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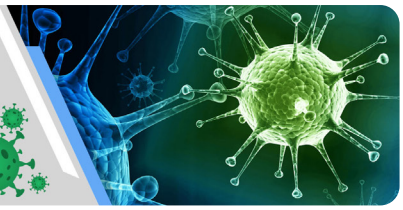

High Rate of anti-SARS-CoV-2 Antibodies along the River Corridor during the COVID-19 Epidemic in the Republic of Congo, 2022

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Abstract

Objectives: Few studies are available on the circulation of SARS-CoV-2 in rural areas, particularly in areas difficult to access during the epidemic in Africa. The aim of the present study was to determine the carriage rate of anti-SARS-CoV-2 antibodies in unvaccinated people living along the river corridor in the Republic of Congo, between July and August 2022.

Methods: DBS were used as sampling method taken and analyzes using the microsphere assay technique with beads coupled to an antibody directed for the search for antibodies against the Sprike protein of SARS-CoV-2.

Results: Of 168 samples analyzed, 143 (85%) were positive, 3 (2%) negative and 22 (13%) equivocal. This seroprevalence varied according to locality, but was not significant (p=0.13). Indeed, the lowest prevalence was observed in Bouemba (75%), while Liranga with 93% had the highest rate.

Conclusion: Despite the relative weakness of data on the circulation of SARS-CoV-2 in remote and landlocked areas, this study demonstrates the intensive circulation of this virus during the epidemic peak even in difficult-to-access regions like that of our study.

Keywords: SARS-CoV-2, Waterway corridor, Republic of Congo

Background

Coronavirus 19 disease (COVID-19) is a severe acute respiratory syndrome caused by coronavirus 2 (SARS-CoV-2) and was first identified in Wuhan, China in December 2019 [1]. Following the December 2019 SARS-CoV-2 outbreak in China, rapid spread occurred leading to a global pandemic [2]. Although the impact has been significant in some parts of the world, the burden has been relatively low in sub-Saharan Africa compared with Europe and America [3]. Two major reasons could explain this observation: such a young population and possible cross-immunity between SARS-CoV-2 and other infectious agents very common in the region. Due to the low socio-economic level of its population, preventive measures against COVID-19 have never been respected [4]. The estimation of virus carriage rates in certain countries has been made possible by the use of numerous tests to assess both the seroprevalence of SARS-CoV-2 infection in the general population, and the level of herd immunity. However, the data available on the continent are difficult to interpret, given the often-non-homogeneous data from hospitalized or symptomatic patients, or from asymptomatic individuals [5].

In the Republic of Congo, a retrospective study of plasma samples from 2019 from people living with HIV in Brazzaville and Pointe Noire showed the

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introduction of SARS-CoV-2 into the country, very shortly after the first case was identified in China [7]. However, at national level, a higher seroprevalence was found in the rural than in the urban region [6]. Vaccine uptake resistance in rural areas, as well as difficulties in transporting vaccines to landlocked areas, were a major problem. That's why a one-off survey was carried out in villages along the river corridor. The aim of this study was to determine the seroprevalence of SARS-CoV-2 infection among unvaccinated people in villages along the river corridor in the Republic of Congo.

Methods

We conducted this study in five localities located on the river corridor, namely: Bouemba, Makotipoko (Plateaux Department), Loukolela and Mossaka (Bassin Department), Liranga (Likouala Department) as illustrated in Figure 1, July to August 2022. We included people of all ages consenting to participate residing in the study localities and not vaccinated against SARS-CoV-2. Whole blood was collected on DBS cards, by saturating dedicated circles of Wathman 903 paper. These spots were dried at room temperature, sealed in bags with desiccators before their transfer to the National Public Health Laboratory in Brazzaville, for analysis. In the laboratory, we reconstituted these samples in 400 µl of phosphate-buffered saline (PBS), then 50 µl of the resulting solution was used for analysis using the coupled-bead microsphere immunoassay technique. to an antibody directed against the Sprike protein of the SARS-CoV-2 binding domain receptor. By measuring fluorescence intensity, we were able to confirm patient samples that had developed anti-SARS-CoV-2 antibodies equal to or above the positivity threshold. To take into account antigenic specificity, seropositivity cutoff values were set at three standard deviations above the mean MFI (mean fluorescence intensity) of the available plasma samples from this cohort, the furthest from the start of the epidemic, i.e. in July and August 2019. Based on this population, the MIA specificity (measurement of antibody intensity) was set at 97.5%. The sensitivity of this test was established using plasma samples from patients confirmed positive for SARS-CoV-2 by PCR, collected within two weeks of the PCR test. Based on this population, the sensitivity of MIA was set to 100%. We calculated the cut-off (positivity threshold) from samples from July and August 2019. To do this, we averaged the intensities obtained on the samples from this period and added 3 times the standard deviation of these intensities. The database was entered into Excel and statistical analyzes were performed using GraphPad Prism 8 software to determine the level of significance.

