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International Journal of Infectious Diseases 108 (2021) 53–56

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Evidence of SARS-CoV-2 reinfection within the same clade in Ecuador: A case study

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ARTICLE INFO

Article history:
Received 1 April 2021
Received in revised form 21 April 2021
Accepted 23 April 2021

Keywords:
SARS-CoV-2
COVID-19
Reinfection
Ecuador

ABSTRACT

Objectives: To date, reported SARS-CoV-2 reinfection cases are mainly from strains belonging to different clades. As the pandemic advances, a few lineages have become dominant in certain areas leading to reinfections by similar strains. Here, we report a reinfection case within the same clade of the initial infection in a symptomatic 28-year-old-male in Quito-Ecuador.
Methods: Infection was detected by reverse transcription-polymerase chain reaction, and immune response evaluated by antibody testing. Whole-genome sequencing was performed and phylogenetic analysis conducted to determine relatedness.
Results: Both the infection and the reinfection strains were assigned as Nextstrain 20B, Pangolin lineage B.1.1 and GISAID clade O. Our analysis indicated 4–6 fold more nucleotide changes than are expected for reactivation or persistence compared with the natural rate of SARS-CoV-2 mutation (~2–3 nucleotide changes per month), thus supporting reinfection. Furthermore, approximately 3 months after the second infection, COVID-19 antibodies were not detectable in the patient, suggesting potential vulnerability to a third infection.
Conclusions: Our results showed evidence of SARS-CoV-2 reinfection within the same clade in Ecuador, indicating that previous exposure to SARS-CoV-2 does not guarantee immunity in all cases.

Introduction

SARS-CoV-2 reinfection cases reported worldwide (Arteaga-Livias et al., 2021; Colson et al., 2020; Prado-Vivar et al., 2020; Tillett et al., 2020; To et al., 2020; VanElslande et al., 2020) are relatively infrequent compared with total reported cases. In Latin America, the number of reinfections is understated as only a small proportion of samples are sequenced (Arteaga-Livias et al., 2021). In Ecuador, only 0.1% of COVID-19 cases are sequenced according to GISAID database (Shu and McCauley, 2017).

In Ecuador, achieving herd immunity is a challenge as only 0.04% of the population has received the COVID-19 vaccine (Google News, 2020; Roser et al., 2020), despite 97.4% of the population being willing to be vaccinated (Sarasty et al., 2020). Surveillance of reinfections in Ecuador is of great importance given the implications for vaccine immunity.

Here, we present a case report of an individual who experienced 2 distinct COVID-19 illnesses from genetically similar SARS-CoV-2 agents.

Methods

Reverse transcription-polymerase chain reaction (RT-PCR) and antibody testing

RNA was extracted from nasopharyngeal swabs using a TANBead Nucleic Acid Extraction Kit (Taiwan Advanced Nanotech Inc., Taiwan). Viral RNA was detected using Allplex2019-nCoV Assay (Seegene Inc., Seoul, Korea).

Immunoglobulin G (IgG) and M (IgM) against SARS-CoV-2 nucleoprotein was detected in the first antibody test using the

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https://doi.org/10.1016/j.ijid.2021.04.073

NovaLisa SARS-CoV-2 (COVID-19) IgG and IgM enzyme-linked immunosorbent assay (Novatec, Dietzenbach, Germany). VIDAS SARS-COV-2 IgM and IgG automated qualitative assays (BioMérieux, Marcy-l'Étoile, Francia) were used in the second test.

Patient fingerprinting

To verify the patient's identity in every sample, we ran trace genomic DNA in our RNA samples on the PowerPlex 21 System (Multiplex STR System for Human Identification) (Promega, Madison, Wisconsin, USA).

