The COVID-19 wave was already here: High seroprevalence of SARS-CoV-2 antibodies among staff and students in a Cameroon University

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Abstract

Background: Seroprevalence studies, to estimate the proportion of people that has been infected by SARS-CoV-2 are importance in African countries, where incidence is among the lowest in the world.

Objective: This study aimed at evaluating the exposure to SARS-CoV-2 within a university setting of Cameroon.

Methods: A cross-sectional study performed in December 2020 - December 2021, among students and staffs of the Evangelical University of Cameroon. COVID-19 antigen rapid detection test (RDT) was performed using Standard Q Biosensor, and one year after SARS-CoV-2 antibody-test was performed within the same population using RDT and chemiluminescence immunoassay (CLIA).

Results: 106 participants were enrolled (80% students), female sex was the most represented. Positivity to SARS-CoV-2 was 0.0% based on antigen RDTs. The seroprevalence of SARS-CoV-2 antibodies was estimated at 73.6% (95% CI. 64.5-81.0) for IgG and 1.9% (95% CI. 0.2-6.8) for IgM/IgG with RDTs, and 91.9% (95% CI. 84.7-96.4) for anti-nucleocapsid with CLIA. 95.3% (101) reported having developed at least one of the known COVID-19 symptoms (cough and headache being the most common). 90.3% (28) of people who experienced at least one of these symptoms developed IgG antibodies. 40.6% (43) of participants took natural herbs, whereas 55.7% (59) took conventional drugs. The most used herb was *Zingiber officinale*, while the most used drugs were antibiotics.

Conclusion: In this Cameroonian University community, SARS-CoV-2 seroprevalence is high, with a greater detection using advanced serological assays. This indicates a wide viral exposure, and the need to adequate control measures especially for those experiencing any related COVID-19 symptoms.

Introduction

The world has experienced several major pandemics and epidemics since time ranging from the plague, Spanish flu, Asian flu, AIDS which is still present, to most recent the COVID-19 pandemic.[1] The latter has appeared since December 2019, and remains a concern for the scientific community, although there are different vaccines and treatments to prevent and manage the disease.[2,3] Nevertheless, the immunological and inflammatory effects associated with its pathophysiology can be controlled with known drugs depending on the patient's immunocompetence and the severity of the symptoms.[4–7] A fundamental challenge in achieving COVID-19 control is understanding transmission dynamics and regional disease prevalence and knowing what proportion of the population has been infected with the virus is an important step to optimize public health interventions, *e.g.,* distancing measures and plan vaccination strategies. The first case of COVID-19 in Cameroon was diagnosed on 6 March 2020. As of 31st of March, and so far, there have been 119 947 confirmed cases of COVID-19 with 1 930 deaths.[8] Given the difficulty and cost of RT-PCR based testing in resource-limited countries like Cameroon mildly affected or asymptomatic individuals are not usually screened, the number of confirmed SARS-CoV-2 infections is likely vastly underestimated.[9]

In this context, seroprevalence surveys are of the most importance to assess the proportion of the population that have already developed antibodies following a previous exposure to SARS-CoV-2 and the immune responses to the virus.[10–12] The spike glycoprotein (S) and the nucleocapsid phosphoprotein (N) are the main viral antigenic targets against which antibodies are detected.[13,14] This Study was to evaluate exposure to SARS-CoV-2 and associated clinical factors within the University community of Cameroon.

Materials and methods

Ethical considerations

The antigenic screening was conducted under the direction of the Bandjoun District Health Services, as part of the epidemiological surveillance of COVID-19 in the municipality of Bandjoun (Cameroon). For the seroprevalence part, authorization was obtained from the Bandjoun District Health Services. All necessary precautions were taken to ensure that the rights, privacy, and freedom of participants were respected. Each participant was informed about the objective and methods of the study and signed free and informed consent before participating to the study.

Study design and population

Following school reopening and classical teaching method resumption, a screening campaign based on rapid antigenic tests was conducted on December 2020 at the Evangelical University of Cameroon (UEC) campus. A year later, a sero-surveillance campaign for anti-SARS-CoV-2 detection was carried out to assess the level of virus circulation within the same student community. The interval time between the two investigations (December 2020-February 2022) has been characterized by 2 successive waves of COVID-19.

