




# Reverse-zoonotic transmission of SARS-CoV-2 lineage alpha (B.1.1.7) to great apes and exotic felids in a zoo in the Czech Republic

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## Abstract

We report an outbreak of SARS-CoV-2 lineage alpha in gorillas and felid species in a zoo in Prague, Czech Republic. The course of illness and clinical signs are described, as are the results of characterization of these particular SARS-CoV-2 variants by next-generation sequencing and phylogenetic analysis. The putative transmission routes are also discussed.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is responsible for the ongoing coronavirus pandemic, apparently originated in a bat reservoir [5, 14] and was transmitted to humans as a zoonotic infection. From the initial outbreak in December 2019, the virus has spread globally and evolved into multiple phylogenetic lineages [12, 15]. During the beginning of 2021, the so-called alpha variant (B.1.1.7), first reported on December 14, 2020 in the United Kingdom [15], was of concern due to its increased transmissibility in the human population and the severity of illness [4]. Since its appearance, the B.1.1.7 lineage has begun to replace the existing variants and started to prevail in the European population.

The constant interaction between humans and animals has also created many opportunities for reverse-zoonotic transmission to other animal species. Accordingly, multiple SARS-CoV-2 spillover events have been reported [5, 11]. Given the higher transmission rate of the B.1.1.7 variant, the probability of reverse-zoonotic events is even increasing. Here, we report infections with the SARS-CoV-2 B.1.1.7 variant in gorillas, Asiatic lions, Sumatran and Malayan

tigers, and an Amur leopard cat in a zoo in the Czech Republic.

After the first clinical signs appeared in gorillas and Asiatic tigers, regular sampling was carried out in the affected pavilions to identify the virus. Samples were analyzed the day after collection. Fecal samples were resuspended in 1 ml of distilled water, and the supernatant was used for extraction. Total nucleic acid was extracted from 200 µl of supernatant, using a MagNAPure Compact Nucleic Acid Isolation Kit (Roche), and eluted to 50 µl. SARS-CoV-2 was detected by RT-qPCR targeting the E gene [3] (QuantiNova Probe RT-PCR Kit, QIAGEN). The SARS-CoV-2 genome was sequenced from five specimens, obtained from a Malayan tiger, an Amur leopard cat, two lions, and two gorillas.

Real-time next-generation sequencing was performed by the nanopore technology using a single Flongle flow cell for each individual specimen (Oxford Nanopore Technologies). Briefly, the SARS-CoV-2 genome was amplified using tiled amplicons [7]. The sequencing libraries were purified (SPRIselect beads; Beckman Coulter) and quantified (QIAxpert; QIAGEN). End preparation and sequencing adapter ligation were performed according to the manufacturer's instructions.

The raw data were basecalled using Guppy, and the reads were reference mapped and assembled using the ARTIC bioinformatic pipeline. The consensus sequences of the five SARS-CoV-2 isolates were submitted to the GISAID EpiFlu (accession numbers EPI\_ISL\_1497613-17) and GenBank (OL752440-OL752444) databases. Phylogenetic and haplotype network analysis were performed using the Nexstrain platform [9] and the PopART program [1], respectively.

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The Prague Zoological Garden is home to eight western lowland gorillas (*Gorilla gorilla gorilla*) (Table 1). Approximately fifty meters away, a big-cat house is situated and is divided into two wings (Fig. 1). Wing A is occupied by a pair of Sumatran (*Panthera tigris sumatrae*) and a pair of Malayan (*P. t. jacksoni*) tigers. Wing B hosts three Asiatic lions (*P. leo persica*) and a pair of fishing cats (*Prionailurus viverrinus*), Amur leopard cats (*P. bengalensis euptilurus*), Palawan leopard cats (*P. b. heaneyi*) and Geoffroy's cats (*Leopardus geoffroyi*). Wing B is occupied by one clouded leopard (*Neofelis nebulosa*) and one Javan leopard (*Panthera pardus melas*). Each of the felid species inhabits its own dormitory, which is separated by glass or solid partitions and is connected by a tunnel to an outdoor area. An overview of all tested animals is provided in Supplementary Table S1.

On February 21, 2021 clinical signs reminiscent of COVID-19 disease, such as tiredness, dry cough and loss of appetite, were observed in a male gorilla, Richard. Fatigue lasted for four days. Similarly, the 47-year-old female, Kamba, developed fatigue, which lasted from February 24 to March 5. On March 17, two females, Bikira and Kijivu, were seen coughing. No clinical signs were observed among the other animals in the gorilla troop.

Concurrently (February 21, 2021), exhaustion, cough, and nasal discharge appeared in two Asiatic lions, Jamvan and Suchi. Within five days, the clinical signs subsided. Meanwhile (February 24, 2021), the Amur leopard cat began to sneeze and developed serous and bloody nasal discharge and rhinitis. Clinical signs were observed for two days. Between March 1 and 2, 2021, the Malayan tigers Johann and Banya started to growl and wheeze, followed by coughing, nasal discharge, lethargy, and loss of appetite. On March 5, 2021, similar signs developed in a Sumatran tiger named Falco, while the other Sumatran tiger, Cinta, showed no signs of illness. Clinical signs in Falco persisted for five days. Generally, the illness was more severe in the tigers than in the lions.

