

with JSF (10). The patient showed no other skin eruptions besides the eschar at the site of the tick bite (Figure). It is highly possible that the eschar on this patient could have been caused by an inflammatory response induced by the local *R. japonica* infection. *R. japonica* did not induce systemic symptoms in this patient for 2 possible reasons. First, the incubation time for SFTS might be shorter than that of JSF. Second, the initiation of antimicrobial drugs in the early phase of disease might have ameliorated the clinical course of the diseases.

In conclusion, we describe a patient with a generalized SFTSV infection and a localized skin lesion caused by *R. japonica* at the site of a tick bite. This study suggests that SFTS patients with eschar at the site of a tick bite should be treated with appropriate antimicrobial drugs, such as doxycycline and minocycline.

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## Imported SARS-CoV-2 Variant P.1 in Traveler Returning from Brazil to Italy

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We report an imported case of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variant P.1 detected in an asymptomatic traveler who arrived in Italy on an indirect flight from Brazil. This case shows the risk for introduction of SARS-CoV-2 variants from indirect flights and the need for continued SARS-CoV-2 surveillance.

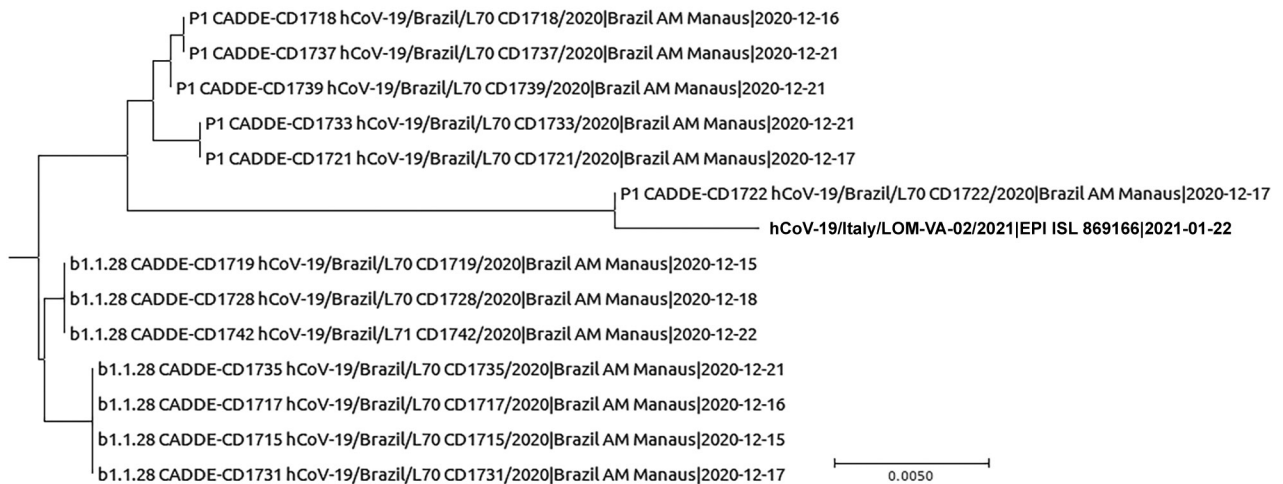
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variant P.1 currently is causing a major outbreak of coronavirus disease (COVID-19) in the Amazonas province of Brazil (N.R. Faria et al., unpub. data, <https://virological.org/t/genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-manaus-preliminary-findings/586>). The P.1 variant also is known as B.1.1.28 in the Phylogenetic Assignment of Named Global Outbreak Lineages (<https://cov-lineages.org/pangolin.html>) and as 20J/501Y.V3 in NextStrain (<https://nextstrain.org>). Preliminary reports have associated several spike protein mutations harbored in the P.1 variant with escape from neutralizing monoclonal antibodies (mAb) and P.1 was detected in convalescent serum collected during previous epidemic waves (Z. Liu et al., unpub. data, <https://www.biorxiv.org/content/10.1101/2020.11.06.372037v1>; S. Jangra et al., unpub. data, <https://www.medrxiv.org/content/10.1101/2021.01.26.21250543v1>).