Whole-genome sequencing and bioinformatic analysis

Whole-genome sequencing of SARS-CoV-2 was performed using CleanPlex® SARS-CoV-2 Panel (Paragon Genomics, Hayward, CA, USA) following the manufacturer's instructions on MiSeq System Illumina Sequencing platforms. FastQC was used to assess the raw read quality (Sah et al., 2020) before cleaning with Trimmomatic 0.39 [PE -phred33 ILLUMINACLIP: TruSeq3-PE. fa:2:30:10:2:keepBothReads (Tillett et al., 2020), LEADING:20 TRAILING:20 SLIDINGWINDOW:4:20 MINLEN:40 (Sah et al., 2020)]. Cleaned reads were mapped to SARS-CoV-2 reference genome Wuhan-Hu-1 (GenBank accession number NC_045512.2) (Sah et al., 2020; Tillett et al., 2020), using BWA-MEM 0.7.17 (Sah et al., 2020). PCR duplicates were marked and removed with Picard 2.23.8 (Tillett et al., 2020). Variant calling was performed with bcftools 1.11 (-q 25 -Q 35) (Li et al., 2020). Only high-quality variants (QUAL $\geq$ 20 and DP $\geq$ 5) (Karamitros et al., 2020; Tillett et al., 2020) were retained and used to generate the consensus sequences. Cross-validation was done with the *de novo* assembly produced by MEGAHIT 1.2.9 (Sah et al., 2020). Finally, mutations were identified in the consensus sequences using GISAID's CoVsurver: Mutation Analysis of hCoV-19 (Shu and McCauley, 2017) and Genome Detection (Vilsker et al., 2019).

Phylogenetic analysis

We downloaded 290 SARS-CoV-2 consensus genomes from GISAID (Shu and McCauley, 2017). Sequences were aligned using MAFFT's online service rapid calculation of full-length multiple sequence alignment of closely related viral genomes (Katoh et al., 2002). A neighbor-joining phylogenetic tree was created (Jukes-Cantor substitution model) and visualized using Phylo.io (Robinson et al., 2016). Clades were assigned using GISAID (Shu and McCauley, 2017), Nextstrain (Tillett et al., 2020), and Pangolin (Rambaut et al., 2020).

Ethical approval

This work was approved under a consent waiver by the Expedited Committee of the General Coordination for Strategic Development in Health as stipulated by the Ecuadorian Ministry of Public Health Resolution N° 074-2020.

Results

Patient

The patient was a 28-year-old male residing in Quito-Ecuador. During the first infection, he had symptoms consistent with viral infection (sore throat, cough, headache, nausea, and diarrhea, anxiety and panic attacks), commencing on July 15, 2020. The diagnosis was confirmed by a positive SARS-CoV-2 RT-PCR test (cycle threshold (Ct) = 29.9) from a nasopharyngeal swab on July 20, 2020. On July 25, 2020, he tested negative for IgG and IgM against SARS-CoV-2. He came out of isolation on August 4, 2020, after 2 negative SARS-CoV-2 RT-PCR tests from nasopharyngeal swabs taken 24 h apart. On August 10, 2020, he tested negative for IgG and IgM against SARS-CoV-2.

Approximately 3 months later, on October 26, 2020, the patient presented with anosmia, ageusia, fever and headache. He tested positive for SARS-CoV-2 RT-PCR (Ct = 17.8) from a nasopharyngeal swab and negative for IgG and IgM against SARS-CoV-2. He came out of isolation on November 6, 2020, after 2 negative SARS-CoV-2 RT-PCR tests from nasopharyngeal swabs taken 24 h apart. On November 6 and 25, 2020, he tested positive for IgG and IgM against SARS-CoV-2. On February 2, 2021, IgG and IgM against SARS-CoV-2 were retested, and negative results were obtained (Figure 1).

Genome analysis

Whole-genome sequencing was performed from RNA collected during the first and second infection. Genomes were registered in GISAID and GenBank databases for the first (GISAID accession number EPI_ISL_660069; GenBank accession number MW294007.1) and second infection (GISAID accession number EPI_ISL_660070; GenBank accession number MW294011.1).

Genomic analysis showed that both infections belonged to GISAID clade O (Other), Next strain clade 20B, and Pangolin Lineage B.1.1 with a probability of 1.0.

Variant calling (reference vs consensus) identified 33 changes (6 common to both infections, 14 first infection only, 13 second infection only). The 2 virus genomes differed by 27 nucleotides, in which 22 were non-synonymous mutations, resulting in amino acid changes (Figure 2).

