SARS-CoV-2 B.1.214.1, B.1.214.2 and B.1.620 are predominant lineages between December 2020 and July 2021 in the Republic of Congo

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ABSTRACT

Background: SARS-CoV-2 variants have been emerging and are shown to increase transmissibility, pathogenicity, and decreased vaccine efficacies. The objective of this study was to determine the distribution, prevalence, and dynamics of SARS-CoV-2 variants circulating in Brazzaville, the Republic of Congo (ROC).

Methods: Between December 2020 and July 2021, a total of n=600 oropharyngeal specimens collected in the community were tested for COVID-19. Of the samples tested, 317 (53%) were SARS-CoV-2 positive. All samples that had a threshold of Ct < 30 (n=182) were sequenced by next-generation sequencing (NGS), and all complete sequenced genomes were submitted to GISAID; lineages were assigned using pangolin nomenclature and a phylogenetic tree was reconstructed. In addition, the global prevalence of the predominant lineages was analysed using data from GISAID and outbreak databases.

Results: A total of 15 lineages circulated with B.1.214.2 (26%), B.1.214.1 (19%) and B.1.620 (18%) being predominant. The variants of concern (VOC) alpha (B.1.1.7) (6%) and for the first time in June delta (B.1.617.2) (4%) were observed. In addition, the B.1.214.1 lineage first reported from ROC was observed to be spreading locally and regionally. Phylogenetic analysis suggests that the B.1.620 variant (VUM) under observation may have originated from either Cameroon or the Central African Republic. SARS-CoV-2 lineages were heterogeneous, with the densely populated districts of Poto-Poto and Moungali likely the epicenter of spread.

Conclusion: Longitudinal monitoring and molecular surveillance across time and space are critical to understanding viral phylogeography, which could have important implications for transmissibility and impact infection prevention and control measures.

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Introduction

The ongoing global Coronavirus disease 2019 (COVID-19) pandemic has caused significant mortality and morbidity (Velavan and Meyer 2020), requiring unprecedented efforts to develop vaccines for COVID-19. The SARS-CoV-2 virus is constantly mutating, leading to new variants. The variants can alter viral properties, leading to increased transmissibility and virulence, compromising treatments, diagnostics, and vaccine efficacy. Currently, emergence of SARS-CoV-2 variants with extensive mutations in the spike (S) protein has raised concerns about increased transmission, vaccines, therapeutics, and diagnostics (Wu et al. 2021; Wang, Nair, et al. 2021). In the early stages of the pandemic, the effects of selective advantage of SARS-CoV-2 S mutation D614G on transmissibility were documented (Korber et al. 2020; Volz et al. 2021). Further it is well known that the S protein mediates SARS-CoV-2 entry into host cells and is crucial for protective antibody responses, also being the antigen component of all the COVID-19 vaccines (Kemp et al. 2021).

Multiple variants of SARS-CoV-2 have been documented worldwide during this pandemic. Particularly, “variants of concern” (VOC), alpha (B.1.1.7), beta (B.1.351), and gamma (B.1.1.28/P1), delta (B.1.617.2) are associated with increased transmission, increased mortality, and decreased vaccine efficacies (Bal et al. 2021; Gaymard et al. 2021; Challen et al. 2021; Davies et al. 2021; Twoghi et al. 2021). It is apparent that the variant alpha, which contains a deletion at position 69-70 of the S protein, leads to an “S gene target failure”, i.e. a loss of amplification (Bal et al. 2021; Gaymard et al. 2021), whereas the results for Beta are more concerning, as this variant is significantly more resistant to neutralization by convalescent plasma and vaccines’ sera, mainly due to the E484K mutation (Wang, Nair et al. 2021). The P.1 and delta is reported to increase transmissibility, virulence, and overall reduction in effective neutralization (Voloch et al. 2020; Felipe et al. 2021; Garcia-Beltran et al. 2021; Voloch et al. 2021). The delta variant with spike mutations P681R, E484Q, and L452R is alarming because the E484Q and L452R mutations are independently resistant to neutralizing antibodies (Focosi and Maggi 2021). Currently, delta has been the most predominant variant circulating around the world (www.gisaid.com) and is known to cause breakthrough infections (Riemersma et al. 2021).

