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SARS-CoV-2 infection and antibody seroprevalence in routine surveillance patients, health care workers and general population in Kita region, Mali

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SARS-CoV-2 infection and antibody seroprevalence in routine surveillance patients, health care workers and general population in Kita region, Mali

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1

Abstract

Objective

To estimate the degree of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) transmission among health care workers (HCWs) and general population in a West African setting.

Design

Routine surveillance in twelve health facilities, HCWs serosurvey in five health facilities and community serosurvey in sixteen villages in or near Kita town, Mali.

Setting

Kita region, western Mali; local health centres around the central (regional) referral health centre.

Participants

Patients in routine surveillance, HCWs in local health centres and community members of all ages in populations associated with study health centres.

Main outcome measures

Seropositivity of ELISA test detecting SARS-CoV-2-specific total antibodies and real time RT-PCR confirmed SARS-CoV-2 infection.

Results

From 2392 routine surveillance at health facilities, 68 samples (2.8%, 95% CI: 2.2% to 3.6%) tested positive for SARS-CoV-2 by RT-PCR. The monthly positivity rate was 0% in June-August 2020 and gradually increased to 6% by December 2020 and 6.2% by January 2021, then declined to 5.5%, 3.3%, 3.6% and 0.8% in February, March, April and May 2021, respectively. From 397 serum samples collected from 113 HCWs, 175 (44.1%, 95% CI: 39.1% to 49.1%) were positive for SARS-CoV-2 antibodies. The monthly seroprevalence was around 10% from September to November 2020 and increased to over 40% from December 2020 to May 2021. For community serosurvey in December 2020, overall seroprevalence of SARS-CoV-2 antibodies was 27.7%. The highest age-stratified seroprevalence was observed in participants aged 60-69 years (45.5%, 95% CI: 32.3% to 58.6%). The lowest was in children aged 0-9 years (14.0%, 95% CI: 7.4% to 20.6%). Attendance at mass gatherings within three months of sample collection was associated with higher seropositivity (RR=1.48, 95% CI: 1.09 to 2.02).

Conclusions

Community spread of SARS-CoV-2 infection started in Kita in October-November 2020, peaked in January 2021 and continued in May 2021. The observation is contrary to the widely expressed view, based on limited data, that Covid-19 infection rates were lower in 2020-2021 in West Africa than in other settings.

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3

Strengths and limitations of this study

- Our study was strengthened by the data available on participants from health facility clients, HCWs and the general population.

- We investigated both active infection and seroprevalence for HCWs and the general population during the same time period.

- First study of its kind in West Africa to provide routine surveillance data capturing.

- Our study was performed in one region and therefore it only reflects the Covid-19 situation of the Kita region.

- Extending active infection RT-PCR testing to non-symptomatic cases, both at the health facility and in the general population, would have provided more robust insights on infection rates as well as the level of asymptomatic cases.

Funding

Competing interests

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: PA had financial support from Bill & Melinda Gates Foundation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

4

Introduction

The global coronavirus disease 2019 (Covid-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has had a devastating impact on the health of populations in all regions of the world, leading to over five million deaths and tens of millions of cases, also causing widespread and long-term social and economic damage. Despite predictions made during the so-called "first wave" (March-May 2020) that Covid-19 would spread widely and cause untold damage to communities in African countries, data during that period suggested that transmission, case, and death rates have been lower in African settings than in other regions and continents.[1]

Whereas the reported numbers of SARS-CoV-2 infection have been low in large parts of Africa, there is some evidence that this may be due to undertesting or underreporting. A recent Zambian post-mortem study suggested that many people who die have an undiagnosed SARS-CoV-2 infection.[2] Unfortunately, there have been very few community-based SARS-CoV-2 studies published so far from Africa and hence the population prevalence in the continent is still largely unknown.

Our study was planned to characterise the epidemiology of Covid-19 epidemic in Kita region in western Mali, an area with very low rates of reported infections. To this end, we monitored time-trends in the proportion of positive SARS-CoV-2 virus test results among patients presenting with suspected SARS-CoV-2 infection in primary health centres over a period of 10 months and complemented the data with seropositivity for SARS-CoV-2 antibodies among personnel working in the same facilities during the same time frame. In the middle of the follow-up period, we conducted a population based viral and serological survey, to establish a point-prevalence of acute infection and past exposure to it, among apparently healthy people of different ages.

5

Methods

Study design and participants

The present study was designed to support the Malian Ministry of Health and Social Affairs'

strategy to strengthen epidemiological surveillance and enhance the national response in Mali.

The study was conducted in Kita region, Western Mali. The area is served by one referral health

centre in Kita centre, while most health areas have a community health centre, offering a range of

basic medical services with fee charged.

Our study targeted three groups –patients at a health facility, HCWs and apparently healthy

community residents. We conducted surveillance for patients presenting at 1 referral health

centre and 11 community health centres, over a period of 12 months. We conducted a HCWs

serosurvey in five of the same health facilities over a period of 9 months and community

serosurvey at one time point in 16 villages. The HCWs samples were from nurses, doctors, office

workers, laboratory personnel and other auxiliary staff. The community samples were selected on

the basis of cluster sampling. We used enumeration areas as clusters and determined the number

of households from each cluster based on its recorded population size. The actual households to

be invited were selected by random sampling.

Data and Specimen collection

For the patient surveillance study, the study nurses filled in a patient screening form, a suspected

infection enrolment form on exposures and symptoms and consenting form, followed by a medical

examination and a flocked nasopharyngeal swab (NPS, flexible minitip, Copan) or oropharyngeal

swab (OPS, flexible minitip, Copan; nasal and throat flocked swab, Dewei Medical Equipment Co.,

6

Ltd) specimen collection from those with a suspected SARS-CoV-2 infection, eg. patients

experiencing fever, cough, shortness of breath and seeking medical advice. For the HCWs sero-

surveillance study, study nurses filled in a screening, consent and enrolment form, a suspected

infection enrolment form on symptoms and exposure questionnaire. Four ml of venous blood

sample (BD vacutainer, Cat. No. 369032) was collected monthly and an NPS or OPS was taken at

enrolment and every 12 weeks thereafter. From each community survey participant, the

nurse/data collector filled in a symptom and exposure questionnaire and collected a nasal swab

specimen and a blood sample. A study nurse or a data collector filled out the symptom and

exposure questionnaire using a REDCap electronic data capture application. The biological samples

were collected by a doctor at the health centre and by a nurse in the community survey. The

collected swab samples were transported at 2-8 °C in 3 ml PBS/VTM from Kita to Bamako lab for

analysis. The blood samples were centrifuged, and serum was aliquoted into cryovials at the Kita

lab and batched samples were transported in dry shipper to Bamako for analysis.

Nucleic acid extraction and real time RT-PCR

RNA was extracted from sample swabs using a QIAamp Viral RNA Mini kit (Qiagen, Germany, Cat.

No. 52906) according to the manufacturer's instructions. Samples were eluted in a final volume of

140 μL.

Real time reverse transcription polymerase chain reaction (rtRT-PCR) assays for SARS-CoV-2 were

carried out at the CVD-Mali Influenza laboratory, a participant in the WHO External Quality

Assessment for the Detection of Influenza and SARS-CoV2 viruses. Briefly, extracted RNA was

amplified using the Superscript One-step RT-PCR kit (Invitrogen, Thermo Fischer Scientific, Cat No.

11732-088) targeting the RdRp gene (Corman VM, 2020) or the Quantabio qScript XLT One-Step

7

RT-qPCR ToughMix targeting the N-gene (N2 primers) (CDC assay,

https://www.fda.gov/media/134922/download).

Reactions (25 µL) were set up as follows: 2x master mix, 0.6 µM forward primer, 0.8 µM of reverse

primer, 0.1µM of each probe, distilled-deionised (dd) H_2O and 5 µL of template. Reactions were

cycled on a 7500 Fast ABI system (Applied Biosystems™) with an initial denaturation step at 95°C

for 3 min followed by 45 cycles at 95°C for 30 s and 55°C for 30 s for the Superscript One-step RT-

PCR kit, and 95°C for 15 s and 58°C for 30 s for the Quantabio qScript XLT One-Step RT-qPCR

ToughMix.

