Multiple Introduction of SARS-CoV-2 C.37 Lambda Lineage in the Southern Brazilian Region

SARS-CoV-2 Lambda Introductions in the Southern Brazilian Region

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Highlight

Deemed a variant of concern by the World Health Organization on June 15th, the Lambda variant of SARS-CoV-2 is a growing epidemiological threat in several South American countries, and initial results suggest it exhibits increased infectivity and immune escape qualities. Here, we present evidence of its multiple introductions in Brazil.

The SARS-CoV-2 variant assigned to PangoLineage C.37 was first reported in late December 2020 in Peru, and by April 2021 was responsible for 97% of new infections in that country.[1] It is currently expanding in Chile and Argentina, and there is evidence of onward transmission in Colombia, Ecuador, Mexico, the USA, Germany, and Israel.[2] C.37 presents 30 molecular signatures, seven nonsynonymous substitutions (G75V, T76I, R246N, L452Q, F490S, D614G, T859N) and a deleted region (Δ247-253) in the Spike protein.[1] Another deleted region is found in the ORF1a gene (Δ3675-3677) also present in variants of concern (VOCs) Alpha, Beta, and Gamma.[1] Due to genomic characteristics and epidemiological relevance, on June 15, 2021, WHO designated C.37 as Variant of Interest (VOI) Lambda.[2,3]

Until July 26th, 2021, six cases of C.37 were notified in Brazil, two in the southern state of Rio Grande do Sul (RS), and four in the southeastern state of São Paulo (SP). The first case in RS (Patient 01), notified in early June, was a 23 years old (yo) male truck driver returning from a trip through Argentina on June 05th.[4] An epidemiological investigation was not able to identify potential infection sources, and even though two contacts also tested positive in antigen tests, samples were unavailable for genomic characterization. The second RS case (Patient 02), notified in early June, was a one-year female resident of Santo Ângelo, near the Argentine border. Only two days before the first symptoms, the patient had resumed pre-school classroom classes. The patient's father, mother, and 4 yo sister presented symptoms compatible with COVID-19, but only the father had positive antigen results, the other two had them undetectable.

The SARS-CoV-2 whole-genome (>99% coverage) of Patient 2 was recovered using Illumina COVIDSeq Test. FastQ reads obtained were imported into the CLC Genomics Workbench version 20.0.4 (Qiagen A/S, Denmark), trimmed, and mapped against the reference

sequence EPI_ISL_402124. Pango Lineages tool v.3.1.7 was used for lineage assignment and CoVSurver (https://mendel.bii.a-star.edu.sg/METHODS/corona/beta/) and Nextclade Web v.1.5.2 (https://clades.nextstrain.org/) tools were used to map the nucleotide and amino acid substitutions.

To understand the spatiotemporal dynamic of RS-identified introductions, we constructed a time-scaled Bayesian phylogenetic tree using the Bayesian Markov chain Monte Carlo (MCMC) approach implemented in BEAST 1.10 with BEAGLE library v3 to improve run-time efficiency.[5] To this end, a sub dataset (n = 175) of all C.37 genomes available in the EpiCoV database of the GISAID initiative (https://www.gisaid.org/) until July 17th was constructed by a local blast search followed by trimming of structured clusters in an ML topology inferred with IQ-TREE v2.1.3.[6,7] The Bayesian MCMC analysis was run for 200×10^6 generations and performed using a strict molecular clock model, a constant prior distribution on the substitution rate ($8–10 \times 10^{-4}$ substitutions per site per year), and the nonparametric Bayesian skyline model as a coalescent tree prior.[8] Viral migrations were reconstructed using a reversible discrete phylogeographic model with a continuous-time Markov chain (CTMC) rate reference prior.[9] Sequences were classified according to their country of origin.

Five Lambda introductions were detected in Brazil, in RS (n = 2) and SP (n = 3) (Supplementary Table 1), with only one introduction in SP aggregating more than one sequence. In the time-scaled Bayesian MCC tree (Figure 1), Patient 01's genome (EPI_ISL_2617911), sampled on June 10th, had its MRCA (Most Recent Common Ancestor) located in Argentina (Posterior State Probability [PSP] = 1.0), emerging on March 14th (95% HPD: 03/28 - 03/03). This observation was in accordance with Patient 01's known traveling pattern in the days before the onset of its symptoms. Patient 02's genome (EPI_ISL_3751412), sampled on June 10th, had its MRCA located in Argentina/Chile (PSP = 0.48/0.34) on March 6th (95% HPD: 02/18 - 03/21). The

family of Patient 02 lives in a city near the Argentine border, an epidemiological link that, in the absence of autochthone transmission, could answer for its lack of traveling history. The relatively low PSP value, however, is most probably due to a genomic subsampling. Other introductions in SP were associated with Peru (n = 2) or the USA (n = 1) (PSP = 1.0). The SP dyad was the one introduction associated with the USA and had its MRCA located in Brazil (PSP = 1.0). The absence of additional identification of C.37 in the state speaks in favor of a limited introduction or limited circulation. The synchronicity of this introduction with the early stages of Gamma dissemination in Brazil, a variant that would lead the largest yet surge of COVID-19 cases in the country may also have limited its reach in the Brazilian population.

The six Brazilian genomes, as analyzed by the Nexclade algorithm (https://clades.nextstrain.org/), exhibited almost all the lineage's 30 synapomorphies.[1] The N gene T366I substitution was not present in three SP sequences (EPI_ISL_2674368, EPI_ISL_1966094, and EPI_ISL_1445272). The genome of Patient 2 bore two additional amino acid substitutions in the Spike protein, T95I, and S151I. Both signatures were not found in any of the sequences the genome is clustered with in the MCC tree topology (Figure 1). The Spike protein deletion (Δ247-253) was absent in the SP dyad, one of them (EPI_ISL_1445272) also not exhibiting the deletion in ORF1 (Δ3675-3677).

Given the relevance to which the VOI Lambda rose in South America, and evidence of increased infectivity and immune escape, genomic surveillance of its spread is essential. Brazil is particularly vulnerable, considering its frontiers with 10 out of all 12 South American countries, with land borders extending for more than 15×10^3Km. The association of both RS introductions detected here with neighboring Argentine illustrates the effect of geographical position in the dissemination of infections diseases such as SARS-CoV-2. Therefore, an increased genomic

surveillance across Brazil's neighboring countries as well as in borderline Brazilian states could lead to the detection of additional introductions and elucidate the structure of clusters that lead to the known occurences. However, despite questions raised by epidemiological surveys, evidence of autochthone C.37 transmission is yet to be detected. Nonetheless, we demonstrated here that, since February, multiple Lambda introductions have occurred in Brazil.

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Author Contributions:

Conceptualization: IA and PCR; Formal Analysis: IA and PCR; Sequencing: ACDP, LA, RSL, ACFM, ASBR, TMM, and ECP; Epidemiological Survey: RSV, TSG, LGM, ALB, and AFM; Supervision: PCR and MMS; Writing – Original Draft: IA, PCR, RSS, TSG, and LGM. All authors took part in the review of the manuscript.

Author statements:

The authors declare no conflict of interest.

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FIGURE 01: Spatiotemporal Characterization of Multiple SARS-CoV-2 C.37 Lineage Introductions in Brazil. Time-scaled Bayesian MCC tree of SARS-CoV-2 C.37 genome sequences (*n* = 175) from Brazil (*n* = 6) and 11 other countries. Branches are colored according to the most probable location state of their descendent nodes as indicated in the legend on the upper right side.