



[Science Archives \(ISSN:2582-6697\)](https://doi.org/10.47587/SA.2023.4109)

Journal homepage: www.sciencearchives.org



<https://doi.org/10.47587/SA.2023.4109>

Research Article



Molecular seroprevalence of Sars-CoV-2 among people seen at the Omar Bongo Ondimba regional hospital of Makokou, in the province of Ogooué-Ivindo, in Eastern-North Gabon

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Received: Mar 1, 2023/ Revised: Mar 29, 2023/ Accepted: Mar 30, 2023

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Abstract

Despite the observation of a considerable decrease in the number of cases of COVID-19 (Coronavirus Disease 2019) in the world, this pandemic whose causal agent has been identified as Sars-CoV-2 remains to this day a threat to world health. In the framework of a permanent health monitoring of this disease, this study was undertaken to evaluate the seroprevalence of this disease among people seen at the Omar Bongo Ondimba regional hospital of Makokou, in the province of Ogooué-Ivindo, in Eastern- North Gabon. This descriptive cross-sectional study, whose data were collected prospectively, took place from 18 September 2021 to 18 February 2022. It enrolled persons of all ages and sexes who came to the laboratory of the Omar Bongo Ondimba regional hospital of Makokou, to be tested for COVID-19. The screening technique used was the molecular RT-qPCR test. Out of a total of 168 patients tested, the presence of the virus was identified in 27 samples, i.e. a prevalence of 16.07%. With a percentage of 96.30%, the most affected locality was that of Makokou, the capital of the Ogooué-Ivindo province. With 25% of positivity, and according to the statistical test, women were much more infected than men ($p=0.04$; OR = 2.5). Residing in an urban locality (Makokou) was a risk factor for Sars-Cov-2 infection. Although no age group was found to be a risk factor, the age group between 25 and 65 years was the most affected by the disease. Thanks to the implementation of the molecular diagnosis of Sars-CoV-2, the persons received at the Omar Bongo Ondimba regional hospital of Makokou, in the province of Ogooué-Ivindo, in Eastern- North Gabon, were able to benefit from a rapid and efficient diagnostic tool, which can help to detect the disease rapidly.

Keywords: Sars-coV-2, Covid-19, RT-qPCR, Omar Bongo Ondimba regional hospital Makokou, Ogooué-Ivindo, Gabon.

Introduction

In December 2019, cases of pneumonia of unknown etiology were reported in Wuhan, China (Huang et al., 2019). It was not until January 2020, that the causative agent was identified as Sars-CoV-2, by the International Committee on Viral Taxonomy (ICTV) (Amir et al., 2020). The rapid human-to-human transmission of this disease has led to a pandemic. In its publication of February 11, 2020, the World Health

Organization (WHO) named the disease caused by the virus: Covid-19 and considered it a global public health emergency (Amir et al., 2020, Nguen et al., 2020). Coronaviruses belong to the family Coronaviridae and the order Nidovirales and are responsible for respiratory, enteric, liver and neurological diseases (Mosites et al., 2020). Despite considerable efforts to contain the disease, the virus has continued its prevalence in many countries with varying degrees of clinical manifestations (Rai et al., 2020). A World Health Organization (WHO) status

report indicated that the Covid-19 pandemic was responsible for the deaths of 13 million to 17 million people by the end of 2021, far more than the officially reported death toll (WHO, 2022). Furthermore, between 2020 and 2021, the number of Africans who would die from SARS-CoV-2 would be 1.24 million, five and a half times more than the 229,197 officially recorded deaths (Bradshaw et al., 2022). In Gabon, the total number of cases recorded to date is 47677 cases with 304 deaths (Our World in Data., 2022). Until very recently, there was no medication against Sars-Cov-2. The arrival of various vaccines has raised great hope for the population. Unfortunately, access to these vaccines for the general public, especially in developing countries South of the Sahara, remains very low. For example, in Gabon, the total number of doses administered is estimated at 558, and the rate of fully vaccinated people at 11.5% (Our World in Data, 2022). Therefore, only a permanent health watch by respecting barrier measures and accurate and early detection of Sars-CoV-2, are essential for the effective prevention and management of cases of COVID-19, in order to stop a resurgence and a spread of this disease in the populations, particularly those in rural areas. It is in this context that this study was undertaken to evaluate the seroprevalence of this disease using RT-PCR diagnosis at the Omar Bongo Ondimba regional hospital of Makokou, in the province of Ogooué-Ivindo, in Eastern- North Gabon.

