RESEARCH ARTICLE

Epidemiology of first cases of SARS-CoV-2 infection, from March to April 2020 and molecular characterization of the spike protein, in Gabon [version 1; peer review: 1 approved, 1 approved with reservations]

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Abstract

Background

After the first cases of coronaviruses disease 2019 (COVID-19) in China in January 2020, we conducted an epidemiological surveillance of COVID-19 in Gabon.

Methods

We led molecular investigations on nasopharyngeal and oropharyngeal samples from the 1161 first suspected cases of COVID-19. We diagnosed the first case of COVID-19 on March, 12 2020.

Results

Among those suspected cases, 83 were confirmed cases. There was no significant difference in prevalence of SARS-CoV-2 between age groups ($p=0.14$). 73% were asymptomatic. The viral loads were

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significantly higher in the nasopharyngeal samples than in the oropharyngeal samples ($p=0.03$). There was no significant difference in viral loads between age groups ($p=0.9895$) and no correlation between clinical symptoms and viral loads ($p=0.06042$). A phylogenetic analysis performed with five sequences of the spike S gene showed that two sequences had the D614G mutation.

Conclusion

In conclusion, this study provides the first molecular data from Gabon concerning the COVID-19 pandemic. The data showed that most of the infected people were asymptomatic. The viral load was higher in the nasopharyngeal samples. The S gene analyzed suggested both introduction of the D614 and G614 variant in Gabon.

Keywords

COVID-19, Diagnostic, Epidemiology, Viral load, S gene, Gabon

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Introduction

In December 2019, an atypical pneumonia emerged in China. A total of 44 case-patients were reported from December 31, 2019, to January 4, 2020, with an unknown etiology (<https://apps.who.int/iris/handle/10665/330760>). In January 2020, a 2019 novel coronavirus was identified, and named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2 caused an outbreak in China and rapidly spread in the whole world.¹ From January 20, 2020, the first cases were reported in North America.² In Europe on January 24, 2020, the first cases were reported by the World Health Organization (WHO).³ On March 11, the WHO declared COVID-19 a pandemic disease⁴ and reported 118,319 confirmed cases and 4292 deaths (<https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200311-sitrep-51-covid-19.pdf>). In Central Africa, Gabon reported its first case of COVID-19 on March 12, 2020. On December 15, 2020, nearly a year after its discovery, this disease continues to rise in the world with 70 million cumulative cases and 1.6 million deaths globally since the beginning of the pandemic (<https://www.who.int/publications/m/item/weekly-epidemiological-update---15-december-2020>).

The literature reports that COVID-19 has an incubation period of 1–14 days and it affects both children and adults. Symptoms present in children are similar to those found in adults with an incubation period of 1–14 days; however, over 90% of affected children are asymptomatic or have no severe form of the disease.⁴ The SARS-CoV-2 virus is detected in several clinical samples, such as nasopharyngeal samples, oropharyngeal samples, sputum, saliva, serum, urine and stool.^{5,6} The viral load can vary between the different clinical samples, throughout the infection after the onset of the first symptoms or could depend on the severity of the disease.^{5,6} The study of the infectivity of SARS-CoV-2 includes the analysis of viral loads, the host's immune response but also the genomic analysis of the strains circulating in order to provide a response to the pandemic and develop a vaccine candidate.^{7–9}

15 first mutations have been identified in the genome, particularly in the main sequence, in the genes encoding non-structural protein, the polymerase, the spike glycoprotein (S), the membrane glycoprotein, and the nucleocapsid phosphoprotein.¹⁰ The mutation D614G in the gene coding S protein could be associated with a more virulent variant leading to a higher mortality rate (<http://dx.doi.org/10.1038/s10038-020-0808-9>). More recently, several mutations were identified in the spike region gene in a new variant of SARS-CoV-2 in the United Kingdom and South Africa (<https://www.who.int/csr/don/21-december-2020-sars-cov2-variant-united-kingdom/en/>).

The first cases of COVID-19 have been widely documented in many countries. However few data are available from sub-Saharan Africa, and more particularly from Central Africa. The aim of this study is to report epidemiological and molecular data on the SARS-CoV-2 virus. Phylogenetic analysis of the S gene will identify the mutations in this genomic region and therefore the strains circulating in Gabon.

