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Mass Screening Using Antigenic and Molecular Diagnostic Tests of COVID-19 in Bangui at the Beginning of the Second Wave in July 2021

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Abstract

Objective: COVID-19 surveillance was established as early as March 2020 in the Central African Republic (CAR), after the WHO statement relating to the identification of several cases outside China. However, given the non-performing molecular biology technical platform in many developing countries in sub-Saharan Africa, the second wave promised to be surprising and formidable. In this context, a mass survey was launched in Bangui to determine the prevalence of COVID-19. **Patients and Methods:** From March 18 to April 2, 2021, a mass screening campaign took place in tourist places, companies and the main hospital infrastructures. Nasopharyngeal swab samples were collected from participants with and without symptoms of Influenza-like illness (ILI) and stored in VTM tubes. The Ag (COVID-19) and RT-PCR tests were carried out in Bangui at the LNBCSP. The sequencing of RT-PCR SARS-CoV-2 positives was carried out at the INRB. **Results:** We included 1480 participants of whom 33 (2.23%) were SARS-COV-2 positive, of whom 24 were male and 9 female. This sex difference was statistically significant (p = 0.012) as the sex ratio M/F was 1.09. Sampling sites located in the 1st arrondissement were the most prolific (p = 0.006) and were sequenced. In addition to the analysis of the 33 samples from the predefined sites under study, 17 control sequences from the provinces generated during the same

period are added. We detected 2 Variants Of Concern (VOC) including the predominant B.1.620 (43.86%) followed by B.1.1.7 or Alpha (5.10%). **Conclusion:** The study showed the importance of surveillance and the availability of means of diagnosis of COVID-19. The identified risk factors were sex and sampling site. This study has shown the importance of setting up sentinel sites for COVID-19 surveillance in all regions of the country and the appropriate use of the anti-COVID-19 vaccine.

Keywords

COVID-19, Variants, Central African Republic

1. Introduction

Starting from Wuhan in China's HUBEI Province in December 2019, COVID-19, with 769,483,224 confirmed cases and 6,954,911 deaths as of August 13, 2023 [1] is the most important pandemic recorded since the Spanish flu in 1918. As soon as March 2020, the WHO (World Health Organization) recommended mass laboratory diagnosis (already available) as the main backbone of the response. Despite significant preventive measures and resources allocated to fight this new disease, its worldwide spread and its dramatic consequences for the population were not prevented [2] [3] [4] [5].

The access to diagnostic tests and vaccine availability have evidenced a wide gap between rich and poor countries and the economic and human consequences were particularly notable in the latter [6] [7].

At the end of 2020, the African continent, has been relatively unaffected by COVID-19, with an incidence of less than 5%.

However, as soon as January 28, 2021, WHO reported a sharp increase in the number of COVID-19 cases and disease-related deaths in the AFRO region.

During the week of January 18-24, 2021, more than 175,000 new COVID-19 cases and 6200 related deaths have been recorded. Moreover, a 50% increase in weekly infection numbers was observed from December 29, 2020 to January 25, 2021 [8] [9] [10]. The Central African Republic (CAR), like other bordering countries, Cameroon and the Democratic Republic of Congo, experienced two waves from April 27th 2020 to June 6th 2021 and April 2020 to September 2021 [11]-[18], respectively.

The first diagnosis of COVID-19 in the Central African Republic (CAR) has been reported on March 14th, 2020. Eventually the disease spread over the country and surveillance screenings and response strategies were implemented. A peak of cases was observed in June-July 2021 and occasional clusters were observed in people from international and military organizations [19]. The "testing" capacities of CAR have been implemented in the two reference laboratories, the National Laboratory of Clinical Biology (LNBCSP) and the Pasteur Institute of Bangui (IPB), which have permitted epidemiological studies. Ge-

nomic surveillance has been programmed consisting of mass sampling campaigns using antigen tests, cheap and easy to use, in which positive samples were eventually submitted to molecular diagnosis (RT-PCR). Thanks to a regional collaboration, 20 RTPCR positive samples may be sent per week to the "Institut National de Recherche Biomédicale" (INRB) Kinshasa, (Democratic Republic of Congo, DRC), a WHO Collaborating Laboratory Center, for viral genome sequencing.