Materials and Methods

Study area, in Eastern- North Gabon

The study took place at the Omar Bongo Ondimba regional hospital of Makokou, in the province (OBORHM), capital of the province of Ogooué-Ivindo, in Eastern-north Gabon. Located 953,1 km from Libreville, the capital of Gabon, Makokou is a town in Gabon, the capital of the Ogooué-Ivindo province. The population of this town is estimated at 16222 inhabitants (2003 census). With a latitude of -1,2194 1° 13' 10" South and a longitude of 12,4667 12° 28' 0" East, the altitude is 531m. The tropical savannah climate consists of two rainy seasons from September to November and from February to May, which are separated by two dry seasons. The inhabitants live in simple houses built with local materials, and their main activities are subsistence farming, small-scale commerce, game hunting, fishing and some employment in the local administrative sector. The vegetation is equatorial.

Type, period and population of the study

This is a prospective cross-sectional study with analytical purposes, conducted from September 18, 2021 to February 18, 2022. The patients in our study were people of all sexes, of all ages, who received a Covid test at the Molecular Biology Laboratory of the Omar Bongo Ondimba regional hospital of Makokou (OBORHM). But also, some samples came from the camps of the Equatorial Wooden Company of Mékambo.

Inclusion and exclusion criteria

In this study, patients were included if they came for the COVID-19 serological test and if their samples were well identified after sampling and had a well-defined result, i.e. positive or negative. Excluded were patients whose specimen tubes were misidentified and those for whom the RT-PCR results were indeterminate. As information (data) on the symptoms of the tested persons was not systematically available, it was collected on the arrival of the patient, on a standardized data collection form. The identification of the patients was done by coding, then transcribed on the transport medium tubes.

Sampling

The COVID-19 RT-qPCR test started with a nasopharyngeal swab. The quality of the nasopharyngeal swab determined the reliability of the result. The collection of the sample was associated with the wearing of appropriate personal protective equipment.

Dressing of the equipment

Before the beginning of the sampling, all the material necessary for the care was provided for the hospital handling agent and the patient. The patient was prepared psychologically, with a detailed explanation of the reasons for the sampling, and it was ensured that the patient had no history of nasal trauma. Then, the hands were rubbed with hydroalcoholic gel, before putting on a single-use gown and an N95 mask, and finally, gloves and single-use protective glasses were put on.

Sampling procedure

The patient was asked to blow his nose to evacuate the nasal secretions. The head was tilted back to facilitate the sampling and the patient was asked to close his eyes to better tolerate the sampling. The swab was gently inserted into the nasal cavity until resistance was felt in the nasopharynx. After rotating the swab on the patient's nasopharyngeal mucosa for approximately 10 seconds, the swab was removed and placed in the transport medium by cutting according to the manufacturer's recommendations.

Routing to the laboratory

Samples from Mékambo in a rural area were transported using a triple packaging system. Once transported to the laboratory, when the samples were analyzed on the same day, the virus was inactivated directly using an incubator at 70°C for one minute. Otherwise, the samples were kept cold, i.e. 24 hours in the refrigerator or freezer for a maximum of 7 days.

Real-time RT-PCR technique

Real-time RT-PCR was performed using a programmable machine, the LineGene 9600 thermal cycler (BIOER, China

2020), with obtained nasopharyngeal swab samples. Microtubes containing the reaction mixture are fed into this machine, which subjects the tubes to selected temperatures and times. A nucleic acid diagnostic kit (PCR-Fluorescence Probing) of the new coronavirus (2019- nCOV), was used for the diagnosis of SARS-COV-2 infection.

