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ORIGINAL ARTICLES

High seroprevalence of anti-SARS-CoV-2 antibodies in the capital of Chad

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Abstract

Background. Since the start of the COVID-19 pandemic, Chad has had 7,417 confirmed cases and 193 deaths, one of the lowest in Africa.

Objective. This study assessed SARS-CoV-2 immunity in N'Djamena.

Methods. In August-October 2021, eleven N'Djamena hospitals collected outpatient data and samples. IgG antibodies against SARS-CoV-2 nucleocapsid protein were identified using ELISA. "Bambino Gesù" Laboratory, Rome, Italy, performed external quality control with chemiluminescence assay.

Results. 25-34-year-old (35.2%) made up the largest age group at 31.9±12.6 years. 56.4% were women, 1.3 women/men. The 7^{th} district had 22.5% and the 1st 22.3%. Housewives and students dominated. Overall seroprevalence was 69.5% (95% CI: 67.7-71.3), females 68.2% (65.8-70.5) and males 71.2% (68.6-73.8). >44-year-old had 73.9% seroprevalence. Under-15s were 57.4% positive. Housewives (70.9%), civil servants (71.5%), and health workers (9.7%) had the highest antibody positivity. N'Djamena's 9^{th} district had 73.1% optimism and the 3rd district had 52.5%. Seroprevalences were highest at Good Samaritan Hospital (75.4%) and National General Referral Hospital (74.7%).

Conclusion. Our findings indicate a high circulation of SARS-CoV-2 in N'Djamena, despite low mortality and morbidity after the first two COVID-19 pandemic waves. This high seroprevalence must be considered in Chad's vaccine policy.

Keywords: COVID-19, Seroprevalence, SARS-CoV-2, Anti nucleocapsid antibodies, N'Djamena community.

INTRODUCTION

Africa is known for its weak health system,[1] and other health problems such as malaria, tuberculosis, Ebola, and HIV/AIDS,[2,3] with COVID-19 pandemic, experts had thought that one of the worst scenarios would be recorded on this continent.[4] However, since the beginning of this pandemic, the low morbidity and mortality due to COVID-19 observed in most African countries,[5,6] is of great interest to the scientific community. Unexpectedly, today, the WHO African Region (WHO Afro) disproportionately records among the regions with the lowest COVID-19 burden particularly in the sub-Saharan region,[7] even though the distribution of cases is heterogeneous across the continent.[8]

Despite its weak health system and the low rate of available qualified health personnel (4.5 doctors per 100,000 inhabitants),[4,9] Chad is one of the countries reporting the fewest cases of COVID-19. As of 20 January 2020 to 25 May 2022, cumulative data showed 7,417 confirmed cases and 193 deaths, a case-fatality rate of 2.6%.[10,11]

This might be at some point due to the lack of large population-based testing procedures or hospital attendance-based regular testing of suspected cases. Most of the reported cases come from travellers entering or leaving the country and the tracing of their contacts for the confirmed positive cases. Very few cases reported from the existing surveillance system.

Several hypotheses have been put forward to understand this African paradox,[8,12,13] and immunological assessments of SARS-CoV-2 circulation in the African population were performed after the different waves of the pandemic.[14,15] Although sub-Saharan Africa reports a low rate of COVID-19 as well as low mortality, some studies have shown that overall anti-SARS-CoV-2 seroprevalence could be high.[14,16–18] These studies prove that a significant proportion of the population in this part of the African continent has been in contact with this virus.[14]

Although Chad is known for its variable demographics, desert, Sahelian and soudanian climate and palaeoanthropology history,[19,20] its context is not excluded from the African COVID-19 paradox. To the best of our knowledge, this study on the evaluation of anti-SARS-CoV-2 seroprevalence in Chad is the first to be conducted in this country. It focuses on N'Djamena, the political capital of Chad, epicentre of the disease, between 12 August and 26 October 2021, after the second wave of the pandemic in the country.[11]

MATERIALS AND METHODS

Ethical consideration

The protocol for this study was finalised in August 2021 and submitted tothe National bioethics committee of Chad (CNBT). Once the ethical clearances were obtained, protocol was presented to 11 focal points in the health facilities selected for the study. Training sessions on the data collection tool were held before the start of data and sample collection. An information sheet was given to each volunteer participant, as well as the signature of informed consent and assent for minors. Each participant was given a code and the data was used in a confidential manner.