Methods

Study design, patients information's and samples

When the worldwide coronavirus pandemic began in January 2020, the “*Centre Interdisciplinaire de Recherches Médicales de Franceville*” (CIRMF) set up a surveillance of COVID-19 in Gabon, according to the guidelines of the Gabonese Ministry of Health, the WHO Region Office for Africa and the Africa Center for Disease Control (Africa CDC). Patients who were enrolled in this study visited health centers for respiratory syndrome comprising fever ($\geq 38^{\circ}\text{C}$) and runny nose, or fever and cough, or fever and sore throat. Epidemiological data, including the name, age, sex, and travel history during the month before onset and clinical data were collected. Other people were asymptomatic but had been in contact with people who tested positive for COVID-19. So, from February 2020, nasopharyngeal and oropharyngeal samples were collected from suspected cases in hospitals: the “*Hôpital d'instruction des Armées Omar Bongo Ondimba*” (HIAOBO), the “*Hôpital d'instruction des Armées d'Akanda*” (HIA A) in Libreville, mobile health centers which collected samples, and health centers in the Haut-Ogooue province. From February 15 to April 30, 2020, 1161 first suspected cases were enrolled in Libreville, Franceville, Port-Gentil and Bitam for the confirmation of SARS-CoV-2 infection with molecular test diagnostics. The size of the study had correlated with samples collected for a month and a half after the first positive case. After collection, samples were stored in transport medium at 4°C until they were sent to the CIRMF along with the patient's medical history mentioned demographical and clinical data such as symptoms and signs of the disease, comorbidities and travel history. The lack of information about age on some files constituted a bias.

Molecular diagnosis and viral load of SARS-CoV-2

The confirmation of SARS-CoV-2 was done by real-time reverse transcriptase polymerase chain reaction (RT-PCR) according to Africa CDC guidelines. Swabs samples processed in a biosafety level-3 laboratory with personal protection equipment were placed in saline (0.9%). RNA extraction was done with the QIAamp® Viral RNA mini Kit (Qiagen) according to the manufacturer's instructions. An extraction control was introduced during this step allowing the validation of the diagnosis by amplifying a gene fragment from Equine Arteritis Virus according to the instructions of

the “TIB MOLBIOL” kit. An extraction control was introduced during this step. RT-PCR was performed using Superscript III RT-PCR kit Invitrogen in simplex, primers and probes targeted the E gene coding envelope and the RdRP gene coding RNA-dependent RNA polymerase (kit Tib-Molbiol), in a 7500 Real Time PCR System (Applied Biosystems).¹¹ The samples were considered negative if the cycle threshold (Ct value) exceeded 36 cycles for the E gene and 40 cycles for the RdRP gene. A person was confirmed as positive case if both nasopharyngeal and oropharyngeal sample were positive or if one of the two was positive for SARS-CoV-2. Viral load was calculated from the Ct value using the standard curve generated by dilution of RNA positive control.

Spike region sequencing

The 3822 nucleotides coding for the 1274 amino acid homotrimeric spike glycoprotein of SARS-CoV-2 (article) were sequenced by the Sanger method with a 3500 Genetic Analyzer (Applied Biosystem) with the BigDye terminator V1.1 kit. The amplification of S gene was made by the technique of walking the genome using overlapping S gene fragments. Ten pairs of forward and reverse primers were designed in order to amplify ten genes fragments from 505 to 753 bp using a conventional RT-PCR method (Supplemental Materials). The fragments were assembled by Chromas Pro and phylogenetic analyses were performed using a multiple sequence alignment with the SARS-CoV-2 isolate Wuhan-Hu-1 reference strain (GenBank Accession number NC_0445512 and MN908947) and twenty five others strains from several countries available in the GenBank database. Sequences were aligned using ClustalX (version 1.81). The phylogenetic tree was built with the PhyML algorithm using the maximum-likelihood method and drawn using FigTree v.1.4.0.^{12,13}

Statistical analysis

Patient records were used as the data sources. Data were analyzed using the Statview version 5.0 software. Pearson’s Chi-squared test and Fisher’s exact test were used to compare variables and to assess the relation between demographical data such as sex, age and clinical data. The R software package (version 4.0.3) was also used to compare the viral load between nasopharyngeal and oropharyngeal clinical samples, the viral load among asymptomatic patients and those with symptoms and the viral load across age groups. Data analysis revealed that the distribution did not follow a normal distribution. So a non-parametric analysis with the Mann–Whitney U test. A p-value less than 0.05 was considered statistically significant.

Results

Epidemiological data of suspected cases

This study took place between February 15, 2020, and April 30, 2020. The first cases of COVID-19 were detected on March 12, 2020, in Gabon. Among the 1161 suspected cases sampled, 517 (48.0%) were male, 560 (52.0%) were female. The sex ratio was 0.92, the median age was 36.0 years (range, 15 days to 82 years) and the mean age was 35.9 ± 12.2 years (Table 1). Information on gender and age was unavailable for 84 and 282 people, respectively, because some data were confidential at the beginning of the pandemic. The most represented age groups were 25–35 and 35–45 years, 32.4% and 34.4%, respectively, while the least represented group was composed of patients aged over 65 years (1.4%) (Table 1). 96% of nasopharyngeal and oropharyngeal samples from the 1161 suspected cases were collected in the capital Libreville, and 19 (1.6%), 10 (0.9%), 16 (1.4%) were collected in Franceville, Port-Gentil and Bitam respectively (Table 1).