Results

A total of 169 people were sampled at the five (05) sites for anti-SARS-CoV-2 antibodies testing, including 52.7% (n=89) women and 47.3% (n=80) men. An overall positivity rate of 85% (n=143) throughout the river corridor was reported and only 2% (n=3) of samples were negative including 1 in Bouemba (female) and 2 in Loukolela (male)

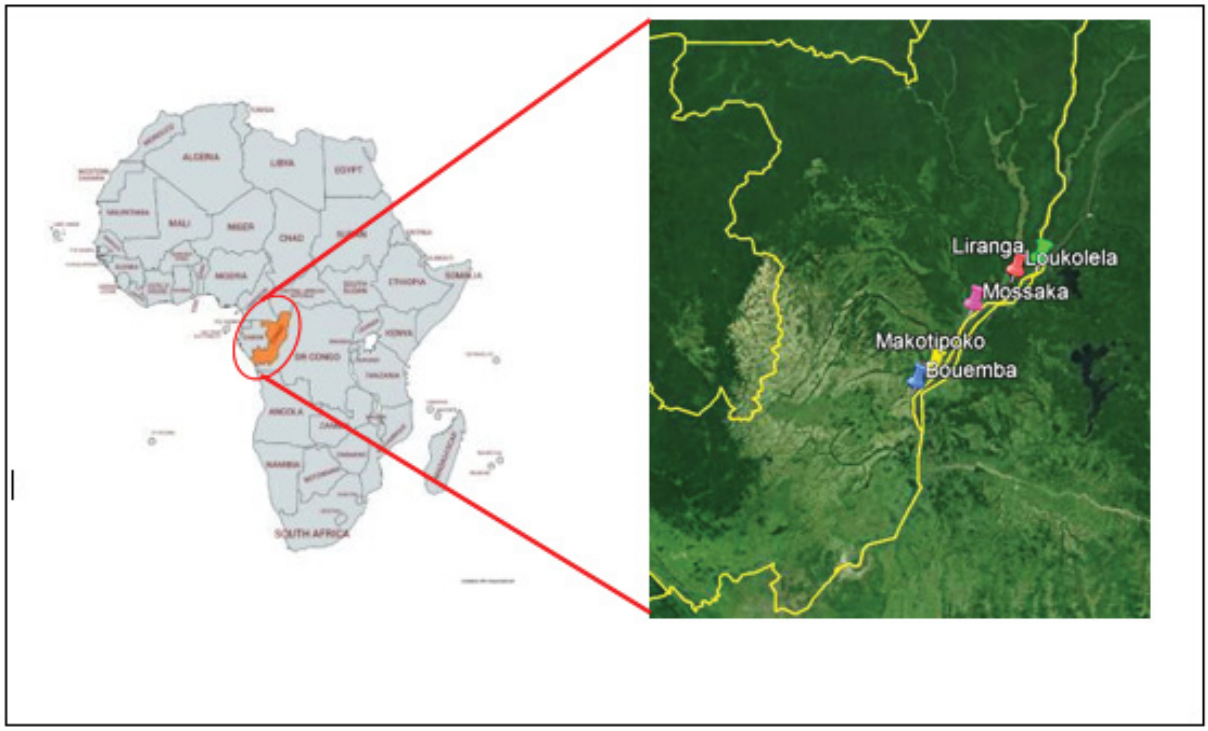

Figure 1: Map of study sites

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and 13% (n=23) were equivocal. Seroprevalence varied considerably between localities: 75% in Bouemba, 88% in Makotipoko, 81% in Loukolela, 88% and 93% in Mossaka and Liranga respectively. No statistical difference was reported between the different localities (p=0.13), nor any difference in seropositivity according to sex (p=0.52) or age groups (p=0.71). The analysis by locality did not make it possible to identify a clear trend according to sex, although in some, a strong representativeness was observed depending on sex. Indeed, in Bouemba, an overall seroprevalence of 75% was reported with a predominance of positivity among men (56% vs 44%) as well as in Liranga with 46% of positive women compared to 54% of men, as well as in Makotipoko was observed for 36.4% of women compared to 63.6% of men. On the other hand, in the localities of Mossaka and Loukolela (88% positivity), women were the most affected by the pandemic with respectively 60% vs 40% and 56% vs 44%.

Discussion

This is the first SARS-CoV-2 seroprevalence study carried out on the river corridor in Congo. In this study, we showed that the unvaccinated population located in this river corridor expressed a very high level of anti-SARS-CoV-2 antibodies, without sex difference. This prevalence, although at a much higher rate in this area, is in line with the conclusions of epidemiological studies conducted in Congo. Indeed, it has been documented that in the Republic of Congo, in a seroprevalence study, that rural areas are the most exposed to this infection, compared to urban areas [6]. Likewise, the prevalences reported in our study, although very high, should not be surprising because in fact, a seroprevalence study conducted at the national level [7], had already shown high infection rates in the departments sheltering this corridor, notably the basin (56.1%) and the Sangha (57.3%). Also, in the same study, a high positivity rate was observed among unvaccinated people