When using the Takara Prime Direct RT-PCR (Takara Bio, Cat. No. RR650B and RR65TW), samples

collected either in PBS or VTM were heated at 95°C for 10 min and used directly in the RT-PCR

reaction. The Takara Prime Direct RT-PCR reaction mix consisted of Prime Takara master mix, 0.25

µM primers, 0.13 µM probe, ddH_2O and 5 µL of template. Cycling conditions were as follows: 90°C

for 3 min, 60°C for 5 min, and 45 cycles of 95°C for 5 s and 60°C for 30 s.

All assays were validated using WHO RNA samples received as part of an external Quality

Assessment Programme and a panel of known positive and negative samples from University

College London, UK.

Serological assays

A laboratory technician performed the Wantai anti-SARS-Cov-2 total antibody ELISA (Beijing

Wantai Biological Pharmacy Ent, Beijing, China) following the manufacturer's instructions. Serum

samples (100 µL) from each participant were tested as a singlet. Ten batches were performed for

community survey samples, six batches for HCW samples and one for repeated samples.

Assay validation was carried out using the WHO international standard (NIBSC code: 20/136), the

WHO International Reference Panel for anti-SARS-CoV-2 immunoglobulin (NIBSC code: 20/268),

8

and an anti-SARSCoV-2 Verification Panel for Serology Assays (NIBSC code: 20/B770). In addition,

100 pre-pandemic samples collected in 2012 were tested to assess the specificity of assay. All of

these pre-pandemic serum samples were from adult women (mean age 26 years old, range 16-41).

Following the manufacturer's instructions, samples giving an absorbance equal to or greater than

the cut-off value (A/CO >=1) were considered positive. Samples with A/CO ratio between 0.9 and

1.1 were considered borderline and were retested.

Statistical analysis

The sample size was originally calculated to estimate the seroprevalence of SARS-Cov-2 antibodies

with an absolute precision (margin of error) of 2.5% and assuming a baseline prevalence of 5%.

Accounting for clustering effects of households within villages and of individuals within

households, we estimated to require a minimum sample size of 3000. Assuming an average village

population of 1446 (based on available regional statistics), average household size of 14, and

average number of under-5 children per household of 3, we decided to visit 215 households to

enrol the study sample.

We compared proportions, means and standard deviations (SD) between groups by using

Student's *t* test for continuous variables and Fisher's exact test for proportions. We calculated risk

ratios (RR) and 95% confidence intervals (CI) with regression model. All statistical analyses were

carried out using R versions 3.4.4 and 4.1.0.

9

Patient and public involvement

Village chefs and health centre leaders were consulted before the study to ensure feasibility of the

planned study procedures. Participants were not involved in the development of the research

design, conduct of the research or preparation of the manuscript.

Results

Between June 2020 and May 2021, a total of 13,104 people presenting at outpatient clinics or

using preventive services at 12 health facilities in or near the town of Kita were screened for

exposure or symptoms of SARS-CoV-2 infection. Of these, 2393 were deemed to have a suspected

SARS-CoV-2 infection and 2392 agreed to provide nasopharyngeal or oropharyngeal samples for

viral detection.

From all the tested samples from health centre patients, 68 (2.8%, 95% CI 2.2% - 3.6%) gave a

positive test result for SARS-CoV-2 by RT-PCR. Prior to August 2020, no SARS-CoV-2 RNA could be

detected, infection rates steadily rose from November 2020 onwards, peaking at 6.2% (95% CI

3.3% - 10.4%) in January 2021, and then gradually declined to 0.8% (95% CI 0.02% - 4.1%) in May

2021 (Figure 1). As shown in the figure, the increase in the proportion of positive samples followed

a similar time trend in nationally reported numbers of SARS-CoV-2 infections and associated

deaths.

Between September 2020 and May 2021, a total of 113 members of staff were approached in five

health facilities and invited to participate in the HCWs SARS-CoV-2 serosurvey. All 113 invited staff

members agreed to participate. Among them, 89 (78.8%) were nurses, 9 (7.9%) were doctors, 7

10

(6.2%) were administrative or laboratory technicians, and 8 (7.1%) were auxiliary staff including housekeepers, cleaners and security staff.

Over the 9-month follow-up period, the participants provided a total of 397 blood samples, with a range of 1-7 samples per participant. Of these samples, 175 (44.1%, 95% CI 39.1% - 49.1%) were positive for SARS-CoV-2 antibody by ELISA. By calendar month, the proportion of positive samples was approximately 10% until November 2020, increasing rapidly to 59.1% (95% CI 38.5% - 79.6%) in January 2021 and remained relatively constant thereafter (Figure 2). For different health professional groups, the proportion of SARS-CoV-2 antibody positive samples was highest among doctors (50.0%, 95% CI 29.9% - 70.1%, 13/26 samples), followed by 49.8% (95% CI 43.4% - 56.2%, 124/249) among nurses, 35.9% (95% CI 21.2% - 52.8%, 14/39) among administrative staff and laboratory technicians and 15.4% (95% CI 1.9% - 45.5%, 2/13) among auxiliary staff.

Of the 101 NPS or OPS samples collected from the health personnel, only one (1.0%) collected in November 2020 gave a positive test result for SARS-CoV-2 by RT-PCR.

In December 2020, a total of 229 randomly selected households were visited in villages in or near the Kita centre and invited to participate in a SARS-CoV-2 community survey. Heads of all the invited households provided a consent and a total of 905 participants were included in the survey. Of the participants, 564 (62.3%) were women, 654 (72.3%) were at least 15 years old, 102 (11.3%) had respiratory symptoms in the preceding 14 days, 496 (55.4%) had attended a mass gathering in the preceding 3 months, none reported a contact with a Covid-19 patient, and 21 (2.3%) had a fever.

Of the 905 community survey participants, 882 provided a blood sample for antibody analysis and 894 provided a nasal swab for viral detection. Among all the participants, the overall

11

seroprevalence was 27.7% (95% CI 24.7% – 30.8%). The seroprevalence was lowest (14.0%, 95%

CI: 7.4% - 20.6%) among 0-9-year-old and highest (45.5%, 95% CI: 32.3% to 58.6%) among 60-69-

year-old participants (Figure 3). Participants who had attended a mass gathering in the preceding

3 months had a higher seroprevalence than those who had not (relative risk 1.48, 95% CI: 1.09 -

2.02). Participant sex, measured fever, and recent history of fever or other respiratory symptoms

were not associated with the presence of SARS-CoV-2 antibodies in the blood samples (Table 1).

A total of 21 (2.4%, 95% CI 1.5% – 3.6%) community survey participants had a positive RT-PCR

result for SARS-CoV-2. The proportion of positive SARS-CoV-2 PCR tests was lowest (0.4%, 95% CI:

0% - 1.3%) among 10-19-year-old and highest (5.8%, 95% CI: 0.3% - 11.3%) among 50-59-year-old

participants (Figure 3).

Of the 100 banked serum samples collected before the Covid-19 pandemic (in year 2012), one

gave a borderline positive result and 99 gave a negative test result in the Wantai SARS-CoV-2

antibody test.

Discussion

This study measured active and past Covid-19 infection in Malian HCWs and the general

population through RT-PCR and seroprevalence testing. The results point towards significant

underestimation of Mali's Covid-19 infection rates, as measured by current testing mechanisms.

The study also provides insights into Covid-19 transmission patterns and risk factors. Extremely

high infection rates were demonstrated by the study's serological testing. By December 2020 over

a quarter (27.7%) of Kita's general population, and more than a third (41.9%) of HCWs had been

infected. By January 2021, this had increased to 60% of HCWs.

12

National routine surveillance morbidity data for the study period (June 2020 – May 2021), indicates that in June, transmission was at its first-wave peak, and that two later larger waves occurred, peaking in early December 2020 and April 2021.[3] Both the study's facility-based client/patient active infection investigation and HCWs seroprevalence findings indicate that Kita region's main transmission started later, in November 2020. This suggests that the region escaped the earlier wave, with Covid-19 imported sometime in late October/early November, perhaps around the celebrations for the Prophet Mohamed's birthday on 29th October. Both study groups indicate a peak in December/January, similar to that seen nationally. The clients' active infection rate then showed a gradual decline to May rather than a second wave in March that was seen nationally.[3] This could be explained by containment measures reducing the emergence of a second peak in the general population.

A comparison of this study's seroprevalence and active infection rates show that seroprevalence is much higher than active cases in both the study's general population and in HCWs. The fact that this high seroprevalence did not translate into a similar expression of morbidity and resultant death, neither in our study nor in the national surveillance system is an interesting area to investigate. This scenario has been documented quite widely in Africa.[4,5] This could be explained by a multitude of interlinking factors. High levels of asymptomatic cases, both for the general population and HCWs, have been documented in African as well as global studies.[6-11] As our study's active infection testing selected suspected cases of Covid-19 only, we could have missed a large proportion of asymptomatic cases. National surveillance would also have missed these cases as, firstly, they wouldn't present to a health centre and, secondly, even those that did would have been missed by diagnostic protocols.