Data and sample collection

Eleven (11) hospitals and health centres in the city of Ndjamena were included in this study. Data collection took place between 12 August and 26 October 2021, after the second wave of the pandemic in Chad.[11] A total of 2,700 samples from voluntary donors who came for routine consultations in these healthcare facilities and referred to the laboratory for blood sampling were included in the study. An electronic based questionnaire using KoboToolBox was used to collect participants data potential associated

Supplementary information The online version of this article (Tables/Figures) contains supplementary material, which is available to authorized users.

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risk factors. The whole blood was collected in BD VACUTAINER K3 EDTA tubes and plasma was obtained by centrifugation at 3000 rpm for 10 minutes at room temperature. Plasma aliquots in cryotubes were stored at -80°C until use. Homolysed blood was excluded. Laboratory analyses were conducted in N'Djamena at the Laboratory for Major Tropical Epidemics (LAGET) and at the Institute for Research on Livestock and Development (IRED).

Nucleocapsid protein anti-SARS-CoV-2 detection

The detection of plasma IgG antibodies against SARS-CoV-2 coronavirus Nucleocapsid (N) antigen, was conducted on each sample using indirect ELISA (enzyme-linked immunosorbent assay) technique. The tests were performed according to the manufacturer's instructions (Diatheva SRL, Cartaceto, Italy). Briefly, the anti-N SARS-CoV-2 IgG antibodies (present in the sample), bind specifically to the N SARS-CoV-2 antigen, then the added horseradish peroxidase (HRP) conjugated secondary antibodies bind to the captured IgG antibodies. The immune complex formed by the bound conjugate was visualised by adding the substrate ABTS (2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), which gives a green reaction product. The absorbance at 405nm was read using an ELISA microplate reader. The presence of specific anti-SARS-CoV-2 IgG antibodies was directly proportional to the colour intensity of the sample. The results of the test were calculated by a ratio between Cut-Off value (Co) and sample absorbance (S), the sample was positive when S/Co $\geq$ 1.5. The sensitivity reported by the manufacturer was 98.80% and the specificity 98.02%.

Internal and external controls of the analysis

Internal and external controls were performed to validate results obtained with the ELISA kit (COVID-19 NP IgG ELISA, Diatheva SRL, Italy). Once the laboratory analysis was completed at IRED, 10% of the randomly selected samples from the positives, negatives and equivocal were analysed at LAGET by another technician and at the same time in the same way at the IRED laboratory. The results were used for internal validation of the results in Chad. The same samples were transferred to the Laboratory of Virology and Immunology of the "Bambino Gesù" Hospital in Rome, for external validation. Chemiluminescence assay (CLIA) was used to identify total antibodies (IgA, IgM, or IgG) directed against the N protein of the SARS-CoV-2. This was the qualitative detection test. The assays were performed according to the manufacturer's recommendations of Elecsys® Anti SARS-CoV-2 immunoassay (Roche Diagnostics, CH) based on the sandwich principle. The sensitivity reported by the manufacturer was 99.5% and the specificity 99.8%. Result was considered anti-N positive if the cut-off index $\geq$1.

Data analysis

All data were analysed using SPSS.20. Pearson's chi 2 Test (with 95% confidence interval) was used for the statistical analysis. A statistical level less than 0.05 was considered statistically significant.

RESULTS

Sociodemographic findings

A total of 2,700 plasma samples were collected and used for the different analyses performed during this study. The biological analyses were performed in the virology and serology laboratories at IRED and LAGET. External and internal quality controls were performed on 10% of the samples.