Principle of the test

Using a sequence-specific fluorescent oligonucleotide probe, the real-time PCR system relies on the visualization of the exponential phase of the reaction. The increase in the fluorescent emission signal is directly proportional to the number of amplicons produced during the reaction. PCR consists of the chain replication of a DNA sequence by DNA polymerase. The RT (reverse transcriptase) is an enzyme that transcribes RNA into DNA. This allows the quantification of RNA. PCR is based on a denaturation-hybridization-elongation cycle, each of these steps being controlled by a different temperature. -Denaturation (95°C for 10 to 15 min) consists in dehybridizing the DNA double-strand, homogenizing the reaction medium by thermal agitation, and activating the DNA polymerases. -Hybridization (56 to 64°C for 2 to 60 sec): sense and antisense primers attach to the template DNAs due to a thermodynamically favorable temperature. - Elongation (72°C for 4 to 120 sec): complementary DNA is synthesized by polymerases from free dNTPs in the medium. In the third cycle: the first amplicons appear (double-stranded DNA bounded by the primers corresponding to the desired DNA fragment).

Preparation of the reaction medium

The preparation of the reaction medium consisted in putting 10µl of each sample in microtubes, followed by 10µl of a lysis solution (incubation 10 minutes after addition of the lysis), and 30µl of the master mix obtained by introducing 96µl of the PCR -Enzyme Mix solution in 624µl of the PCR Mix solution. The mixture was homogenized with a vortex and all the manipulations were done under an oven. NB: a control is always made in which the DNA is replaced by water (negative control) in order to detect a possible contamination. A positive control will allow to validate the good conservation of the reagents and the performances of the thermocycler. An internal control present in the reaction medium will provide information on the presence or absence of amplification inhibitors. After preparation, the microtubes were placed in the thermal cyler programmed according to the instructions mentioned above.

Data entry and analysis

Data were entered and analyzed on Excel 2013. Fisher's exact test was performed to calculate the p-value, in order to understand the different associations between the different factors considered in the study and Sars-CoV-2 infection.

Results and Discussion

Socio-demographic characteristics of the patients in the study

Of a total of 168 patients enrolled in this study, 69.64% were female for a sex ratio (F/H) of 2.3. With a mean age of 36.71 ± 12.94 years and a percentage of 79.76%, the majority of patients were between 25 and 65 years old. The number of patients from Mékambo and Makokou was the same (50%) [Table1](#).

Table 1. Socio-demographic characteristics of the patients in the study

Caractéristiques	Numbers	Percentages
Sex		
Male	51	30.36%
Female	117	69.64%
Age groups		
14 - 24 ans	34	20.24%
25-65 ans	134	79.76%
Localities		
Makokou	84	50%
Mékambo	84	50%

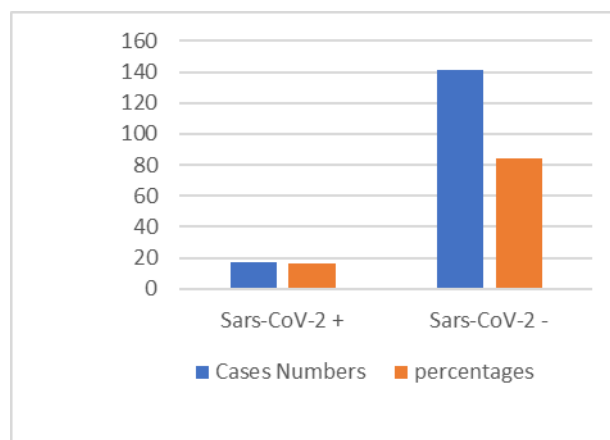


Fig. 1 Seroprevalence of Sars-cov-2 infection in the study population

The different symptoms that led to consultation

All the symptoms that led people to consult, fever (n= 23, i.e. 12% of patients) was the first symptom, followed by cough (9.95% n = 19) and dyspnoea (8.9% n = 17) ([Table 2](#)).

