2 among Health Care Workers in Kenya. *Clinical Infectious Diseases* 2021. DOI:10.1093/cid/ciab346.
- 12 Chibwana MG, Jere KC, Kamng'ona R, *et al.* High SARS-CoV-2 seroprevalence in health care workers but relatively low numbers of deaths in urban Malawi. *Wellcome Open Research* 2020; **5**. DOI:10.12688/wellcomeopenres.16188.2.
- 13 Halatoko WA, Konu YR, Gbeasor-Komlanvi FA, *et al.* Prevalence of SARS-CoV-2 among high-risk populations in Lomé (Togo) in 2020. *PLoS ONE* 2020; **15**. DOI:10.1371/journal.pone.0242124.
- 14 Milleliri JM, Coulibaly D, Nyobe B, et al. SARS-CoV-2 infection in ivory coast: A serosurveillance survey among gold mine workers. American Journal of Tropical Medicine and Hygiene. 2021; 104. DOI:10.4269/ajtmh.21-0081.
- 15 Kempen JH, Abashawl A, Suga HK, *et al.* SARS-CoV-2 serosurvey in Addis Ababa, Ethiopia. *American Journal of Tropical Medicine and Hygiene* 2020; **103**. DOI:10.4269/ajtmh.20-0816.
- 16 Wiens KE, Mawien PN, Rumunu J, *et al.* Seroprevalence of anti-SARS-CoV-2 IgG antibodies in Juba, South Sudan: a population-based study. *medRxiv : the preprint server for health sciences* 2021; **27**: 1598–606.
- 17 Mulenga LB, Hines JZ, Fwoloshi S, *et al.* Prevalence of SARS-CoV-2 in six districts in Zambia in July, 2020: a cross-sectional cluster sample survey. *The Lancet Global Health* 2021; **9**. DOI:10.1016/s2214-109x(21)00053-x.
- 18 Dia N, Lakh NA, Diagne MM, *et al.* COVID-19 outbreak, Senegal, 2020. *Emerging Infectious Diseases* 2020; **26**. DOI:10.3201/eid2611.202615.
- 19 Ministère de la Santé et de l'Action Social du Sénégal. COVID-19 weekly report (Bulletin SITREP 98, June 7, 2021). https://www.sante.gouv.sn/activites/sitrep-98-coronavirus-riposte-à-lépidémie-dunouveau-coronavirus-covid-19-sénégal-rapport.
- 20 (WHO) WHO. Population-based age-stratified seroepidemiological investigation protocol for coronavirus 2019 (COVID-19) infection.
- 21 Staines HM, Kirwan DE, Clark DJ, *et al.* IgG seroconversion and pathophysiology in severe acute respiratory syndrome coronavirus 2 infection. *Emerging Infectious Diseases* 2021; **27**. DOI:10.3201/EID2701.203074.
- Krüttgen A, Cornelissen CG, Dreher M, Hornef MW, Imöhl M, Kleines M. Determination of SARS-CoV-2 antibodies with assays from Diasorin, Roche and IDvet. *Journal of Virological Methods* 2021;
 287. DOI:10.1016/j.jviromet.2020.113978.
- GeurtsvanKessel CH, Okba NMA, Igloi Z, *et al.* An evaluation of COVID-19 serological assays informs future diagnostics and exposure assessment. *Nature Communications* 2020; 11. DOI:10.1038/s41467-020-17317-y.