The average age of the participants was 31.9±12.6 years, ranging from 1 to 86 years, and the most represented age group was 25-34 years (951) 35.2% (Fig. 1A). Female sex represented 56.4% (1524) while male was 43.6% (1176) for a female/male ratio of 1.3. Among the participants, 94.7% (2557) had not received a vaccine against COVID-19 at the time of the roll-up and 1.3 % (36) of participants were previously known COVID-19 cases (Table 1).

Almost half of participants were from the 7th district (607) 22.5% and the 1st district was the less represented with 22.3% (602) participants (Fig. 1B). Several socio-professional categories were included, housewives (786) 29.1% and students (734) 27.2% were most represented categories (Fig. 1C). In addition, the majority of participants 86.3% (2329) reported not experiencing any symptoms associated with COVID-19 in the months prior to the study.

Among whom reported experiencing at least one of the symptoms associated with COVID-19, fever was the most common (290) 10.7% (Fig. 1D). Blood group O was the most represented 20.5% (553) while more than half of the respondents (55.7%, 1504) did not know their blood group (Fig. 1E). In the figure 1F data showed that, most the participants came from King Faisal Hospital (KFH), 14.1% (381) and "Notre Dame des Apôtres" hospital (NDAH) 13.5% (365).

Anti-Nucleocapsid (anti-N) IgG seroprevalence

The overall reported seroprevalence was 69.5% (95% CI 67.7-71.3), corresponding to 1,877 individuals out of the 2,700 participants tested. Table 2 gives full details of the seroprevalences, observed among participants according to different factors. It is interesting to note that the seroprevalence for females was 68.2% (95% CI: 65.8-70.5) compared to 71.2% (95% CI: 68.6-73.8) for males. Of the 143 (5.3%) participants vaccinated against COVID-19, 68.5% (98) showed anti-N positivity related to breakthrough infections. The age group with highest seropositivity was that composed by individuals aged >44 years, with a seroprevalence rate of 73.9% (n=300). Participants under 15 years of age had the lowest positivity rate, 57.4% (54). The positive socio-professional categories with highest seropositivity were civil servants 71.5% (148) followed by the housewives 70.9% (551). Healthcare personnel, who constituted 9.7% of the respondents, had anti-N seroprevalence of 67.8% (177) (Table 2). The highest number of positivity was recorded in participants from the 9thdistrict (73.1%, 258); whereas the 3rd district had the lower recorded positivity (52.5%, 31). The Good Samaritan Hospital (75.4%, 215) and the National General Reference Hospital (74.7%, 189) reported the highest seroprevalences. However, among the 24 and 78 participants reported by the Provincial Farcha Hospital of and the Training Military Hospital, 79.2% (19/24) and 76.9% (60/78) were seropositive, respectively (Table 3).

Factors associated with SARS-CoV-2 antibody positivity

By analysing data according to anti-N IgG seroprevalence, risk factors were evaluated. Being aged of >44 years was associated to anti-N positivity [OR: 1.3 (1.0-1.6), p=0.038], whereas being younger than 15 years was protective versus the infection (negativity of anti-N) [OR: 0.6 (0.4-0.9), p=0.010]. For all other factors (sex, comorbidities, profession, blood group), no statistically significant association were found (Table 2).

DISCUSSION

This cross-sectional study was, to our knowledge, the first to report the seroprevalence of SARS-CoV-2 in the Chadian population. It was a pilot survey that used ELISA and CLIA to detect antibodies to SARS-CoV-2 and was conducted after the second wave of the epidemic in Chad. The study was conducted to assess community immunity to SARS-CoV-2 in the population of the city of N'Djamena, which has the highest incidence of COVID-19 in Chad (approximately 88% of national cases; SITREP No. 448, Chad).

