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RESEARCH LETTER

SARS-CoV-2 Delta variant in Cartagena de Indias, Colombia, August 2021

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1 | INTRODUCTION

The severe acute respiratory syndrome (SARS)-CoV-2 Delta variant virus was first detected in India on 1 October 2020. Since then, the Delta variant has rapidly spread and displaced other SARS-CoV-2 variants circulating in several locations, becoming the dominant variant. The Delta variant, which includes B.1.617.2 and AY lineages, has been reported in >190 countries and has been the predominant SARS-CoV-2 variant detected in the Americas as of 2 October 2021

On 24 July 2021, the first case of the Delta variant was detected in the Colombian city of Cali by routine genomic surveillance conducted by the Colombian National Institute of Health.[1] However, despite subsequent reports of Delta in other Colombian regions,[2] knowledge and description of SARS-CoV-2 variants remained scarce and limited to the first case of the Delta variant from Cali. In August 2021, additional Delta variant cases (n = 5) were identified in Cartagena, and herein, we described their genetic and epidemiological investigation.

2 | METHODS

Participants were recruited by an ongoing surveillance study conducted by Unidad de Investigación Molecular (UNIMOL) that enrolls individuals with acute respiratory symptoms since 2014 under an institutional review board-approved protocol (UDC2014-70). Epidemiological information, including demographic characteristics, co-morbidities, vaccination status, and clinical data, were collected at the time of enrollment using a standard form provided by the National Institute of Health of Colombia. Nasopharyngeal swabs were routinely collected, and real-time reverse-transcriptase polymerase chain reaction (RT-PCR)[3] and antigen (Abbott; Panbio COVID-19 Ag Rapid Test Device) tests were performed to detect SARS-CoV-2 RNA and protein, respectively.

The SARS-CoV-2 spike gene was sequenced using a targeted amplification approach of overlapping segments. Briefly, previously described sets of 12 PCR primers (SC2M1-54 to SC2M1-65) and a set of primers (SC2M1-55_ LEFT and SC2M1-56_RIGHT) were selected for the entire and partial gene sequencing, respectively.[4] Viral RNA was extracted from swabs using the QIAamp Viral RNA Mini kit (QIAGEN; 52906), and cDNA and amplicons were generated as previously described[4] in a one-step RT-PCR using 20 μL reaction volume that contained 1.5 μL nuclease-free water, 10 μL 2X buffer, 0.5 μL enzyme mix (Invitrogen; 12574026), 1.5 μL 10 μM of forward and reverse primers, and 5 μL of RNA. Amplicon products were purified using ExoSAP-IT (Applied Biosystems; 78200.200.UL). The Sanger sequencing amplification was performed using the BigDye Terminator v3.1 kit (ThermoFisher; 4337455), products were purified by BigDye XTerminator kit (ThermoFisher; 4376486), and the capillary electrophoresis was performed in the SeqStudio genetic analyzer. The analysis and quality control were performed using the Sequencing Analysis Software v6.0 (Applied Biosystems; 4474950), and trimmed and high-quality sequences were assembled to the SARS-CoV-2 reference genome (GenBank accession number: NC_045512.2) by the SeqScape Software v3.0 (Applied Biosystems; reference 4474978). The SARS-CoV-2 genome was generated as described in the ARTIC v3 protocol (https://www.protocols.io/view/ncov-2019-sequencing-protocol-v3-locost-bh42j8ye) and next-generation sequencing (NGS) reads were

analyzed using the bioinformatics pipeline described by Oxford Nanopore (https://artic.network/ncov-2019/ncov2019-bioinformatics-sop.html). Consensus sequences of the spike gene were deposited in GenBank (MZ836848, MZ836849, OL354418, OL354419, and OL354420) and the genome in GISAID (EPI_ISL_4081113).