- 24 Diop F, Diop G, Niang M, *et al.* The value of local malaria strains for serological studies: Local strains versus Palo Alto reference strain. *Malaria Journal* 2015; **14**. DOI:10.1186/s12936-015-0734-z.
- 25 Weinstein S, Obuchowski NA, Lieber ML. Clinical evaluation of diagnostic tests. American Journal of Roentgenology. 2005; 184. DOI:10.2214/ajr.184.1.01840014.
- 26 Emmerich P, Murawski C, Ehmen C, et al. Limited specificity of commercially available SARS-CoV-2 IgG ELISAs in serum samples of African origin. *Tropical Medicine and International Health* 2021; 26. DOI:10.1111/tmi.13569.
- 27 Steinhardt LC, Ige F, Iriemenam NC, *et al.* Cross-Reactivity of Two SARS-CoV-2 Serological Assays in a Setting Where Malaria Is Endemic. *Journal of Clinical Microbiology* 2021; **59**. DOI:10.1128/jcm.00514-21.
- 28 Lapidus S, Liu F, Casanovas-Massana A, *et al.* Plasmodium infection induces cross-reactive antibodies to carbohydrate epitopes on the SARS-CoV-2 Spike protein. *medRxiv* 2021.
- 29 Sagara I, Woodford J, Kone M, et al. Rapidly increasing SARS-CoV-2 seroprevalence and limited clinical disease in three Malian communities: a prospective cohort study. medRxiv: the preprint server for health sciences 2021. DOI:10.1101/2021.04.26.21256016.
- 30 Sykes W, Mhlanga L, Swanevelder R, *et al.* Prevalence of anti-SARS-CoV-2 antibodies among blood donors in Northern Cape, KwaZulu-Natal, Eastern Cape, and Free State provinces of South Africa in January 2021. *Research square* 2021. DOI:10.21203/rs.3.rs-233375/v1.

	Overall (N=1.463)
Age (years)	(1,1,1,00)
Missing (n)	17
Mean (SD)	29 20 (20 64)
Range	0.20 - 84.8
A go group (yoars)	0.20 - 04.0
Age group (years)	17
	1/
	283 (19.0%)
]5,15]	1/9 (12.4%)
]15,30]	337 (23.3%)
]30,45]	297 (20.5%)
]45,60]	233 (16.1%)
> 60	117 (8.1%)
Sex	
Female	860 (58.8%)
Male	603 (41.2%)
Occupation	
Missing	281
Traders	81 (6.9%)
Schoolchild - student	230 (19.5%)
Housewife	325 (27.5%)
Workers	65 (5.5%)
Jobless	364 (30.8%)
Others	117 (9.9%)
Level of education	. ,
Missing	52
None	615 (43.6%)
Primary school	251 (17.8%)
Secondary school	298 (21.1%)
Koranic school	247 (17 5%)
Regions	
Dakar	302 (20.6%)
Diourbel	121 (8 3%)
Fatick	76 (5 2%)
Kaffrine	80 (5.5%)
Kaolack	116(7.0%)
Kadaugau	(7.970)
Kedougou	21(1.470)
Lauga	(4.170)
Louga	119(8.1%)
Matam	45 (3.1%)
Saint-Louis	94 (6.4%)
Sedhiou	57 (3.9%)
Tambacounda	112 (7.7%)
Thies	210 (14.4%)
Ziguinchor	50 (3.4%)
COVID-19 symptomatic*	
Missing	41
NO	464 (32.6%)
YES	958 (67.4%)
COVID-19 symptoms	
Fever	707 (73.8%)
Headache	311 (32.4%)
Cough	286 (30.0%)
Rhinorrhea	187 (19.5%)
Fatigue	148 (15.4%)

Table 1. Main characteristics of the study population

Myalgia	72 (7.5%)
Sore throat	34 (3.5%)
Diarrhea	33 (3.4%)
Taste or smell lost	27 (2.8%)

* History of symptoms compatible with COVID-19 less than 6 months before the survey.

 Table 2. Seroprevalence of anti-SARS-CoV-2 IgG per age group and gender: combined results obtained with OMEGA/IDVet and Wantai ELISA

			Crude seroprevalence			Standardized seroprevalence		Adjusted seroprevalence	
	All samples	Seropositive samples	%	(95%CI)	Senegal population (2020)	%	(95%CI)	%	(95%CI)
Age group									
[0-5]	283	45	15.9	11.8-20.7	3,224,935	15.9	11.8-20.7	16.1	12.2-21.1
]5-15]	179	44	24.6	18.5-31.6	4,096,096	24.6	18.5-31.6	24.9	19-32.2
]15-30]	337	124	36.8	31.6-42.2	4,423,193	36.5	31.3-41.9	37.3	32.2-42.8
]30-45]	297	81	27.3	22.3-32.7	2,687,369	29.9	24.8-35.5	27.6	22.6-33.1
]45-60]	233	70	30.0	24.2-36.4	1,418,953	30.0	24.2-36.4	30.4	24.6-36.8
>60	117	29	24.8	17.3-33.6	855,062	24.9	17.3-33.6	25.1	17.3-33.6
Sex									
Female	850	235	27.6	24.7-30.8	8,391,358	27.6	24.7-30.8	28.0	25-31.2
Male	596	158	26.5	23-30.2	8,314,250	27.2	23.6-30.9	26.9	23.3-30.6

