Reinfection with two genetically distinct SARS-CoV-2 viruses within 19 days

To The Editor,

Increasing detection of reinfections and waning neutralizing antibody (Nab) titers as early as 23 days following initial infection\(^1\) raises concerns for herd immunity and the durability of vaccine efficacy.\(^2,3\) Since the first reported reinfection case in August 2020,\(^4\) at least 70 confirmed cases have emerged as of April 27, 2021.\(^5\) In October 2020, the US Centers for Disease Control and Prevention (CDC) published investigative criteria for suspected SARS-CoV-2 reinfections.\(^6\) These criteria included any individuals testing positive ≥90 days after their first laboratory-confirmed SARS-CoV-2 infection or symptomatic individuals testing positive 45–89 days after initial infection with paired respiratory specimens.\(^6\) Here, we describe a patient infected with two genetically distinct SARS-CoV-2 strains detected 19 days apart, indicating that reinfection can occur within a short period.

Ninety-two SARS-CoV-2 positive nasopharyngeal samples (CDC 2019 Novel Coronavirus Real-Time Reverse Transcriptase-PCR Diagnostic Panel\(^7\)) were collected in Columbia, Missouri from March to May 2020. Two samples, collected 19 days apart, were from the same patient. SARS-CoV-2 virus isolates were recovered from each of the two samples. The SARS-CoV-2 viruses from both clinical swabs were sequenced using Access Array microfluidic (Fluidigm Corporation) and MiSeq systems (Illumina).\(^8\) Phylogenetic analyses were performed using BEAST2 (see Supporting Information Appendix for Materials and Methods).

This patient was a female in her 20 s with asthma, obesity, anxiety, and depression, who reported cough, chills, exertional dyspnea, sore throat, dizziness, rhinorrhea, and fever during her initial COVID-19 diagnosis in March 2020. She tested positive 1 day after symptom onset and was instructed to self-isolate at home. Nineteen days following her initial positive test, she returned for another COVID-19 test due to return-to-work requirements. Despite her symptoms waning to encompass only productive cough and fatigue, she tested COVID-19 positive again. She continued to experience persistent cough, fatigue, and dyspnea until 55 days after her initial positive test.

Phylogenetic analyses showed that the two samples contained SARS-CoV-2 viruses from two distinct lineages (Figure 1); Sample 1 (GenBank accession No.: MW521480.1; cycle threshold \([C_\text{t}] \text{ value} = 17.76\)) belonged to the PANGOLIN A.3 lineage, whereas the Sample 2 (MW521502.1; \([C_\text{t}] \text{ value} = 20.36\)) belonged to the PANGOLIN B.1.1 lineage. Additionally, we compared the sequences between viral isolates and clinical samples. Results showed that sequences from each isolate were identical to the corresponding clinical sample, but those at the first sample and at the second sample were distinct. The virus sequences had 21 nucleotide substitutions relative to each other, encoding 11 nonsynonymous amino acid mutations across five genes (ORF1ab (D75E on nonstructural protein 1 (NSP1), P971L on NSP3, P4715L on NSP12, F6158L on NSP14), ORF8 (V62L, L84S), ORF7a (S81L) ORF10 (I4L), S (D614G) and N (R202K, G203R)). The average sequence depth was 3960 (Day 1 virus) and 3233 (Day 19) reads, and each of those 21-variation positions had a minimum raw read depth of 1978 reads (Table 1). No diverse polymorphisms were identified among the sequences of the viruses from each clinical sample, suggesting true reinfection rather than a coinfection.

This report is limited by the unavailability of sera samples to study Nab titers and lack of information regarding the patient's potential contacts with others during the 2-week isolation period. Nevertheless, this case showed a patient who unknowingly became reinfected with two genetically distinct viruses within 19 days and may have still been infectious after the CDC-recommended 10 day isolation period.\(^9\) Additionally, the CDC has encouraged symptom-based strategies for ending isolation rather than viral retesting for asymptomatic individuals or for individuals without new symptoms during 90 days after illness onset due to findings that detectable but noninfectious SARS-CoV-2 RNA can persist in respiratory samples.\(^9\) Larger studies are necessary to test whether the prevalence of reinfection within a short period is high, as shown in this case: if yes, this may pose a challenge of infection control, especially as variants of concern continue to emerge and immune evasion increases despite vaccination efforts.

Reinfections are likely underreported due to lack of multiple sample collections and sequencing from the same individuals. A pressing question remains of whether immunity developed from initial infection protects against other strains. The E484K spike mutation, present in the B.1.351 and P.1 variants of concern, has raised fears over their potential to impact immune escape and reinfection.\(^10\)
With the mass rollout of COVID-19 vaccinations, other urgent unknowns include the true occurrence of reinfection, the health impact of subsequent infections, and the duration of immunity generated from infections and vaccinations. Expanding sequencing and surveillance of COVID-19 reinfections will help address many of these questions.