13

Mali has gaps in demographic, disease morbidity, and death surveillance, including for Covid-19. A recent Zambian study showed that nearly 20% of people who had died had a positive SARS-CoV-2 PCR test in a postmortem NP swab sample. These people had not been tested before death, even though some of them had presented with Covid-19 symptoms.[2] A similar situation is likely present in Mali where diagnostic capacity is limited, at 7191 tests per 1 million people (compared to that of South Africa, 113 756) (Salyer, ym., 2021), and where Covid-19 stigma exists.[12,13]

This study showed that 1.5 times more HCWs in Kita had been infected with SARS-CoV-2 by December 2020 compared to the general population. In fact, half of patient facing cadres such as doctors and nurses had been exposed to the virus and this was higher than laboratory, administrative (both 36%) and auxiliary staff (15.4%). Measures of increased risk of infection in HCWs vs the general population have been demonstrated in many global studies, with adequate PPE provision and use being key to minimizing this risk.[4,6,11,14] In resource-constrained settings such as Kita, key tools for protecting HCWs, along with early disease detection, are often limited or absent in Africa.[15,16] Measures to improve protection for doctors and nurses, especially considering low levels of health staff availability in the country – in 2017 Mali had only six qualified providers per 10,000 inhabitants – would be pertinent.[17]

In the general population, risk factors included increased age and attendance at mass gatherings. Seroprevalence was lowest in the very young, and highest in 60-69-year-olds but again lower in the 70+ year olds. Mass gatherings are likely sources of infection given Covid-19's transmission dynamics and the difficulty of ensuring appropriate prevention control measures (mask wearing, social distancing, hand washing) at such events. Interestingly, neither fever (current or recent) nor other respiratory symptoms were seen to be significant risk factors, which again infers a high asymptomatic population.

14

The study's principal strength is that we included health facility clients, HCWs and the general population. We also investigated both active infection and seroprevalence for HCWs and the general population during the same time period. Study limitations include restriction to one region and therefore this study only reflects the Covid-19 situation of the Kita region. Extending active infection RT-PCR testing to non-symptomatic cases, both at the health facility and in the general population, would have provided more robust insights on infection rates as well as the level of asymptomatic cases. Widening the study to investigate exposure/transmission factors for study participants, such as PPE provision and use, social distancing, Covid-19 interventions, and behaviour change would have allowed the study to explore risk factors to better inform policy. When interpreting the RT-PCR results, possible sources of bias could have occurred within the sample collection regarding sample types (oropharyngeal/nasopharyngeal swabs), sample quality, transportation and temperature, and also in the use of 3 different RT-PCR assays. The latter is unlikely to have affected the results as all RT-PCR assays were validated in-house and by others.[18] Previous reports have suggested that some SARS-CoV-2 ELISA kits lack specificity when used on serum samples originating from Africa and in particular exhibit pre-existing cross-reactivity to SARS-CoV-2.[19,20] Our assay showed a 99% specificity when pre-pandemic samples were analysed and previous work has shown the Wantai assay to be highly sensitive (93%) and specific (100%).[21]

The main finding of this study is that SARS-CoV-2 in rural Mali is much more widespread than assumed by national testing data and particularly in the older population and frontline health workers. Institutional preparedness as well as public risk assessment and personal protection capacity should be augmented, and behaviours in high-risk activities such as mass gatherings improved, if cessation of such events is not possible. Improving the understanding of Covid-19 in Mali through surveillance will enable the country to reduce the economic and social impacts of the disease. Further studies which provide accurate estimates of SARS-CoV-2 infection rates and

15

seroconversion in the population would enable the development of a national transmission model,

mapping of transmission hotspots, identification of national/regional risk factors, and the

prioritization of equitable and effective responses. Such studies could also direct policy

determinations in terms of disease prioritization, ensuring that resources reallocated for Covid-19

control measures are used appropriately.

16

Contributors

DA and FCH contributed equally and share first authorship. CD and YMF contributed equally and share last authorship. JL performed statistical analysis. PA, UA, NK, SS, KW, CD and YMF initiated the project and preliminary design. DA, FCH, LA, HB, ECG, FD, RI, OM, UM, OS, AT developed the design and methods. DA, FCH, CD and YMF wrote the first draft of the manuscript. All authors contributed to the design of the study and interpretation of its results, and revised and approved the manuscript. PA and YMF are the guarantors and accept full responsibility for the work and the conduct of the study, had access to the data, and controlled the decision to publish. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Data sharing

Deidentified participant data might be available on reasonable request to the corresponding author.

Ethics

The study protocol was approved by the Faculté de Médecine, Pharmacie et d'Odonto-stomatologie (FMPOS) institutional review board in Mali and only participants who provided informed consent were enrolled in the study. All consents were requested verbally and recorded in the electronic data capture system. The minors' caregivers gave consent for their children to participate in the study.

17

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Table 1. Seroprevalence of SARS-CoV-2-specific total antibody by background characteristics

	Sample size, n	Seropositive participants, n	Seroprevalence, %	OR (95% CI)
Participants, *n*	875	242	27.7%	
Sex				
Male	330	98	29.7%	Ref
Female	543	142	26.2%	0.84 (0.62-1.14)
Fever during past 14 days				
No fever	832	229	27.5%	Ref
Fever	41	11	26.8%	0.97 (0.48-1.96)
Fever (body temperature over 38 °C)				
No fever	852	238	27.9%	Ref
Fever	21	2	9.5%	0.27 (0.06-1.17)
Respiratory symptoms in the past 14 days				
No symptoms	773	216	27.9%	Ref
Any symptoms	100	24	24.0%	0.81 (0.50-1.32)
Mass gatherings in the past 3 months				
No attendance	370	86	23.0%	Ref
Attendance	487	151	31.0%	1.48 (1.09-2.02)

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Legends of Figures:

Figure 1. Percentage of SARS-CoV-2 positive RT-PCR test by month in routine surveillance patients from health facilities. Grey area and values in parentheses indicate 95% confidence interval from the proportion of SARS-CoV-2 RNA containing samples. Yellow lines indicate nationally reported numbers of SARS-CoV-2 infections ((WHO https://covid19.who.int/region/afro/country/ml). Values above the x-axis indicate number of study samples in each month. PS = pharyngeal swab, SARS-CoV-2 = Severe acute respiratory syndrome coronavirus-2, RNA = ribonucleic acid.

Figure 2. Seroprevalence of SARS-CoV-2-specific total antibody by month in health care worker serosurvey. Grey area and values in parentheses indicate 95% confidence interval from the proportion of SARS-CoV-2-specific antibody containing samples. Values above the x-axis indicate number of study samples in each month. SARS-CoV-2 = Severe acute respiratory syndrome coronavirus-2.