throughout the national territory (45%). Thus, the populations living in these localities whose primary activity is commerce, could explain this infection rate, a reflection of a silent circulation and the dynamics of transmission at the local level of this virus. It may also be linked to access to clean water, perhaps in combination with other factors not investigated in this study, which may increase their vulnerability to infection. Indeed, a study in Tanzania found that patients who did not have access to clean water but relied on river water were more likely to have had a previous SARS-CoV-2 infection [8]. This could be the case for populations living on the river corridor included in this study and having shown a high seropositivity rate. Possibly, the demonstration of the early circulation of SARS-CoV-2 in Congo [9] could also explain an intensive and early circulation of this virus in these local populations.

Table 1: Summary of socio-demographic data

Localities/Sex	Female n (%)	Male n (%)	P value
Bouemba	**5**	**5**	
Non reactive	1 (10%)	0	0,14
Reactive	4 (40%)	5 (50%)	
Liranga	**12**	**14**	
Non reactive	0	0	0,10
Reactive	12 (46%)	14 (54%)	
Loukolela	**20**	**18**	
Non reactive	0	2 (5%)	0,07
Reactive	20 (53%)	18 (47%)	
Makotimpoko	**8**	**14**	
Non reactive	0	0	0,15
Reactive	8 (36%)	14 (64%)	
Mossaka	**30**	**20**	
Non reactive	0	0	0,05
Reactive	30 (60%)	20 (40%)	
Total	**75**	**71**	

Conclusion

This study made it possible to document a surprising epidemiological reality of SARS-CoV-2 in an environment with low attendance due to its difficult access and highlights the need to conduct broader studies by looking for possible risk factors.

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Authors' Contributions

EYBDA analyzed the samples, coordinated data analysis, and wrote the draft manuscript. DYLR and FRN designed and coordinated the study and reviewed the manuscript. FKK, and OIF, BBCN coordinated sample analysis read and reviewed the manuscript. KJM and PIM reviewed the manuscript. All authors approved the final version of the manuscript.

Conflicts of Interest

The authors declare no potential conflicts of interest for this study.

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Ethical Approval Statement

The study protocol was approved by the National Ethics Committee (Authorization 342/MRSIT/IRSSA/CERSSA)

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