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CONFLICT OF INTERESTS
The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS
Xiu-Feng Wan and Cynthia Y. Tang conceived this study, designed the analysis, and wrote the paper. Tao Li, Yang Wang, and Cynthia Y. Tang collected the data. Jun Hang, Richard Hammer, Detlef Ritter, and Grace M. Lidl contributed data or analysis tools. Cynthia Y. Tang performed the data analyses. Yang Wang, Jane A. McElroy, Richard Hammer, Detlef Ritter, Grace M. Lidl, Richard Webby, and Jun Hang revised the paper.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are openly available in GenBank: The GenBank Accession Numbers are MW521480.1 (Sample 1) and MW521502.1 (Sample 2).
TABLE 1  Pairwise comparison of nucleotide and amino acid substitutions

| Sample | Ct-Value | Gene 5'-UTR** | ORF1ab NSP1 | NSP3 | NSP4 | NSP12 | NSP14 |
|--------|----------|--------------|-------------|------|------|-------|-------|
|        |          | Nucleotide Position |             |      |      |       |       |
| SARS-CoV-2/human/USA/20×1029/2020 (Day 1) | 17.8 | 160 | 241 | 313 | 490 | 3037 | 3177 | 8782 | 14,408 | 18,736 |
|        |          | Amino Acid Position |             |      |      |       |       |
|        |          | NA | NA | 47 | 75 | 924 | 971 | 2839 | 4715 | 6158 |
|        |          | Nucleotide (% reads*) |             |      |      |       |       |
|        |          | G (99.82) | C (99.74) | C (99.36) | A (99.09) | C (99.74) | T (99.46) | T (99.38) | C (99.94) | C (99.76) |
|        |          | Amino Acid |             |      |      |       |       |
|        |          | NA | NA | K | E | F | L | S | P | L |
|        |          | Sequence Coverage (reads) | 2855 | 4252 | 4892 | 4619 | 5395 | 6310 | 4259 | 3363 | 3034 |
|        |          | Nucleotide (% reads*) |             |      |      |       |       |
|        |          | T (99.36) | T (99.12) | T (99.29) | T (99.29) | T (99.70) | C (99.82) | C (99.59) | T (99.75) | T (99.70) |
|        |          | Amino Acid |             |      |      |       |       |
|        |          | NA | NA | K | D | F | P | S | L | F |
|        |          | Sequence Coverage (reads*) | 2372 | 3422 | 3972 | 3832 | 4376 | 5187 | 3934 | 2902 | 2396 |

| Sample | Ct-Value | Gene 3'-UTR** | S | M | ORF7a | ORF8 | N | ORF10 |
|--------|----------|--------------|---|---|-------|------|---|-------|
|        |          | Nucleotide Position | 21,658 | 23,403 | 24,034 | 26,729 | 27,635 | 28,077 | 28,144 | 28,881 | 28,883 | 29,567 | 29,700 |
| SARS-CoV-2/human/USA/20×1029/2020 (Day 1) | 17.8 | Amino Acid Position | 32 | 614 | 824 | 69 | 81 | 62 | 84 | 203 | 203 | 204 | 4 | NA |
|        |          | Nucleotide (% reads*) | T (99.80) | A (99.52) | T (99.77) | C (99.74) | T (99.81) | C (99.79) | C (99.30) | G (99.87) | G (99.86) | G (99.38) | C (99.76) | G (99.26) |
|        |          | Amino Acid | F | D | N | A | L | L | S | R | R | G | L | NA |
|        |          | Sequence Coverage (reads) | 7016 | 2538 | 4482 | 4256 | 4852 | 3935 | 5331 | 2316 | 2299 | 2293 | 5025 | 3260 |
|        |          | Nucleotide (% reads*) | C (99.68) | G (99.30) | C (99.52) | T (99.56) | C (99.62) | G (99.59) | T (99.82) | A (99.29) | A (99.34) | C (99.39) | A (99.48) | A (99.73) |
|        |          | Amino Acid | F | G | N | A | S | V | L | K | K | R | I | NA |
|        |          | Sequence Coverage (reads*) | 6328 | 2161 | 3812 | 3665 | 3972 | 3247 | 4446 | 1989 | 1985 | 1978 | 4108 | 2647 |

Note: Variants were identified using CLC Workbench and validated using Bowtie 2. Sequence coverage and read counts were calculated using pysamstats. SARS-CoV-2/human/USA/20×1029/2020 (GenBank Accession No: MW521480.1). SARS-CoV-2/human/USA/20×1104/2020 (GenBank Accession No: MW521502.1).

Abbreviations: M, membrane glycoprotein; NA, not applicable; NSP, nonstructural protein; ORF, open reading frame; S, surface glycoprotein; UTR, untranslated region.

*% Depth calculated by (read count)/coverage × 100.

**Noncoding region.
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SUPPORTING INFORMATION
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