Infection and Drug Resistance

Dovepress
open access to scientific and medical research

Open Access Full Text Article

CASE REPORT

First Report of SARS-CoV-2 Lineage B.1.1.7 (Alpha Variant) in Ecuador, January 2021

Andres Carrazco-Montalvo,[1,*] Alfredo Bruno,[2,3,*] Domenica de Mora,[2,*] Maritza Olmedo,[2] Jimmy Garces,[2] Michelle Paez,[2] Mary Regato-Arrata,[4] Manuel Gonzalez,[4,5] Jorge Romero,[6] Orson Mestanza,[7] Byron Freire-Paspuel,[8] Anibal Gaviria,[9] Solon-Alberto Orlando,[1,3] Miguel Angel Garcia-Bereguiain,[8] Leandro Patino[1]

[1]Dirección de Investigación, Desarrollo e Innovación, Instituto Nacional de Investigación en Salud Pública "Leopoldo Izquieta Pérez", Guayaquil, Ecuador; [2]Centro de Referencia Nacional de Influenza y otros Virus Respiratorios, Instituto Nacional de Investigación en Salud Pública "Leopoldo Izquieta Pérez", Guayaquil, Ecuador; [3]Universidad Agraria del Ecuador, Guayaquil, Ecuador; [4]Centro de Referencia Nacional de Virus Exantemáticos, Gastroentéricos y Transmitidos por Vectores, Instituto Nacional de Investigación en Salud Pública "Leopoldo Izquieta Pérez", Guayaquil, Ecuador; [5]Universidad de Guayaquil, Guayaquil, Ecuador; [6]Ministerio de Salud Pública, Zonal 5, Guayaquil, Ecuador; [7]Instituto Nacional del Niño San Borja, Lima, Perú; [8]One Health Research Group, Universidad de Las Américas, Quito, Ecuador; [9]Laboratorio Cruz Vida, Cruz Roja Ecuatoriana, Quito, Ecuador

*These authors contributed equally to this work

Correspondence: Miguel Angel Garcia-Bereguiain; Leandro Patino
Email magbereguiain@gmail.com; lpatino@inspi.gob.ec

Abstract: On January 5 2021, Ecuadorian COVID-19 genomic surveillance program detected a suspicious case of the B.1.1.7 lineage (alpha variant) of SARS-CoV-2 in Los Rios province, later confirmed by genome sequencing. The patient travelled from the UK by the end of December 2020. By contact tracing, several new cases were detected confirming B.1.1.7 transmission and spreading in Ecuador.

Keywords: SARS-CoV-2, Ecuador B.1.1.7 lineage, alpha variant

Introduction

Genomic analysis of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have led to identification of multiple lineages worldwide and tracking of geographical spread. A current priority of genomic surveillance is the detection of variant of concerns (VOC) defined by the Centers for Disease Control and Prevention (CDC) from US as a variant with an increase in transmissibility, causing more severe disease (e.g., increased hospitalizations or deaths), significant reduction in neutralization by antibodies generated during previous infection or vaccination, reduced effectiveness of treatments or vaccines, or diagnostic detection failures. Up to date, four VOCs have been named by the World Health Organization as follows: lineage B.1.1.7 or alpha, first identified in the United Kingdom; lineage B.135 or beta, first identified in South Africa; lineage B.1.617.2 or delta, first identified in India; and lineage P.1 or gamma, first identified in Brazil and Japan. Among those VOCs, the lineage B.1.1.7 (clade 501.YV1) or alpha is characterized by 17 unique mutations and was first detected in southeastern England in late September 2020, and by January 17 2021 it was confirmed in 38 countries.[1,2] Genetic and epidemiologic studies suggest that the explosive growth of alpha is caused by its enhanced transmissibility, estimated to be 56–70% higher than other SARS-CoV-2 lineages,[3] that has been potentially associated to mutations like N501Y that increased the binding affinity to human ACE2 receptor.[4] B.1.1.7 or alpha lineage has been associated to the dramatic increase of the SARS-CoV-2 incidence in countries like UK and Portugal pushing their public health systems close to collapse during January 2021.