Recent studies have shown that the combined seroprevalence of anti-SARS-CoV-2 antibodies in Africa is 22% with remarkably high heterogeneity. Seroprevalence was higher in Central Africa (41%, CI: 14-72) as opposed to Southern Africa (34%, CI: 13-59), West Africa (25%, CI: 13-39), North Africa (13%, CI: 2-32) and East Africa.[14] We found an overall prevalence of 69.5% in N'Djamena, based on the results of this survey conducted between 12 August and 26 October 2021.This was high compared to other Central African countries such as Cameroon (Yaoundé) which reported an overall seroprevalence of 29.2% in November 2020,[21] in the Republic of Congo (Brazzaville) which reported 17.6% in July 2020,[18] and in Gabon (Libreville) from July to October 2020 was found 36.2%.[16]

Similar studies in other cities in Chad's neighbouring countries have found lower seroprevalence than in N'Djamena. This is the case of the state town in Niger (Nigeria) where seroprevalence was 25.41% in June 2020,[22] and the town of Juba in Southern Sudan which recorded 38.3% seropositivity in October 2020.[23]

All the above studies were conducted directly after the first wave of the pandemic in each of these countries mentioned, but our study was conducted

later on, after the second wave (12 August to 26 October 2021) of pandemic in Chad. Probably, after several waves of virus circulation, it would be likely that seroprevalence would be higher as shown in a prospective study conducted in January-February and April-May 2021 in Cameroon (Yaoundé).[24] The increased seroprevalence after several viral waves could be due to a possible higher transmissibility of circulating new variants that have emerged, to a lower immune protection secondary to previous infection with different viral variants, or to the different movements in the community.[16]

The seroprevalence value found proves a high circulation of SARS-CoV-2 in the population of N'Djamena, despite the low morbidity and mortality reported in the country since the first local transmission was declared on 6 April by the government (SITREP N° 460, Chad).[11] This result also confirms the higher incidence of COVID-19 noted in N'Djamena according to the Chadian Ministry of Health report.

Our results indicated that, IgG seropositivity had a trend to be associated with male. Nwosu et al in 2021, Yaoundé (Cameroon) found that SARS-CoV-2 IgG revealed significantly higher odds of seropositivity for men,[21] although this difference was not statistically significant in our study. Our results showed a higher anti-N positivity within the age group >44 years, the review by Chisale et al in 2022 also showed that seropositivity in Africa was numerically influenced by sex and age with males and those under 50 years of age being the most affected.[14] In N'Djamena, the GSH and NRGH collection sites and the 9th and 1st districts reported the highest anti-N rates. Two of campuses of N'djamena University are located in the 9th district where the seroprevalence was 73.1% (68.2-77.4). This result proves that the circulation of the virus is intense in the university community, as shown in a similar study by Deutou et al, 2022 (in press) at the Evangelical University of Cameroon, IgG SARS-CoV-2 seropositivity within the campus, 73.6% (95% CI 64.5-81.0).

Chronic underlying diseases (diabetes, hypertension, asthma) although present, did not show a significant association with SARS-CoV-2 seropositivity. Also reported blood group and profession of participants were not statistically associated with SARS-CoV-2 seropositivity.

Although most people remain asymptomatic, SARS-CoV-2 has spread widely in N'Djamena with low associated mortality. Several hypotheses have been put forward to understand this paradox of COVID-19 in Africa as the results observed in this study. For example, some authors believe that this contradiction is due to genetic and immunity factors, due to the multiple viruses that have circulated before in Africa, a relatively young population, lower rates of comorbidity or simply limited testing and the late arrival of the pathogen on the continent.[12] Other hypotheses include a pre-existing immune state and differences in cytokine induction patterns. In addition, the central role of the nuclear factor-κB (NF-κB) signalling pathway via artemisinin has been explored, as artemisinin is thought to have a negative effect on NF-κB and other pro-inflammatory cytokine synthesis,[25–29] however, data on the artemisin-based antimalarial treatment possibly taken by participants was not collected.

The paradox is real, as several studies have shown a high circulation of SARS-CoV-2 in African countries despite the low mortality and morbidity observed. This clearly shows that most Africans have been in contact with SARS-CoV-2 but have manifested mostly less severe or asymptomatic forms of COVID-19. More specifically designed studies are needing to assess and justify this difference between circulation and pathogenicity of SARS-CoV-2 in the African context.

Like everywhere else Chad has adopted and procured COVID-19 vaccines, which may not be available to the entire population.