Figure 3. Seroprevalence of SARS-CoV-2-specific total antibody and percentage of SARS-CoV-2 positive RT-PCR test by 10-year age interval group in community participants. Red bars indicate proportion (95% CI) of serum samples containing SARS-CoV-2-specific antibodies (primary y-axis), blue bars indicate proportion (95% CI) of PS samples containing SARS-CoV-2 RNA (secondary y axis). PS = pharyngeal swab, SARS-CoV-2 = Severe acute respiratory syndrome coronavirus-2, RNA = ribonucleic acid.
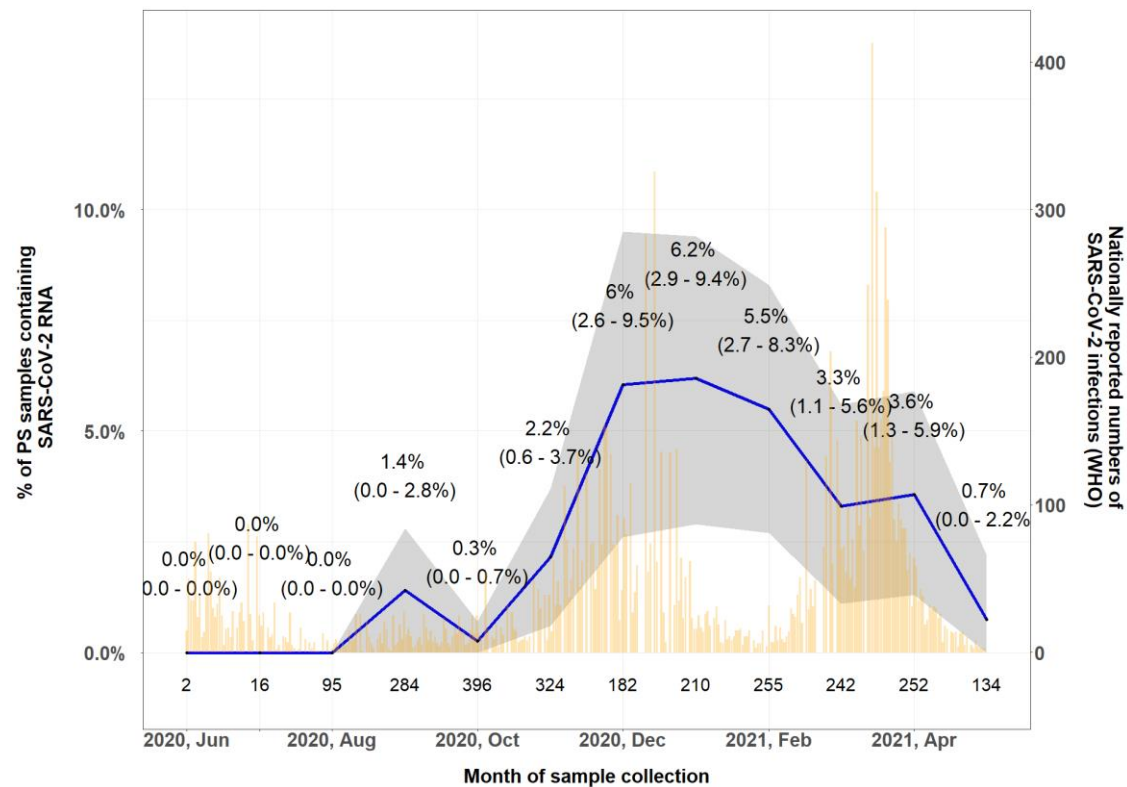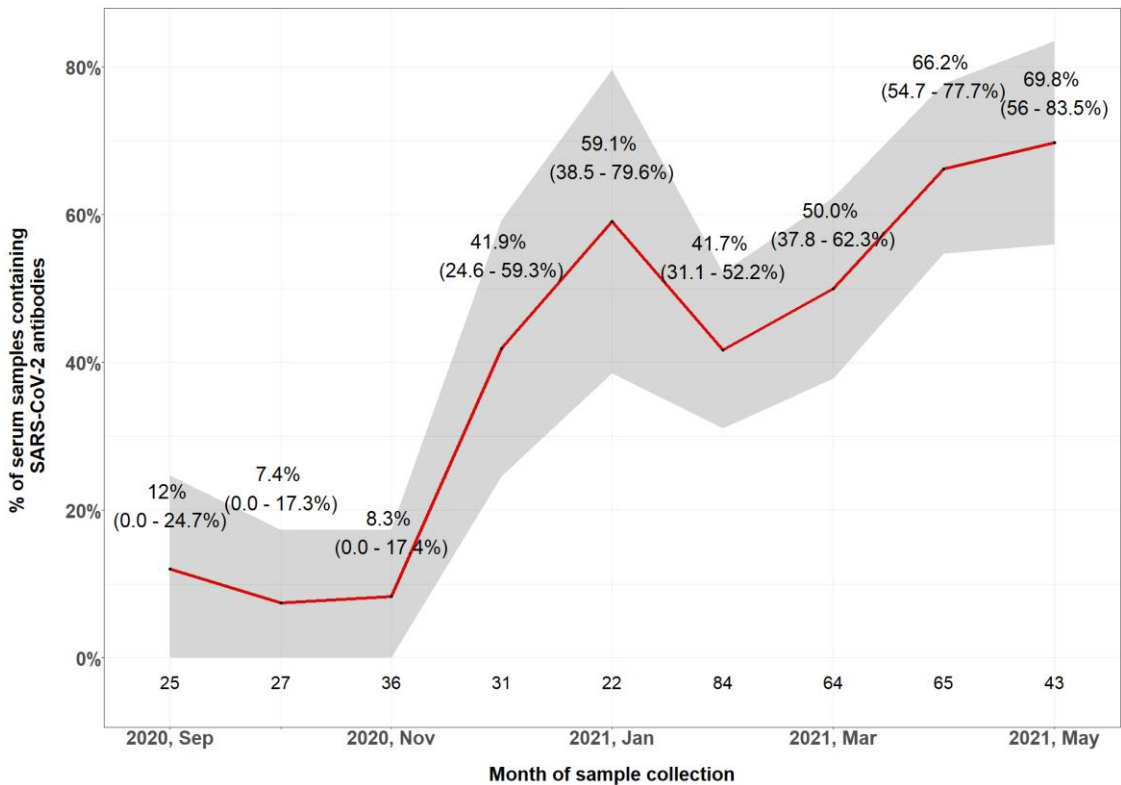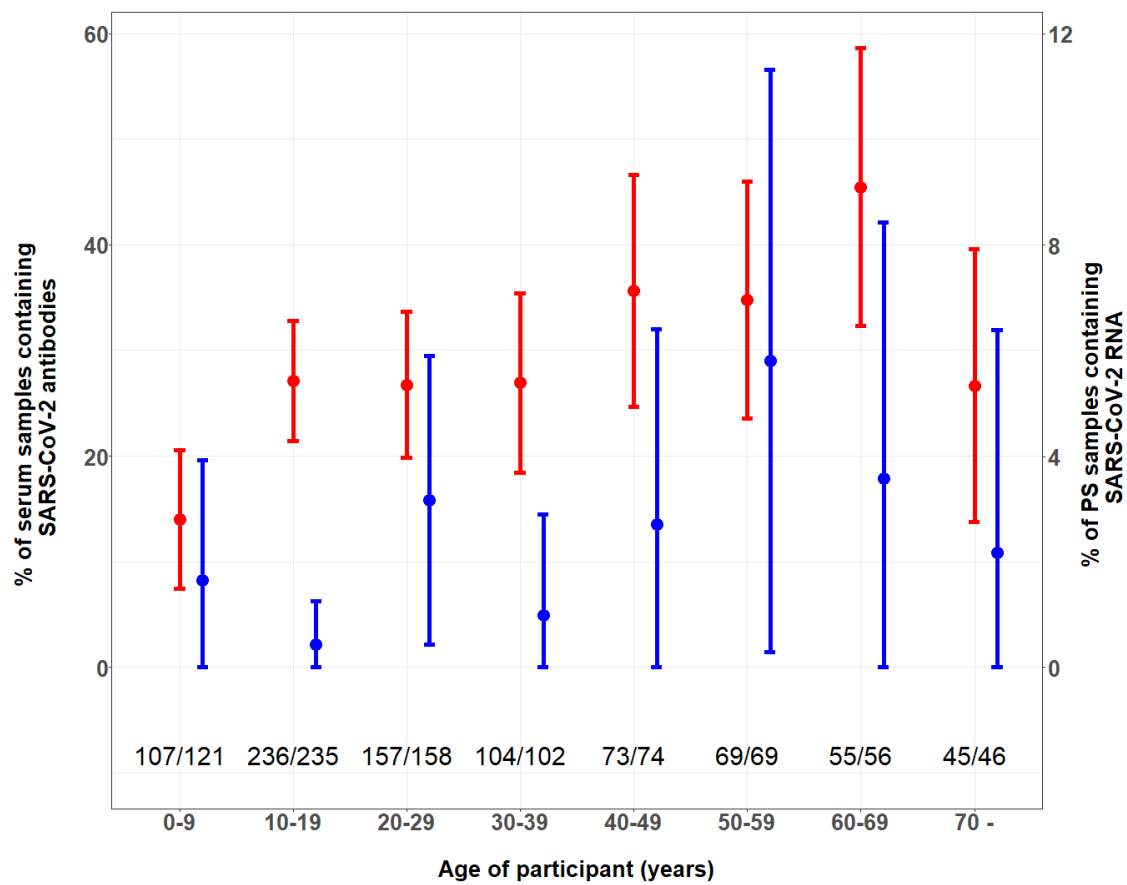
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Figure 1.

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Figure 2.

