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

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. 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. 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, 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-CoV-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.

Reinfections in west Africa, a region with a lower toll of infections and deaths 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. 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. 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 healthcare 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. 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                        |
| Age, years                    | 31                            |
| Comorbidities                 | None                          |
| First infection               |                               |
| Date of infection             | Aug 30, 2020                  |
| Clinical presentation         | WHO criteria for mild infection* |
| Ct value ORF-1/N gene         | 36/7/34 1                     |
| Lineage                       | B.1 (discovered in March, 2020, in the UK, Mexico, and USA) |
| Second infection              |                               |
| Date of infection             | Jan 21, 2021                  |
| Clinical presentation         | WHO criteria for mild infection* |
| Ct value E gene/N gene        | 34/0/33 1                     |
| Lineage                       | B.1.1.74 (discovered in March, 2020, in the UK, Ireland, and Belgium) |

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
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Online for appendix

1. Fontanet A, Cauchemez S, Fontanet A, Cauchemez S. COVID-19 herd immunity: where are we? Nat Rev Immunol 2020; 20: 583–84.
2. Lee JS, Kim SY, Kim TS, et al. Evidence of severe acute respiratory syndrome coronavirus 2 reinfection after recovery from mild coronavirus disease 2019. Clin Infect Dis 2020; published online Nov 21. https://doi.org/10.1093/cid/ciaa1421.
3. Hansen CH, Michlmayr D, Gubbels SM, Melbak E, Eitelberg S. Assessment of protection against reinfection with SARS-CoV-2 among 4 million PCR-tested individuals in Denmark in 2020: a population-level observational study. Lancet 2021; 397: 1204–12.
4. Sabino EC, Buss LF, Carvalho MPS, et al. Resurgence of COVID-19 in Manaus, Brazil, despite high seroprevalence. Lancet 2021; 397: 452–55.
5. Worldometer. COVID-19 coronaviruses pandemic. https://www.worldometers.info/coronavirus/ (accessed April 5, 2021).
6. GISAID. Phylodynamics of pandemic coronavirus in west Africa. 2021. https://www.gisaid.org/phylodynamics/west-africa/ (accessed April 5, 2021).
7. Quick J. nCoV-2019 sequencing protocol v3 (LoCost) V.3. 2020. https://www.protocols.io/ncov2019-bioinformatics-sop.html (accessed April 5, 2021).
8. Loman N, Rowe W, Rambaut A. nCoV-2019 novel coronavirus bioinformatics protocol. 2020. https://artic.network/ncov-2019/ncov2019-bioinformatics-sop.html (accessed March 1, 2021).
9. Thoirvaldsdottir H, Robinson JT, Missoev JP. Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. Brief Bioinform 2013; 14: 178–92.
10. GISAID CoVsurver: mutation analysis of hCoV-19. https://www.gisaid.org/epiinfo-applications/covsurver-mutations-app/ (accessed Feb 17, 2021).
11. GitHub. Pangolin. 2020. https://github.com/cov-lineages/pangolin (accessed March 1, 2021).
12. Huang Y, Yang C, Xu XF, Xu W, Liu SW. Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19. Acta Pharmacol Sin 2020; 41: 1141–49.

(11) Bioinformatics pipeline for SARS-CoV-2 genome analysis. 11 Lineage assignment was done using Pangolin (version 2.3.0). 11 The Gambian Government and MRG 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.

**Table 1: Details of the two confirmed reinfections**

| Case | Date of Infection | Time Lag | Symptoms | Genotype |
|------|-------------------|----------|----------|----------|
| A    | 2020-03-01        | 5 months | Mild     | B1.1.74  |
| B    | 2020-09-15        | 6 months | Mild     | B1.1.74  |

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. 11 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.

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13 Zhang L, Jackson CB, Mou H, et al. SARS-CoV-2 spike-protein D614G mutation increases virion spike density and infectivity. Nat Commun 2020; 11: 6013.
14 Ozono S, Zhang Y, Ode H, et al. SARS-CoV-2 D614G spike mutation increases entry efficiency with enhanced ACE2-binding affinity. Nat Commun 2021; 12: 848.
15 Weisblum Y, Schmidt F, Zhang F, et al. Escape from neutralizing antibodies by SARS-CoV-2 spike protein variants. eLife 2020; 9: 9.
16 Long QX, Tang XJ, Shi QL, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat Med 2020; 26: 1200–04.