Given the low rate of reported SARS-CoV-2 infection and the high seroprevalence observed, as well as the limited availability of COVID-19 vaccines, local health authorities should design vaccination campaign accordingly. Availability of SARS-CoV-2 serologic data may help local health authorities to improve immune coverage of the population considering the protection attained because of previous infections and focusing vaccination on individuals being seronegative for SARS-CoV-2.

LIMITATIONS OF THE STUDY

The main limitation of this study was the poor completing of questionnaire administered. It would have been preferable if the collection of samples had also been done in the community places like markets, bus stations, incarcerated environment, airports or households, in order to facilitate the generalisation of the results. Similarly, if the spike test was carried out on all samples, as well as the external control that was initiated. Further analysis for anti-Spike and neutralizing antibodies are in progress.

CONCLUSIONS

As in other sub-Saharan African populations, our finding reveals that while the apparent health impacts of the COVID-19 pandemic have been lower than other parts of the world, the virus has spread extensively, and the high level of the various antibodies would suggest some immune memory. These data can be taken in account by health policy makers for the vaccine campaign.

INFORMATION

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Contributions: MFA, HF, SA, AMM, ALDW and GRTD conceived the study protocol and supervised data collection; FAA and ALDW wrote original draft preparation. FA, AZN, DD, ALDW, PK, NN, GRTD, LG, AY, HF and MFA contributed to data collection and lab analysis. Statistical analyses were done by MFA, ALDW and NN. Interpretation and writing of the manuscript were done by all authors, led by AMM, SA, CR, HKT, CFP, VC and OC. All authors approved the final version and made the decision to submit for publication.

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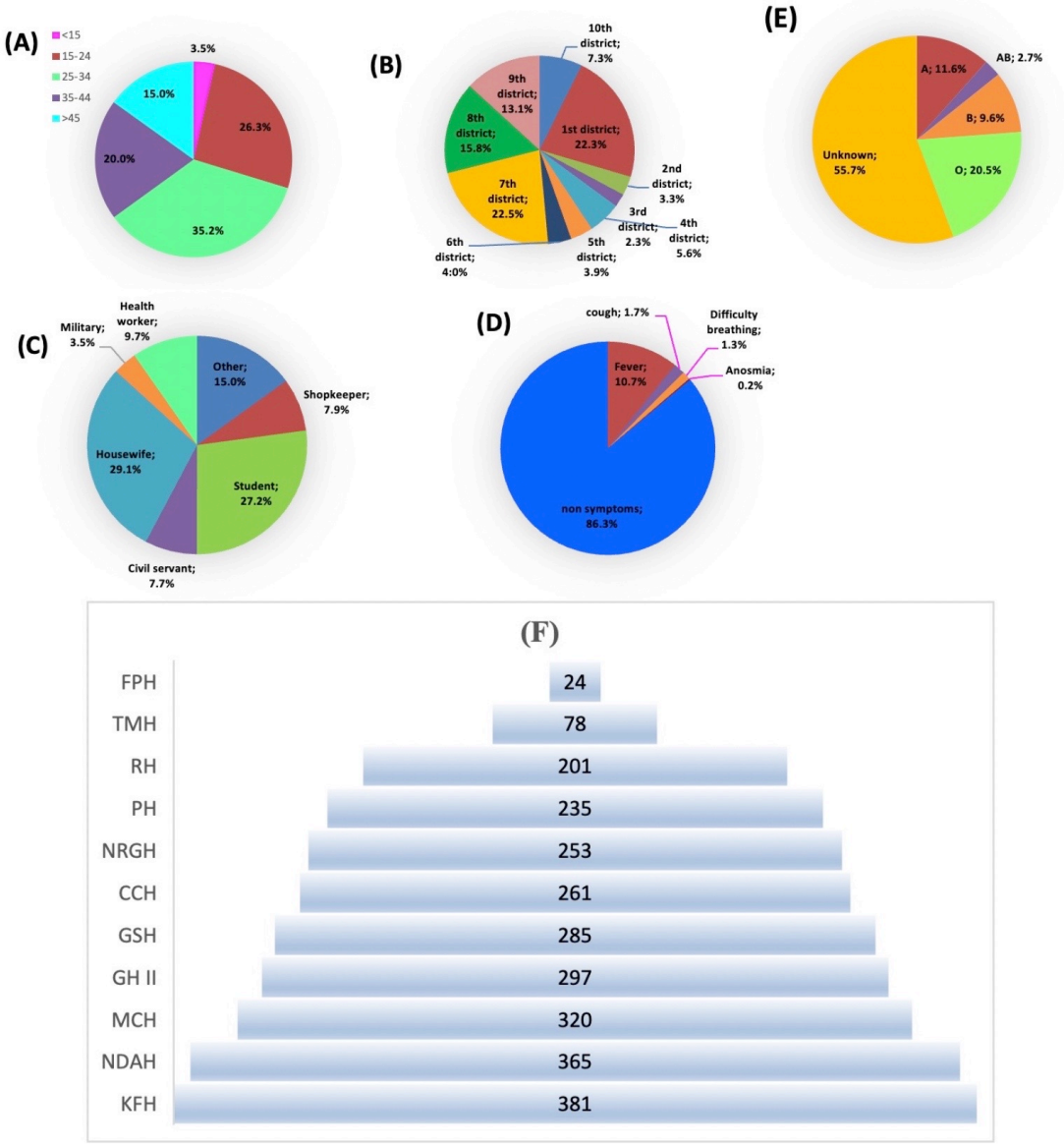