Similarly, there are other emerging variants of SARS-CoV-2 that are also gaining attention as they are known as “variants of interest” (VOI) - lambda (C.37) and Mu (B.1.621) (WHO 2021c), which also appear to be associated with transmissibility and warrant further functional studies. The variants suspected of posing a future risk are defined as “variants under monitoring” (VUM) - B.1.620, B.1.214.2, B.1.525, B.1.526, B.1.617.1 and B.1.1.318 (WHO 2021c).

By early November 2021, Africa reported about 8.5 million COVID-19 cases with about 218 thousand deaths, with most deaths in South Africa, Egypt, and Tunisia (WHO 2021a). Regarding the vaccination status of COVID-19 in Africa, of the 54 countries, 15 countries have a 10% full vaccination coverage, and in half of these countries, the coverage rate is only 2% or less (WHO 2021b). To track and identify the variants, 49414 SARS-CoV-2 genomes have been sequenced and deposited from Africa (as of Oct. 2021) (Khare et al., 2022). Phylogenetic analysis of these genomes revealed that most transmission occurred within African countries and between Africa and Europe (Wilkinson et al. 2021). Recent reports suggest that the B.1.620 lineage, which is UVM and has several mutations and deletions in the S protein that resemble VOCs, originated in west central Africa (Dudas et al. 2021). Because of low vaccination coverage and the high prevalence of immune-compromised diseases such as HIV, tuberculosis, and other infectious diseases, the virus may re-emerge and continue to spread. Therefore, genomic surveillance of SARS-CoV-2 variants is essential to understanding viral dynamics, particularly transmissibility, virulence, and pathogenesis.

In Republic of Congo the first cases of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections were reported on 14th March 2020 (Ntoumi, 2020; Ntoumi and Velavan 2020), and as of Oct. 2021 a total of 15,962 cases were reported with 231 deaths (WHO). The ROC was the first country in Central Africa to report a very high exposure of its population to SARS-CoV-2 through a seroprevalence study (Batchi-Bouyou et al. 2021). Since then, the high exposure of the Congolese population, the emergence and rapid spread of VOCs across the globe have emphasized the need for genomics surveillance that can help guide interventions to mitigate the COVID-19 pandemic in the country by informing prevention strategies. Since Brazzaville, the capital of RoC, concentrates more than 50% of reported cases (SITREP 153, June 2021) the objective of this study was to determine the distribution, prevalence, and dynamics of SARS-CoV-2 variants circulating in Brazzaville, ROC, between December 2020 and July 2021.

Materials and methods

Sampling and screening

Oropharyngeal swabs from symptomatic and asymptomatic individuals (n=600) were obtained and screened for COVID-19 between December 2020 and July 2021 in Brazzaville, Republic of Congo (ROC). RNA was extracted from swabs using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) and subjected to RealStar® SARS-CoV-2 real-time PCR targeting the S gene of SARS-CoV-2 (Altona Diagnostics, Hamburg, Germany) by using a LightCycler® 480 Instrument II (Roche diagnostics, Mannheim, Germany). Out of 317 individuals tested positive for COVID-19, 182 were Next-Generation Sequencing (NGS) with a Ct <30.

NGS sequencing

For Illumina sequencing, libraries were prepared as described in the COVID-19 ARTIC v3 Illumina protocol V.5 (DNA Pipelines R&D 2020), using the NEBNext Ultra II DNA Library Prep Kit (New England Biolabs) and for Oxford Nanopore sequencing Technology (ONT), libraries were prepared as described in Freed et al., (RAPID barcoding, 1200bp amplicon) (Freed et al. 2020). The libraries were quantified (Qubit DNA BR, Thermo Scientific), and sequenced on Illumina MiSeq V2 (500 cycles) and ONT. The FastQ files obtained from sequencing were analysed using the nf-core/viralrecon (Ewels et al., 2020) and artic network field bioinformatics pipeline for ONT data. The 171 genomes were deposited in GISAID (Khare et al., 2021) and the lineages of these genomes were annotated by the Pangolin online tool (O’Toole et al., 2021).