Epidemiological data of confirmed cases

Among the 1161 suspected cases, 83 (7.15%) were confirmed cases of COVID-19. There was no significant difference between males (48.2%) and females (51.8%) according to prevalence, 7.74 and 7.68, respectively ($X^2=0.001$, $p=0.97$). The median age was 37.5 years (range, 3 years to 68 years) and the mean age was 37.1 ± 12.9 years (Table 1). Regarding to the age group distribution, the highest percentages were in the 25–35 (24.1%) and 35–45 years (32.5%) age groups. However, there was no significant difference in prevalence of SARS-CoV-2 between age groups ($X^2=9.58$, $p=0.14$). No confirmed cases were found in the 47 suspected cases of the 15-25 years age group (Table 1). All the positive samples were collected in Libreville, except, one which came from Bitam.

Clinical characteristics of patients with COVID-19

73% of the confirmed cases were asymptomatic (Table 2). However the prevalence of COVID-19 in patients with a respiratory syndrome (18.9%) was higher than in asymptomatic cases (5.8%), ($X^2=25.17$, $p<0.0001$). Among the 22 symptomatic cases, 20 (90.9%) had an influenza-like illness (ILI) defined as fever ($\geq 38^\circ\text{C}$) and a runny nose, cough, or sore throat while two (9.1%) patients had respiratory distress. ILI symptoms were accompanied by anosmia and ageusia for two patients, one patient suffered from diarrhea and another had asthenia (Table 2). 94% of people infected with the coronavirus did not have comorbidity. One confirmed case had asthma, two had sinusitis, one had diabetes and another had hypertension (Table 2). The positive rate in people with chronic disease and no chronic disease was 6.3% and 7.2%, respectively. So, no association was found between comorbidities and SARS-CoV-2 infection ($X^2=0.004$, $p=0.95$).

Table 1. Demographic characteristics of suspected cases and prevalence of confirmed COVID-19 cases.

Characteristics	Suspected cases		Confirmed cases	
	n (%)	95% CI	N(p)	95% CI
Sex				
Male	517 (48.0)	45.0-51.0	40 (7.74)	5.38-9.98
Female	560 (52.0)	49.0-55.0	43 (7.68)	5.53-9.95
ND	84			
Mean age	35.9 ± 12.2	-	37.1 ± 12.9	-
Median age	36.0	-	37.5	-
Interquartile	13.0	-	12.0	-
Age group (years)				
[0-15]	59 (6.7)	5.0-8.4	7 (11.9)	3.6-20.2
[15-25]	47 (5.3)	3.8-6.8	0 (0.0)	-
[25-35]	285 (32.4)	29.3-35.5	20 (7.0)	4.0-10.0
[35-45]	302 (34.4)	31.3-37.5	27 (8.9)	5.7-12.1
[45-55]	136 (15.5)	13.1-17.9	16 (11.8)	6.4-17.2
[55-65]	38 (4.3)	2.9-5.7	2 (5.3)	-1.8-12.4
[65-82]	12 (1.4)	0.6-2.2	2 (16.7)	-4.4-37.8
ND	282	-	9	-
Towns (Province)				
Libreville (Estuaire)	1116 (96.1)	95.0-97.2	82 (7.4)	5.9-8.9
Franceville (Haut-Ogooué)	19 (1.6)	0.9-2.3	0 (0.0)	-
Port-Gentil (Ogooué-Maritime)	10 (0.9)	0.4-1.4	0 (0.0)	-
Bitam (Woleu- Ntem)	16 (1.4)	0.7-2.1	1 (6.2)	-5.6-18.0
TOTAL	1161	-	83 (7.1)	5.6-8.6

CI: confidence interval, ND: no data, (p): prevalence.

Table 2. Clinical characteristics of confirmed COVID-19 cases.

	n (%)	95% CI
Clinical characteristic		
Asymptomatic	61 (73.5)	64.0-83.0
Symptomatic	22 (26.5)	17.0-36.0
Symptoms		
Influenza-like-illness	16 (19.3)	10.8-27.8
Influenza-like-illness + anosmia + ageusia	2 (2.4)	-0.9-5.7
Influenza-like-illness + diarrhoea	1 (1.2)	-1.1-3.5
Influenza-like-illness + asthenia	1 (1.2)	-1.1-3.5
Respiratory distress	2 (2.4)	-0.9-5.7
Chronic comorbidities		
Asthma	1 (1.2)	-1.1-3.5
Sinusitis	2 (2.4)	-0.9-5.7
Diabetes	1 (1.2)	-1.1-3.5
Hypertension	1 (1.2)	-1.1-3.5
None	78 (94.0)	88.9-99.1
TOTAL	83	-

CI: confidence interval.

Recent history of travel and context of COVID-19 screening

The surveillance of COVID-19 in Gabon revealed that more than three-quarters of positive cases (87.9%) had no recent travel history, 67 (80.7%) were in contact with confirmed cases, and 6 (7.2%) persons participated in voluntary screening (Figure 1A). The 10 (12.1%) people who had traveled from affected areas came from France (n=9) and Senegal (n=1) (Figure 1B).