Table 2. Frequency distribution of symptoms related to COVID-19

Symptoms	Number (n)	Percentages (%)
Asthenia	14	7.33
Choking	4	2,1
Coughing	19	9.95
Respiratory distress	6	3,14
Dyspnea	17	8.9
Fever	23	12.0
Diarrhoea	3	1.57
Tired	1	0.52
Coma	2	1.04
Contact subject	4	2.1
Mycosis	1	0.52
Loss of taste	2	1.04
Loss of sense of smell	2	1.04
Hernia operation	1	0.52
Tachycardia	1	0.52
Pregnancy	1	0.52
Vertigo	3	1.57
No data	16	8.37
Palpitation	1	0,52
Altered consciousness	3	1.57
Shivering	2	1.04
Epigastralgia	10	5.23
Chest pain	12	6.28
Caesarean section	2	1.04
Polypnea	1	0.52
Céphalées	12	6.28
Headache	4	2.1
Polyaltragia	2	1,04
Vomiting	1	0.52
Anthrax	1	0.52
Joint pain	1	0.52
Diabetes	7	3.79
HTA	12	6.28

Seroprevalence of Sars-cov-2 infection in the study population

Of the 168 patients collected for this study, only 27, i.e. a prevalence of 16.07%, were diagnosed as positive for Sars-cov-2 infection, compared to 141, i.e. 83.92%, negative.

Analysis of the association between different factors and the seroprevalence of Sars-Cov-2 infection

Statistical correlations between gender and Sars-Cov-2 infection

The frequency of infected women was significantly higher than that of infected men (25% vs. 12%; $p=0.04$), so according to the statistical test men were much more susceptible to infection ($p=0.04$; $OR=2.5$). Specifically, men were 2.5 times more likely to contract the disease. Therefore, male gender was a risk factor that increased the likelihood of disease occurrence.

Table 3. Measure of association between gender and infection

Gender	Sars-cov-2+ (%)	Sars-cov-2- (%)	Total	Test
Male	13 (25%)	38 (75%)	51	$p=0.04$
Female	14 (12%)	103 (88%)	117	$OR=2.5$
Total	27	141	168	$IC95\% [0.9849; 6.3385]$

Statistical correlations between localities and infection

The frequency of infected patients was significantly higher in patients from Makokou (urban locality) compared to those from Mékambo (rural locality) (1.12% vs 39.95%; $p= 4.9 \times 10^{-8}$). Urban location was significantly associated with the risk of contracting the disease, unlike rural location ($OR= 0.03$). Thus, there is a relationship between locality and the risk of developing the disease or not.

Table 4. Measure of association between locality and infection

Localities	Sars-cov-2+ (%)	Sars-cov-2- (%)	Total	Test
Mékambo (Rural)	1 (1.19%)	83 (98.81%)	84	$p= 4.9 \times 10^{-8}$
Makokou (Urban)	26 (39.95%)	58 (60.05%)	84	$OR = 0.03$
Total	27	141	168	$IC95\% [0.0007;0.1757]$

Statistical correlation between age and infection

The frequency of infection in adolescents did not differ significantly from that in adults ($p= 0.3$). Therefore, there was no statistical association between the age factor and the risk of contracting the disease ($OR= 0.34$).

Table 5. A measure of the association between age and infection

Age groups	Sars-cov-2+ (%)	Sars-cov-2- (%)	Total	Test
14 - 24 years	2 (5.9%)	32 (94.1%)	34	$p= 0.3$
25-65 years	25 (18.7%)	139 (81.3%)	134	$OR=0.34$
Total	27	141	168	$IC95\% [0.0299; 1.2016]$