To investigate the global prevalence of B.1.214.1, B.1.214.2, and B.1.620 predominated lineages in this study, we obtained data of these lineages from GISAID (www.gisaid.com) and Outbreak.info (Julia L. Mullen 2020). SARS-CoV-2 genomes between December 2020 and July 2021 vary from African and European countries and Wuhan reference sequence were retrieved from GISAID and as reported most of the transmissions happened within Africa and between Africa and Europe (Wilkinson et al. 2021). Inclusion criteria for the reference genomes were used to construct the final phylogenetic tree; a separate phylogenetic tree was constructed for each variant using the Congo genomes and the genomes from Europe and Africa for that variant. The genomes that clustered closely with the Congo variants were used to create the final phylogenetic tree. Not all ROC genomes for lineages B.1.214.2; B.1.214.1; and B.1.620 were included because many of the genomes were genetically identical. The sequences that have the quality according to selected criteria (complete genome, high quality control and coverage of the sequence) were aligned using a multiple sequence alignment (MAFFT) (Katoh et al., 2002). Subsequently, a phylogenetic tree was reconstructed with the maximum likelihood method using the general time-reversible (GTR) model with rate heterogeneity (GTR+G) in the IQ-TREE server (Trifinopoulos et al., 2016). A 1000 bootstrap iterations by ultrafast bootstrap was computed (Hoang et al. 2018) and were classified into Pangolin lineages (Rambaut et al., 2020). The final dataset
was displayed with the Interactive Tree of Life (iTOL) v6 (Letunic and Bork 2019).

Results

Demographic characteristics

The characteristics of the SARS-CoV-2 infected patients from whom the 171 genomes were sequenced are summarized in Table 1. The median age of the participants was 34 years (IQR: 25 to 47) and 67% (115/171) were male. The distribution of the genomes deposited between December 2020 to July 2021 are summarized as follows: 18% (31/171), 12% (20/171), 33% (57/171), 6% (10/171), 4% (7/171), 2% (3/171), 17% (29/171) and 8% (14/171) respectively (Table 1).

SARS-CoV-2 distribution

The 171 genomes were assigned to different pangolin lineages (Table 2). A total of 15 lineages circulated during the study period, and their prevalence includes B.1.214.2 (26%), B.1.214.1 (19%), B.1.620 (18%), B.1.544 (6%), B.1.1.7 (alpha) (6%), B.1 (5%), C.16 (5%), B.1.617.2 (delta) (4%), B.1.214; B.1.221; B.1.214.3; B.1.1.349; B.1.1.318; B.1 and A.23.1 (1%). For 6% of the genomes, the lineages were not assigned. Lineages B.1.214.2, B.1.214.1, and B.1.620 were the most predominantly circulated in Brazzaville, and the identified alpha and delta VOC and B.1.620 VUM were also introduced and circulated in the country. Monthly distribution of lineages in the country between December 2020 and July 2021 is shown in the figure (Figure 1).

During the period from December 2020 to March 2021, most circulated lineages were B.1.214.2, B.1.214.1, B.1.544, and C.16, and from April 2021 to July 2021, most circulated lineages were B.1.620, B.1.1.7, and B.1.617.2. These results show the dynamics of several lineages circulated in the ROC.

Distribution of SARS-CoV-2 lineages

The distribution of lineages among the different districts includes 19% (32/171), 15% (26/171), 12% (21/171), 11% (19/171), 8% (13/171), 8% (14/171), 8% (14/171), 7% (12/171), and 5% (8/171), respectively from Mounagali, Poto-poto, Makélékélé, Diiri, Bacongo, Ouenze, Talangai, Madibou, and Mfliou. Twelve (7%) samples were from other regions of the country (Figure 2). Most variants were detected in the districts of Poto-poto (10) and Mounagali (12), while the viral diversity was low in the main part of Brazzaville: Mfliou (4 variants), Diiri (7 variants), Talangai (7 variants), Ouenze (5 variants), Bacongo (8 variants), Makélékélé (7 variants), and Madibou (6 variants). Boima and Sibit host 3 and 9 variants, respectively (Figure 2).

Global prevalence of B.1.214.1, B.1.214.2 and B.1.620 lineages and their key mutations

To understand the prevalence of the three predominant lineages (B.1.214.1, B.1.214.2 and B.1.620) worldwide, we explored the data retrieved from GISAID (Khare et al. 2021) and Outbreak.info (Julia L. Mullen 2020). The data showed there are around n=47, n=1569 and n=1058 genomes of B.1.214.1, B.1.214.2 and B.1.620 lineages respectively deposited (until 15.10.2021) (Table 3). The list of top 10 countries that have deposited these three lineages is shown in Table 3 from highest to lowest. Countries with the highest number of deposited genomes for lineages B.1.214.1, B.1.214.2, and B.1.620 include ROC, Belgium, and South Korea, respectively (Table 3). The prevalence of these lineages in ROC, Belgium and South Korea was 12%, 1% and 3%, respectively based on the total number of sequences deposited by these countries. The number of deposited genomes and their prevalence for these three lineages (Table 3) in Africa includes B.1.214.1 [ROC n=30 (12%) and Angola n=6 (1%)], B.1.214.2 [ROC n=41 (16%) and DRC n=12 (2%)], and B.1.620 [ROC n=47 (18%) and CAR n=19 (34%)].