The prevalence was similar in the three categories of confirmed cases, the contact cases (6.7%), the voluntary screening cases (6.3%) and people who reported having traveled recently (13.7%), ($\chi^2=5.07$, $p=0.08$). All positive cases who presented themselves for voluntary screening were asymptomatic, whereas all the confirmed cases who had a recent travel history abroad had respiratory symptoms. Among the 67 contacts cases 55 were asymptomatic.

Viral load of SARS-CoV-2 in oropharyngeal and nasopharyngeal clinical samples

The viral loads in oropharyngeal samples ranged from $2.86 \log_{10}$ copies/mL (7.25×10^3 copies/mL) to $7.51 \log_{10}$ copies/mL (3.21×10^7 copies/mL) with a median of $3.96 \log_{10}$ copies/mL (9.13×10^3 copies/mL) (Figure 2A). In the nasopharyngeal samples the viral load ranged from $2.50 \log_{10}$ copies/mL (3.16×10^3 copies/mL) to $7.70 \log_{10}$ copies/mL (4.98×10^7 copies/mL) with a median of $4.68 \log_{10}$ copies/mL (47.67×10^3 copies/mL). The viral loads were significantly higher in the nasopharyngeal samples than in the oropharyngeal samples ($p=0.03$) (Figure 2A).

There was no significant difference in viral loads between children under 15 years old and people over 15 years old ($p=0.9895$) (Figure 2B). Likewise, there was no correlation between clinical symptoms and viral loads ($p=0.06042$) (Figure 2C).

SARS-CoV-2 sequences

The phylogenetic analysis was performed with five sequences of 3822 nucleotides of the spike S gene obtained from the collected samples in five patients. Among the patients, two had symptoms and came from France (62GAB_S, accession

Figure 1. (A-B): Context of COVID-19 screening. A. Causes of COVID-19 screening. B. Travel history of confirmed cases.

Figure 2. (A-C): Viral load of SARS-CoV-2 in clinical samples. A: Viral load from oropharyngeal and nasopharyngeal samples. B: Comparison of viral loads in two age groups. C: Comparison of viral loads in symptomatic and asymptomatic people.

Figure 3. Phylogenetic tree based on nucleotides sequences of the S gene. Legend: The red rectangles indicates the sequences of SARS-CoV-2 strain circulating in Gabon.

number MW512913), including the first case diagnosed in Gabon (34GAB_S, accession number MW512911), one had symptoms and a travel history in Senegal (121GAB_S, accession number MW512914), and finally, the other two were contact cases without travel history, one of which had symptoms (56GAB_S, accession number MW512912) and the other was asymptomatic (297GAB_S, accession number MW512915). We compared these sequences with 27 sequences of SARS-CoV-2 of the GenBank database which were diagnosed in several countries of the five continents (China, South Korea, Malaysia, Russia, Sweden, France, Italy, USA, Brazil, Guatemala, Tunisia, Egypt, Morocco, South Africa and Benin) (Figure 3). The five sequences were distributed in two clusters. The sequences 56GAB_S and 121GAB_S clustered with the sequences of the reference strain (NC_045512, MN908947) and ten sequences registered in the GenBank database (Figure 3). Six sequences including, 56GAB_S, displayed 100% identity at the nucleotide level with the sequences of the reference strain in this cluster. On the other hand, three sequences MT499216-Tunisia, MT093571-Sweden and 121GAB_S displayed 99.97% identity with the reference strain. The alignment of SARS-CoV-2 sequences showed that the nucleotide substitution (G906T) on the S gene of the 121GAB_S sequence did not generate an amino-acid substitution (Threonine). The alignment of the 18 sequences of the second cluster, including the three others sequences of Gabon (34GAB_S, 62GAB_S and 297GAB_S), showed a nucleotide substitution (A1841G) generated a synonymous D614G mutation, with an amino-acid substitution of aspartic acid Asp (D) with glycine Gly (G), at position 614 (Figure 3). Furthermore, there was a nucleotide substitution (G3621T) in the 34GAB_S sequence which displayed 99.95% identity with the reference sequence strain. This substitution generated a mutation at the amino-acid sequence position 1207, glutamic acid Glu (E) was substituted by aspartic acid Asp (D) (Figure 3).

Discussion

Between, March 12, 2020, and April 30, 2020, 83 confirmed cases of COVID-19 were detected in Gabon. During the 50-day period, the progression of the disease was slow and comparable to the spread of COVID-19 in Nigeria during the first 45 days. However, in several countries in Africa, such as Cameroon, South Africa, Tunisia, Morocco, the burden of COVID-19 was higher, with more than 500 confirmed cases reported 35 days after the first case.¹⁴ This difference could probably be due to the population density which would be higher in these countries. Several studies which described more confirmed cases showed that more than three quarters of patients were found in the [30–79] age group.^{15,16} Despite the fact that all ages were susceptible, others studies showed that the majority of cases were over 30 and more specifically between 30 and 60 years old.^{16,17} The 30-years-olds in our study were one of the most represented age groups (32.4%).