Figure 3.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5-8*
Bias	9	Describe any efforts to address potential sources of bias	5-8
Study size	10	Explain how the study size was arrived at	8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	

Results

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9-10
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9-10
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*9-10*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	

Discussion

Key results	18	Summarise key results with reference to study objectives	11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11-13
Generalisability	21	Discuss the generalisability (external validity) of the study results	14

Other information

Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	15

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

SARS-CoV-2 infection and antibody seroprevalence in routine surveillance patients, health care workers and general population in Kita region, Mali: an observational study 2020 - 2021

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SARS-CoV-2 infection and antibody seroprevalence in routine surveillance patients, health care workers and general population in Kita region, Mali: an observational study 2020 - 2021

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1

Abstract

Objective

To estimate the degree of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) transmission among health care workers (HCWs) and general population in Kita region of Mali.

Design

Routine surveillance in twelve health facilities, HCWs serosurvey in five health facilities and community serosurvey in sixteen villages in or near Kita town, Mali.

Setting

Kita region, western Mali; local health centres around the central (regional) referral health centre.

Participants

Patients in routine surveillance, HCWs in local health centres and community members of all ages in populations associated with study health centres.

Main outcome measures

Seropositivity of ELISA test detecting SARS-CoV-2-specific total antibodies and real time RT-PCR confirmed SARS-CoV-2 infection.

Results

From 2392 routine surveillance samples, 68 (2.8%, 95% CI: 2.2% to 3.6%) tested positive for SARS-CoV-2 by RT-PCR. The monthly positivity rate was 0% in June-August 2020 and gradually increased to 6% by December 2020 and 6.2% by January 2021, then declined to 5.5%, 3.3%, 3.6% and 0.8% in February, March, April and May 2021, respectively. From 397 serum samples collected from 113 HCWs, 175 (44.1%, 95% CI: 39.1% to 49.1%) were positive for SARS-CoV-2 antibodies. The monthly seroprevalence was around 10% from September to November 2020 and increased to over 40% from December 2020 to May 2021. For community serosurvey in December 2020, overall seroprevalence of SARS-CoV-2 antibodies was 27.7%. The highest age-stratified seroprevalence was observed in participants aged 60-69 years (45.5%, 95% CI: 32.3% to 58.6%). The lowest was in children aged 0-9 years (14.0%, 95% CI: 7.4% to 20.6%).

Conclusions

SARS-CoV-2 in rural Mali is much more widespread than assumed by national testing data and particularly in the older population and frontline HCWs. The observation is contrary to the widely expressed view, based on limited data, that COVID-19 infection rates were lower in 2020-2021 in West Africa than in other settings.

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Strengths and limitations of this study

- Our study was strengthened by the data available on participants from health facility clients, HCWs and the general population.

- We investigated both active infection and seroprevalence for HCWs and the general population during the same time period.

- First study of its kind in West Africa to provide routine surveillance data capturing.

- Our study was performed in one region and therefore it only reflects the COVID-19 situation of the Kita region.

- Extending active infection RT-PCR testing to non-symptomatic cases, both at the health facility and in the general population, would have provided more robust insights on infection rates as well as the level of asymptomatic cases.

3

Introduction

The global coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has had a devastating impact on the health of populations in all regions of the world, leading to over five million deaths and tens of millions of cases, also causing widespread and long-term social and economic damage.[1] Despite predictions made during the so-called "first wave" (March-May 2020) that COVID-19 would spread widely and cause untold damage to communities in African countries, data during that period suggested that transmission, case, and death rates have been lower in African settings than in other regions and continents.[2,3]

Whereas the reported numbers of SARS-CoV-2 infection have been low in large parts of Africa, there is some evidence that this may be due to undertesting or underreporting. A recent Zambian post-mortem study suggested that many people who die have an undiagnosed SARS-CoV-2 infection.[4] Various studies have determined the seroprevalence in Africa.[5–13] A recent systematic review has estimated it to be approximately 22%, with highest seroprevalence in central Africa.[5] A recent study in Mali, conducted between November 2020 and June 2021, has reported a seroprevalence of 61.8% in health care workers (HCWs) who were in direct contact with COVID-patients in Bamako Hospital.[12] However, little is known about the spread of SARS-CoV-2 in rural areas among HCWs and the general population.

The objective of our study was to characterise the epidemiology of COVID-19 epidemic in Kita region in western Mali, an area with very low rates of reported infections. To this end, we monitored time-trends in the proportion of positive SARS-CoV-2 virus test results among patients presenting with suspected SARS-CoV-2 infection in primary health centres over a period of 10 months and complemented the data with seropositivity for SARS-CoV-2 antibodies among personnel working in the same facilities during the same time frame. Furthermore, in the middle

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of the monitored time period, we conducted a population based viral and serological survey, to establish a point-prevalence of acute infection and past exposure to it, among apparently healthy people of different ages.

Methods

Study design and participants

The present study was designed to support the Malian Ministry of Health and Social Affairs' strategy to strengthen epidemiological surveillance and enhance the national response in Mali. The study was conducted in Kita region, Western Mali. The area is served by one referral health centre in Kita centre, while most health areas have a community health centre, offering a range of basic medical services with fee charged.

Our study targeted three groups –patients at a health facility, HCWs and apparently healthy community residents. We conducted surveillance for patients presenting at 1 referral health centre and 11 community health centres, over a period of 12 months. These patients presenting with symptoms of COVID-19 (respiratory symptoms and fever) were tested for active SARS-CoV-2 virus infection.

We conducted a HCWs serosurvey in five of the same health facilities over a period of 9 months and community serosurvey at one time point in 16 villages. Between September 2020 and May 2021, a total of 113 HCWs were approached in five health facilities and invited to participate in the HCWs SARS-CoV-2 serosurvey. The HCWs samples were from healthy nurses, doctors, office

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workers, laboratory personnel and other auxiliary staff who consented to participate in the study. Among them, 78.8% were nurses reflecting their high proportion among HCWs.

The community samples were selected on the basis of cluster sampling. We used enumeration areas as clusters and determined the number of households from each cluster based on its recorded population size. The actual households to be invited were selected by random sampling. The sample size was originally calculated to estimate the seroprevalence of SARS-Cov-2 antibodies with an absolute precision (margin of error) of 2.5% and assuming a baseline prevalence of 5%. Accounting for clustering effects of households within villages and of individuals within households, we estimated to require a minimum sample size of 3000. Assuming an average village population of 1446 (based on available regional statistics), average household size of 14, and average number of under-5 children per household of 3, we decided to visit 215 households to enrol the study sample. In December 2020, a total of 229 randomly selected households were visited in villages in or near the Kita centre and invited to participate in a SARS-CoV-2 community survey. Heads of all the invited households provided a verbal consent and a total of 905 participants presented in the house during the visit were included in the survey.

Data and Specimen collection

For the patient surveillance study, the study nurses filled in a patient screening form, a suspected infection enrolment form on exposures and symptoms and consenting form, followed by a medical examination and a flocked nasopharyngeal swab (NPS, flexible minitip, Copan) or oropharyngeal swab (OPS, flexible minitip, Copan; nasal and throat flocked swab, Dewei Medical Equipment Co., Ltd) specimen collection from those with a suspected SARS-CoV-2 infection, eg. patients experiencing fever, cough, shortness of breath and seeking medical advice. For the HCWs sero-surveillance study, study nurses filled in a screening, consent and enrolment form, a suspected

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infection enrolment form on symptoms and exposure questionnaire. Four ml of venous blood

sample (BD vacutainer, Cat. No. 369032) was collected monthly and an NPS or OPS was taken at

enrolment and every 12 weeks thereafter. From each community survey participant, the

nurse/data collector filled in a symptom and exposure questionnaire and collected a nasal swab

specimen and a blood sample. A study nurse or a data collector filled out the symptom and

exposure questionnaire using a REDCap electronic data capture application. The biological samples

were collected by a doctor at the health centre and by a nurse in the community survey. The

collected swab samples were transported at 2-8 °C in 3 ml PBS/VTM from Kita to Bamako lab for

analysis. The blood samples were centrifuged, and serum was aliquoted into cryovials at the Kita

lab and batched samples were transported in dry shipper to Bamako for analysis.

Nucleic acid extraction and real time RT-PCR

RNA was extracted from sample swabs using a QIAamp Viral RNA Mini kit (Qiagen, Germany, Cat.

No. 52906) according to the manufacturer's instructions.