The SARS-CoV-2 genome was submitted to the Pangolin COVID-19 Lineage Assigner (https://pangolin.cog-uk.io/), and the genome and spike sequences were submitted to Nextclade v1.7.1 (https://clades.nextstrain.org/) for clade assignment and mutation calling. Differences at the nucleotide and amino acid levels were assessed by aligning consensus sequences using MUSCLE in Mega 10.0.5.[5] To confirm the clade assignment, the first Delta variant reported for Colombia (EPI_ISL_3065505), and three other non-Delta variants were downloaded from GISAID (https://www.gisaid.org/) and used as references in the phylogenetic analysis.

3 | RESULTS

On 9 August 2021, Subject A was enrolled and tested positive for SARS-CoV-2 (Table 1). Subject A was a 53-year-old female who reported no co-morbidities, being fully vaccinated with a two-dose mRNA COVID-19 vaccine 2.9 months prior to enrollment. She did report of a headache since 6 August. Additionally, Subject A reported to have attended a work-related social event on August 4. Subject A reported no suspected cases of COVID-19 among her close contacts.

On 10 August 2021, Subject B was enrolled and tested positive for SARS-CoV-2 (Table 1). Subject B was a 46-year-old male with no co-morbidities who reported being fully vaccinated with a two-dose mRNA COVID-19 vaccine 2.9 months before enrollment. He reported a sore throat and rhinorrhea since 7 August. Interestingly, Subject B reported being at the same event as Subject A, since they are coworkers. Subject B had reported symptomatic suspected COVID-19 cases among his household contacts.

All household contacts of Subject B—a 47-year-old wife (Subject B.1), two daughters aged 20 (Subject B.2) and 2 years (Subject B.3), and a 7-year-old son (Subject B.4)—were enrolled on 10 August. Subjects B.1, B.3, and B.4 tested positive for SARS-CoV-2 (Table 1) and were symptomatic. Subjects B.1 and B.4 reported headache and fever since August 9, respectively, and Subject B.3 reported cough and fever since August 8. Subject B.2 had negative antigen and RT-PCR results, and reported no acute respiratory symptoms at and 3 days after enrollment. Additionally, subjects B.1 and B.2 reported complete

TABLE 1 Laboratory diagnosis of SARS-CoV-2 and genetic sequencing results

Cases	A	B	B.1	B.3	B.4
RT-PCR (Ct value)[a]	Positive (24.9)	Positive (26.3)	Positive (25.2)	Positive (22.6)	Positive (28.8)
Antigen test	Positive	Positive	Positive	Positive	Negative
Sequencing approach					
Spike	Partial	Partial	Partial	Full	Partial
Whole genome	No	No	No	Yes	No
Nextstrain clade/Pangolin lineage	21A (Delta)/-	21A (Delta)/-	21A (Delta)/-	21A (Delta)/AY.46.6[b]	21A (Delta)/-
Spike aa changes[c]					
T19R	Yes	Yes	Yes	Yes	N.D.
T95I	Yes	Yes	Yes	Yes	Yes
G142D	Yes	Yes	Yes	Yes	Yes
R158G	Yes	Yes	Yes	Yes	N.D.
L452R	N.T.	N.T.	N.T.	Yes	N.T.
T478K	N.T.	N.T.	N.T.	Yes	N.T.
D614G	N.T.	N.T.	N.T.	Yes	N.T.
P681R	N.T.	N.T.	N.T.	Yes	N.T.
D950N	N.T.	N.T.	N.T.	Yes	N.T.
Deletions					
DEL156	Yes	Yes	Yes	Yes	N.D.
DEL157	Yes	Yes	Yes	Yes	N.D.

[a]Cycle threshold for the RNA-dependent RNA polymerase (RdRp) gene described in the Charité/Berlin protocol (doi: 10.2807/1560-7917.ES.2020.25.3.2000045).

[b]Spike mutations were described in the table. Additional mutations in ORF1a (T403I, A1306S, P2046L, P2287S, V2930L, T3255I, T3646A, A3889V, and T4265I), ORF1b (P314L, G662S, P1000L, A1219S, A1918V, and V2345A), ORF3a (S26L), M (I82T), ORF7a (V82A, and T120I), ORF7b (T40I), ORF8 (DEL119, and DEL120), N (D63G, R203M, G215C, and D377Y), and ORF9b (T60A) were detected across the AY.46.6 genome.