More than 70% of the cases were asymptomatic in our study. Two studies which reported the first cases of COVID-19 in China and Europe mentioned that 5% of case were asymptomatic.^{3,18} The proportion of asymptomatic cases varied

according to the studies, values ranged from 1% to 78%^{7,19,20} (<http://dx.doi.org/doi:10.1136/bmj.m1375>). The percentage of asymptomatic cases (73.5%) in Gabon during this period was within the range of the value reported worldwide meaning that asymptomatic carrier transmission was effective in Gabon. Moreover, there may have been presymptomatic transmission in Gabon such as the one described in Germany where an asymptomatic businessman from China transmitted the virus to a healthy German businessman. The Chinese visitor reportedly had symptoms of COVID-19 upon his return.²¹ Several reports cases have recorded similar facts and shown asymptomatic and presymptomatic transmission.^{22,23} Approximately 98% of patients would have had an incubation period of 5 days on average ranging from 2 to 14 days, although this period could be up to 24 days.⁴ These data could explain the spread of the virus in Gabon despite the high number of asymptomatic cases (73%).

Review authors described symptoms of COVID-19 as predominantly flu-like symptoms such as fever (80–90%), cough (50%), lethargy (20–40%) and in some cases diarrhea.⁴ In some more vulnerable patients including the elderly and people with chronic diseases, the symptoms progress to pneumonia and respiratory distress, requiring in 20% of cases hospital treatment.⁴ In addition, 80% of COVID-19 infections in China were mild flu-like symptoms.⁴ Our data corroborated those of this study. Indeed, a large number of symptomatic patients developed mild flu-like symptoms (90% of symptomatic patients) (Table 2). Only two patients had respiratory distress and 78 (94%) had no chronic disease (Table 2). However, since our data only considered the first cases in Gabon, the number of cases was low and probably underestimated.

In the 50 days following the report of the first case of COVID-19 on March 12, 2020, 87% of confirmed cases declared that they had not made a recent trip (Figure 1A), which would suggest that the virus was introduced in Gabon before March. The WHO declared the COVID-19 pandemic on March 11, 2020 meaning that a large number of countries across the African continent were affected by this period. The flow of travelers and international trade between Gabon and various affected countries would explain the introduction of the virus in Gabon. The slow spread is said to be linked to the fact that the government acted by closing borders, closing schools, quarantine of all suspected and confirmed cases and restricting movement as early as the end of March.

Some studies report a very high SARS-CoV-2 viral load in nasal swabs and others in throat swabs.⁸ We found that the viral loads were significantly higher in the nasopharyngeal samples than in the oropharyngeal samples ($p=0.03$) (Figure 2A). Our data is similar to a study which compared the viral load in different clinical samples (saliva, spectrum, urine, nasopharyngeal and oropharyngeal samples) and showed that the viral load was the highest in the nasopharynx.⁵ One review based on seven studies measured and compared the viral load in pre-symptomatic, asymptomatic and symptomatic patients. It reported little to no difference between the SARS-CoV-2 viral load in the three categories of patients.⁸ Our results which showed no correlation between the viral load and the clinical symptom of patients are in accordance with these studies. Some studies compared the SARS-CoV-2 viral loads in patients of different age groups in order to establish a possible link between the viral load, the duration of symptoms and the age of the patients. They concluded that there was no significant difference with regard to viral load or the duration of virus detection between adults and children.⁸ The number of patients in each age group and the relationship between viral load and infectivity should be considered. Our study shows that there is no difference between patients under 15 and patients over 15 (Figure 2).

It is important to characterize the virus SARS-CoV-2 by identifying the genotype circulating in Gabon in order to set up an efficient response to this pandemic. The first SARS-CoV-2 genome of the isolate Wuhan-Hu-1 was sequenced in China (accession number NC_045512 or MN908947) in January 2020. The 1273 amino acid spike glycoprotein (S) located on the virion surface is essential for the entry of the virus in host cells. Thus, we compared the 3822 nucleotide fragments of the S gene with sequences published in the GenBank database. The alignment of the sequences and the phylogenetic tree showed that the sequences were distributed in two clusters. One cluster was composed of the SARS-CoV-2 reference strain sequences without the D614G mutation, including two sequences of Gabon, 56GAB_S and 121GAB_S. The second cluster included sequences without the D614G mutation. The three other sequences 34GAB_S, 62GAB_S and 297GAB_S were in the second cluster (Figure 3). The literature reported a frequent mutation in the S gene (position 23403A>G) coding the variant of S protein D614G¹⁰ (<http://dx.doi.org/10.1038/s10038-020-0808-9>). This mutation emerged in Europe and North America on January 29 and February 28, 2020 respectively.²⁴ Initially, the D614 and G614 variants reached an equal ratio in Europe and North America at the end of February, then in March, the G614 variant started to circulate predominantly in both continents from March.²⁴ The sequence 34GAB_S isolated from the sample of the first case of SARS-CoV-2 diagnosed in Gabon on March 12, 2020, had the D614G mutation. Among the five samples collected at the beginning of the pandemic in Gabon, three had a SARS-CoV-2 strain with the D614G mutation. These results corroborated that of a study which reported the emergence of the G614 variant in Africa on March 13, 2020.²⁴ At this time, the circulation of both the D614 and G614 variants in Europe and the world suggested a simultaneous