Case Report

We reported the index case of alpha variant in Ecuador on January 11 2021 confirmed by whole genome sequencing. We received a nasopharyngeal sample from one patient for genomic sequencing as part of the SARS-CoV-2 surveillance

Received: 12 May 2021
Accepted: 16 October 2021
Published: 6 December 2021

strategy at the National Institute of Research and Public Health (INSPI), located in Guayaquil. The patient was an Ecuadorian citizen, male of 50 years of age, residing in Los Rios province in the Coastal Region of Ecuador, who reported to arrive to Ecuador from the United Kingdom on December 12 2020, with a flight stop in Spain. Upon arrival, he reported a negative SARS-CoV-2 RT-PCR test and did not present symptoms of COVID-19. His symptoms began on December 28 2020 and by January 4 2021 he required hospitalization diagnosed with acute respiratory disease. SARS-CoV-2 infection was confirmed on December 7 at the National Reference Center for Influenza and other Respiratory Viruses of INSPI using SARS-CoV-2 RT-PCR (Cobas technology by Roche, Switzerland) targeting E and Orf1ab genes. Other five RT-PCR kits were used in order to find a potential discrimination of the variant. These kits comprised Allplex TM 2019-nCoV Assay (Seegene, South Korea), ECUGEN SARS-CoV-2 RT-PCR kit (UDLA-StarNewCorp, Ecuador), "TaqMan 2019 nCov Assay Kit v1", 'TaqMan 2019 SARS-CoV-2 Assay Kit v2' and 'TaqPath COVID-19 RT-PCR Kit' (ThermoFisher Scientific, USA). The target genes and the Ct values obtained are shown in Table 1. The expected dropout of S gene amplification associated to alpha lineage with "TaqPath COVID-19 RT-PCR Kit" and "TaqMan SARS-CoV-2 Assay Kit v2" was found (Table 1).

Alpha variant was confirmed by whole-genome sequencing at the laboratory of the Direction of Research Development and Innovation of INSPI using nanopore technology in a MinION device (Oxford Nanopore Technologies) and following the ARTIC V2 protocol for amplicon sequencing.[5–8] MinION was used given its cost effectiveness in low resource setting such as low and middle income countries. By January 2021 this was the only sequencing technology available at INSPI. The genome was processed using the Artic bioinformatics pipeline, assembled in the online tool Genome Detective using default parameters[9] and submitted to Nextclade (https://clades.nextstrain.org/) for preliminary taxonomic analysis. Analysis in Genome Detective showed 99.86% identity with the reference genome with a breadth of coverage of 85% and a median depth of coverage of 98 X (number of reads that align to a given position in the genome). Following the Nextclade analysis, the annotated genome clustered into 501.YV1 clade which correspond to Alpha VOC. Alignment with the reference genomes "isolate Wuhan-Hu-1" GenBank (ID: NC_045512.3) and "VOC Alpha 202012/01 first detected in the UK" GISAID (ID: EPI_ISL_601443) showed that the sequence obtained contains the 17 mutations defining the Alpha variant or 501.YV1 clade,[10] as well as other 4 mutations shared with the Wuhan-Hu isolated (Figure 1). The sequence is available at GISAID, accession ID: EPI_ISL_806544. Phylogenetic analysis with a selection of alpha genomes from Ecuador, North America, Latin America and Europe, using RAxML-NG under a GTR substitution model and 1000 bootstrap replicates,[11] confirms the genome identity as a proximal descendant from EPI_ISL_601443, a genetically similar sequence reported in England in September 2020 (Figure 2), and show different clusters of the variant in Ecuador (Figure 2). Following the detection of the index case different sequencing laboratories in Ecuador reported an increasing number of alpha genomes to GISAID, alpha variant was the most frequent VOC until June 2021, before gamma and delta were detected in the country (Figure 3).