The aim of the present survey was to provide information on the circulation of SARS-CoV-2 variants from samples collected in the city of Bangui using antigen tests, in which positive results were submitted to a RT-PCR and eventually sent to INRB.

2. Materials and Methods

2.1. The Framework of the Study

Previous communication campaign: An extensive communication campaign preceded the launch of COVID-19 mass testing. The communication channel of the Ministry of Health and Population was used with the permission of the Government as part of the COVID-19 response.

The recruiting of patients was on a voluntary basis for those who wanted to know their infectious status to Severe Acute Respiratory Syndrom-Coronavirus-2 (SARS-CoV-2). For children under the age of 12 the presence of an accompanying adult was necessary.

The National Laboratory of Clinical Biology and Public Health (LNCBPH) of Central African

Republic (CAR) coordinated the survey, from the collection of samples to the molecular diagnosis.

Site selection. The selection of sampling sites was carried out on the basis of their experiences and their involvement in the pandemic management process, either directly, either as a health facility or as a support a member of the WHO representative mission.

Site teams. Under the supervision of the Ministry of Health and in collaboration with its partners, 185 civil protection officers have been trained to sample and carry out COVID-19 tests in the community at the NLCBPH. These officers were familiar with such large-scale projects.

The sites were organized into 3 teams of 10 people including a site manager. The latter was responsible for sending the samples to the NLPH, and making a daily report of the activities of his site to the laboratory activities' coordination at the NLCBPH.

Data management. A form was completed for each sampled volunteer. Patients were asked to present themselves with a copy of their identity form completed with their phone number and exact address. These personal data were transmitted to the data management team at the LNCBPH where they were treated anonymously.

Duration of the project. The survey lasted two weeks in May 2021 situated at the beginning of the second wave of COVID-19 in the CAR. Operationally, a wave corresponds to a rapid, exponential increase in the number of contaminations over a sustained period.

Size of sample. The sample size consisted of people tested during the campaign.

2.2. Sampling and Analysis Techniques and Methods

2.2.1. Antigenic Tests

The rapid immunochromatographic Standard Q Covid-19 Ag® antigen test (Biosensor SD, Chungcheongbuk-Do, Republic of Korea) was used on the collected nasopharyngeal samples. The test has been introduced in November 2020 and its quality determined against RT-PCR by the LNBCSP Quality Assurance team (Sensitivity 82%, Specificity = 98%). Two negative and three positive samples were used as internal controls for each new batch of reagents used (ISO 15 189).

These rapid tests were performed and read on the spot in accordance with the manufacturer's recommendations. If the patient had a positive result, a second sample was taken and collected in virological transport medium (VTMs) and sent within an hour to the LNCBPH for molecular diagnosis. In case of impossibility, the patient was asked to come at the laboratory.

2.2.2. Molecular Diagnosis

RNA extraction was performed manually with the RNA kit Qiagen (Hilden, Germany) following the manufacturer's instructions. After reverse transcriptione, PCR was performed on ABI FAST platforms (Thermo Fisher Scientific, Waltham, MA USA) or CFX96® (BIORAD, Marnes-laCoquette, France) using QIAamp's® DAAN GENE PCR kits (Qiagen). Only the RT-PCR confirmed positive samples were sent to the INRB for sequencing.

In case of RT-PCR negativity, people were presumed positive, treated as such, and put in quarantine from which they were liberated only in case of a negativity of a RT-PCR tests done 10 days later. Arrangements were also made to test by RT-PCR the possible contacts of all positive patients.

2.2.3. Sample Selection and Sequencing

All the samples positive for both Antigen and molecular tests were eligible for sequencing and provided they did not fulfill the rejection criteria of the WHO:

- SARS-CoV-2 infection in the days following vaccination (not applicable at this time);
- A discrepancy between molecular and antigen tests the latter being positive while RT-PCR remains negative (none observed);
- Patient with a severe or devastating forms of COVID-19 (none observed);
- Low viral load (none observed).