Fecal specimens collected both from the gorilla and the big-cat houses during the course of the infection mostly showed weak positivity by RT-qPCR [3] (Table 1).

Complete coding genomic sequences of SARS-CoV-2 from the female gorillas Shinda and Kamba, from a pooled sample obtained from the Asiatic lions Jamvan and Suchi, from the Malayan tiger Johann, and from an Amur leopard cat were determined by next-generation sequencing [7, 13] (Table 1). All of the SARS-CoV2 isolates obtained from felids had identical nucleotide sequences. Similarly, no variability was observed between the isolates obtained from gorillas.

Phylogenetic [9] and haplotype network [1] analysis (Supplementary Figs. S1 and S2) suggested that all of the zoo strains belonged to the B.1.1.7 lineage. Furthermore, a clear separation of the SARS-CoV-2 genome sequences

**Table 1** The matrix of RT-qPCR results.

	Gorilla keeper	Gorilla Richard	Gorilla Shinda	Gorilla Kamba	Gorilla Kijivu	Gorilla Bikira	Cat keeper 1	Cat keeper 2	Asiatic Lions*	Amur Leopard cat	Malayan Tiger Johan	Malayan Tiger Banya	Sumatran Tiger Falco
21 Feb							+ve						
22 Feb	+ve												
23 Feb							+ve						
25 Feb		33.0						26.1					
1 March										26.1			
3 March			33.1								31.1		33.2
5 March												31.1	
11 March			29.7		32.1								
15 March													34.4
17 March				33.1		33.1							

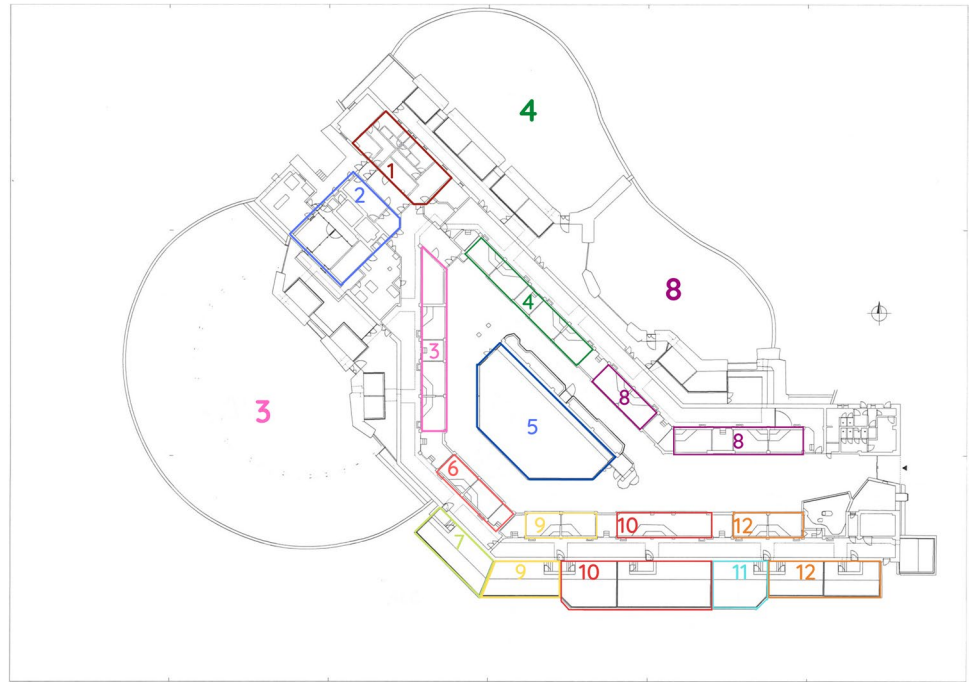
\*Pooled samples from 2 individuals

+ve - positive

Dark gray fields indicate specimens for next generation sequencing.

Numbers indicate Cq values.

**Fig. 1** Big-cat house: 1, feeding preparation; 2, keeper's space; 3, Asiatic lion; 4, Malayan tiger; 5, reptiles; 6, Palawan leopard cat; 7, Amur leopard cat; 8, Sumatran tiger; 9, Javan leopard; 10, clouded leopard; 11, Geoffroy's cat; 12, fishing cat



between the gorilla and felid isolates was observed, with six nucleotide differences between the gorilla and felid strains.

Epidemiological investigation of the zoo employees revealed that one gorilla keeper and two cat keepers were diagnosed with COVID-19 shortly before the outbreak in the animals, i.e., February 21–23, 2021 (Table 1). A schematic timeline is shown in Supplementary Fig. S1. All of them remained in isolation. In addition, the B.1.1.7 SARS-CoV-2 lineage was identified using a specific PCR assay (data provided by National Institute of Public Health) in one gorilla keeper and one cat keeper. The SARS-CoV-2 lineage of the second SARS-CoV-2-positive cat keeper is unknown. Unfortunately, clinical specimens were not available for next generation-sequencing and back-tracing.