Table 1 SARS-CoV-2 RT-qPCR Commercial Kits and Ct Values for the First B.1.1.7 or Alpha Strain Identified in Ecuador

SARS-CoV-2 RT Kit	Gene Targets	Ct Values
Cobas SARS-CoV-2 test	E/Orf1ab	27.7/28.2
ECUGEN SARS-CoV-2 RT-PCR kit	N1/N2	25.1/25.6
Allplex TM 2019-nCoV Assay	E/RdRp	25.4/28.8
TaqMan 2019 nCov Assay Kit v1	S/Orf1ab/N	26.0/ 26.1/ 24.0
TaqMan 2019 SARS-CoV-2 Assay Kit v2	S/Orf1ab/N	NA/ 23.3/ 25.3
TaqPath COVID-19 RT-PCR Kit	S/Orf1ab/N	NA/ 25.9/24.6

Abbreviation: NA, Not Amplification.

Discussion

Alpha variant was the first VOC identified in Ecuador in January 2021. Once this index case was confirmed, the Ministry of Health (MoH) implemented a contact tracing strategy including 14 family members under quarantine and surveillance of 2560 people from the same neighborhood. Four additional cases of alpha variant were reported from samples sent by the MoH to the University San Francisco de Quito, showing the local transmission of this variant in Ecuador (two of those sequences are shown in blue in Figure 2). The five initial cases of the alpha VOC in Ecuador were detected in members of the same family, four of them without record of travel to other countries or within Ecuador. By epidemiological nexus the transmission occurred from the index case. These samples

