In May 2021, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was detected in Asiatic lions in a zoological park in India. Sequence and phylogenetic analyses showed the SARS-CoV-2 strains were the B.1.617.2 (Delta) variant. To reduce transmission of variants of concern, surveillance of SARS-CoV-2 in wild animal populations should be increased.
India, where the zoological park is located, during January 1–June 11, 2021. To generate a set of representative sequences, we used a UCLUST algorithm (4) to select sequences that clustered at the 99.9% identity threshold. We used MAFFT version 7.475 (5) to align representative SARS-CoV-2 sequences from GISAID with sequences from the lions; then we constructed a phylogenetic tree by using the general time reversible plus gamma model in RAxML version 8.2.12 (6) (Figure).

The amino acid substitutions and deletions in the spike protein of SARS-CoV-2 in lions typically matched with the SARS-CoV-2 Delta variant (Appendix Table 2). We noted amino acid changes in the N terminal domain (NTD), including T19R, G142D, E156del, F157del, R158G; in the receptor binding motif (RBM), including L452R and T478K; and in D614G of subdomain 2. We also noted a substitution close to SI/ S2 protease cleavage site at P681R and heptad repeat 1 at D950N (Appendix Figures 1, 2). In addition, the lion sequences had the K77T substitution in the NTD, which has been detected in SARS-CoV-2 genomes from 24 countries. In India, frequency of the K77T substitution generally is low (0.44%) but occurred in 27.42% (65/237) of sequences in the B.1.167.2 lineage collected in Tamil Nadu state (Appendix Table 2).

The changes in the spike protein, including E156del, F157del, and R158G, of lion sequences were not found in human SARS-CoV-2 sequences from the same geographic area, nor were changes in nonstructural protein 3 (NS3) V88I, possibly because SARS-CoV-2 sequencing is limited in the region. Furthermore, these changes in spike and NS3 were not seen in previously reported lion SARS-CoV-2 sequences, ruling out the possibility that these are host-adapted mutations (7) (Appendix Figure 3). Further investigations could delineate whether changes in the spike protein, namely E156del, F157del, R158G, and K77T, are escape mutants or are associated with increased transmissibility or pathogenicity.

A nucleotide similarity comparison of the 4 lion SARS-CoV-2 sequences against the sequences available in GISAID and phylogenetic analysis revealed that the lion sequences closely matched with a representative human SARS-CoV-2 sequence of B.1.617.2 lineage, GI-SAID accession no. EPI_ISL_2463770, that comprises 152 viral genome pools collected from the same geographic region during the same month that the lions' samples were collected (Figure; Appendix Figure 4). The park's management strictly adhered to COVID-19 guidelines and did not introduce any new animals to the zoo during India’s widespread COVID-19 pandemic. The primary source of SARS-CoV-2 infection in the lions might have been an asymptomatic or paucisymptomatic person. Among the 9 infected lions, 7 were in the lion safari and shared a common habitat, shelter, feeding spaces, and water sources. The other 2 infected lions were on display in separate enclosures that shared a common moat. Because shared habitats offered opportunities for close physical contact, identifying genetically identical SARS-CoV-2 infections in these lions in a short period of time indicates the possibility of lion-to-lion transmission.

In conclusion, evidence of confirmed natural SARS-CoV-2 Delta variant infections in Asiatic lions in India justifies need for increased SARS-CoV-2 surveillance in wild animal species. In addition, strict biosecurity measures should be implemented for wild animals kept in captivity.

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SARS-CoV-2 Variants in Immunocompromised Patient Given Antibody Monotherapy

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A 72-year-old immunocompromised man infected with severe acute respiratory syndrome coronavirus 2 received bamlanivimab monotherapy. Viral evolution was monitored in nasopharyngeal and blood samples by melting curve analysis of single-nucleotide polymorphisms and whole-genome sequencing. Rapid emergence of spike receptor binding domain mutations was found, associated with a compartmentalization of viral populations.