introduction of the two strains in Gabon. A Japanese study showed a significant positive correlation between the S protein 614G variant and fatality rates (<http://dx.doi.org/10.1038/s10038-020-0808-9>). They analyzed the frequency of mutations in the SARS-CoV-2 genome in 28 countries which they grouped into three clusters in which they show a correlation between the mutations and the fatality rate (<http://dx.doi.org/10.1038/s10038-020-0808-9>). The full genome of a large number of our Gabonese strains would provide more information on the multiple mutations and their impact on the COVID-19 disease in Gabon.

In conclusion, this study reported the first data on the COVID-19 pandemic in Gabon. The data showed that most of the infected people were asymptomatic. The viral load was higher in the nasopharyngeal samples. The S gene analyzed suggested both introduction of the D614 and G614 variant in Gabon. However analysis of the full genome in order to identify possible mutations, as well as immunological investigations would provide additional data and increase knowledge about the circulation and the impact of the virus in Gabon and more generally in Africa.

Data availability

BioStudies: “Epidemiology of first cases of SARS-CoV-2 infection, from March to April 2020 and molecular characterization of the spike protein, in Gabon”. Accession number S-BSST800: <https://identifiers.org/biostudies:S-BSST800>

NCBI Gene: Accession numbers:

MW512911: <https://www.ncbi.nlm.nih.gov/nuccore/MW512911>

MW512912: <https://www.ncbi.nlm.nih.gov/nuccore/MW512912>

MW512913: <https://www.ncbi.nlm.nih.gov/nuccore/MW512913>

MW512914: <https://www.ncbi.nlm.nih.gov/nuccore/MW512914>

MW512915: <https://www.ncbi.nlm.nih.gov/nuccore/MW512915>

Consent

Informed consent for publication of the participants/patients’ details was obtained from the participants/patients/parents/guardian/relative of the participant/patient.’

Acknowledgments

We would like to thank the staffs of all the health centers. We also wish to thank the members of the CIRMF’s public health emergencies group, Kumulungui Brice, Yala Jean Fabrice, Mouinga Ondeme Augustin, Ngoubangoye Barthelémy, Onanga Richard, Kengne Pierre, Oyegue Lydie Sandrine, Kassa Kassa Roland Fabrice, for their suggestions concerning the management of the pandemic. We also thank Lekouna Lady Charlène and Mombo Illich Mandred for their participation in the meeting.

We acknowledge Heidi Lançon for the English revision of the manuscript.

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Version 1

Reviewer Report 08 August 2022

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Anggraini Dwi Sensusiati

Department of Radiology, Universitas Airlangga Hospital, Surabaya, Indonesia

This article is about the epidemiological data of the first SARS-Cov-2 infection case in Gabon and the molecular characteristics data of the spike protein. Based on the results of the study, it was explained that the first COVID-19 case in Gabon was found on March 12, 2020. The characteristics of confirmed COVID-19 patients are mostly asymptomatic. While symptomatic patients mostly have influenza-like symptoms (ILI symptoms), namely fever; runny nose; cough; sore throat; and some of them experience respiratory distress. Some patients with ILI symptoms are also accompanied by anosmia, ageusia, diarrhea, or asthenia. The results of the study also stated that there was no relationship between comorbidities and SARS-Cov-2 infection. Based on travel history, all samples confirmed to have COVID-19 who traveled abroad had respiratory symptoms.

In the results of the study related to the molecular characteristics of spike proteins, it was explained that the viral load was higher in nasopharyngeal samples than in oropharynx; there was no significant difference in the viral load of children under 15 years of age with people aged 15 years and over; and there is no correlation between the clinical symptoms of confirmed COVID-19 patients and viral loads.

Phylogenetic analysis in this study was carried out with five sequences of 3822 spike S gene nucleotides, obtained from 5 patients (2 symptomatic patients originally from Paris; the first case in Gabon; and 2 contact cases without travel history). The S genes that have been analyzed suggest the introduction of the D614 and G614 variants in Gabon.

In general, this research is good. The results of the study are important to enrich data and literature related to the epidemiology of COVID-19 in all countries around the world, including Gabon. The results of molecular research of the virus are also useful to enrich the genome database of the COVID-19 virus, which can be used for other molecular research in the future.

The systematic of writing this article has also been good. The writing of research methods is good and complete. Especially in the study design section and patient information. The number of samples used is also sufficient. The writing of the results and discussion have also answered the

purpose of the study.