RNA extracts or samples were sent in triple layer boxes to the INRB using the

UNHAS (United Nations Humanitarian Airlift service), which ensure a shuttle link between Bangui and Kinshasa, and with the support of WHO logistic (transportation in ice).

2.2.4. Sequencing Technique

Positive samples with cycle threshold (CT) values < 30 were sent for sequencing to the INRB (Kinshasa, RDC). Seventeen RNA obtained from the laboratory routine diagnosis during the same period than the survey were sent in parallel.

Libraries were prepared according to the Illumina COVID-19 ARTIC v3 V.5 library construction and sequencing protocol, using the NEBNext Ultra II DNA Library Prep Kit (New England Biolabs Inc., Ipswich, MA, USA). Libraries were quantified (Qubit DNA BR, Thermo Scientific, Waltham, MA, USA), normalized and pooled, and sequencing was performed using an Illumina MiSeq 100 (Illumina, San Diego, CA, USA). The complete genome was sequenced when possible. Otherwise the available obtained partial sequences were used (Si le génome complet est obtenu à chaque fois supprimer ces deux phrases).

2.2.5. Bioinformatics Analysis

The raw FASTQ files were quality checked using FASTQC and MultiQC to generate a single quality report for all samples. To avoid introducing errors, vcf files were generated by filtering with read depth greater than 7 and mapping quality greater than 10 using bcf tools, and only SNPs of high quality and with depth of high site coverage were considered for the downstream analysis. Our sample sequences and SARS-CoV-2 FASTA sequences were deposited on the GISAID database. The reads from FASTQ samples were assembled using the Flye tool, the resulting assembled contigs were joined using contigMerger to generate a single scaffold per sample. The per-sample scaffold was combined into a single multifasta file that was used in the phylogenetic analysis. Multiple sequence alignment was performed using MAFFT version 7.310 and the phylogenetic tree was constructed using the maximum likelihood method in MEGA version 11.

The generated resulting tree was manipulated using the Figtree package.

2.3. Data Analysis

The collected personal were entered into Epi-info version 3.3.7 (CDC ATLANTA). Statistical analyses were performed using the chi-2 test and the Odds Ratios.

3. Results

During the survey period 1480 antigen tests have been performed of which 33 were antigen positive (**Table 1**) (2.2%). They were all confirmed positive by RT-PCR with a CT value between 16 and 29. The average age of volunteers was 41 (**Table 1**). There was a small difference of positivity between the age groups. Male patients were more infected than women (OR = 2.43). The difference between districts was important and the 6th had the higher prevalence of positive. On the contrary none positive test was found in the 7th district (**Table 2**).

Table 1. Prevalence of SARS-Cov-2 positive antigenic tests in volunteers according to age and sex (survey July 2021).

Patients	Antigen test positive	n
Mean age (year) ± DS	40.45 ± 11.24	40.66 ± 11.08
Median age (years)	41.00	40.00
Age categories (years)		
1 - 20	1 (2.5%)	40
21 - 30	7 (2.7%)	255
31 - 40	8 (1.7%)	475
40 - 50	10 (2.5%)	402
>50	7 (2.3%)	309
Gender (OR = 2.47)		
Male	24 (3.1%)	774
Female	9 (1.3%)	706
Sex-ratio M/F = 1.09		
Total	33 (2.2%)	1480

Table 2. Prevalence of SARS-Cov-2 positive antigenic tests according to the different districts of |Bangui (RCA) volunteers reside.

Collection sites	Antigenic tests positive	n	p-value
1st District	9 (3.5%)	259	
2nd District	9 (2.4%)	376	
3rd District	3 (1.3%)	224	
4th District	2 (2.0%)	99	
5th District	1 (0.6%)	163	**<0.006**
6th District	6 (9.8%)	61	
7th District	0 (0%)	61	
9th District	3 (1.3%)	237	
Total	**33 (2.2%)**	**1480**	

In addition to the analysis of the 33 samples from the predefined sites under study, 17 control sequences from the provinces generated during the same period are added. Thus, among these 50 strains, the B.1.620 variant was predominant (46 strains, 86%) and the same prevalence was found for the sample suveys. (25 strains, 86%). The 4 remaining strains belong the B.1 (2 strains, 4%) and B.1.1.7 (5 strains, 10%) variants (Figure 1 and Figure 2).