Table 3. Seroprevalence of anti-SARS-CoV-2 IgG per regions: combined results obtained with OMEGA/IDVet and Wantai ELISA

			Crude seroprevalence		Adjusted seroprevalence						
Regions	All samples (n)	Seropositive samples (n)	%	(95% CI)	%	(95% CI)	Population per region	Estimates COVID-19 infection	Confirmed COVID-19 cases	Ratio of reported cases to estimated infection	
Dakar	295	128	43.4	37.7-49.3	44.0	38.3-49.9	3,835,011	1,687,405	11,016	1:153	
Diourbel	117	22	18.8	12.2-27.1	19.0	12.2-27.1	1,859,503	353,306	754	1:469	
Fatick	75	13	17.3	9.6-27.8	17.5	9.6-27.8	900,800	157,640	325	1:485	
Kaffrine	79	21	26.6	17.3-37.7	26.9	17.3-37.7	728,951	196,088	20	1:9,804	
Kaolack	113	37	32.7	24.2-42.2	33.1	24.2-42.2	1,191,577	394,412	291	1:1,355	
Kedougou	21	4	19.0	5.4-41.9	19.2	05.4-41.9	190,509	36,578	219	1:167	
Kolda	52	12	23.1	12.5-36.8	23.4	12.5-36.8	822,003	192,349	216	1:891	
Louga	118	13	11.0	6-18.1	11.1	6-18.1	1,061,612	117,839	75	1:1,571	
Matam	45	5	11.1	3.7-24.1	11.2	3.7-24.1	732,863	82,081	51	1:1,609	
Saint-Louis	94	15	16.0	9.2-25	16.2	9.2-25	1,091,735	176,861	319	1:554	
Sedhiou	57	27	47.4	34-61	48.0	34-61	572,101	274,608	151	1:1,819	
Tambacounda	106	25	23.6	15.9-32.8	23.9	15.9-32.8	872,155	208,445	111	1:1,878	
Thies	200	48	24.0	18.3-30.5	24.3	18.7-31.1	2,162,833	525,568	1,974	1:266	
Ziguinchor	50	28	56.0	41.3-70	56.7	41.3-70	683,955	387,802	567	1:684	
National	1,422	398	27.9	25.7-30.4	28.4	26.1-30.8	16,705,608	4,744,393	16,089	1:295	









SAINT-LOUIS

С





1.85 (1.07-3.21, p=0.028) 3.06 (1.95-4.92, p<0.001) 1.82 (1.12-3.00, p=0.018) 2.06 (1.22-3.50, p=0.007) 1.38 (0.70-2.68, p=0.342) 0.25 (0.14-0.43, p<0.001) 0.22 (0.09-0.49, p=0.001) 0.44 (0.23-0.79, p=0.007) 0.53 (0.30-0.91, p=0.024) 0.90 (0.21-3.51, p=0.873) 0.34 (0.16-0.69, p=0.004) 0.19 (0.09-0.37, p<0.001) 0.07 (0.01-0.23, p<0.001) 0.20 (0.10-0.38, p<0.001) 1.07 (0.53-2.17, p=0.848) 0.35 (0.18-0.65, p=0.001) 0.40 (0.25-0.62, p<0.001) 1.14 (0.55-2.34, p=0.720)

[0,5] (5,15] (15,30] (30,45] (45,60] (60,100] DAKAR DIOURBEL FATICK KAFFRINE KAOLACK KEDOUGOU KOLDA LOUGA MATAM SAINT-LOUIS SEDHIOU TAMBACOUNDA THIES ZIGUINCHOR

Region

Age group

POS or NEG: OR (95% CI, p-value)