The difference in the amino acids between the 2 genomes was located in the spike protein, nucleocapsid protein, nonstructural proteins (NSP2, NSP3, NSP12, NSP13, NSP14), accessory protein (NS3/ORF3a), and membrane protein.

GISAID's CoVsurver: Mutation Analysis of hCoV-19 (Shu and McCauley, 2017) showed that the first infection had 99.84% AA identity with the reference hCoV-19/Wuhan/WIV04/2019 owing to

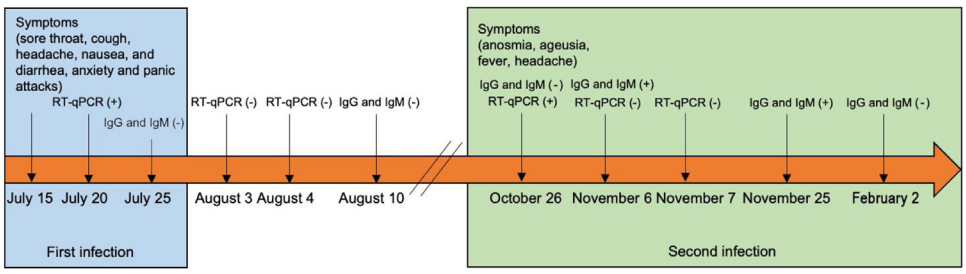

Figure 1. Timeline of a reinfection case, symptoms and diagnostic tests are shown.

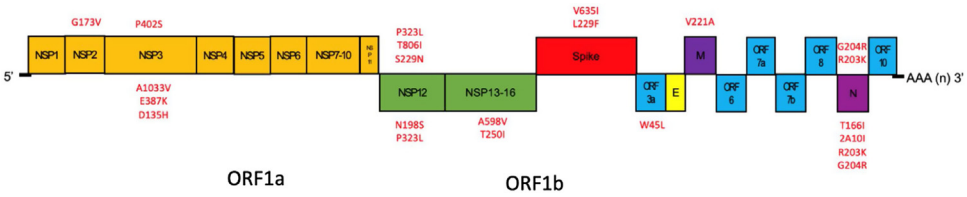

Figure 2. Schematic diagram of SARS-CoV-2 open reading frames showing differences in amino acids between the first and second infection. The first infection is shown at the top and the second infection is at the bottom.

2 aa changes, L229F and V635I. The second infection had 100% AA identity with hCoV-19/Wuhan/WIV04/2019.

Phylogenetic analysis showed the relatedness of both infections to each other and their relative distance from other Ecuadorian COVID-19 samples. Both samples are closely related to GISAID's clade GR, Pangolin lineage B.1.1.29; however, the first infection is closely related to a sample isolated from Quito, while the second infection is closely related to a sample isolated from Guayaquil (Figure 3).

DNA fingerprinting confirmed the identity of the patient.

Discussion

A reinfection case with different clades has previously been reported in Ecuador (Prado-Vivar et al., 2020), and a reinfection case with the same clade was reported in the USA (Tillett et al., 2020). However, unlike these cases, we were able to assess the immune response of the first infection of SARS-CoV-2.

According to the Pan American Health Organization definitions (Pan American Health Organization, 2020) and European Centre for Disease Prevention and Control guidelines (European Centre for Disease Prevention and Control, 2020), suspected reinfection happens if: the minimal interval between 1 confirmatory negative test and a subsequent positive PCR test is 45–90 days; the initial confirmed case has an interval without symptoms or a negative laboratory test (epidemiological criteria); the secondary infection belongs to a different clade or lineages (laboratory criteria), regardless of the number of single-nucleotide variants; the viral load Ct is <37 in both cases—a Ct of <37 is defined as a positive test, while a Ct >40 is defined as a negative test (Xing et al., 2020); and the mutation rate within the host makes it possible to rule out persistence and double infection through sequence phylogenetic analysis.

Our case had an interval of 82 days between the first and second infection; during the interval, he had no symptoms and had 2 negative laboratory tests, thus ruling out the phenomenon known as re-positivity where asymptomatic patients carry a small quantity of the virus (Lan et al., 2020; Xing et al., 2020). Therefore, true reinfection is more likely than a re-activation or re-positivity. Our patient had symptoms of COVID-19 in both cases, so we discarded false positivity. Both infections had Ct <37; for the first Ct = 29.9, in the second Ct = 17.8.