To make the discussion section more comprehensive, it would be better if the authors add more information related to the profession and the activities of the sample. It is also necessary to add more detailed information related to comorbidities such as the level of blood sugar in diabetic patients.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: COVID-19, Radiology, Laboratory Findings, Neuroradiology, and Head & Neck Imaging, Artificial Intelligence for COVID-19.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 31 Oct 2022

Sonia LEKANA-DOUKI

Responses to reviewer 2

Manuscript title: Epidemiology of first cases of SARS-CoV-2 infection, from March to April 2020 and molecular characterization of the spike protein, in Gabon

We were unable to obtain some detailed data such as sugar levels in diabetic patients because some data were missed during the implementation of monitoring at the beginning of the pandemic.

We changed the title and removed the sequencing section to better accommodate the other reviewer's suggestions.

Title: "Epidemiology of first cases of SARS-CoV-2 infection, from March to April 2020" (line 26).

The keywords have been modified: "COVID-19, Diagnostic, Epidemiology, Viral load, Gabon" (line 52).

Since the first data presented in this study, we have carried out another study on the complete genomes of samples from the first wave of the pandemic in Gabon (line 273, 286). Thus, we have deleted the paragraph on Sanger's method in the methods section as well as the paragraph SARS-CoV-2 sequences in the results. We have modified the discussion in this way Three samples for which we had done sequencing by the Sanger method were selected in this other study to obtain the complete genome. These were samples 34, 56 and 62. We have therefore added information on their lineage to the discussion (line 273). We deleted the Figure 3.

As a result, the title of the study has been modified because the data concerning the characterization of the strains by the genotyping of the S gene are no longer provided in this study but constitute a comment in the discussion which provided additional information on the lineage of the first cases of COVID-19 in Gabon.

Competing Interests: No competing interests were disclosed.

Reviewer Report 18 July 2022

<https://doi.org/10.5256/f1000research.78125.r141590>

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Babatunde Motayo

Department of Virology, College of Medicine, Federal Medical Centre Abeokuta, University of Ibadan, Ibadan, Nigeria

I thank the handling Editor of the F1000research for selecting me as a reviewer for the paper titled "Epidemiology of the first cases of SARSCoV-2 infection from March to April 2020 and molecular characterization of the Spike protein in Gabon". I have gone through the paper and have made a decision of major revision. My comments and suggested revision are outlined below.

MAJOR COMMENTS

ABSTRACT: The background section of the Abstract needs to be re-written to summarize actual events that led to the study. The method section is not explicit enough nothing was mentioned about how the study was carried out or actual techniques used such as RT-PCR and sequencing,

please re-cast to reflect the actual techniques used to carry out the study.

METHODS: The authors did not mention anything about ethical approval and consent, this should be stated clearly if it was sought. The authors did not apply the generally acceptable classification system to properly genotype the Gabonese isolates identified, that is the PANGO-lineage and Nexclade classification. These two genotyping tools should be done and reported appropriately. Also, the associated genomic data that was generated to generate sequence alignments and the phylogenetic tree is not enough to conclusively derive any significant phylogenetic relationship, considering the enormity of data already available in databases such as GenBank and GISAID, at least a reasonable number of sequences from each sub-region of Africa and major continents, including China should be included. The authors should update with more sequences from diverse SARSCoV Lineages including major variants of concerns, variants of interest, and relevant global sequences and re-do the alignment and ML tree construction. The authors are also advised to improve on the tree visualization the labels should be bolder and more informative, I suggest the authors make use of tree visualization tools such as FigTree, or ggTree for better representation, authors are also advised to construct an MCC time tree showing lineage assignments of the Gabonese isolates along with relevant circulating lineages in Africa and their dates of introduction.

RESULTS: The results in the first two sub-sections should be merged since they both report the same thing in different sub-populations. The sub-heading of the last sub-section SARSCoV-2 sequences should be re-casted e.g Molecular characterization of S-gene of SARSCoV-2. The molecular data from the suggested analysis such as PANGO-lineage should be included also; results of phylogenetic analysis should reflect lineage clustering, geographic diversification from previous/more recent related isolates, e.t.c. A time tree will also show introductions of same lineage, or multiple lineages within the time frame under study.

DISCUSSION: The discussion aspect has to be re-written to include the data generated from the suggested additional analysis particularly the genotype and potential implications. Also, the sample size of the sequences is too small and should be included as a limitation to the study. Also, other limitations should be clearly stated such as the inability to sequence the complete genome of the Gabonese SARSCoV-2 strains.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Virology; Molecular Epidemiology; Evolutionary Biology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 31 Oct 2022

Sonia LEKANA-DOUKI

Responses to reviewer 1

Manuscript title: Epidemiology of first cases of SARS-CoV-2 infection, from March to April 2020 and molecular characterization of the spike protein, in Gabon

Manuscript ID :