[c]The amino acid (aa)-level mutation calling in the spike gene was performed in Nextclade v.1.7.2 (https://clades.nextstrain.org/). N.T., not tested because the sample was selected for the partial sequencing approach. N.D., not detected because the Sanger sequencing resulted in a short-read sequence.

vaccination with a two-dose mRNA COVID-19 vaccine 1.9 and 2.3 months before enrollment, respectively.

All subjects described here reported no co-morbidities, no domestic or international travel, and no exposure to suspected or confirmed COVID-19 individuals in the past 15 days. Subjects A and B reported being in constant isolation as a preventive measure, and having socialized the day of the event with people outside their social circle in the last 15 days. The epidemiological investigation is summarized in Figure 1A.

The swab from Subject B.3 was selected for full-length spike and whole-genome sequencing as the specimen had the highest viral load, whereas specimens from subjects A, B, B.1, and B.4 were selected for the partial sequencing approach (Table 1). Mutations in the SARS-CoV-2 spike gene are summarized in Table 1. Nextstrain clade assigned for all sequences was 21A (Delta), and Pangolin lineage for the genome was AY.46.6 (Table 1). Given that all reported sequences had the same mutations in spike, and subjects were epidemiologically linked, the phylogenetic analysis only included the AY.46.6 genome (EPI_ISL_4081113). The phylogenetic analysis validated that the genome reported here clustered and was genetically related to other Delta variants (Figure 1B).

4 | DISCUSSION

Since its initial report in October 2020, the Delta variant has evolved and become the most dominant variant in several locations worldwide.[6-8] However, it was not until July 2021 that the Delta variant increased exponentially in the Americas.[9] In Colombia, Delta variant reports increased similarly to those observed in the rest of the Americas. August was the month in which these reports specifically increased (https://covariants.org/per-country).

While studying respiratory viruses in Cartagena, two epidemiologically linked Delta-positive subjects were detected. A subsequent epidemiological investigation revealed three additional cases of the Delta variant among household contacts from one of the first two cases. In view of the genetic results and epidemiological information, it is very likely that the reported social event was the source of infection for subjects A and B, and SARS-CoV-2 subsequently spread within household contacts of Subject B.

Sanger sequencing of the spike gene is a rapid and low-cost strategy that allows it to identify signature mutations, and to distinguish and monitor circulating variants.[10,11] In this study, the mutation calling and Nextstrain clade assignment were successfully performed using