A 72-year-old immunocompromised man in France who had chronic lymphocytic leukemia associated with hypogammaglobulinemia for 4 years experienced diarrhea, asthenia, fever, and cough associated with coronavirus disease (COVID-19). Although he had received 1 injection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) mRNA vaccine (BNT162b2; Pfizer/BioNTech, https://www.pfizer.com) 20 days earlier, we confirmed a diagnosis of COVID-19 by using a semiquantitative SARS-CoV-2 reverse transcription PCR (RT-PCR) viral load assay. This assay showed a cycle threshold (Ct) value of 27 for a nasopharyngeal swab specimen. His most recent monoclonal antibody (mAb) chemotherapy treatment (venetoclax and rituximab) had been conducted 17 days earlier. Because of his immunocompromised status, treatment with bamlanivimab (LY-CoV555), a neutralizing IgG1 mAb, was initiated at day 0, 4 days after onset of symptoms (Table). The patient received an infusion of 700 mg in a single dose and was discharged.

Analysis of samples showed a high viral load in a nasopharyngeal swab specimen (Ct, 20) and a blood sample (Ct, 37) (Table). Three days after the mAb infusion, the patient’s symptoms worsened, and he was hospitalized in the Infectious Diseases Department at Grenoble Hospital (Grenoble, France) on day 6. The condition of the patient had deteriorated; he had an additional need for oxygen, which resulted in a convalescent-phase plasma transfusion on day 10.

After this treatment, the condition of the patient continued to deteriorate, and he was transferred to...
SARS-CoV-2 Delta Variant among Asiatic Lions, India

Appendix

Supplementary Methods

SARS-CoV-2 Whole-Genome Sequencing on the Oxford Nanopore MinION platform

We performed whole-genome sequencing of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) directly from nasal swabs of 4 Asiatic lions (Panthera leo persica) using the MinION (Oxford Nanopore Technologies, https://nanoporetech.com) sequencing platform. The lions tested included Jeya, a 3-year-old female; Shankar, an 18-year-old male; Niranjana, a 2-year-old female; and Pradeep, a 2-year-old male.

In brief, we performed tiling PCR spanning the whole genome of SARS-CoV-2 by using the Artic (https://artic.network) network primers. We performed cleaning and quantification of PCR products and used 100 ng of each sample to create a barcoded sequencing library by using the PCR Barcoding Kit (Oxford Nanopore). We used an Oxford Nanopore Technologies MinION with a R9.4.1 flow cell for sequencing, which yielded a total of 300 MB of data. To assemble the whole genome, we used guppy version 5.0.7 (Oxford Nanopore Technologies) for base calling and demultiplexing; and then we used Porechop (https://github.com/rrwick/Porechop) for adaptor removal. We mapped the readings to the SARS-CoV-2 reference genome (GenBank accession no. NC 045512) by using Minimap2 version 2.17 (r941) (1), and the called variations by using Nanopolish version 0.13.2 (https://github.com/jts/nanopolish) (2). After 2 rounds of Nanopolish, we generated 4 complete genomes.

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**Appendix Table 1.** Characteristics and results of quantitative reverse transcription PCR on samples collected from 11 Asiatic lions, Arignar Anna Zoological Park, Chennai, India*

| Lion identity | Age, y/sex | Sample type | E gene | RdRp | N | Results |
|---------------|------------|-------------|--------|------|---|---------|
| Jeya          | 3/F        | Nasal swab  | 20.16  | 19.65 | 19.97 | Positive |
| Padmnabhan    | 12/M       | Nasal swab  | 18.90  | 18.10 | 18.50 | Positive |
| Kavitha       | 18/F       | Rectal swab | 27.41  | 26.75 | 27.79 | Positive |
| Neela         | 9/F        | Rectal swab | 22.71  | 22.11 | 22.57 | Positive |
| Shankar       | 18/M       | Fecal sample | –      | –     | –     | Negative |
| Niranjana     | 2/F        | Rectal swab | 38.61  | 38.14 | 33.69 | Negative |
| Pradeep       | 2/M        | Fecal sample | 29.05  | 28.60 | 29.02 | Positive |
| Vishnu        | 4/M        | Fetal sample | 24.34  | 23.80 | 24.72 | Positive |
| Jeya@Bhuvana  | 12/F       | Rectal swab | 28.11  | 27.39 | 28.49 | Positive |
| Veera         | 10/M       | Fecal sample | –      | –     | –     | Negative |
| Shiva         | 12/M       | Nasal swab  | –      | –     | –     | Negative |
|               |            | Throat swab | –      | –     | –     | Negative |
|               |            | Trachea sample | –     | –   | –     | Negative |
|               |            | Lymph node sample | –  | –   | –     | Negative |
|               |            | Lung sample  | –      | –     | –     | Negative |

*Ci, cycle threshold; E, envelope gene; N, nucleocapsid; RdRP, RNA-dependant RNA polymerase; –, not detected.

