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

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**Short Report**

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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. In this short communication, we present epidemiological and genomic information of the first case of P1 lineage in Rio Grande do Sul state, in a patient with no reported travel history and a tracked transmission chain. These findings occurred in a tourist destination representing an important hub receiving tourists from diverse places.

Introduction
In December 2019, the 2019 novel coronavirus disease (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in China and now has spread around the world [1]. 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).

Currently 18 countries have reported the presence of P.1 lineage, but only Brazil and Colombia described local transmission (https://cov-lineages.org/global_report_P.1.html). Although there is a lack of consistent data pointing to an increased transmissibility of P.1 lineage, it shares numerous mutations with the B.1.1.7 (N501Y) and the B.1.325 (K417N/T, E484K, N501Y) lineages that emerged in the United Kingdom and South Africa and were associated to increased transmissibility [2, 3]. All along that, 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 an 1,240,319 estimated population, including Gramado and Canela, also known as Caxias do Sul Region, since Caxias do Sul is the densely populated city from this region (https://cidades.ibge.gov.br/brasil/rs/panorama).

Case Report
An 88-years-old male patient presented acute respiratory symptoms on Jan 29th, 2021 and was hospitalized at Arcanjo São Miguel Hospital, Gramado city, on Feb 3rd after medical examination. At the
same day of hospitalization, the patient was admitted at the intensive care unit with fever of 39°C, no leg movements, chest pain, besides flu-like symptoms/Acute Respiratory Syndrome (sore throat, dyspnea, oxygen desaturation). Respiratory secretion was collected on Feb 1st, the RT-qPCR for COVID-19 was positive (Ct 22) and 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 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 [4] 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[5] (Mean coverage: 2.158,216). Consensus fasta was obtained with SAMtools [6], and analyzed with Pangolin (github.com/cov-lineages/pangolin) to determine SARS-CoV-2 lineage.

The sequenced genome was assigned to P.1 lineage on Pangolin. Thus, we aligned the consensus fasta with other 156 P.1 genomes from worldwide available on GISAID [7] as of February 16, 2021 with MAFFT [8] under default parameters. The aligned multi-fasta was used to construct a maximum-likelihood tree in IQ-Tree v.2.1.2 [9] (GTR + G4 + F -alrt 1000 -nt AUTO), annotated in the iTOL web-based tool [10] 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, Rondonia, Roraima and São Paulo state, along with sequences found in Japan and Colombia (Fig. 1). Interestingly, the sequences originated from three patients transferred from Manaus to be hospitalized in Rio Grande do Sul in the same period (February 2021), fell on different branches of the P.1 phylogeny, suggesting distinct transmission chains.

**Epidemiological Investigation**

The patient had no travel history, and the epidemiological investigation revealed a plausible 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 (A). This person had previous contact in a diner with an individual (B) on Jan 23rd. Individual (B), a tourism worker who used to have close contact with tourists, was positive to COVID-19 with onset symptoms on Jan 21st. The person (A) presented her first symptoms on Jan 26th and had a positive RT-qPCR test for SARS-CoV-2 on Jan 31st. The person (A) presented mild disease and the individual (B) to date remains hospitalized with severe disease symptoms.

Colleagues from individual (B) who shared the same workplace 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. Before that, the highest number of hospitalizations in Caxias do Sul Region, occurred in
November, when the number of cases of the disease in the state peaked. The number of hospitalizations
has declined since the beginning of January. So far, the increase in hospitalizations seems to be
restricted to Gramado and Canela, compared to cities from same region.

Conclusions

In order to monitor the SARS-CoV-2 transmission and spread, genomic sequencing represents an
essential component of larger surveillance that includes timely epidemiological investigation and
accordingly standardized and well-established data collection protocols. Our findings indicate a potential
local transmission of P.1 lineage occurring in Gramado city, along with an important increase in the
number of hospitalizations in the city which differs from what has been observed in other cities in the
same region.

There are potential limitations in our study: the dataset used in the analyses is based on a limited number
of cases, also, more genomic data is urgently needed to address the transmissibility of P.1 lineage in this
setting. Nevertheless, our study revealed a worrying emergence of severe COVID-19 cases in this region
and calls for future use of genomic surveillance as a regular 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 from the high-risk group
(older than 60 years with comorbidities) practicing self-isolation, living in a remote area.

Our analysis highlights that 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. Using
the Google database - COVID-19: Community Mobility Report
(https://www.gstatic.com/covid19/mobility/2021-02-
12_BR_State_of_Rio_Grande_do_Sul_Mobility_Report_pt-BR.pdf), it is possible to notice a 21% increase in
public transport in Gramado city from January 1st to February 12nd, which could help explain an
increased movement of people and facilitating local spread of SARS-CoV-2. 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
associated with population mobility can assist the community in COVID-19 control measures.

Declarations

Competing Interests: The authors declare no competing interests.

Ethical Approval/participant consent: Not required, since the samples were processed as part of SARS-
CoV-2 epidemiological state surveillance at State Central Laboratory (LACEN-RS).
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.

Authors contribution: RSS and TSG conceived the study and its design; RSS and AASC conducted phylogenetic analysis; LVC, MJV, TMSR, SV, LGM and EVS analyzed epidemiological data; ERP and AB conducted epidemiological investigation; LFB performed laboratorial experiments, contributed to data analysis and manuscript writing; SS, TRMM, IMB, RR, CFP and ZMAN were in charge for clinical sample and laboratorial experiments; CGMB read the manuscript and revised it critically. All authors read and approved the manuscript.

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