A nucleic acid sequence alignment involving 2529 cocirculating human SARS-CoV-2 strains collected in the Czech Republic between March and April 2021 revealed two nucleotide changes in the feline SARS-CoV-2 strains and four in gorilla SARS-CoV-2 sequences that were sporadically observed among the human strains included in the

dataset (Table 2). The nucleotide mutations in the feline SARS-CoV-2 genome resulted in one amino acid change in the Orf1ab protein and one synonymous mutation in the Orf10 coding region. Protein BLAST analysis of the felid-like polyprotein 1ab (pp1ab) containing the 894G/S mutation indicated the sporadic but consistent occurrence of the 894G/S mutation in human SARS-CoV-2 isolates. Therefore, we did not consider this change to be a specific feature of feline SARS-CoV-2 variants detected in the Prague Zoo.

In the case of the gorilla-derived SARS-CoV-2 genomes, two synonymous nucleotide changes were observed within pp1ab. A sequential two-nucleotide change at pp1ab positions 21,305 – 21,306 leading to a CGC/AAC codon change and thus to an R/N substitution at aa 7016 is of particular interest. A nucleotide sequence alignment revealed the sporadic presence of this dinucleotide change among the Czech strains collected from humans between March and April 2021. Therefore, the 7016 R/N substitution seems not to be a specific feature of the gorilla SARS-CoV-2 strain that emerged during circulation in the Prague Zoo.

**Table 2** Nucleotide and amino acid sequence differences of the felid and gorilla SARS-CoV-2 genomes compared to human SARS-CoV-2 dataset.

Species	Genomic position#	Region/codon	Amino acid position/Change#
Cats	2945 G/A	Orf1ab GGC/AGC	894 G/S
	29614 C/T	Orf10 TGC/TGT	19 synonymous
Gorillas	9487 C/T	Orf1ab TAC/TAT	3075 synonymous
	20529 T/C	Orf1ab GTT/GTC	6755 synonymous
	21304 C/A	Orf1ab CGC/AAC	7014 R/N
	21305 G/A		

# Numbering according to MN908947

Detection of SARS-CoV-2 in domestic and other animal species [5, 11] suggests its reverse-zoonotic potential. The likelihood of this back-spillover may be enhanced by the emergence of novel variants with higher infectivity [4]. The outbreak of lineage B.1.1.7 in the Prague Zoo correlates with the rise of a new variant in the human population of the Czech Republic.

Sequence differences between the gorilla and felid SARS-CoV-2 genomes strongly suggest two independent incursion events, each from a human host. Subsequently, the virus strains spread to secondary individuals by direct contact: the gorilla-like strain to other members of the troop and the felid-like strain among other felid species. However, detailed infection routes are difficult to elucidate. The zoo had been closed since the middle of December 2020, and all preventive measures, like using KN95 or FFP2/3 masks, gloves or hand disinfectants, and disinfection mats had already been in place weeks before the appearance of the B.1.1.7 variant in the Czech Republic. Nevertheless, the reverse-zoonotic transmission from the infected employees represents the most plausible explanation despite the absence of direct proof in the form of SARS-CoV-2 genomic sequences. We hypothesize that a combination of factors, such as the higher infectivity of the B.1.1.7 lineage, a subclinical infection of some keepers, or inattention or negligence in the application of protective equipment, led to the infection of both the great apes and the exotic felids. Hence, the only way to prevent a SARS-CoV-2 re-occurrence is strict adherence to hygiene measures, limiting human-animal interactions as much as possible, and increasing the frequency of virus screening among the zoo staff.

On the other hand, our findings suggest that not only gorillas and big cats are capable of being actively infected with SARS-CoV-2 but that they also develop clinical signs and efficiently spread the virus to other animals. This is in agreement with a recent report from the Bronx Zoo [10]. However, development of clinical signs in the Amur leopard cat is at odds with previous reports in which no clinical signs of COVID-19 were observed in domestic cats [2, 8].

The susceptibility of felid species, and more importantly, domestic cats as popular pets, may have important epidemiological consequences. Felids kept in captivity, or those that live in close quarters to people may serve as an alternative evolutionary niche where SARS-CoV-2 may undergo important genetic [2] or even antigenic changes. This necessitates continued genomic surveillance for new SARS-CoV-2 variants, both in human and animal populations.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s00705-022-05469-9>.

**Author contributions** All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Alexander Nagy, Martina Stará, Roman Vodička, Lenka Černíková, Helena Jiřincová, Vlastimil Krívda, and Kamil Sedlák. The first draft of the manuscript was written by Alexander Nagy, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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**Data availability** The consensus sequences of five SARS-CoV-2 strains were submitted to the GISAID EpiFlu (accession numbers EPI\_ISL\_1497613-17) and GenBank (OL752440-OL752444) databases. The data generated during the current study are available from Alexander Nagy.

## Declarations

**Conflict of interest** The authors declare that there is no conflict of interest.

**Ethical approval** The analysed specimens were not collected for the purpose of the presented study. The ethical standards of animal welfare are under the supervision of the State Veterinary Administration of the Czech Republic.

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