The common mutations of the SARS-CoV-2 genes for B.1.214.1, B.1.214.2 and B.1.620 lineages are shown in Figure 3 (Khare et al., 2021; Julia L. Mullen 2020). First, mutations of B.1.214.1 include: ORF1a (I1398V, T1881I, and A4016V), ORF1b (P314L), S (D614G), ORF8 (S84L), and N (T205I). Second, mutations of B.1.214.2 include ORF1a (I1398V, T1881I, and A4016V), ORF1b (P314L, R574G), S (Q441K, N450K, D614G, T716I), ORF8 (S84L), and N (D31L, T205I). Third, mutations of B.1.620 include ORF1a (T403I, V1991I, del3675/3677), ORF1b (P314L, A1215S), S (P266, del69/70, V126A, del144/144, del241/243, H245Y, S477N, E484K, D614G, P681H, I1027I, D118H), ORF7b (del14/15), ORF8 (S84L). The B.1.620 lineage has the highest number of nonsynonymous mutations and deletions in the S protein compared to the other two variants (Figure 3).

Phylogenetic analysis

For phylogenetic analysis, 107 genomes from this study were used, representing 13 of 15 strains and containing 110 reference genomes from European and African countries (Figure 4). The ROC genomes were divided into nine distinct clades in the phylogenetic tree (Figure 4), seven of which had a single lineage and two of which had two or more lineages. Of these two clades the lineages B.1.544 and B.1 formed a single clade, while B.1.214, B.1.214.1, B.1.214.2, and B.1.214.3 sister lineages formed a single clade (Figure 4). Nearly all genomes from this study clustered with genomes from European and African countries.

The majority of genomes from the ROC in the phylogenetic tree are from lineages B.1.214.2, B.1.214.1, and B.1.620, which circulated most frequently during the study period. The tree shows that the genomes of the B.1.214.2 lineage from ROC form two monophyletic clusters with genomes from European and African countries, including sequences from neighbouring DRC. This indicates that there were two separate introductions into the country that were locally transmitted. Next, the
Table 3
SARS-CoV-2 lineages and the prevalence of B.1.214.1, B.1.214.2 and B.1.620 genomes

| Lineage       | Number of Genomes (Country) | Percentage |
|---------------|-----------------------------|------------|
| B.1.214.1     | 47 (Republic of Congo)      | 47(12)     |
| B.1.214.2     | 1569 (Belgium)              | 1569(1)    |
| B.1.214.3     | 1058 (South Korea)          | 1058(3)    |
| B.1.214.1     | 8 (France)                  | 8(<0.1)    |
| B.1.214.1     | 267 (Switzerland)           | 267(<0.1)  |
| B.1.214.1     | 216 (Belgium)               | 216(<0.1)  |
| B.1.214.1     | 1 (Angola)                  | 1(1%)      |
| B.1.214.1     | 19 (Central African Republic)| 19(34)    |
| B.1.214.1     | 398 (South Korea)           | 398(3)     |
| B.1.214.1     | 8 (France)                  | 8(<0.1)    |
| B.1.214.1     | 27 (Canada)                 | 27(<0.1)   |
| B.1.214.1     | 12 (Portugal)               | 12(<0.1)   |
| B.1.214.1     | 14 (Portugal)               | 14(<0.1)   |
| B.1.214.1     | 11 (Lithuania)              | 11(1)      |
| B.1.214.1     | 1 (Belgium)                 | 1(<0.1)    |
| B.1.214.1     | 41 (Republic of Congo)      | 41(16)     |
| B.1.214.1     | 6 (Angola)                  | 6(1%)      |
| B.1.214.1     | 29 (Indonesia)              | 29(<0.1)   |
| B.1.214.1     | 15 (Ireland)                | 15(<0.1)   |
| B.1.214.1     | 1 (Belgium)                 | 1(<0.1)    |
| B.1.214.1     | 12 (Central African Republic)| 12(2)      |
| B.1.214.1     | 1 (Democratic Republic of Congo)| 1(2)      |