Description of the study site

The Evangelical University of Cameroon is located in the Mbouo district of Bandjoun, Pomougne district, Koung-Khi department, Western Cameroon region. The town of Bandjoun is located on the right bank of the South Mifi, 17 km south of the regional capital Bafoussam.[15] The district in which the UEC is located is one of the most popular and busiest because, in the same area, there is the large Protestant hospital of Mbouo, a nursery and primary school, a secondary school, as well as the evangelical church.

Enrolment of study participants

This study took place at the UEC campus at two different periods, Staff as well as students, were included. Antigenic RDTs (nasopharyngeal swabs) were performed in December 2020, while blood collection for seroprevalence testing took place between December 2021 and February 2022.The questionnaire was the main instrument for data collection. Whole blood was collected in BD VACUTAINER K3 EDTA tubes, plasma was obtained by centrifugation at 3000 rpm for 10 minutes at room temperature and stored at -20°C until use.

Laboratory procedures

Detection of SARS-CoV-2 antigen by rapid antigen test

The SARS-CoV-2 RDTs in nasopharyngeal specimens was performed with the STANDARD Q COVID-19 Ag Test (SD Biosensor Inc, KR). It is a Lateral-flow Immunochromatographic Assay (LFIA) for the qualitative detection of specific antigens to SARS-CoV-2 present in the human nasopharynx. The analysis was performed following the recommendation of the manufacturer. The result was considered negative when only the control line C was present. It was considered positive when there was presence of both test line T and control line C in the window result. It was invalid when the control line did not appear. The sensitivity reported by manufacturer was 88.7% and the specificity 97.6%.

Detection of antibodies SARS-CoV-2 by rapid diagnostic test

The Pharmact SARS-CoV-2 IgM/IgG Rapid Test kit (Pharmact, GmbH, DE) was used to screen for IgG and IgM antibodies against the SARS-CoV-2 virus on serum/plasma, following the manufacturer's instructions. It is a LFIA for the qualitative detection of IgG and IgM antibodies against SARS-CoV-2 nucleocapsid (N) protein. Test results were categorized into one of the five categories: IgM positive only (indicating that recent infection within the first 7 to 10 days), IgG positive only (indicating that the body has been exposed to the virus in the past), IgG and IgM positive (also indicating recent infection between 7 and 21 days), negative (indicating that there are no such antibodies in the blood) and invalid/inconclusive. Results were repeated and graded accordingly. The manufacturer rates the sensitivity for IgM at 98.1% and for IgG at 98.2%, IgM specificity estimated at 99.5% and IgG 99.7% IgM precision estimated at 99.0% and IgG 99.2%.

Anti- Nucleocapsid Protein (anti-N) detection

The determination of SARS-CoV-2 anti-N antibodies to the nucleocapsid antigen, was performed in the Virology and Immunology Laboratory of the Bambino Gesù Hospital in Rome. 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%. The sample was considered anti-N positive if the cutoff index ≥1.

Statistical 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. The graphs were performed with GraphPad Prism 6.0.

Results

Detection of SARS-CoV-2 antigen with Rapid antigen test

Of the 106 participants in December 2020, 84.9% (90) were students and 15.1% (16) were staff; 56.6% (60) were female while 43.4% (46) were male. The mean age of the participants was 24.0±1 years. The results of the antigen RDTs indicated the absence of SARS-CoV-2 in all participants (100%, 106) (Table 1).

Detection of SARS-CoV-2 antibodies

General characteristics of participants

Between December 2021 and February 2022, one year after the SARS-COV-2 antigen screening campaign within the UEC campus, a SARS-CoV-2 seroprevalence investigation was conducted on the same community. 106 volunteers (80% were students while 20% were staff) participated to the seroprevalence screening. The female gender was the most represented (62.3%, 66) and 29.2% (55) of the participants declared they have been in contact at least once with confirmed COVID-19 positive patient. 95.3% (101) of participants claimed to have developed at least one of the known symptoms of COVID-19 since the last campaign in December 2020 (Table 2).

To fight against these symptoms, 62.6% (66) of the participants took drugs such as analgesics, anti-inflammatories, antibiotics, and others, with antibiotics (46.2%, 49) as the most widely used drugs class. 50.9% (54) used natural and local plants including *Zingiber officinale* (Ginger), *Artemisia afra Jacq* (Artemisia), *Cymbopogon flexuosus* (Lemongrass), *Combretum micranthum* (Kinkeliba). Ginger was the most widely used plant followed by Lemongrass and Artemisia (Table 3).