Figure 1. General characteristics of participants. (A) The age of the participants grouped by age range; The <15-year-olds were the least numerous, while the 25–34-year-olds were the most represented. (B) Districts organised into 10 circuits; this figure represents the frequency of participation in each district. (C) Socio-professional categories present in this study. (D) Symptoms related to COVID-19 manifested by the participants in the months preceding data collection. (E) Frequency of blood group represents among the participants, 55.7% (1504) did not know their blood group. (F) Data collection site and samples, a total of 11 sites participated: Farcha provincial hospital and the military training hospital had the lowest participation rates.
NRGH: National Referral General Hospital, GSH: Good Samaritan Hospital, MCH: Mother and Child Hospital, RH: Renaissance Hospital, CCH: Chad-China Hospital, NDAH: "Notre Dame des Apôtres" Hospital, FPH: Farcha Provincial Hospital, PH: Peace Hospital, KFH: King Faisal Hospital, TMH: Training Military Hospital, GH2: Guinebor II Hospital.

Table 1. Socio-demographic characteristics of the participants.

Variable	N	%
Age Mean (years)	31.9 ± 12.6	-
Sex	-	-
Female	1524	56.4
Male	1176	43.6
Symptom	371	13.7
No symptoms	2329	86,3
Vaccine	143	5.3
Non vaccine	2557	94.7
Contact case	76	2.8
Non case contact	2624	97.2
Old COVID-19 case	36	1.3
Non old COVID-19 case	2664	98.7
Chronic disease	137	5.1
Asthma	20	0.7
Diabetes	31	1.1
Hypertension	40	1.4
Obesity	4	0.1
Hepatitis B	5	0.2
HIV	3	0.1
No chronic disease	2563	94.9

Table 2. Seroprevalence and associated factors.