(%) Percentage is calculated based on the number of genomes deposited by a country of a specific lineage to the total number of genomes deposited from that country. Highlighted are those with significant proportion of sequences originating from Africa.
ROC genomes of the B.1.214.1 lineage formed a single cluster with genomes from Angola and France, locations from which few cases with this lineage have been reported. In addition, the genomes of the B.1.620 lineage from the ROC formed a monophyletic cluster with genomes from Cameroon, CAR, France, Belgium, Germany, and England, suggesting single introduction and local transmission. (Figure 4). The genomes of B.1.1.7 lineage from ROC formed two distinct clusters in a monophyletic group with the genomes from Europe and Africa (Figure 4). Next, the genomes of the Delta lineage clustered with the genomes from France, Germany, Belgium, Angola, and the CAR suggest that the variants may have been introduced from European and/or West African countries bordering the ROC.

Discussion

The findings of the present study were the identification of 15 lineages found in Brazzaville, ROC and 11 lineages were reported for the first time. The study reports the alpha and delta VOCs and B.1.214.2 and B.1.620 VUMs for the first time in ROC and found that B.1.214.2, B.1.214.1, and B.1.620 were the predominant lineages in circulation. The lineages B.1.620 and B.1.617.2 first appeared in May and June, respectively, and were still circulating at the end of the study. The genomes in this study formed tight clusters with genomes from Europe and Africa and strengthened earlier findings that most of the introductions of the variants happened within Africa and between Africa and
Europe. As SARS-CoV-2 transmission lineages are growing and spreading, they will become more difficult to eliminate, underscoring the importance of timely intervention to reduce transmission. This will add to the public health burden in resource-poor areas in ROC and Africa in general. An important aspect of early epidemiological and genomic surveillance is essential in understanding the lineages introduced into a region.

Lineages B.1.214.1 and B.1.214.2 are the predominant sister lineages of B.1.214 that were in circulation during the first three months of this study. The B.1.214.2 lineage was by far the most widespread in Europe in early 2021 and was the predominant lineage in the ROC. In this study, we show that the lineage B.1.214.1 was first identified in ROC and 70% of the deposited sequences in GISAID (Khare et al., 2021) originated from ROC and had circulated from December 2020 to February 2021. Phylogenetic inference and metadata clearly suggest that the lineage may have originated in ROC, was transmitted locally, and then subsequently spread to France and Angola, where few out-of-country cases have been reported. The B.1.214.2 circulated in Brazzaville were reported for the first time in ROC from January 2021 to April 2021, and the first cases associated with this lineage were reported from Switzerland in November 2020. In Europe, the lineage transmitted in the community in Belgium, where it was first detected in early January 2021 at the University of Liège, Belgium. From Europe, the first cases of this lineage were reported from Switzerland, England, and France, and from Africa, the first cases were reported from Togo, the Democratic Republic of Congo (DRC), and Benin during late December 2020 and early January 2021. Later, the lineage was also reported in other African countries (South Africa, Gabon, Angola, Rwanda, and Senegal) indicating the wide presence of this strain in Africa (Khare et al., 2021). The B.1.214.2 is related to the parental lineage B.1.214, which spread to the DRC region during the early phase of the pandemic (Ntoumi et al. 2021; Gerdol, Dinhia, and Giorgetti 2021), and the lineage may have been introduced into the ROC from the neighbouring DRC. Subsequent introduction and spread of the alpha, delta, and B.1.620 lineages led to the disappearance of B.1.214.2 and B.1.214.1 lineages from the ROC, with the last record of B.1.214.2 lineage from South Africa reported October 17, 2021 (Khare et al., 2021).