The seroprevalence of anti-SARS-CoV-2 antibodies found with RDTs in our study population was 73.6% (95% CI 64.5-81.0) for IgG and 1.9% (95% CI 0.2-6.8) for IgM/IgG. About 90.3% (28) had contact with a COVID-19 case and were SARS-CoV-2 IgG positive (Table 3).

Table 1. General data detection of SARS-CoV-2 antigen.

Characteristics	N	%
Study population		
Students	90	84.9
Staff	16	15.1
Age (years) : Mean 24.0 ± 1	-	-
Sex	-	-
Female	60	56.6
Male	46	43.4
RDTs (Ag) COVID-19	-	-
Negative	106	100 (CI 96.5-100)
Positive	0	0

RDTs: Rapid Diagnostic Test, Ag: Antigen, N: frequency, %: percentage, CI: 95% Confidence Interval

Table 2. General characteristics of antibodies detection.

Characteristics	N	%
Study population		
Students	85	80
Staff	21	20
Age (years) Mean 26.9 ±11.0	-	-
Sex	-	-
Female	66	62.3
Male	40	37.7
Non Social distancing	55	52.0
Contact with COVID-19 case	31	29.2
Symptoms	101	95.3
COVID-19 vaccine	5	4.7

N: Frequency, %: Percentage

Student population

For this study, 80% (85) were UEC students, with the largest proportion (28.2%, 24) from biomedical sciences (BMS). Level 3 (L3) students were the most represented (38.8%, 33), as well as female gender (67.1%, 57). The age of the participants ranged between 18-29 years, and the average age was 22.0± 2.1 years (Table 4).

In this young population, 28.2% (24) indicated have being in contact with positive cases of COVID-19. 93% (79) of these students claimed to have experienced at least one of the known symptoms of COVID-19 during the year 2021. 56.5% (48) took medication for these symptoms while 40.0% (34) took natural local plants.

The results of rapid anti-SARS-CoV-2 antibody test showed that 80.0 % (CI 70.3-87.2) of students who participated in this study developed IgG antibodies against SARS-CoV-2 (Table 4). All biomedical engineering (BME) and reproductive health (RH) students tested positive of anti-SARS-CoV-2 and Agronomy and biomedical science (BMS) students showed the highest positive percentage 83.3% (05) and 79.2% (19) respectively (Figure 1A).

Clinical factors associated with the seroprevalence of SARS-CoV-2

More than half of the participants (75.2%, 76) who experienced symptoms were positive for the detection of IgG antibodies SARS-COV-2, while all individuals who had no symptoms tested positive for IgG detection (100.0%, 5). The most common symptoms reported among the IgG seropositive participants were headache, cough, rhinitis, and fever (Figure 1B).

The comparison between the results obtained from SARS-COV-2 antigen detection in 2020 and seroprevalence investigation after one year clearly shows that, between December 2020 and December 2021 the virus strongly circulated through this community. In December 2020 there were no antigen positive cases in this population. However, the antibodies detection one year later shows that these people have indeed been in contact with the virus, although there have been no cases of serious symptoms or hospitalization (Table 5).

Detection of anti-N proteins

The detection of antibodies against protein N was done by the CLIA method, which is known as a gold standard compared to LFIA.[16,17] Analyses were performed of 99 samples previously tested for IgG detection by LFIA (the remaining 07 samples were excluded because the quality and quantity did not meet the requirements of the kit). The results showed that of these selected participants, 78.8% (95% CI 69.4-86.4) were positive for IgG by LFIA while 91.9% (95% CI 84.7-96.4) were positive for anti-N (Table 5). Of the 78.8% positive, 2% (2/99) had not detected for anti-N, while 76.8% (76/99) were detected for total anti-N against SARS-CoV-2, and 15.5% (15/99) of the IgG negative samples were detectable by CLIA. When the two types of analysis (LFIA and CLIA) were combined, 6.1% (6/99) of the participants had no antibodies against N protein at the time of the study.

Discussion

This study aimed to evaluate the circulation of SARS-CoV-2 within the community of the UEC.

Indeed, the perception of action in relation to COVID-19 is still mixed and dominated by hesitation in the Cameroonian community,[18] as we observed a low rate of acceptance of participation by students from other departments, except biomedical sciences.