Real time reverse transcription polymerase chain reaction (rtRT-PCR) assays for SARS-CoV-2 were

carried out at the CVD-Mali Influenza laboratory, a participant in the WHO External Quality

Assessment for the Detection of Influenza and SARS-CoV2 viruses. Briefly, extracted RNA was

amplified using the Superscript One-step RT-PCR kit (Invitrogen, Thermo Fischer Scientific, Cat No.

11732-088) targeting the RdRp gene (Corman VM, 2020) or the Quantabio qScript XLT One-Step

RT-qPCR ToughMix targeting the N-gene (N2 primers) (CDC assay,

https://www.fda.gov/media/134922/download).

All assays were validated using WHO RNA samples received as part of an external Quality

Assessment Programme and a panel of known positive and negative samples from University

College London, UK.

7

Serological assays

A laboratory technician performed the Wantai anti-SARS-Cov-2 total antibody ELISA (target: RBD, qualitative assay detecting IgM and IgG., Beijing Wantai Biological Pharmacy Ent, Beijing, China) following the manufacturer's instructions. The sensitivity and specificity reported was 93% and 100%, respectively. Serum samples (100 µL) from each participant were tested as a singlet. Ten batches were performed for community survey samples, six batches for HCW samples and one for repeated samples.

Assay validation was carried out using the WHO international standard (NIBSC code: 20/136), the WHO International Reference Panel for anti-SARS-CoV-2 immunoglobulin (NIBSC code: 20/268), and an anti-SARSCoV-2 Verification Panel for Serology Assays (NIBSC code: 20/B770). In addition, 100 pre-pandemic samples collected in 2012 were tested to assess the specificity of assay. All of these pre-pandemic serum samples were from adult women (mean age 26 years old, range 16-41). Following the manufacturer's instructions, samples giving an absorbance equal to or greater than the cut-off value (A/CO >=1) were considered positive. Samples with A/CO ratio between 0.9 and 1.1 were considered borderline and were retested.

Statistical analysis

We compared proportions, means and standard deviations (SD) between groups by using Student's t test for continuous variables and Fisher's exact test for proportions and Chi-squared test for global difference with $p < 0.05$ indicating statistical significance. We calculated odds ratios (OR) and 95% confidence intervals (CI) with regression model. All statistical analyses were carried out using R versions 3.4.4 and 4.1.0.

8

Patient and public involvement

Village chefs and health centre leaders were consulted before the study to ensure feasibility of the planned study procedures. Participants were not involved in the development of the research design, conduct of the research or preparation of the manuscript.

Results

Between June 2020 and May 2021, a total of 13,104 people presenting at outpatient clinics or using preventive services at 12 health facilities in or near the town of Kita were screened for exposure or symptoms of SARS-CoV-2 infection. Of these, 2393 were deemed to have a suspected SARS-CoV-2 infection and 2392 agreed to provide nasopharyngeal or oropharyngeal samples for viral detection.

From all the tested samples from health centre patients, 68 (2.8%, 95% CI 2.2% - 3.6%) gave a positive test result for SARS-CoV-2 by RT-PCR. Prior to August 2020, no SARS-CoV-2 RNA could be detected, infection rates steadily rose from November 2020 onwards, peaking at 6.2% (95% CI 3.3% - 10.4%) in January 2021, and then gradually declined to 0.8% (95% CI 0.02% - 4.1%) in May 2021 (Figure 1). As shown in the figure, the increase in the proportion of positive samples followed a similar time trend in nationally reported numbers of SARS-CoV-2 infections and associated deaths.

 Among the 113 invited staff members who all agreed to participate, 89 (78.8%) were nurses, 9 (7.9%) were doctors, 7 (6.2%) were administrative or laboratory technicians, and 8 (7.1%) were auxiliary staff including housekeepers, cleaners and security staff.

9

Over the 9-month follow-up period, the participants provided a total of 397 blood samples, with a

range of 1-7 samples per participant. Of these samples, 175 (44.1%, 95% CI 39.1% - 49.1%) were

positive for SARS-CoV-2 antibody by ELISA. By calendar month, the proportion of positive samples

was approximately 10% until November 2020, increasing rapidly to 59.1% (95% CI 38.5% - 79.6%)

in January 2021 and remained relatively constant thereafter (Figure 2). For different health

professional groups, the proportion of SARS-CoV-2 antibody positive samples was highest among

doctors (50.0%, 95% CI 29.9% - 70.1%, 13/26 samples), followed by 49.8% (95% CI 43.4% - 56.2%,

124/249) among nurses, 35.9% (95% CI 21.2% - 52.8%, 14/39) among administrative staff and

laboratory technicians and 15.4% (95% CI 1.9% - 45.5%, 2/13) among auxiliary staff.

Of the 101 NPS or OPS samples collected from the health personnel, only one (1.0%) collected in

November 2020 gave a positive test result for SARS-CoV-2 by RT-PCR.

Of the 905 community survey participants, 564 (62.3%) were women, 654 (72.3%) were at least 15

years old, 102 (11.3%) had respiratory symptoms in the preceding 14 days, 496 (55.4%) had

attended a mass gathering in the preceding 3 months, none reported a contact with a COVID-19

patient, and 21 (2.3%) had a fever. 882 provided a blood sample for antibody analysis and 894

provided a nasal swab for viral detection. Among all the participants, the overall seroprevalence

was 27.7% (95% CI 24.7% – 30.8%). The seroprevalence was lowest (14.0%, 95% CI: 7.4% - 20.6%)

among 0-9-year-old and highest (45.5%, 95% CI: 32.3% to 58.6%) among 60-69-year-old

participants (Figure 3). Participants who had attended a mass gathering in the preceding 3 months

had a higher seroprevalence than those who had not (relative risk 1.48, 95% CI: 1.09 - 2.02).

Participant sex, measured fever, and recent history of fever or other respiratory symptoms were

not associated with the presence of SARS-CoV-2 antibodies in the blood samples (Table 1).

10

A total of 21 (2.4%, 95% CI 1.5% – 3.6%) community survey participants had a positive RT-PCR

result for SARS-CoV-2. The proportion of positive SARS-CoV-2 PCR tests was lowest (0.4%, 95% CI:

0% - 1.3%) among 10-19-year-old and highest (5.8%, 95% CI: 0.3% - 11.3%) among 50-59-year-old

participants (Figure 3).

Of the 100 banked serum samples collected before the COVID-19 pandemic (in year 2012), one

gave a borderline positive result and 99 gave a negative test result in the Wantai SARS-CoV-2

antibody test.

Discussion

This study measured active and past COVID-19 infection in Malian HCWs and the general

population through RT-PCR and seroprevalence testing. The results point towards significant

underestimation of Mali's COVID-19 infection rates, as measured by current testing mechanisms.

The study also provides insights into COVID-19 transmission patterns and risk factors. Extremely

high infection rates were demonstrated by the study's serological testing. By December 2020 over

a quarter (27.7%) of Kita's general population, and more than a third (41.9%) of HCWs had been

infected. By January 2021, this had increased to 60% of HCWs.

National routine surveillance morbidity data for the study period (June 2020 – May 2021),

indicates that in June, transmission was at its first-wave peak, and that two later larger waves

occurred, peaking in early December 2020 and April 2021.[14] Both the study's facility-based

client/patient active infection investigation and HCWs seroprevalence findings indicate that Kita

region's main transmission started later, in November 2020 and at the beginning it was very low,

but increased gradually. Both study groups indicate a peak in December/January, similar to that

seen nationally. The clients' active infection rate then showed a gradual decline to May rather

11

than a second wave in March that was seen nationally.[14] This could be explained by containment measures reducing the emergence of a second peak in the general population.

A comparison of this study's seroprevalence and active infection rates show that seroprevalence is much higher than active cases in both the study's general population and in HCWs. The fact that this high seroprevalence did not translate into a similar expression of morbidity and resultant death, neither in our study nor in the national surveillance system is an interesting area to investigate. This scenario has been documented quite widely in Africa.[15,16] This could be explained by a multitude of interlinking factors. High levels of asymptomatic cases, both for the general population and HCWs, have been documented in African as well as global studies.[11,17–21] As our study's active infection testing selected suspected cases of COVID-19 only, we could have missed a large proportion of asymptomatic cases. National surveillance would also have missed these cases as, firstly, they wouldn't present to a health centre and, secondly, even those that did would have been missed by diagnostic protocols.