The first cases of the B.1.620 lineage were reported from CAR, Cameroon, Equatorial Guinea, and France in early February 2021 (Elbe and BucKland-Merrett 2017). The first cases of this lineage were reported from ROC in May 2021. A recent study based on phylogenetic data and metadata including travel information concluded that this lineage probably originated in CAR and spread to neighbouring countries Cameroon, ROC, Equatorial Guinea in Africa and then to Europe and other countries (Dudas et al. 2021). This lineage has been introduced into several countries and transmitted locally in Lithuania, France, Spain, Italy, Germany, and ROC. In Africa, most sequences were deposited, and the highest prevalence was reported from ROC (this study) and the neighbouring country CAR. The phylogenetic tree and data suggest that transmission of this lineage may have occurred through Cameroon and/or the CAR, as both countries share a border with the ROC. This clearly indicates that transmission across the borders could have occurred during economic activities between the countries. Most of the sequences in this lineage were introduced from non-African countries such as South Korea, Lithuania, France, and Germany (Khare et al., 2021). However, relative to the total number of the sequences deposited in GISAID, the overall prevalence is low in these countries. For several reasons this lineage may be of concern (Dudas et al. 2021): 1) it has a large number of VOC-like mutations and deletions; 2) it is reported to be 2.4-fold more frequent in vaccine breakthrough cases, possibly due to escaping antibody-mediated immunity; 3) mutations of B.1.620 in the NTD (N-terminal domain) may result in partial loss of neutralization of convalescent serum and NTD-targeted monoclonal antibodies (Wang, Casner, et al. 2021). It is suggested that this lineage may have arisen through escape from antibody-mediated immunity (Jiu et al. 2021). Mutation studies have shown that substitutions increase the affinity of the RBD for the ACE2 receptor (Starr et al. 2020). Overall, close monitoring of this variant is needed in ROC and in other African countries, as it is potentially of concern.

It has been reported that the alpha and delta VOCs are the predominant variants circulating in almost all countries of the world, including in ROC (Khare et al., 2021). In the present study, we show low frequencies of these variants, probably due to their recent introduction into the ROC. The individuals who exhibited the VOCs in the present study had no recent travel history. Nevertheless, both variants showed tight clusters with genomes of the lineage from European and African countries, which makes it difficult to predict the introductions and transmission patterns. Phylogenetic analysis of the sequences in this study show high similarities between the sequences, suggesting a single introduction and local spread within a shorter period.

The distribution of variants in Brazzaville was heterogeneous, with the Poto-Poto and Moungali districts harbouring the most variants and representing an epicenter of viral genetic diversity. The highest diversity of SARS-CoV-2 in the Poto-Poto district may be due to the fact that the population of this district is cosmopolitan and has high economic activity. Indeed, the district has a large market that attracts not only people from all parts of the country but also foreign traders. However, the escalation of the disease in the neighbouring Democratic Republic of Congo (DRC) explains the rapid transmission of the variants in the Republic of Congo due to the proximity of the cities (Brazzaville and Kinshasa are the closest capitals in the world). Since the Poto-Poto district is located on the border, this explains the fact that it is the epicentre. The data illustrate the high dynamics of SARS-CoV-2 variants in Congo.

Limitations of our study include that the lineages and predominance may be highly biased by differences in Ct values (only the samples with low Ct values were included for sequencing) and also several districts in ROC were not covered, which may not reveal the true perspective of the variants circulating in the country. Also, our efforts to reconstruct the dynamics of SARS-CoV-2 in ROC and Africa are certainly biased by the uneven sampling.

Our previous study from the ROC reported limited SARS-CoV-2 diversity from sequences between April and July 2020. In that study, all SARS-CoV-2 genomes were reported to carry the S mutation D614G and only lineage B.1 was observed, with two separate introductions of SARS-CoV-2 virus likely from DRC (Ntoumi et al. 2021). Together, the results from the present study indicate that many SARS-CoV-2 variants are circulating in Brazzaville, ROC, and the detection of alpha and delta variants for the first time in the country has the raised alarm to health authorities. Thus, the spatiotemporal genomic surveillance of SARS-CoV-2 variants will be useful in monitoring viral evolution and emerging variants, transmissibility, transmission dynamics, assessing vaccine efficacy, and evaluating infection prevention and control measures in the ROC.

Ethics statement

Informed written consent was obtained from all participants. The study was approved by the ministry of scientific research and technological innovation, ROC (Approval Nr. 049/MRSIT-CAB) and by institutional ethics committee of the Congolese foundation for medical research (Approval Nr. 027/CIE/FCRM/2020).

Consent for publication

All authors agreed with the results and conclusions. All authors approved this version of the manuscript to be published.

Availability of data and materials

All related data supporting the results reported in the article is available within the manuscript.
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