Table 3. Seroprevalence of SARS-CoV-2 and other data.

	Variables	N	%	P-Value
Prevalences	Anti-SARS-CoV-2 IgG positive (RDTs)	78	73.6 (CI 64.5-81.0)	
	Anti-SARS-CoV-2 IgM/IgG positive (RDTs)	2	1.9 (CI 0.2-6.8)	
	Contact with COVID-19 case and IgG positive	28	90.3	0.416
	Symptom and IgG positive	76	75.2	0.261
	Symptom and IgG negative	25	24.8	0.261
	Symptom and plant	43	40.6	0.039
	Symptom and drug	59	55.7	0.207
Drugs	Drugs used	66	62.3	
	Antibiotics	49	46.2	
	Anti-inflammatory	14	13.2	
	Paracetamol	34	32.1	
	Hydrochloroquine	6	5.6	
	Others	3	2.8	
Plants	Local natural plants used	54	50.9	
	Zingiber officinale (Ginger)	32	30.1	
	Artemisia afra Jacq (Artemisia)	12	11.3	
	Cymbopogon flexuosus (Lemongrass)	23	21.6	
	Honey	12	11.3	
	Combretum micranthum (Kinkeliba)	11	10.3	
	Others	16	15.1	

A P-Value less than 0.05 is statistically significant. IgG: immunoglobulin G, IgM: immunoglobulin M, RDTs: Rapid Diagnostic Tests, N: frequency, %: percentage, CI: 95% Confidence Interval

Table 4. General characteristic of student's participants.

Characteristics	N	%
Age (years) Mean: 22.0 ± 2.1		
Sex		
Female	57	67.0
Male	28	32.9
Specialty		
Agronomy	6	7.0
BME	5	5.9
PH	4	4.7
BMS	24	28.2
RH	10	11.8
NS	22	25.9
MAT	14	16.5
Level of study		
L1	21	24.7
L2	22	25.9
L3	33	38.8
M1	2	2.4
M2	7	8.2
Drugs used	48	56.5
Natural Plant used	34	40.0
COVID-19 vaccine	3	3.5
Contact with COVID-19 case	24	28.2
Symptoms	79	93.0 (CI 86-97.9)
SARS-CoV-2 IgM/IgG	2	2.4 (CI 0.3- 8.2)
SARS-CoV-2 IgG	68	80.0 (CI 70.3-87.2)

L1,2,3: level 1,2,3; M1,2: master 1,2; BME: biomedical engineering, PH: public health., BSM: biomedical sciences, RH: reproductive health, NS: nursing students, MAT: medical analysis technician, N: frequency, %: percentage, CI: 95% Confidence Interval

Table 5. Comparison of COVID-19 antigen and antibody detection.

SARS-CoV-2 detection	Methods	Result	N N total = 106	%	Screening
Ag	LFIA	Negative	106	100	December 2020
			N total = 106		
IgG	LFIA	Positive	78	73.6 (CI 64.5-81.0)	December 2021-Febuary 2022
IgG/IgM	LFIA	Positive	2	1.9 (CI 0.2-6.8)	
IgG or IgM		Negative	26	24.5 (17.3-33.5)	
			N total =99 accepted on 106		
	LFIA	IgG positive	78	78.8 (CI 69.4-86.4)	December 2021-Febuary 2022
		IgG negative	21	21.2 (CI. 13.6-30.6)	
Total anti-N	CLIA	Positive	91	91.9 (CI. 84.7-96.4)	
		Negative	8	8.0 (CI. 3.6-15.3)	

Anti-N: anti Nucleocapsid, LFIA: Lateral Flow Immunochromatographic Assay, CLIA: Chemiluminescent Immunoassay, Ag: Antigen, IgG: immunoglobolin G, IgM: immunoglobulin M N: frequency, %: percentage, CI: 95% Confidence Interval, The SARS-CoV-2 rapid antigen detection campaign showed no positive results among the participants. For COVID-19 screening, several rapid and molecular tests have been recently developed and quickly adopted in several countries across the globe including Cameroon.[19,20] Availability and low cost guided the choice of Ag RDTs for this campaign, although it would have been best to combine them with molecular tests for SARS-CoV-2 to avoid false negatives.[21]