4. Discussion

This study, conducted as part of a mass campaign using COVID-19 antigen tests was limited to BANGUI and do not preclude of what happens elsewhere in the country [14] [16]. The samples have been obtained at the time of an epidemic episode, during which the virus actively circulated in the city. The difference of prevalence among the different districts may be explained by population concentration and socio-economic factors. As men are more prone to have a professional

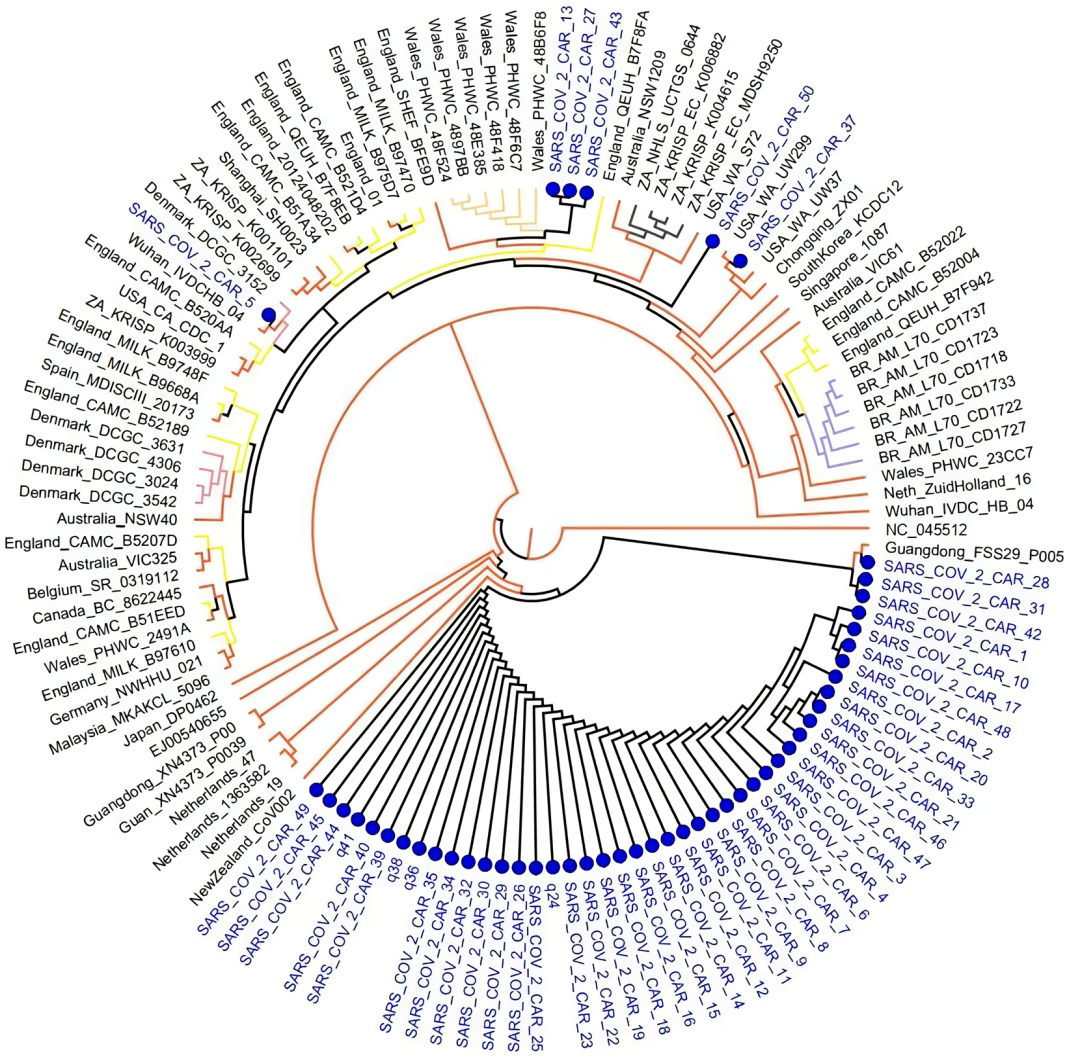

Figure 1. Phylogenetic tree of strains detected during the first mass campaign in C.A.R. Figure represents the variants circulating both in the CAR and to countries around the world such as England, Germany, Belgium, Denmark, the United States, etc. We note here that the samples from other countries are the sequences collected on GISAID from the same study period. In blue, fifty (50) samples from RCA 43 are B.1.620 variants, materialized by the massive grouping on this branch at the bottom of the figure of the phylogenetic tree. The rest of the 7 sequences are divided between sub-variants B.1 and B.1.1.7 which were already circulating in Europe in the rest of the world.

occupation and women tend to be more occupied at home may explain the difference between sexes. However an hormonal factor have been suggested [20] [21].

The prevalence of SARS-CoV-2 infections in our sample was 2.23%. Much higher prevalences have been reported by Manirakiza and Lango Yaya during the first and second waves [19] [20].

As the molecular tests have been conducted solely in antigen positive patients some positive results may have been missed because antigenic tests are less sensitive than the molecular ones [22] [23]: their number is likelty limited and estimated as not more than 20.