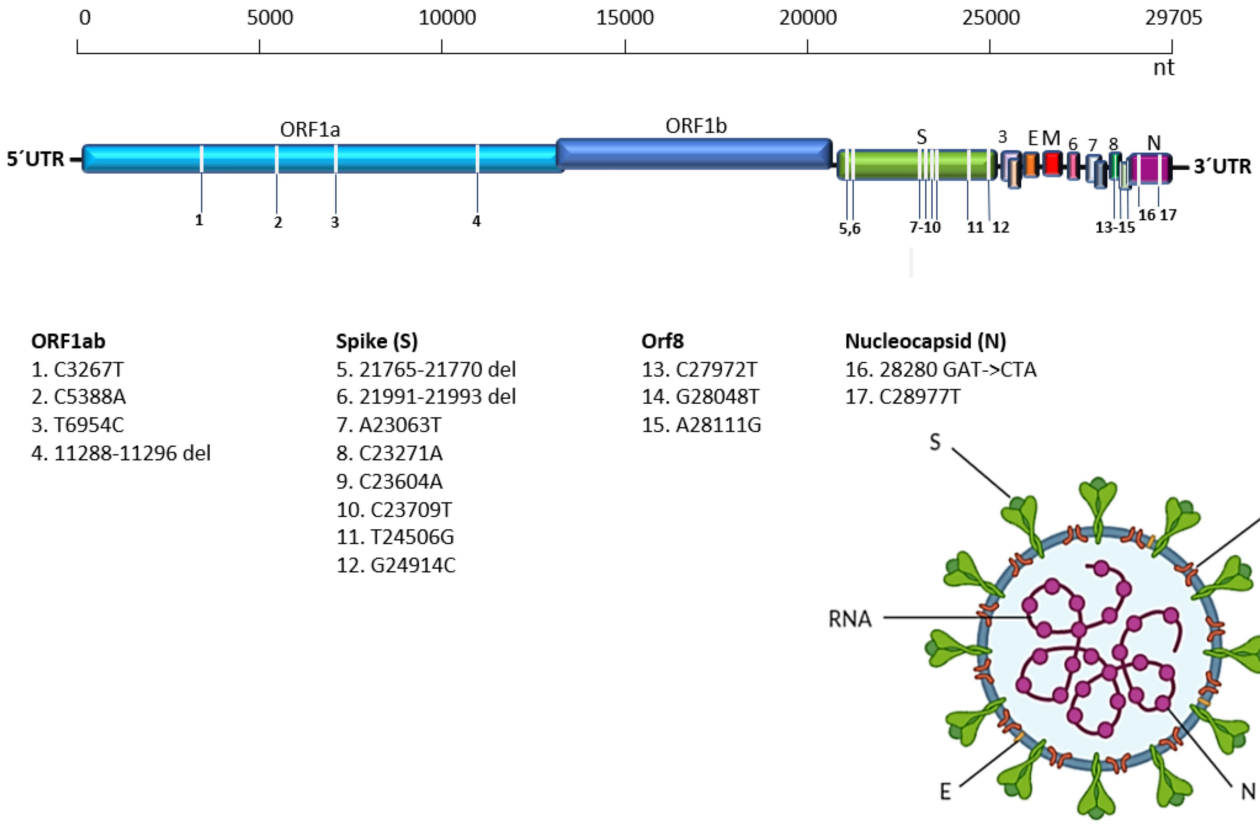

Figure 1 The 17 mutations defining lineage B.1.1.7 or alpha (clade 501.YV1). All the mutations are shared between the genome assembled for the first record of this variant in Ecuador and the reference genome for the clade.

were sequenced using MinION technology in two different laboratories, the index case in INSPI and the other four at the University San Francisco de Quito, in both cases the genomes were assigned to the alpha variant, the independent clustering in the phylogenetic analysis could be due to the different assembly methodology. Although current average base error rate of MinION reads is between 10–5%, mapping a genome reference with a cutoff ≥ 30 X depth of coverage provide reliable sequence annotation, this depth can be achieved by amplicon sequencing.[6–9] Difference in assembly methods is still a challenge that needs to be addressed, we are normalizing our methodology locally and regionally. MinION technology has been widely adopted for SARS-CoV-2 genome sequencing;[6–9] in Ecuador, up to date 50% of sequences reported to GISAID have been obtained with this platform. The extent of transmission of alpha variant in Ecuador found by genomic sequencing ranged up to the 37% of genomes uploaded in GISAID by May 2021.

Latin America has been severely affected by COVID-19 pandemic. By January 25 2021 Ecuador, in particular, reported a total of 221,506 SARS-CoV-2 infection cases and 14,184 deaths, even before the first SARS-CoV-2 VOCs was confirmed in the country. Weak public health system in the region could be easily overflowed by waves of new highly transmissible SARS-CoV-2. Under this scenario, the surveillance of variants that carry mutations of potential biological significance (such as VOCs and variants of interest [VOI]) and the measures to reduce community transmission needs to be improved.[12] For example, the strategies for tracking VOC might include an initial screening by RT-qPCR assays such as the described here failing to amplify the S gene, sequencing subsets of random positive samples and establishing inter institutional collaborations in order to increase the number of samples sequenced as well as standardize the methods for genome assembly at local or regional scale. Finally, it is important to notice that the introduction of alpha into Ecuador happened despite the travelling requirement of a SARS-CoV-2 RT-qPCR negative test within 10 days of departure or negative SARS-CoV-2 antigen test upon arrival. SARS-CoV-2 incubation period may be as long as two weeks or more like on this case of study,[13] so quarantine upon arrival must be improved for an effective control and

Figure 2 Maximum likelihood phylogeny of a sample of B.1.1.7 Pango lineage/VOC Alpha genomes from Europe, Latin America and North America. Names of key sequences from Ecuador are featured; the first sequence from Ecuador is shown in red, a selection of additional sequences produced by the national genomic surveillance scheme are shown in blue, and genomes from international locations are shown in grey.