Sonia Etenna Lekana-Douki^a, Nadine N'dilimabaka^{a,b}, Elvire Mbongo-Kama^c, Marisca Kandet Yattara^d, Armel Mintsia Ndong^e, Audrey Michel Ngonga Dikongo^a, Julia Cyrielle Andeko^a, Ornella Zong Minko^a, Danielle Styvie Koumba Mavoungou^a, Abdoulaye Diané^f, Arsène Mabika Mabika^g, Telstar Ndong Mebaley^a, Nal Kennedy Ndjangangoye^{a,h}, Octavie Banga Mve-Ella^a, Linda Bohou Kombila^a, Joa Braithe Mangombi^a, Jeordy Dimitri Engone Ondo^f, Gaël Darren Maganga^{a,i}, Jean-Bernard Lekana-Douki^{h,j}

Reviewer 1

APPROVED WITH RESERVATIONS

I thank the handling Editor of the F1000research for selecting me as a reviewer for the paper titled "Epidemiology of the first cases of SARSCoV-2 infection from March to April 2020 and molecular characterization of the Spike protein in Gabon". I have gone through the paper and have made a decision of major revision. My comments and suggested revision are outlined below.

MAJOR COMMENTS

ABSTRACT: *The background section of the Abstract needs to be re-written to summarize actual events that led to the study. The method section is not explicit enough nothing was mentioned about how the study was carried out or actual techniques used such as RT-PCR and sequencing, please re-cast to reflect the actual techniques used to carry out the study.*

We have re-written the section "ABSTRACT" with information about events that led to the study and information about the PCR and the sequencing (line 31 to 40).

METHODS: *The authors did not mention anything about ethical approval and consent, this should be stated clearly if it was sought.*

We added the following information about ethical approved and consent in the section Ethics declaration:

"This study was approved by the national ethics committee (N°0003/2020/CNER/SG/P) and

was performed in accordance with the ethical standards of the Declaration of Helsinki of 1964. Consent was obtained before sampling.” (line 305).

The authors did not apply the generally acceptable classification system to properly genotype the Gabonese isolates identified, that is the PANGO-lineage and Nexclade classification. These two genotyping tools should be done and reported appropriately. Also, the associated genomic data that was generated to generate sequence alignments and the phylogenetic tree is not enough to conclusively derive any significant phylogenetic relationship, considering the enormity of data already available in databases such as GenBank and GISAID, at least a reasonable number of sequences from each sub-region of Africa and major continents, including China should be included. The authors should update with more sequences from diverse SARSCoV Lineages including major variants of concerns, variants of interest, and relevant global sequences and re-do the alignment and ML tree construction. The authors are also advised to improve on the tree visualization the labels should be bolder and more informative, I suggest the authors make use of tree visualization tools such as FigTree, or ggTree for better representation, authors are also advised to construct an MCC time tree showing lineage assignments of the Gabonese isolates along with relevant circulating lineages in Africa and their dates of introduction.

Since the first data presented in this study, we have carried out another study on the complete genomes of samples from the first wave of the pandemic in Gabon (line 273, 286). Thus, we have deleted the paragraph on Sanger's method in the methods section as well as the paragraph SARS-CoV-2 sequences in the results. We have modified the discussion in this way. Three samples for which we had done sequencing by the Sanger method were selected in this other study to obtain the complete genome. These were samples 34, 56 and 62. We have therefore added information on their lineage to the discussion (line 273). We deleted the Figure 3.

As a result, the title of the study has been modified because the data concerning the characterization of the strains by the genotyping of the S gene are no longer provided in this study but constitute a comment in the discussion which provided additional information on the first cases of COVID-19 in Gabon :

“Epidemiology of first cases of SARS-CoV-2 infection, from March to April 2020” (line 26).

The keywords have been modified: “COVID-19, Diagnostic, Epidemiology, Viral load, Gabon” (line 52).

RESULTS: The results in the first two sub-sections should be merged since they both report the same thing in different sub-populations. The sub-heading of the last sub-section SARSCoV-2 sequences should be re-casted e.g Molecular characterization of S-gene of SARSCoV-2. The molecular data from the suggested analysis such as PANGO-lineage should be included also; results of phylogenetic analysis should reflect lineage clustering, geographic diversification from previous/more recent related isolates, e.t.c. A time tree will also show introductions of same lineage, or multiple lineages within the time frame under study.

We merged the first two sub-sections and changed the title of the sub-section: Epidemiological data of suspected and confirmed cases (line 146).
We deleted the sub-section SARS-CoV-2 sequences for the reasons previously mentioned.

DISCUSSION: The discussion aspect has to be re-written to include the data generated from the suggested additional analysis particularly the genotype and potential implications. Also, the sample size of the sequences is too small and should be included as a limitation to the study. Also, other limitations should be clearly stated such as the inability to sequence the complete genome of the Gabonese SARSCoV-2 strains.

We have re-written the discussion by mentioning the whole genomes made in another study which provides information on the lineages that circulated at the beginning of the pandemic (line 273, 286).

Competing Interests: No competing interests were disclosed

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