Mali has gaps in demographic, disease morbidity, and death surveillance, including for COVID-19. A recent Zambian study showed that nearly 20% of people who had died had a positive SARS-CoV-2 PCR test in a postmortem NP swab sample. These people had not been tested before death, even though some of them had presented with COVID-19 symptoms.[4] A similar situation is likely present in Mali where diagnostic capacity is limited, at 7191 tests per 1 million people (compared to that of South Africa, 113 756)and where COVID-19 stigma exists.[22,23]

This study showed that 1.5 times more HCWs in Kita had been infected with SARS-CoV-2 by December 2020 compared to the general population. In fact, half of patient facing cadres such as doctors and nurses had been exposed to the virus and this was higher than laboratory, administrative (both 36%) and auxiliary staff (15.4%). Measures of increased risk of infection in

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HCWs vs the general population have been demonstrated in many global studies, with adequate

PPE provision and use being key to minimizing this risk.[15,17,21,24] In resource-constrained settings

such as Kita, key tools for protecting HCWs, along with early disease detection, are often limited or

absent in Africa.[25,26] Measures to improve protection for doctors and nurses, especially

considering low levels of health staff availability in the country – in 2017 Mali had only six qualified

providers per 10,000 inhabitants – would be pertinent.[27]

In the general population, risk factors included increased age and attendance at mass gatherings.

Seroprevalence was lowest in the very young, and highest in 60-69-year-olds but again lower in

the 70+ year olds. The possible reasons for lower seroprevalence in young children might be rapid

waning of antibodies following mild symptoms and less exposures compared with adults. Mass

gatherings are likely sources of infection given COVID-19's transmission dynamics and the difficulty

of ensuring appropriate prevention control measures (mask wearing, social distancing, hand

washing) at such events. Interestingly, neither fever (current or recent) nor other respiratory

symptoms were seen to be significant risk factors, which again infers a high asymptomatic

population.

The study's principal strength is that we included health facility clients, HCWs and the general

population. We also investigated both active infection and seroprevalence for HCWs and the

general population during the same time period. Study limitations include restriction to one region

and therefore this study only reflects the COVID-19 situation of the Kita region. Extending active

infection RT-PCR testing to non-symptomatic cases, both at the health facility and in the general

population, would have provided more robust insights on infection rates as well as the level of

asymptomatic cases. Widening the study to investigate exposure/transmission factors for study

participants, such as PPE provision and use, social distancing, COVID-19 interventions, and

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behaviour change would have allowed the study to explore risk factors to better inform policy.

When interpreting the RT-PCR results, possible sources of bias could have occurred within the

sample collection regarding sample types (oropharyngeal/nasopharyngeal swabs), sample quality,

transportation and temperature, and also in the use of 3 different RT-PCR assays. The latter is

unlikely to have affected the results as all RT-PCR assays were validated in-house and by others.[28]

Previous reports have suggested that some SARS-CoV-2 ELISA kits lack specificity when used on

serum samples originating from Africa and in particular exhibit pre-existing cross-reactivity to

SARS-CoV-2.[29,30] Our assay showed a 99% specificity when pre-pandemic samples were analysed

and previous work has shown the Wantai assay to be highly sensitive (93%) and specific (100%).[31]

In conclusion, SARS-CoV-2 in rural Mali is much more widespread than assumed by national testing

data and particularly in the older population and frontline health workers. The observation is

contrary to the widely expressed view, based on limited data, that COVID-19 infection rates were

lower in 2020-2021 in West Africa than in other settings.

Institutional preparedness as well as public risk assessment and personal protection capacity

should be augmented, and behaviours in high-risk activities such as mass gatherings improved, if

cessation of such events is not possible. Improving the understanding of COVID-19 in Mali through

surveillance will enable the country to reduce the economic and social impacts of the disease.

Further studies which provide accurate estimates of SARS-CoV-2 infection rates and

seroconversion in the population would enable the development of a national transmission model,

mapping of transmission hotspots, identification of national/regional risk factors, and the

prioritization of equitable and effective responses. Such studies could also direct policy

determinations in terms of disease prioritization, ensuring that resources reallocated for COVID-19

control measures are used appropriately.

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Contributors

DA and FCH contributed equally and share first authorship. CD and YMF contributed equally and share last authorship. JL performed statistical analysis. PA, UA, NK, SS, KW, CD and YMF initiated the project and preliminary design. DA, FCH, LA, HB, ECG, FD, RI, OM, UM, OS, AT developed the design and methods. DA, FCH, CD and YMF wrote the first draft of the manuscript. All authors contributed to the design of the study and interpretation of its results, and revised and approved the manuscript. PA and YMF are the guarantors and accept full responsibility for the work and the conduct of the study, had access to the data, and controlled the decision to publish. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Data sharing

Deidentified participant data might be available on reasonable request to the corresponding author.

Ethics

The study protocol was approved by the Faculté de Médecine, Pharmacie et d'Odonto-stomatologie (FMPOS) institutional review board in Mali and only participants who provided informed consent were enrolled in the study. All consents were requested verbally and recorded in the electronic data capture system. The minors' caregivers gave consent for their children to participate in the study.

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Competing interests

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: PA had financial support from Bill & Melinda Gates Foundation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

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18

Table 1. Seroprevalence of SARS-CoV-2-specific total antibody by background characteristics

	Sample size, n	Seropositive participants, n	Seroprevalence, %	OR (95% CI)
Participants, *n*	875	242	27.7%	
Sex				
Male	330	98	29.7%	Ref
Female	543	142	26.2%	0.84 (0.62-1.14)
Fever during past 14 days				
No fever	832	229	27.5%	Ref
Fever	41	11	26.8%	0.97 (0.48-1.96)
Fever (body temperature over 38 °C)				
No fever	852	238	27.9%	Ref
Fever	21	2	9.5%	0.27 (0.06-1.17)
Respiratory symptoms in the past 14 days				
No symptoms	773	216	27.9%	Ref
Any symptoms	100	24	24.0%	0.81 (0.50-1.32)
Mass gatherings in the past 3 months				
No attendance	370	86	23.0%	Ref
Attendance	487	151	31.0%	1.48 (1.09-2.02)

19

Legends of Figures:

Figure 1. Percentage of SARS-CoV-2 positive RT-PCR test by month in routine surveillance patients

from health facilities. Grey area and values in parentheses indicate 95% confidence interval from

the proportion of SARS-CoV-2 RNA containing samples. Yellow lines indicate nationally reported

numbers of SARS-CoV-2 infections ((WHO https://covid19.who.int/region/afro/country/ml).

Values above the x-axis indicate number of study samples in each month. PS = pharyngeal swab,

SARS-CoV-2 = Severe acute respiratory syndrome coronavirus-2, RNA = ribonucleic acid.

Figure 2. Seroprevalence of SARS-CoV-2-specific total antibody by month in health care worker

serosurvey. Grey area and values in parentheses indicate 95% confidence interval from the

proportion of SARS-CoV-2-specific antibody containing samples. Values above the x-axis indicate

number of study samples in each month. SARS-CoV-2 = Severe acute respiratory syndrome

coronavirus-2.

Figure 3. Seroprevalence of SARS-CoV-2-specific total antibody and percentage of SARS-CoV-2

positive RT-PCR test by 10-year age interval group in community participants. Red bars indicate

proportion (95% CI) of serum samples containing SARS-CoV-2-specific antibodies (primary y-axis),

Chi-squared global age group difference, p=0.002; blue bars indicate proportion (95% CI) of PS

samples containing SARS-CoV-2 RNA (secondary y axis). PS = pharyngeal swab, SARS-CoV-2 =

Severe acute respiratory syndrome coronavirus-2, RNA = ribonucleic acid.

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Figure 1.

Figure 2.

