PUBLICAÇÃO OFICIAL DO NÚCLEO HOSPITALAR DE EPIDEMIOLOGIA DO HOSPITAL SANTA CRUZ E PROGRAMA
DE PÓS GRADUAÇÃO EM PROMOÇÃO DA SAÚDE - DEPARTAMENTO DE BIOLOGIA E FARMÁCIA DA UNISC

Revista de Epidemiologia e Controle de Infecção

Hospital Santa Cruz

BRIEF COMMUNICATIONS

Epidemiological investigation reveals local transmission of SARS-CoV-2 lineage P.1 in Southern Brazil

Investigação epidemiológica revela transmissão local da variante P.1 do SARS-CoV-2 no Sul do Brasil

Una investigación epidemiológica revela la transmisión local de la variante P.1 del SARS-CoV-2 en el sur de Brasil

https://doi.org/10.17058/reci.v1i1.16335

Received: *03/19/2021*
Accepted: *04/05/2021*
Available online: *04/06/2021*

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Please cite this article as: *SALVATO, Richard Steiner et al. Epidemiological investigation reveals local transmission of SARS-CoV-2 lineage P.1 in Southern Brazil. Revista de Epidemiologia e Controle de Infecção, [S.l.], v. 11, n. 1, abr. 2021. ISSN 2238-3360. Available at: <https://online.unisc.br/seer/index.php/epidemiologia/article/view/16335>. Date accessed: 13 set. 2021. doi:https://doi.org/10.17058/reci.v1i1.16335.*

ABSTRACT

Since its detection in December of 2020, the SARS-CoV2 lineage P.1, descendent of B.1.1.28 lineage, has been identified in several places in Brazil and abroad. This Variant of Concern was considered highly prevalent in Northern Brazil and now is rapidly widening its geographical range. Here, we present epidemiological and genomic information of the first case of P1 lineage in Rio Grande do Sul state, in a patient without reported travel history and a tracked transmission chain. These findings occurred in a tourist destination representing an important hub receiving tourists from diverse places.

Keywords: *SARS-CoV-2. COVID-19. transmission. B.1.1.28.*

RESUMO

Desde a sua detecção em dezembro de 2020, a linhagem P.1 do SARS-CoV2, descendente da linhagem B.1.1.28, foi identificada em diversos locais no Brasil e no mundo. Essa variante de preocupação era considerada altamente frequente no Norte do Brasil e agora está ampliando rapidamente sua distribuição geográfica. Aqui, apresentamos informações epidemiológicas e genômicas do primeiro caso da linhagem P.1 no Rio Grande do Sul em um paciente sem histórico de viagens relatado e com cadeia de transmissão identificada. Esses achados ocorreram em um destino turístico que representa um importante pólo de recepção de turistas de diversas localidades.

Descritores: *SARS-CoV-2. COVID-19. transmissão. B.1.1.28.*

RESUMEN

Desde su detección en diciembre de 2020, del linaje P.1 del SARS-CoV2, derivada de la B.1.1.28, hay sido ampliamente identificada en Brasil y en todo el mundo. Esta variante preocupante es muy frecuente en el norte de Brasil y ahora está ampliando rápidamente su distribución geográfica. Aquí, presentamos información epidemiológica y genómica del primer caso de P.1 en Rio Grande do Sul en un paciente sin antecedentes de viaje y con una cadena de transmisión identificada. Estos datos se han obtenido en un destino turístico que representa un importante centro de acogida de turistas de diferentes lugares.

Palabras clave: *SARS-CoV-2. COVID-19. transmisión. B.1.1.28.*

Recently, a new variant first detected in Manaus/Amazonas in the North Region of Brazil has become a concern worldwide. The named P.1 lineage is descendent of B.1.1.28 lineage and carries a set of mutations with important biological significance, mainly at region encoding spike protein (E484K, K417T and N501Y) (N.R. Faria, et al., unpub. data, https://virological.org/t/genomic--characterisation-of-an-emergent-sars-cov-2-lineage--in-manaus-preliminary-findings/586).

The P.1 lineage emerged with high frequency in a short period of time, spreading fast in North of Brazil, and in the recent weeks in other cities from Southeastern Brazil (F. Naveca et al., unpub. data, *https://virological.org/t/sars-cov-2-reinfection-by-the-new-variant-of--concern-voc-p-1-in-amazonas-brazil/596)*. Here we described the first detected case of SARS-CoV-2 P.1 lineage in Rio Grande do Sul, the southernmost state in Brazil. The case occurred in Gramado city, a mountain town with German colonization that receives 6.5 million tourists every year. Gramado belongs to the 5th Regional Health Coordination (an administrative division) that comprises 49 municipalities with a 1,240,319 estimated population, also known as Caxias do Sul Region (*https://cidades.ibge.gov.br/brasil/panorama*).