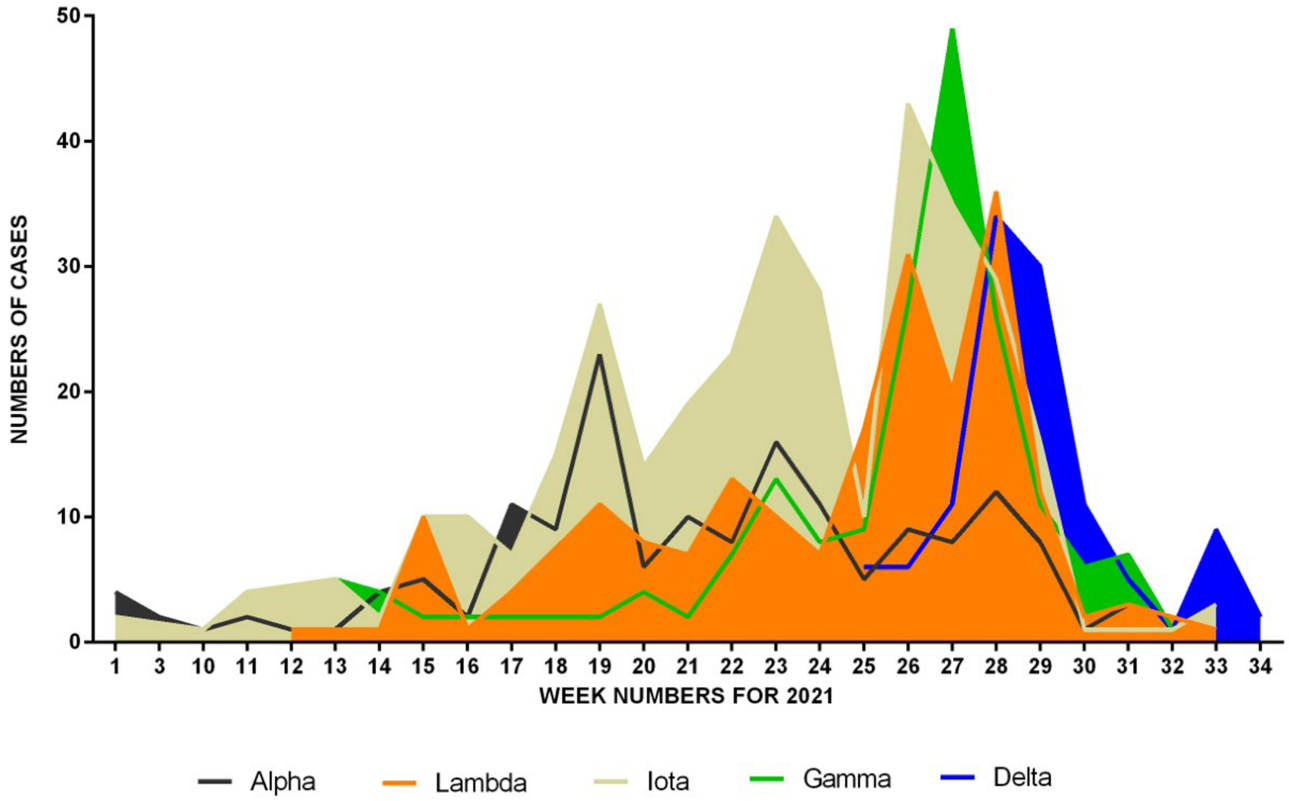

Figure 3 Number of SARS-CoV-2 =VOC and VOI submitted to GISAID from Ecuador from January 2021. The figure shows the number of cases detected by week.

prevention of SARS-CoV-2 VOCs spread, at least for travelers coming from countries with high VOCs prevalence.

Ethics Statement

Written consent was obtained from sample collection and case report publication from the patient; the study was authorized by the Scientific Board of Instituto Nacional de Investigación en Salud Pública "Leopoldo Izquieta Pérez."