Factors	Seropositive	N.	Seroprevalence (95% CI)	OR (95% CI)	P-Value
Overall	1877	2700	69.5 (67.7-71.3)	-	-
Sex	-	-	-	-	-
Male	838	1176	71.2 (68.6-73.8)	1.2 (1.0-1.3)	0.084
Female	1039	1524	68.2 (65.8-70.5)	References	
COVID-19 vaccine	98	143	68.5 (60.5-75.5)	1.0 (0.7-1.4)	0.792
Age group	-	-	-	-	-
<15	54	94	57.4 (47.3-66.9)	0.6 (0.4-0.9)	0.010
15-24	502	709	70.8 (67.3-74.0)	1.1 (0.9-1.3)	0.387
25-34	645	951	67.8 (64.7-70.7)	0.9 (0.7-1.0)	0.158
35-44	376	540	69.6 (65.6-73.4)	1.0 (0.8-1.2)	0.950
>44	300	406	73.9 (69.4-78.0)	1.3 (1.0-1.6)	0.038
Professions					
Shopkeepers	149	212	70.2 (63.8-76.0	1.0 (0.8-1.4)	0.801
Students	503	734	68.5 (66.8-73.4)	0.9 (0.8-1.1)	0.495
Civil servants	148	207	71.5 (65.0-77.2)	1.1 (0.8-1.5)	0.520
Housewives	551	786	70.9 (67.6-73.9)	1.0 (0.9-1.2)	0.673
Militaries	65	95	68.4 (58.5-76.9)	0.9 (0.6-1.5)	0.813
Healthcare workers	177	261	67.8 (61.9-73.2)	0.9 (0.7-1.2)	0.530
Other works	284	405	70.1 (65.5-74.4)	1.0 (0.8-1.3)	0.774
Blood group					
O	386	553	69.8 (65.8-73.4)	1.0 (0.8-1.2)	0.871
A	222	312	71.2 (65.8-75.9)	1.1 (0.8-1.4)	0.505
B	183	259	70.7 (64.8-75.9)	1.1 (0.8-1.4)	0.676
AB	44	72	61.1 (49.5-71.5)	0.7 (0.4-1.1)	0.116
Contact with case	52	76	68.4 (57.3-77.7)	0.9 (0.6-1.6)	0.833
Old COVID-19 case	26	36	72.2 (56.0-84.1)	1.1 (0.5-2.4)	0.723
Diabetes	22	31	71.0 (53.4-83.9)	1.2 (0.5-2.7)	0.648
Hypertension	24	40	60.0 (44.6-73.7)	0.7 (0.4-1.3)	0.275
Asthma	12	20	60.0 (38.6-78.1)	0.5 (0.2-1.3)	0.157
Non symptoms	1631	2329	70.0 (68.1-71.8)	1.2 (0.9-1.5)	0.148
Symptom	246	371	66.3 (61.4-70.9)	-	-
Fever	189	290	65.2 (59.5-70.4)	0.8 (0.6-1.0)	0.089

N: total number tested, n: number of seropositive, %: Percentage, OR: Odds Ratio, CI: confidence interval, P.value <0.05 is significant.

Table 3. Districts and collection sites and seroprevalences.

Factors	n	N	Seroprevalence (%) (95% CI)
Neighborhood			
1st district	426	601	70.8 (67.1-74.3)
2nd district	56	86	65.1 (54.6-74.3)
3rd district	31	59	52.5 (40.0- 64.7)
4th district	104	150	69.3 (61.5-76.1)
5th district	72	102	70.5 (61.1-78.5)
6th district	69	105	65.7 (56.2-74.0)
7th district	422	605	69.7 (65.9-73.3)
8th district	297	425	69.8 (65.4-74.0)
9th district	258	353	73.1 (68.2-77.4)
10th district	129	195	66.1 (59.2-72.4)
Other Place	13	19	68.4 (46.0-84.6)
Collection sites			
NRGH	189	253	74.7 (69.0-79.6)
GSH	215	285	75.4 (70.1-80.1)
RH	143	201	71.1 (64.5-76.9)
MCH	200	320	67.6 (57.1-67.6)
NDAH	228	365	62.5 (57.4-67.2)
FPH	19	24	79.2 (59.5-90.8)
PH	151	235	64.3 (57.9-70.1)
KFH	262	381	68.8 (63.9-73.2)
TMH	60	78	76.9 (66.4-84.9)
GH2	217	297	73.1 (67.7- 77.8)
CCH	193	261	73.9 (68.3-78.9)

N: total number, n: number of seropositive, %: Percentage, CI: confidence interval.
NRGH: National Referral General Hospital GSH: Good Samaritan Hospital MCH: Mother and Child Hospital, RH: Renaissance Hospital, CCH: Chad-China Hospital, NDAH: "Notre Dame des Apôtres" Hospital, FPH: Farcha Provincial Hospital, PH: Peace Hospital, KFH: King Faisal Hospital, TMH: Training Military Hospital, GH2: Guinebor II Hospital.