An 88-years-old male patient (A) presented acute respiratory symptoms on Jan 29th, 2021 and was hospitalized on Feb 3rd after medical examination. At the same day, the patient was admitted at the intensive care unit with fever of 39ºC, no leg movements, chest pain, besides flu-like symptoms/Acute Respiratory Syndrome. Respiratory secretion was collected on Feb 1st and RT-qPCR for SARS-CoV-2 detection was performed at Central Laboratory of Public Health from Rio Grande do Sul (LACEN--RS). The RNA was extracted from respiratory clinical sample through MagMAX Viral/Pathogen II Isolation kit on KingFischer Flex extractor (Thermo Fisher Scientific, Waltham, USA). The multiple detection of 3 target genes (E gene, RdRP gene and N gene) from pathogen was performed on Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher Scientific, Waltham, USA), using Allplex SARS-CoV-2 Assay (Seegene Inc, Seoul, Republic of Korea). The SARS-CoV-2 RT-qPCR was positive (Ct 22) and the result released on Feb 2nd, the patient died on Feb 10th, nine days after hospitalization.

As part of SARS-CoV-2 genomic surveillance in Rio Grande do Sul State, the collected sample, along with samples from different cities, were sequenced aiming to obtain the current scenario of SARS-CoV-2 genomic diversity in this region. Whole genome library preparation of SARS-CoV-2 was performed using QIAseq SARS-CoV-2

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Primer Panel paired with QIAseq FX DNA Library UDI Kit, according to the manufacturer instructions. The sequencing was performed on an Illumina MiSeq machine using MiSeq Reagent Kit v3 (600-cycle). Raw FASTQ files from genome sequencing were firstly trimmed to remove adapter sequences and low-quality reads using trimmomatic[1] the read data quality assessed in fastQC (www.bioinformatics.babraham.ac.uk/projects/fastqc/) and then mapped against the reference genome Wuhan-Hu-1 (GenBank Accession MN908947.3) using the BWA-MEM algorithm[2] (Mean coverage: 2.158,216). Consensus fasta was obtained with SAMtools.[3]

The sequenced genome was assigned to P.1 lineage on Pangolin (github.com/cov-lineages/pangolin). Thus, we aligned the consensus fasta with other 156 P.1 genomes from worldwide available on GISAID[4] as of Feb 16, 2021 with MAFFT[5] under default parameters. The aligned multi-fasta was used to construct a maximum-likelihood tree in IQ-Tree v.2.1.2[6] (GTR+G4+F -alrt 1000 -nt AUTO), annotated in the iTOL web-based tool[7] and rooted on Wuhan-Hu-1 reference genome. The maximum-likelihood phylogenetic analyses revealed that the sequence from Gramado, Rio Grande do Sul, is branched in a monophyletic clade that comprises 25 genomes including sequences from Amazonas, Rondônia, Roraima and São Paulo state, along with sequences found in Japan and Colombia (Figure 1).

The patient (A) had no travel history, and the epidemiological investigation revealed a transmission chain as follows (linkages were omitted for privacy-preserving): he lived in a rural area, nearby the city, under self-isolation, and had daily contact with a person (B). This person had previous contact in a diner with an individual (C) on Jan 23rd, a tourism worker who used to have close contact with tourists and was positive to COVID-19 with onset symptoms on Jan 21st. (B) presented her first symptoms on Jan 26th and had a positive RT-qPCR test for SARS-CoV-2 on Jan 31st, presenting mild disease; (C) to date remains hospitalized with severe disease symptoms. Colleagues who shared the same workplace to (C) were also diagnosed with COVID-19 and some were admitted to the ICU with a severe disease presentation. In this same period the hospitalizations have risen in Gramado and more than 3-fold in the neighboring city, Canela, also a touristic city.

Our findings indicate a local transmission of P.1 lineage occurring in Gramado, and revealed a worrying emergence of severe COVID-19 cases in this region. It calls for future use of genomic surveillance as a regular