Acknowledgments

We thank to our colleagues from The USFQ SARS-CoV-2 Consortium: Paul Cardenas, Sully Marquez, Belen Prado-Vivar, Juan José Guadalupe, Bernardo Gutierrez, Mónica Becerra-Wong, Michelle Grunauer, Gabriel Trueba, Patricio Rojas-Silva, Veronica Barragán. They were responsible for the sequencing of several of the alpha variant genomes included in this study. This institution is making a crucial contribution to SARS-CoV-2 surveillance in Ecuador.

Author Contributions

"All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work." AC, MAGB and LP wrote the first draft of the manuscript.

Funding

This study was supported by Instituto Nacional de Investigación en Salud Pública and Universidad de Las Américas.

Disclosure

The authors declare no conflicts of interest in this work.

References

1. virological.org. Preliminary genomic characterisation of an emergent SARS-CoV-2 lineage in the UK defined by a novel set of spike mutations. ARTIC Network; 2020 [updated December 5 2020]. Available from: https://virological.org/t/preliminary-genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-the-uk-defined-by-a-novel-set-of-spike-mutations/563. Accessed November 30, 2021.

2. cov-lineages.org; 2021 [updated November 30 2021. Available from: https://cov-lineages.org/global_report_B.1.1.7.html. Accessed November 30, 2021.
3. Kupferschmidt K. Mutant coronavirus in the United Kingdom sets off alarms, but its importance remains unclear. *Science*. 2020. doi:10.1126/science.abg2626.
4. European Centre for Disease Prevention and Control. Rapid increase of a SARS-CoV2 variant with multiple spike protein mutations observed in the United Kingdom. Stockholm: ECDC; 2020.
5. Quick J, Grubaugh ND, Pullan ST, et al. Multiplex PCR method for MinION and Illumina sequencing of Zika and other virus genomes directly from clinical samples. *Nat Protoc*. 2017;12:1261–1276. doi:10.1038/nprot.2017.066
6. Hourdel V, Kwasiborski A, Balière C, et al. rapid genomic characterization of SARS-CoV-2 by direct amplicon-based sequencing through comparison of MinION and Illumina iSeq100TM system. *Front Microbiol*. 2020;11. doi:10.3389/fmicb.2020.571328
7. Li J, Wang H, Mao L, et al. Rapid genomic characterization of SARS-CoV-2 viruses from clinical specimens using nanopore sequencing. *Sci Rep*. 2020;10(1):1–10. doi:10.1038/s41598-019-56847-4
8. Freed NE, Vlková M, Faisal MB, Silander OK. Rapid and inexpensive whole-genome sequencing of SARS-CoV-2 using 1200 bp tiled amplicons and Oxford Nanopore Rapid Barcoding. *Biol Methods Protoc*. 2020;5(1):bpaa014. doi:10.1093/biomethods/bpaa014
9. Bull RA, Adikari TN, Ferguson JM, et al. Analytical validity of nanopore sequencing for rapid SARS-CoV-2 genome analysis. *Nat Commun*. 2020;11:6272. doi:10.1038/s41467-020-20075-6
10. Vilsker M, Moosa Y, Nooij S, et al. Genome Detective: an automated system for virus identification from high-throughput sequencing data. *Bioinformatics*. 2019;35(5):871–873. doi:10.1093/bioinformatics/bty695
11. Kozlov AM, Darriba D, Flouri T, Morel B, Stamatakis A. RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics*. 2019;35(21):4453–4455. doi:10.1093/bioinformatics/btz305
12. Public Health England. Investigation of novel SARS-CoV-2 variant, variant of concern 202012/01 technical briefing 2–28. London: PHE; December 2020. Available from: https://www.gov.uk/government/publications/investigation-of-novel-sars-cov-2-variant-variant-of-concern–20201201. Accessed November 23, 2021.
13. Lauer SA, Grantz KH, Bi Q, et al. The incubation period of coronavirus disease 2019 (COVID-19) from publicly reported confirmed cases: estimation and application. *Ann Intern Med*. 2020;172(9):577–582. doi:10.7326/M20-0504