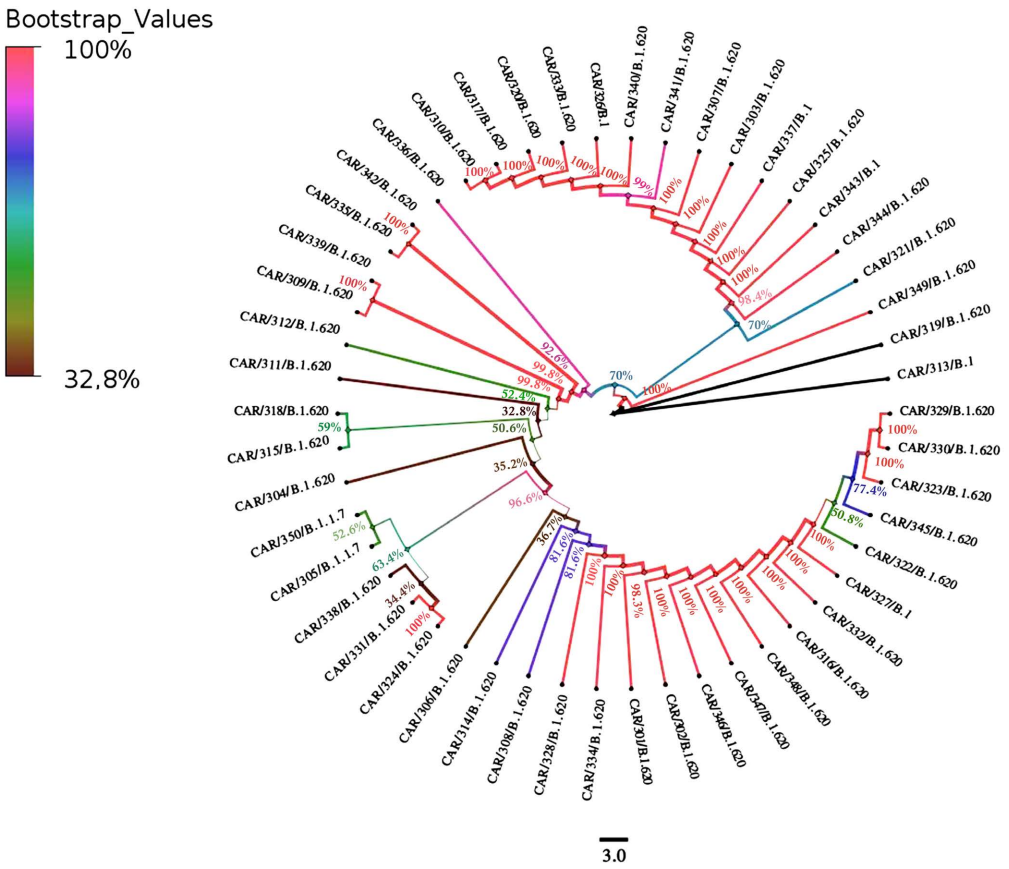

Figure 2. Affiliation of mutants detected during the first mass campaign in C.A.R. The figure shows the distribution of variants according to their genetic proximities or affiliations. The distance which separates the different sub-lineages is the ratio between the size of the genome and the number of mutations undergone by the reference strain and the variant. Here the variant is Delta (lineage) and the strain is Wuhan, the distance is 3.0. The closest sublineages are indicated by the boostrap color variation. The distance is indicated by the percentage values in the cladograms. Here we have several "B.1.620" subvariants which are not located on the same branch, although close.

The network collaboration of sub-regional [11] [24] laboratories have made possible the characterization of the circulating variants of SARS-CoV-2 in the sub-region and identified the strains responsible of the variants that influenced the associated with the second wave of COVID19 .Thus despite limited resources it is possible to establish a local genomic surveillance useful for the fight against this disease [25].

Similar studies at the start of the pandemic reported the same trends in Africa and around the world. Several variants of the "B.1.620" parent strain have been evidenced as they are on separate branches but remain closely related, These differences are due to mutations which may modify the properties (virulence, escape to the immune system, loss of efficacy of the vaccine…) as compared to the parent strain and their appearances are facilitated by the rapid circulation of the virus in the population [26]. The two SARS-Coronavirus-2 variants B.1.1.7 and B.1.620, in addition to the original strain from China evidenced in CAR were also present in Cameroon, and DRC [17] [18] [27] [28]. The B.1.620 va-

riant was identified primarily in Spain although a phylogenetic study is in favor of an African origin possibly from RCA or Cameroon. The presence of the same variants in neighboring countries may be explained by crossings of the borders (common habits) as well as air travels.

5. Conclusion