Since the beginning of COVID-19, low contaminations prevalence are declared in many countries, especially in Africa.[22–24] In contrast to that, many seroprevalence studies showed important circulation of the virus although asymptomatic forms are recurrent.[25] In this study, the seroprevalence of IgG anti-SARS-CoV-2 was 73.6% with 80% in young people aged 18-29 years. Similar studies were conducted in general population of the central part of Cameroon (Yaoundé) between June-August 2020 and October-November 2020, with an IgG seroprevalence of 24% in the first study and 29.2% in the second.[26,27] The seroprevalences obtained in these studies are lower than those found in our study, this difference can be justified by the fact that our study was conducted in a young and student environment unlike theirs. In the same region, a more recent study (January-February and April-May 2021) showed an SARS-CoV-2 IgG seroprevalence increased significantly from 18.6% in the first survey to 51.3% in the second, illustrating high community transmission during the second wave of COVID-19.[28] These results show that from one wave of

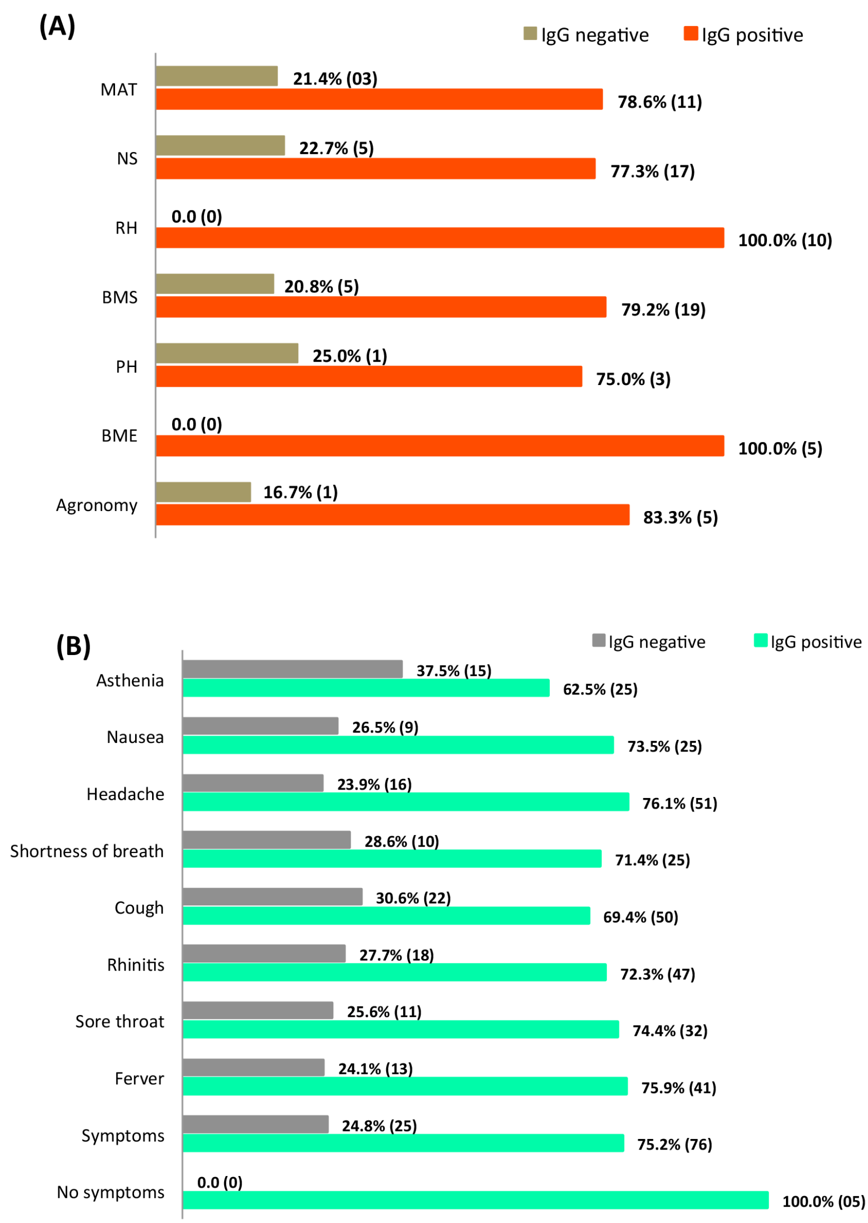

Figure 1. (A) SARS-CoV-2 IgG seroprevalence by specialty. The percentage of seropositivity in relation to each specialty present at the Evangelical University of Cameroon. The Biomedical sciences account for the highest percentage of positivity. BME: biomedical engineering. BMS: biomedical sciences. RH: reproductive health. NS: nursing students., PH: Public health, MAT: medical analysis technician. (B) IgG seroprevalence and symptoms. The different symptoms in relation to IgG antibodies SARS-CoV-2 results show that all participants who did not claim any symptoms were detected IgG positive. In addition, participants who claimed at least one symptom had IgG positive. All P. values were not significant.

COVID-19 to the next in Cameroon, the immune response is higher in the community, proving that the SARS-CoV-2 virus has circulated widely in this population, despite the low morbidity and mortality observed. Seroprevalence was highest in studies conducted in Central Africa, as well as among African-Americans, and antibody prevalence was highest among those under 30 years of age.[13,29] It has been shown that LFIA assays may have a lower performance than enzyme-linked immunosorbent assays (ELISA) as well as chemiluminescent immunosorbent assays (CLIA), and the sensitivity of LFIA tests may be related to the profile of the infected patients.[30–32] To address this gap, anti-N was also performed by the CLIA method, and the results revealed that 91.9% of the participants developed antibodies against protein N. This value was higher than those found by LFIA which could be justified as the CLIA method is more efficient and it has been shown that the sensitivity of LFIA is higher in symptomatic hospitalised patients than in asymptomatic patients,[30] which constitutes the population of this study.