This study was conducted from September 18, 2021, to February 18, 2022, on 168 samples taken with nasopharyngeal swabs. The objective of this study was to evaluate the prevalence of Sars-CoV-2 using a molecular diagnosis called RT-PCR, at the regional hospital of Makokou, in order to monitor the health situation against a possible resurgence of this pandemic. Among all the different symptoms that led patients to consult the hospital, fever was the first symptom, with 12% of cases followed by cough (9.95%) and dyspnea (8.9%). These results are very similar to those found by the Belgian Institute of Health "sciensano" which showed in their work that the characteristic symptoms of COVID-19, were fever (61%), cough (52%), shortness of breath (49%), and generalized weakness (39%). In contrast, in some previous studies, it was reported that asthenia was the most frequent clinical sign (80.2%) (Gattinoni et al., 2020, Louhaichi et al., 2020), dyspnea (29.1%) was ranked tenth (Ketfi et al., 2020). This variability of results can be explained by the difference in sampling and geographical locations where these studies were conducted. This study revealed a higher seroprevalence of Sars-coV-2 in the urban locality (Makokou) compared to the rural locality (Mékambo). These results are quite similar to those obtained elsewhere, where it was shown that there were fewer cases of infection in small towns than in large towns (Pollán et al., 2020, Rocha et al., 2015). This could be explained by the fact that in large cities, the promiscuity between populations and carelessness offer a material on which many pathologies (infectious or not) can easily develop (Hervé et al., 2020). Also, urbanization and metropolization with their factories, are responsible for air pollution, and global warming which can make populations more vulnerable to different infections. The statistical analysis of the results of this study indicated a greater sensitivity to infection in men than in women. These results are not only similar to those found in China (Hervé et al., 2020), but also to those obtained in one of the most affected countries in Europe, Italy, where, according to the national public health agency, 60% of Covid-19 cases were men, and men constituted almost 70% of the deceased patients (Italy's national public health agency, 2020). These results indicate an immune advantage for women, which can be explained by the fact that after a disease, women keep their protective antibodies longer (France Public Health, 2020). According to the results of a study by the University Hospital of Strasbourg and the Pasteur Institute on 308 caregivers, a hypothesis put forward by the University Hospital of Strasbourg stated that the X chromosome would carry one immune gene, whereas women have two. Moreover, unlike testosterone, estrogen promotes immunity. More fundamentally, there is also the role of androgens, the male sex hormones, which act on an enzyme that facilitates the entry of the virus into the cells. Finally, the immune response is better in women than in men. The behavior of men is also sufficient to explain why they develop more severe forms of the disease. They smoke more than women and are more obese. Weakened lungs and being overweight are important risk factors for severe forms of COVID-19 (LCI Editorial Board – Published, 2020). The results of this study also revealed a statistical association between locality and.

Contrary to works that indicated that 90% of the patients with Sars-Cov-2 infection who died were older than 64 years and the risk was higher in men than in women (Ketfi et al., 2020), this one, found no statistical association between age and the probability of contracting the disease. However, these results are similar to those obtained in previous studies which showed that all age groups could be infected with Sars-Cov-2 (Vakili et al., 2020). This could be explained by the fact that the virus infects type 2 pneumocytes, which have the receptor for the virus on their surface, namely, angiotensin-converting enzyme 2 (ACE-2) (Wadman et al., 2020). Indeed, the peak protein of the virus binds to ACE-2 allowing the entry of the virion into the host cell. Therefore, all age groups can contract the virus (Xia et al., 2020).

Conclusions

This study showed that gender and locality were risk factors associated with the disease. Indeed, male patients were the population most at risk of contracting the disease compared to females. Also, patients living in the city were more exposed to the infection compared to those living in the village. However, no association was found between the different age groups and the risk of infection. However, it would be interesting to extend the study to look at the immunological parameters to see if there is a broader immune response that would be triggered in women or in men. We could also see if there is a development of a collective immunity within the population. Because it could be that some patients could be positive for Sars-CoV-2 antibodies. Thus we would have better results describing the epidemiological situation of our study area. Even if some people think that the pandemic is over, it will be necessary to maintain respect of the barrier measures, to fight effectively against the Sars-CoV-2 infection. It is up to the decision-makers to put in place appropriate facilities for early diagnosis against this persistent pandemic.

Acknowledgment

The authors would like to thank the staff of the Koula-Moutou regional hospital for their cooperation and assistance during this work.

Conflict of Interest

The author hereby declares no conflict of interest.

Consent for publication

The author declares that the work has consent for publication.

Ethical consideration

In order to preserve the anonymity of the participants, a unique coded number was assigned in the database to all patients included in the study. Under no circumstances will the identity of the patient be disclosed.

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How to cite this article

Mba, T. N., Kenguele, H. M. and Pambo-Pambo, B. A. (2023). Molecular seroprevalence of Sars-CoV-2 among people seen at the Omar Bongo Ondimba regional hospital of Makokou, in the province of Ogooué-Ivindo, in Eastern- North Gabon. *Science Archives*, Vol. 4(1), 53-58.
<https://doi.org/10.47587/SA.2023.4109>

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