This survey, although limited to Bangui, took place at the beginning of the second wave of COVID-19 and gave a low prevalence of the disease but also provided a picture of the circulating strains of SARS-Coronavirus-2. These strains were the same as the ones observed in the neighbor countries (**Cameroun, DRC, Gabon**) where a burst of COVID-19 cases was observed at the same time. Such random informative surveys should be undertaken regularly for the government to adopt adequate epidemiological and preventive measures. This study also evidenced the benefit of an international collaboration to tackle the surveillance of infectious diseases despite limited resources.

Ethical and Administrative Procedures

This study which was part of the national response against Covid-19 was approved by the Institutional Ethical Review Committee of the Ministry of Health and Population of the Central African Republic (CAR). Administrative authorization was obtained from the Minister of Health and Population of the CAR. Voluntary informed consent was obtained from the patients. In addition, we received ethical clearance from the Ethics and Scientific Committee of the Faculty of Health Sciences from University of Bangui (N32/FACSS/CES.2020).

Consent for Publication

All authors read and approved the manuscript before publication.

Availability of Data and Materials

Given the sensitive nature of the data, access to the database is only possible with authorization from the Ministry of Health and Population. The same is true of the preliminary report of this mass campaign available in the archives of the Department of Health.

Data on GISAID can however be consulted if necessary (https://www.epicov.org/epi3/frontend#5aa6aa)

Financement

The mass campaign benefited from COVID-19 Funds provided by the World Bank via the government. The tests were provided by WHO, recipient of these Logistics Funds, and by Africa CDC and FIND.

Authors' Contributions

We certify that all authors have contributed to the realization of the article

whether in the technique, coordination and document review.

CDR, ELY, PS, SP, EJK, RMS, BK: project design and laboratory techniques.

CDR, NK, LSH, OS, WSN, FXMK, GG, JDDL, BKPS: data analysis and outcome interpretations and coordination of screening activities.

CDR, NK, LSH, WSN, FXMK, GG, JDDL, BK, UV, MRDB, AS, CDMK: Elaboration of the manuscript and document review.

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Conflicts of Interest

The authors declare themselves free from any conflict of interest.

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