The SARS-CoV-2 N protein is one of the major immunogenic elements of the virus,[33] and anti-nucleocapsid protein IgG antibodies are the best markers of infection and exposition.[34,35] Furthermore, it has been shown that responses against N protein seem to attenuate over time, [36–38] and the high level of this antibody found in our study population would therefore indicate exposure to SARS-CoV-2 infection.

West Cameroon and precisely in Bandjoun where this study was conducted is a cold and dusty area with high prevalence of infectious diseases (respiratory and gut infections, parasitosis, and other) among inhabitants.[39] This situation could have conferred to the population. a high adaptability for inflammation, one of the symptoms implicated in COVID-19 physiopathology in the lungs,[4,7]explaining the high circulation of SARS-CoV-2 without serious forms. In addition, our study population was young people, and their strong immune system could have prevented serious forms development.

At the start of the pandemic, many people rushed to self-medication,[40] and took, among others, natural plants,[41–43] to prevent or treat symptoms of COVID-19. Several combinations of natural plants were used, perhaps these aspects could also justify the asymptomatic forms observed in this study, as several reports reviewed by *Attah et al,*[44] confirmed antiviral properties of some of these plants to SARS-CoV-2 with cross-kingdom activity.[45] The regulation of plant across kingdoms is a novel concept confirmed in other studies in which anti-inflammatory and anti-tumor activity was observed due to epigenetic alterations.[46–49] Nevertheless, the excessive consumption of drugs in our study, like antibiotics, could soon raise another pandemic linked to antibiotic resistance.

More than half of the participants (52.0%) said they no longer respect social distancing, this observation had also been made previously by *Metchum et al* in the same setting.[50] This non-respect of social distancing could be one of the factors that favoured the circulation of the virus among these students. In addition, most of the participants were health science students who, since December 2020 have completed several internships in a hospital environment. This undoubtedly increased their contamination risk as it is well known that the prevalence of COVID-19 is high among healthcare workers.[51]

Limitations of the study

The main limitation of this study was the small number of participants and the poor completing of questionnaire administered to each participant. The lack of adjusted seroprevalence data based on the pre-pandemic antibody survey. The need to perform the Ag detection by RDT in 2021 to strengthen the antibody findings.

Conclusions

Our finding shows high seroprevalence of SARS-CoV-2 in UEC young students and staff. These results may signify that after the first wave of COVID-19 with lockdown, the resumption of face-to-face school activities would have facilitated collective immunity within populations. SARS-CoV-2 already circulated widely among staff and students at a university in Cameroon. Despite a low level of COVID-19 vaccination there were no serious symptoms or hospitalisations reported. Several participants resorted to self-medication and local natural plants as a means of prevention from serious forms.

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