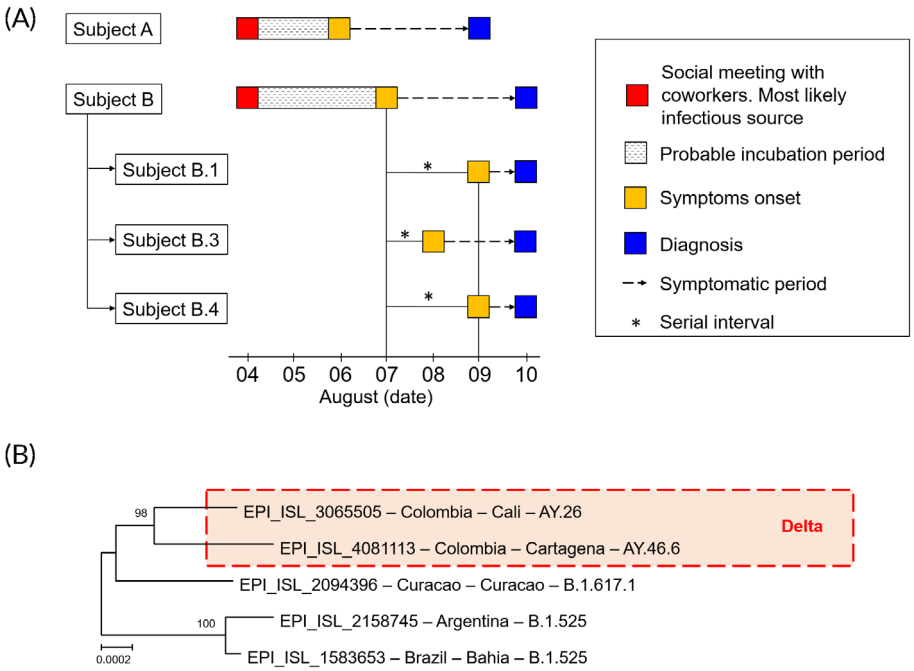

FIGURE 1 Epidemiological investigation and phylogenetic analysis of the first cases detected with the SARS-CoV-2 Delta variant in Cartagena. (A) Case map of Delta variant-positive cases and their relationship. The probable infectious source was reported by Subjects A and B. Incubation periods are probable assuming that the infectious source coincided with the day of the social event. Subjects B.1, B.3, and B.4 are considered secondary transmissions from Subject B given the close contact in the household. The serial interval was defined as the duration between symptom onset of Subject B and symptom onset of its household contacts. (B) The first Delta variant reported for Colombia (EPI_ISL_3065505), the B.1.617.1 variant from Curacao (EPI_ISL_2094396), and two B.1.525 variants from Argentina (EPI_ISL_2158745) and Brazil (EPI_ISL_1583653) were downloaded from GISAID (https://www.gisaid.org/) and used as references in phylogenetic analysis. The analysis included the AY.46.6 genome reported in this study. Sequences were labeled using the GISAID identification number (https://www.gisaid.org/), Country and City (if available) of detection, and the Pango lineage. The analysis was conducted using 29 673 nt (99.2% of the whole genome) with the maximum likelihood method under a Tamura 3-parameter substitution model with 1000 bootstrap replicates. The scale bar denotes the genetic distance in nucleotide substitution per site

partial or complete spike gene information generated by Sanger sequencing; however, such information was not sufficient for lineage assignment by Pangolin. Interestingly, regardless of the use of Sanger or NGS, the mutations detected were useful for variant classification.[8,12] Hence, in settings with scarce resources for whole-genome sequencing, Sanger may represent a suitable characterization tool.

The whole-genome sequencing revealed an AY.46.6 lineage, which is the first report for its presence in Cartagena and Colombia. The lineage was first documented in October 2020, and given its wide distribution in Europe, it is considered a European lineage (https://cov-lineages.org/lineage.html?lineage=AY.46.6, accessed on 30 October 2021). Nearly all reported mutations identified in the AY.46.6 are signature characteristics of its lineage (https://outbreak.info/situation-reports?pango=AY.46.6), and several have previously been associated with increased transmissibility, immune evasion, and resistance to neutralizing antibodies.[13-15]

The cases described herein illustrate cryptic transmission of the Delta variant in Cartagena residents with no travel history. However, since subjects were recruited through passive surveillance, we are unable to determine community transmission. The information generated here, however, could contribute to further transmission reconstruction in Cartagena. In addition, we were unable to establish the incubation period for contacts of Subject B, nor to describe transmission chains within household contacts. However, given the high transmissibility and short incubation period of the Delta variant described even for vaccinated individuals,[15-17] transmission from Subject B to its contacts likely occurred in the pre-symptomatic phase.

The integration of epidemiological and genomic surveillance is critical for generating a comprehensive public health response strategy oriented to local needs.[18,19] In summary, a familial cluster of four cases and an additional case related to the index case of the cluster were identified with the Delta variant in Cartagena during August 2021. This report constitutes the first description of Delta in Cartagena, highlights the relevance of genomic and epidemiological surveillance for monitoring variants, and provides genomic information that will be useful for future studies in settings with limited sequencing capacity.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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All authors have read and approved the final version of the manuscript.

All authors had full access to the data and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

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