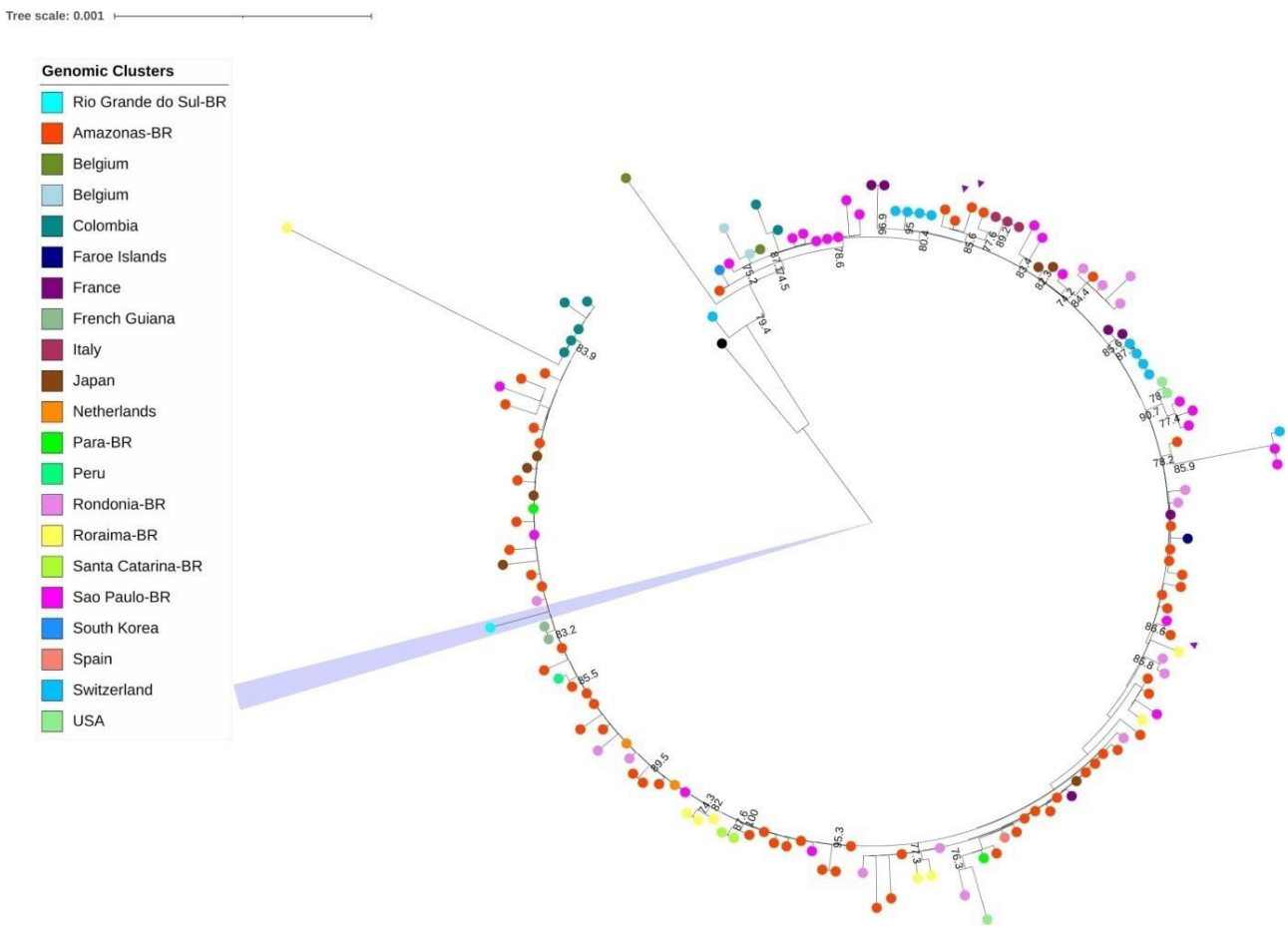

Figure 1. Maximum-likelihood tree from the first SARS-CoV P.1 genome identified in Rio Grande do Sul (Brazil) contextualized on 156 P.1 genomes from worldwide. The aLRT support values are shown in key nodes. Tip colors indicate the origin of samples. Triangles are indicating the samples from three patients from Amazonas (Brazil), transferred to Rio Grande do Sul to receive hospital care. The first SARS-CoV P.1 genome identified in Rio Grande do Sul it is highlighted in purple.

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and permanent tool to identify the underlying events as in the present case, where, due to genomic surveillance, it was possible to detect an unsuspected variant of concern P.1 on a case of COVID-19 death in a patient practicing self-isolation, living in a remote area.

Our analysis highlights monitoring protocols for new variants must consider key social mobilization sites in the state. From all cities of Rio Grande do Sul state, Gramado and Canela are by far the most important from the point of view of social mobility, as they receive tourists from Brazil and abroad. The Capital and the border/port regions must also be constantly monitored in order to guarantee success in monitoring new circulating strains. The joint observation of epidemiological and laboratory surveillance findings can assist the community in COVID-19 control measures.

DATA AVAILABILITY

Whole genome sequence from SARS-CoV-2 genome sequenced in this work it is available on GISAID database under accession ID EPI_ISL_983865.

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AUTHORS' CONTRIBUTIONS

Richard Steiner Salvato and **Tatiana Schäffer Gregianini** conceived the study and its design;

Richard Steiner Salvato and **Aline Alves Scarpellini Campos** conducted phylogenetic analysis;

Lara Villanova Crescente, **Marcelo Jostmeier Vallandro**, **Tani Maria Schilling Ranieri**, **Sabrina Vizeu**, **Letícia Garay Martins** and Ed**uardo Viegas da Silva** analyzed epidemiological data;

Ellen Regina Pedroso and **Andreia Burill**e conducted epidemiological investigation; **Ludmila Fiorenzano Baethgen** performed laboratorial experiments, contributed to data analysis and manuscript writing;

Sun Hee Schiefelbein, **Taís Raquel Marcon Machado**, **Irina Marieta Becker**, **Raquel Ramos**, **Cláudia Fasolo Piazza** and **Zenaida Marion Alves Nunes** were in charge for clinical sample and laboratorial experiments;

Cynthia Goulart Molina Bastos read the manuscript and revised it critically. All authors read and approved the manuscript. The authors declare no conflict of interests.

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