The B.1.1.28 lineage emerged in Brazil during February 2020, and 2 subclades recently evolved separately (C.M. Voloch et al., unpub. data, <https://doi.org/10.1101/2020.12.23.20248598>; 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>). During January 2021, SARS-CoV-2 variant P.1 was reported in 4 travelers returning to Japan from Amazonas state in Brazil (1). The strain identified in the travelers was associated with E484K, K417N, and N501Y mutations as noted in the the B.1.351 line 20I/501.V2 clade of South African lineage (1). In addition, 1 case of reinfection has been documented months after a B.1 primary infection (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>). Another lineage, P.2, was reported in Rio de Janeiro, Brazil, but has been associated with spike mutations only in E484K;  $\geq 2$  cases of reinfection have been documented several months after primary B.1.1.33 infections (P. Resende et al., unpub. data, <https://virological.org/t/spike-e484k-mutation-in-the-first-sars-cov-2-reinfection-case-confirmed-in-brazil-2020/584>; C.K. Vasques Nonaka et al., unpub. data, <https://doi.org/10.20944/preprints202101.0132.v1>). Among the spike mutations, E484K is considered the main driver of immune evasion to mAbs and convalescent serum (A.J. Greaney et al., unpub. data, <https://doi.org/10.1101/2020.12.31.425021>). Of note, many of the most potent mRNA vaccine-elicited mAbs were 3- to 10-fold less effective at neutralizing pseudotyped viruses carrying E484K (K. Wu et al., unpub. data, <https://doi.org/10.1101/2021.01.25.427948>), which has unknown implications for protection. We report an asymptomatic traveler from Brazil who tested positive for the SARS-CoV-2 P.1 variant in a screening nasopharyngeal swab sample.

After visiting São Paulo, Brazil, during November 23, 2020–January 16, 2021, a family, including a 33-year-old man, his 38-year-old wife, and his 7-year-old daughter, flew back to their home in Italy. During their time in Brazil, the family did not travel outside of São Paulo, which is  $>2,000$  miles from Amazonas. The family took an indirect return flight; they flew from São Paulo/Guarulhos International Airport in Brazil to Madrid, Spain, and from there flew to Milan Malpensa Airport in Italy. Molecular tests were performed on all 3 family members at the departure airport in Brazil, and all were SARS negative.

The family arrived in Milan on the afternoon of January 17 and took a train and a car to their home, 30 miles from Milan. Under current recommendations in Italy, all persons entering the country can decide to be screened for SARS-CoV-2. After consulting a general practitioner on January 21, the father went to the hospital for a screening nasopharyngeal swab sample. The sample was tested by using the Alinity platform (Abbott, <https://www.abbott.com>), which returned a positive result for SARS-CoV-2 RNA with a cycle threshold of 23. Reverse transcription PCR (RT-PCR) fragments corresponding to the receptor-binding domain (RBD) in the spike gene of SARS-CoV-2 were amplified from purified viral RNA by using a OneStep RT-PCR Kit (QIAGEN, <https://www.qiagen.com>). We used a reference sequence from GSAID (<https://www.gisaid.org>; accession no. EPI\_ISL\_402124) and



**Figure.** Phylogenetic tree of severe acute respiratory syndrome coronavirus 2 variant P.1 sequences from a male traveler returning from Brazil to Italy and reference sequences from Brazil. Bold text indicates sequence from the traveler. Scale bar indicates nucleotide substitutions per site.

nucleotide sequences of primer sets to map genome locations (Figure; Appendix, <https://wwwnc.cdc.gov/EID/article/27/4/21-0183-App1.pdf>). The sequence of RBD from the patient included the P.1 barcoding mutations K417T, E484K, and N501Y. We deposited these data in GenBank (accession no. MW517286) and GISAID (accession no. EPI-ISL-869166).