**Appendix Table 2.** Amino acid substitutions and functional roles identified in different proteins encoded by severe acute respiratory syndrome coronavirus 2 detected in lions, India*

| Amino acid substitutions | No. times reported (%)† | No. of countries‡ | Mo. collected§ | Accession no.¶ | Functional roles of substitutions |
|--------------------------|--------------------------|-------------------|----------------|----------------|----------------------------------|
| M_182T                   | 51,777 (2.75)            | 99                | Apr            | hCoV-19/Indonesia/GO-NIHRD-PME2020/2020 | – |
| N_D377Y                  | 72,190 (3.83)            | 106               | Feb            | hCoV-19/USA/OH-ODH-SC1040172/2020 | – |
| N_D63G                   | 36,273 (1.93)            | 62                | Apr            | hCoV-19/Indonesia/GO-NIHRD-PME2020/2020 | Antigenic drift; viral oligomerization interfaces |
| N_R203M                  | 40,703 (2.16)            | 73                | Mar            | hCoV-19/Spain/NCPV-001370/2020 | – |
| NS3_S26L                 | 42,135 (2.24)            | 83                | Mar            | hCoV-19/USA/NY-NYCHL-000016/2020 | – |
| NS3_V88I                 | 71 (0.00)                | 14                | Mar            | hCoV-19/Austria/CeMM0388/2020 | Viral oligomerization interfaces |
| hCoV-19/Indonesia/GO-NIHRD-PME2020/2020 | hCoV-19/Indonesia/GO-NIHRD-PME2020/2020 | hCoV-19/India/PB-ICMR-148040/2020 | EPON0016 (B.136.8, Punjab, JUL-2020) |
| hCoV-19/Scotland/CVR01/2020 | hCoV-19/Italy/MAR- UnivPM30_45476/2020 | hCoV-19/India/PB-ICMR-148040/2020 | EPON0016 (B.136.8, Punjab, JUL-2020) |
| hCoV-19/Scotland/CVR01/2020 | hCoV-19/Italy/MAR- UnivPM30_45476/2020 | hCoV-19/India/PB-ICMR-148040/2020 | EPON0016 (B.136.8, Punjab, JUL-2020) |
| NS7a_T120I               | 40,866 (2.17)            | 77                | Feb            | hCoV-19/Scotland/CVR01/2020 | – |
| NS7a_V82A                | 39,371 (2.09)            | 66                | Apr            | hCoV-19/Scotland/CVR01/2020 | – |
| NSP12_P323L              | 1,802,848 (95.76)        | 182               | Oct            | hCoV-19/Italy/MAR- UnivPM30_45476/2020 | Antigenic drift |
| NSP15_K259R              | 4,692 (0.25)             | 54                | Jul            | hCoV-19/USA/CA-IIG-0320/2020 | – |
| NSP2_P129L               | 13,166 (0.70)            | 91                | Mar            | hCoV-19/Japan/PJ-1597/2020 | – |
| NSP3_P822L               | 14,468 (0.77)            | 77                | Feb            | hCoV-19/USA/CA-CDPH018/2020 | Host cell protein/RNA interaction; viral oligomerization interfaces |
| NSP4_D217N               | 2,516 (0.13)             | 55                | Mar            | hCoV-19/England/BIRM-61F00/2020 | – |
| Amino acid substitutions | No. times reported (%)† | No. of countries‡ | Mo. collected§ | Accession no.¶ | Functional roles of substitutions |
|--------------------------|-------------------------|------------------|---------------|----------------|----------------------------------|
| NSP4_F375S               | 1,136 (0.06)            | 33               | Jun           | hCoV-19/USA/WA-UW-10769/2020 |                                |
| NSP6_H11Q                | 3,639 (0.19)            | 48               | Apr           | hCoV-19/Italy/LOM-Pavia-41147/2020 |                                |
| NSP6_H11Q                | 3,639 (0.19)            | 48               | Apr           | hCoV-19/Italy/MAR-UnivPM30_45476/2020 |                                |
| Spike_D614G              | 1,839,357 (97.70)       | 185              | Oct           |                              | Antigenic drift; virulence and host change; ligand binding; viral oligomerization interfaces |
| Spike_D950N              | 36,277 (1.93)           | 70               | Mar           | hCoV-19/Iran/K1r-108/2020   | Viral oligomerization interfaces |
| Spike_E156G              | 32,810 (1.74)           | 62               | Mar           | hCoV-19/USA/Panama/328688/2020 |                                |
| Spike_F157del            | 33,063 (1.76)           | 63               | Jul           | hCoV-19/USA/TX-HMH-MCoV-40913/2020 |                                |
| Spike_G142D              | 25,756 (1.37)           | 61               | Mar           | hCoV-19/England/BRIS-124CD4/2020 |                                |
| Spike_K77T               | 777 (0.04)              | 24               | Dec           | hCoV-19/Switzerland/ZH-ETHZ-431373/2020 |                                |
| Spike_L452R              | 101,257 (5.38)          | 108              | Mar           | hCoV-19/Denmark/ALAB-HH65/2020 | Host and other changes; antigenic drift; antibody recognition sites |
| Spike_P681R              | 45,877 (2.44)           | 91               | Apr           | hCoV-19/Indonesia/GO-NIHRD-PME20208/2020 | Increased rate of membrane fusion, internalization, and thus better transmissibility |
| Spike_R158del            | 33,071 (1.76)           | 63               | Jul           | hCoV-19/USA/TX-HMH-MCoV-40913/2020 | Antibody recognition sites |
| Spike_T19R               | 320,114 (1.07)          | 64               | Apr           | hCoV-19/Indonesia/GO-NIHRD-PME20208/2020 | Removes a potential N-glycosylation site that might also affect antigenic and other properties of this strain |
| Spike_T478K              | 56,250 (2.99)           | 79               | Apr           | hCoV-19/Indonesia/GO-NIHRD-PME20208/2020 | Host and other changes; antigenic drift; host surface receptor binding; antibody recognition sites; viral oligomerization interfaces |