The second infection came from the same clade. However, our analysis showed more nucleotide changes than expected for persistence, approximately 4–6 fold more than the natural rate of mutation of SARS-CoV-2 of ~2–3 nucleotide changes per month (Callaway, 2020; Candido et al., 2020). Reinfection is indicated where genetic discordance between the specimens is greater than can be accounted for by *in vivo* evolution (Tillett et al., 2020).

The patient presented different COVID-19 symptoms, with the first episode more aggressive than the second. Similar findings were reported for reinfection cases in Belgium and Hong Kong (To et al., 2020; VanElslande et al., 2020). COVID-19 antibodies were detected in the second infection but not in the first, similar to the Hong Kong case (To et al., 2020). We can speculate that the first infection was caused by a virus that caused worse disease owing to its ability to evade the immune system through spike mutations (Weisblum et al., 2020). Interestingly, approximately 3 months after the second infection COVID-19 antibodies were not detectable in the patient, suggesting potential vulnerability to a third infection. However, analysis of the patient's immune system is yet to verify this.

Several mutations in the spike protein receptor-binding domain and N-terminal domain have been shown to confer reduced susceptibility to neutralizing antibodies (Weisblum et al., 2020). For our patient, 2 amino acid residues differed in the spike protein between the first and second infection: L229F and V635I. L229F was first only found in North America (Grabowski et al., 2021; Guruprasad, 2021); however, variants have now extended worldwide (Shu and McCauley, 2017). In addition, NSP12_P323L, the mutation present in both infection samples, is frequently found worldwide (Coppée et al., 2020).

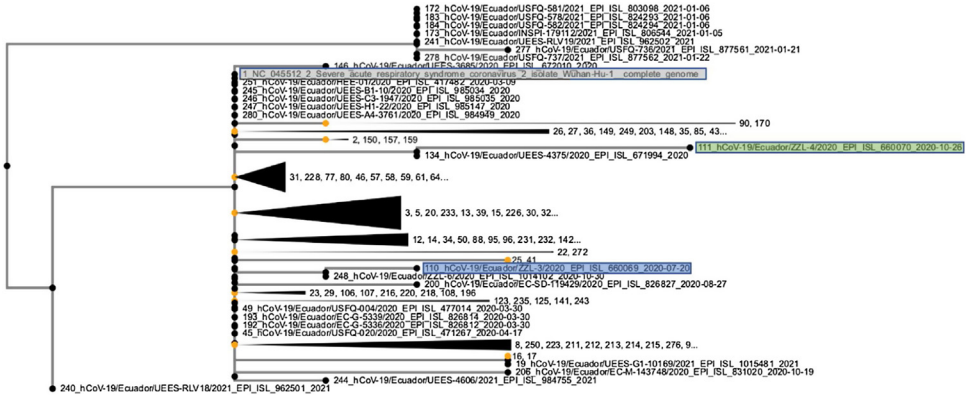

Figure 3. Phylogenetic analysis of whole SARS-CoV-2 genomes of Ecuador showing the relationships between the 2 strains identified from the patient. The first infection (shown in blue) is closely related to a sample isolated from Guayaquil, and the second (shown in green) is closely related to a sample isolated from Quito. The reference genome is shown in grey.

Conclusions

Some patients do not acquire natural immunity from COVID-19 after infection, which allows them to be reinfected by very similar SARS-CoV-2 strains. These cases are probably rare, but the present case shows that this phenomenon can be observed.

Limitations

The role of reinfection within the same clade on transmission could not be investigated as contact tracing and follow-up were not possible. Despite the similarity of the outputs, depending on the approach taken to analyze SARS-CoV-2 Illumina genomes, slight variations can happen, including the number of reads used for assembly, the number of mutations, and the branches of the phylogenetic trees.

Conflict of interest

All authors declare no competing interests

Funding

This work was supported by the Unidad de Investigaciones en Biomedicina from Zurita and Zurita Laboratorios, Quito-Ecuador [grant number MIC-018].

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