SARS-CoV-2 variant P.1 is characterized by K417N, but K417T also has been reported in several cases before our patient (1), suggesting ongoing evolution. On January 22, 2021, after we reported the sequencing results, the patient was admitted to the infectious and tropical diseases unit of ASST dei Sette Laghi-Ospedale di Circolo e Fondazione Macchi (Varese, Italy) for observation. The patient remained asymptomatic and was discharged on January 29. The patient's spouse also tested positive for SARS-CoV-2 RNA via a nasopharyngeal swab sample. Antibody tests conducted by using Liaison Analyzer (DiaSorin, <https://www.diasorin.com>) were negative for SARS-CoV-2 S1/S2 IgG in serum of both the man and his wife, suggesting a primary infection.

Direct flights from Brazil to Italy were canceled upon the unilateral decision of the government of Italy on January 16, 2021, but our findings confirm the risk for introducing SARS-CoV-2 variants from indirect

flights if no surveillance measures are implemented at arrival. This case also suggests wider circulation of SARS-CoV-2 variant P.1 in areas other than Amazonas in Brazil. P.1-specific primer sets recently have been designed (A. Lopez-Rincon et al., unpub. data, <https://doi.org/10.1101/2021.01.20.427043>) and will aid in development of large-scale screening programs for this variant.

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# Imported SARS-COV-2 Variant P.1 Detected in Traveler Returning from Brazil to Italy

## Appendix

### Supplementary Methods

Reverse transcription PCR (RT-PCR) fragments corresponding to the receptor-binding domain (RBD) in the spike gene of SARS-CoV-2 were amplified from purified viral RNA collected from the patient's nasopharyngeal swab by using a OneStep RT-PCR Kit (QIAGEN, <https://www.qiagen.com>). We performed 2 nested PCR (nPCR) reactions the amplified viral RNA, nPCRA and nPCRB, in 50  $\mu$ L according to the manufacturer's instructions. Amplification conditions were 50°C for 30 min followed by 94°C for 15 min plus 40 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 1 min with a final extension step of 72°C for 10 min. After the first PCR reaction, we used 5  $\mu$ L of amplified product for the second nPCR reaction. Amplification conditions for nPCRB were 95°C for 3 min plus 30 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 1 min with a final extension step of 72°C for 10 min. We purified the amplified products by using QIAquick PCR Purification kit (QIAGEN) to prepare for Sanger sequencing analysis. We included negative RNA samples and template controls in every assay and found all to be negative. We used BigDye Terminator v.1.1 (Applied Biosystems, <https://www.thermofisher.com>) cycle sequencing kit to sequence the purified RT-PCR products. We purified the sequencing reactions by using Centri-Sep Spin Columns (Princeton Separations, Inc., <https://www.prinsep.com>) and analyzed on a SeqStudio Genetic Analyzer (Applied Biosystems). We identified sequence variants by using CLC main workbench 7.0.0 (QIAGEN). We used reference sequence GSAID accession no. EPI\_ISL\_402124 and nucleotide sequences of primer sets to map genome locations (Appendix Table) The sequence of receptor binding domain from the patient included the P.1 barcoding mutations K417T, E484K, and N501Y. We

deposited these data in NCBI (<https://www.ncbi.nlm.nih.gov/genbank>; GenBank accession no. MW517286) and GISAID (<https://www.gisaid.org>; GISAID accession no. EPI-ISL-869166).

**Appendix Table.** List of oligonucleotide primers used for amplification of severe acute respiratory syndrome coronavirus 2 receptor-binding domain\*

nPCR	Name	Sequence, 5'-3'	Genome location, nt†	Amplicon size, bp
A	RBD_F1	GTACGTTGAAATCCTTCACTGTAGA	22464–22488	936
	RBD_R1	GATAAAGAACAGCAACCTGGTTAGAAG	23399–23373	
B	RBD_F2	CAAACCTTCTAACTTTAGAGTCCAACC	22502–22527	863
	RBD_R2	CCTGGTGTTATAACACTGACACCA	23364–23341	

\*bp, base pairs; nPCR, nested PCR; nt, nucleotide.

†Genome location within the hCoV-19/Wuhan/WIV04/2019 genomic sequence (GISAID accession no. EPI\_ISL\_402124).