*Reference sequence Wuhan-Hu-1 (GISAID accession no. EPI_ISL_402124) was used for comparison. –, no role identified.
†Percentage of sequences with this gene showing the same mutation.
‡Number of countries reporting the same mutation.
§Month first strain with this mutation was collected during 2020.
¶Accession number of the first strain to show the mutation.
Appendix Figure 1. Comparison of amino acid changes detected in the spike protein (SP) of SARS-CoV-2 detected in Asiatic lions (Panthera leo persica), India. Wuhan-Hu-1 (GISAID accession no. EPI_ISL_402124) was used as the reference sequence. CT, cytoplasmic tail; FP, fusion peptide; HR, heptad repeat; NTD, N terminal domain; RBD, receptor binding domain; RBM, receptor binding motif; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TM, transmembrane domain.
Appendix Figure 2. Mapping of amino acid substitutions noted on the structural model of the spike protein of SARS-CoV-2 detected in Asiatic lions (*Panthera leo persica*), India. Model represents spike protein of SARS-CoV-2 from Asiatic lion (GenBank accession no. MZ363851) from the top (left) and the front (right). Model was built by using I-TASSER (Yang Zhang Lab, https://zhanglab.ccmb.med.umich.edu/I-TASSER) and the PDB:6acc template. Red indicates T19R; green indicates K77T; and blue indicates L452R in both views; all other areas of interest are labeled with arrows. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
Appendix Figure 3. Maximum likelihood tree showing the phylogenetic relationship among SARS-CoV-2 detected in Asiatic lions (*Panthera leo persica*), India. Blue text indicates SARS-CoV-2 sequences from this study. Comparison sequences were selected from available lion and tiger sequences in the GISAID. Scale bar indicates nucleotide substitutions per site. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
Appendix Figure 4. The phylogenetic analysis of SARS-CoV-2 detected in Asiatic lions (*Panthera leo persica*) and all available SARS-COV-2 sequences from Tamil Nadu, India. Colors represent different PANGO lineages; sequences in gold highlighting represent SARS-CoV-2 from lions in this study. Scale bar indicates nucleotide substitutions per site. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.