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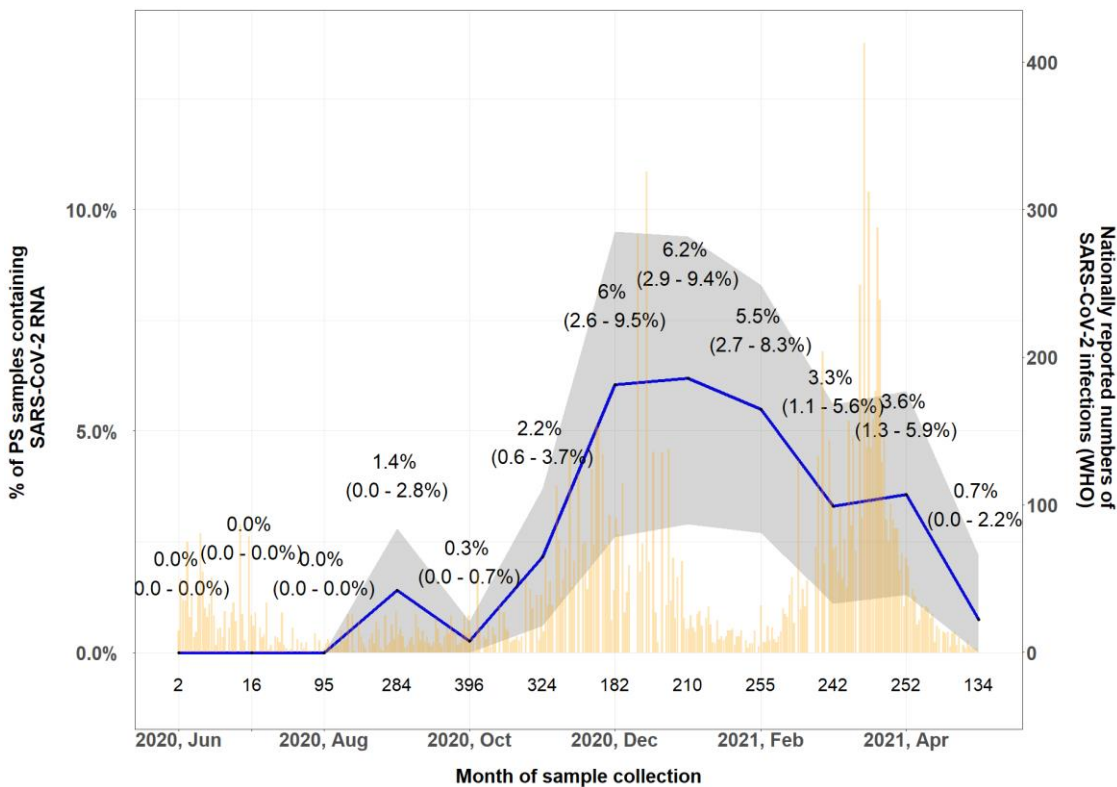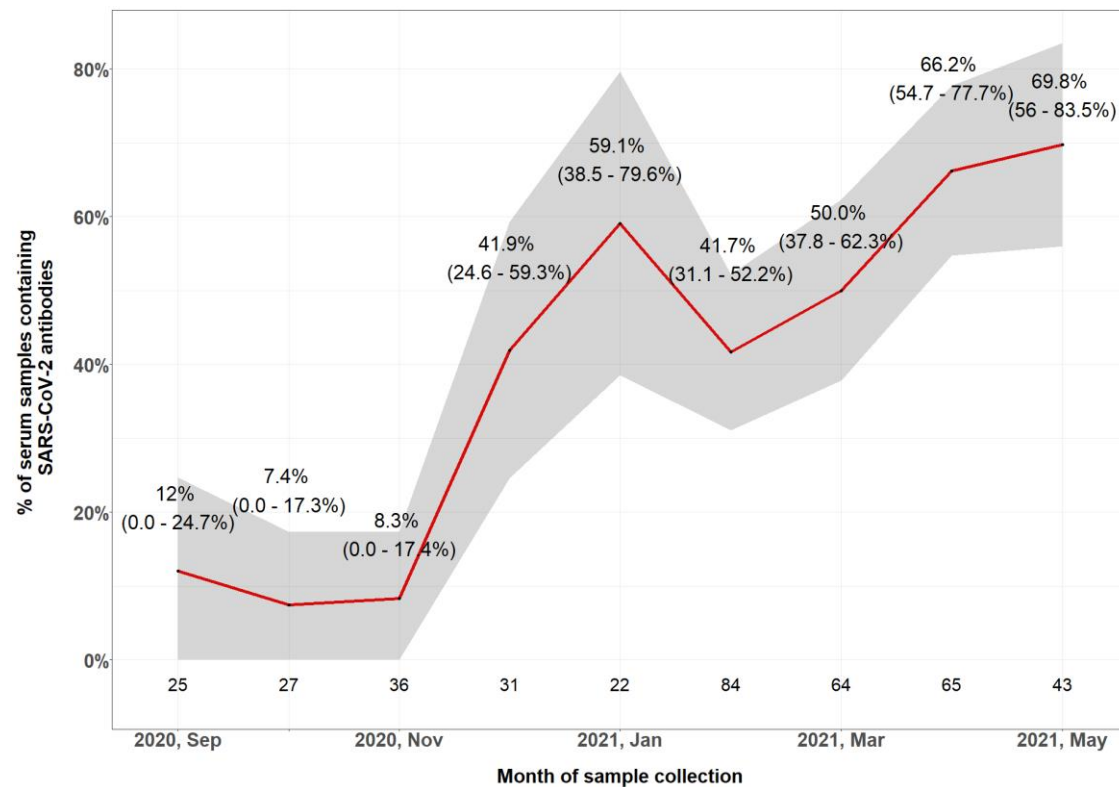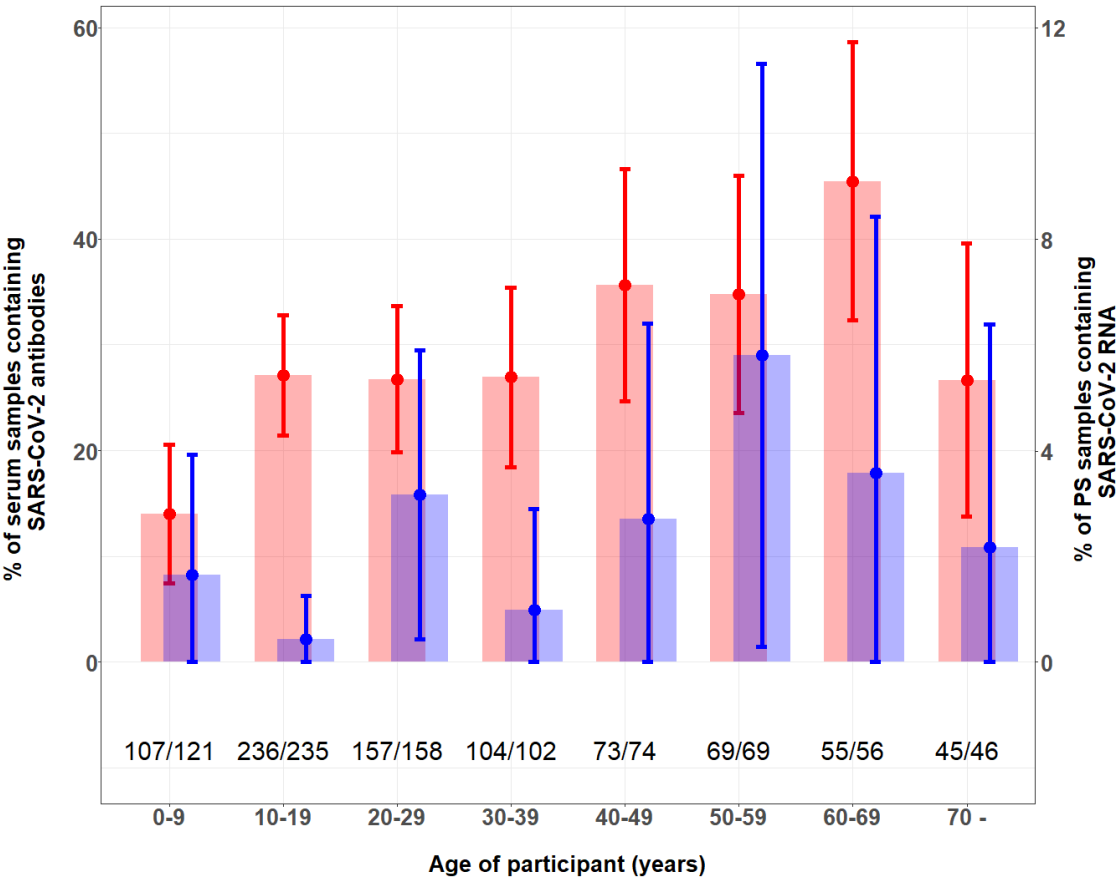38
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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5-6
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5-8*
Bias	9	Describe any efforts to address potential sources of bias	5-8
Study size	10	Explain how the study size was arrived at	5-6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	

Results

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9-10
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9-10
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*9-10*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	

Discussion

Key results	18	Summarise key results with reference to study objectives	11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13-14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11-13
Generalisability	21	Discuss the generalisability (external validity) of the study results	14

Other information

Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.