COVID-19 reinfections in The Gambia by phylogenetically distinct SARS-CoV-2 variants—first two confirmed events in west Africa

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At the beginning of the COVID-19 pandemic, in early 2020, the scientific community hypothesised that SARS-CoV-2 transmission would eventually be hindered by herd immunity, conferred by natural infection, vaccination, or both.[1] However, essential questions about whether infection with SARS-CoV-2 confers protection against reinfection and the length of time the protection lasts after either infection or vaccination remain open. These answers are crucial for the development of appropriate health control measures worldwide and become more important as new viral variants spread.

By March 15, 2021, fewer than 100 reinfections had been reported worldwide, mainly in countries with a high mortality burden. In most cases, reinfections were less severe than the initial infection.[2,3] However, recent reports from Brazil, a country that in parts surpassed the threshold of herd immunity after the first wave but had a similarly strong second wave,[4] are worrisome. Such resurgence of COVID-19 cases in the second wave can be explained by rapid waning immunity, the expansion of the new SARS-Co-V-2 variants that might evade immunity generated in response to previous infections (ie, B.1.1.7, B.1.351, and P.1), higher transmissibility of new lineages that require a larger herd immunity, or a combination of all these factors.[4]

Reinfections in west Africa, a region with a lower toll of infections and deaths[5] than Europe, North America, or South America, are yet to be described. We aimed to ascertain whether any reinfections had occurred in The Gambia.

The Gambia is the smallest country in west Africa, with 4712 cases and 150 deaths reported by March 1, 2021, although the number of cases is probably underestimated.[5] At that time, 460 SARS-CoV-2 genomes from confirmed cases had been sequenced in the country, with 430 (93·5%) genomes already submitted on the GISAID database.[6] Among these samples, only two main lineages of the virus, lineages A and B, have been detected. Lineage B constitutes almost 98% of the total genomes sequenced, with the sub-lineage B.1 being the most prevalent, found in 20% of the sequences. Naso-oropharyngeal samples are collected as part of the national surveillance and by the Medical Research Council Unit The Gambia (MRCG) at the London School of Hygiene and Tropical Medicine through clinical, occupational health, and research activities, including the PaTS trial (NCT04703608), from symptomatic individuals or contacts of known COVID-19 cases. Screening of asymptomatic health-care workers and those proposing to travel across international borders also takes place. The standard test for COVID-19 diagnosis in The Gambia, as of March 15, 2021, is real-time PCR of SARS-CoV-2 specific viral gene sequences.

Confirmation of reinfections were done by genomic analysis. Library preparation and sequencing were done using the ARTIC (version 3) protocol for SARS-CoV-2 that targeted whole genome sequencing.[7] Libraries were pooled in multiplexes of 24 per flow cell and sequenced on a GridION platform (Oxford Nanopore Technologies, UK). Bioinformatics analysis was done using the ARTIC

	Patient A	Patient B
Sex	Female	Female
Age, years	31	36
Comorbidities	None	None
First infection		
Date of infection	Aug 30, 2020	July 31, 2020
Clinical presentation	WHO criteria for mild infection*	Asymptomatic
Ct value ORF-1/N gene	36·7/34·1	Negative/38·0
Lineage	B.1 (discovered in March, 2020, in the UK, Mexico, and USA)	B.1.235 (discovered January, 2020, in the UK, USA, and Spain)
Second infection		
Date of infection	Jan 21, 2021	Feb 1, 2021
Clinical presentation	WHO criteria for mild infection*	WHO criteria for mild infection*
Ct value E gene/N gene	34·0/33·1	20·9/19·1
Lineage	B.1.1.74 (discovered in March, 2020, in the UK, Ireland, and Belgium)	B.1 (discovered in March, 2020, in the UK, Mexico, and USA)

Ct=cycle threshold. ORF-1=open reading frame 1. *Symptomatic patient meeting the case definition for COVID-19 without evidence of viral pneumonia or hypoxia.

Table: **Clinical, epidemiological, and molecular characteristics of the two individuals with SARS-CoV-2 reinfections**

(version 3) bioinformatics pipeline for SARS-CoV-2 genome analysis.[8] Genome alignments against the reference were visualised on the Interactive Genomics Viewer.[9] Mutations were confirmed using the GISAID CoVsurver.[10] Lineage assignment was done using Pangolin (version 2.3.0).[11] The Gambian Government and MRCG joint ethics committee approved the study presented here (Ref L2021.E04).

We have phylogenetically confirmed two reinfections among healthy Gambian individuals aged 31 years and 36 years, with a time lag of 5 months and 6 months, respectively. Both individuals had mild symptoms during the second infection that lasted less than 1 week. For the initial infection, one individual had mild symptoms, whereas the other was asymptomatic (tested as a contact of a positive case). Epidemiological, clinical, and molecular details of both infections are shown in the table.

Genome-wide sequence comparisons for the two viruses of each patient to the reference shows mutations on the S gene, which encodes for the virus spike protein (appendix p 3). The spike protein mediates receptor recognition and binding during infection, playing an important part in viral transmission.[12] Although it is still unclear as to what extent immune responses against a previous variant will protect against reinfection by a different variant, mutations of the viral spike protein are predicted to enhance infectivity.[13] In both patients, we have seen more mutations in the S gene in the variants from the second infection episodes than in those from the first episode (two amino-acid changing mutations in the first infection and four in the second infection). One of these mutations associated with enhanced infectivity (D614G)[14] was present in all four infections. Certain mutations on the receptor-binding domain of the spike protein confer resistance to commonly elicited antibodies during SARS-CoV-2 infection in vitro.[15] One such mutation is N440K, detected in patient A's reinfection. Other mutations identified include A1020S, which is involved in viral oligomerisation interfaces; and G946V, detected in the last quarter of 2020 among different variants, including B.1.1.7. Overall, these reinfections are potentially a consequence of divergent mutations enhancing transmission of the virus, but this will need to be assessed in a larger pool of reinfected individuals. Two (B.1·235 and B.1.1.74) of the three lineages involved in these two reinfections are uncommon in the country, representing 3% and less than 1% of the Gambian isolates, respectively.[6]

In summary, our data conclude that at least two reinfections have occurred in The Gambia. These events have occurred in healthy young individuals infected with similar viral variants in the first and second episode. If reinfections with similar strains are possible, herd immunity in west Africa could take longer than expected as a large majority of cases are asymptomatic or with mild disease and these probably develop weaker immune responses.[16] In the absence of widespread vaccination, reinfections could become more common when the new variants become predominant in the region. Community-based immunological studies are urgently needed.

See Online for appendix

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