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Anthropogenic Transmission of SARS-CoV-2 from Humans to Lions, Singapore, 2021

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In Singapore, 10 captive lions tested positive for SARS-CoV-2 by real-time PCR. Genomic analyses of nanopore sequencing confirmed human-to-animal transmission of the SARS-CoV-2 Delta variant. Viral genomes from the lions and zookeeper shared a unique spike protein substitution, S:A1016V. Widespread SARS-CoV-2 transmission among humans can increase the likelihood of anthroponosis.

We investigated natural SARS-CoV-2 infection in captive African (*Panthera leo*) and Asiatic (*Panthera leo persica*) lions at a zoo in Singapore during increased Delta variant community infections. Understanding virus dynamics in different hosts is crucial for preventing interspecies transmission and protecting endangered species (*1*,*2*).

We studied 14 lions, 9 Asiatic and 5 African, that were housed in separate enclosures. On November 6, 2021, respiratory signs developed in a male Asiatic lion (AS-M1) (Appendix 1, https://wwwnc.cdc.gov/EID/article/29/12/22-1916-App1.pdf). On November 7, three Asiatic lionesses (AS-F1, AS-F2, and AS-F3) in the same enclosure exhibited similar clinical signs. A male African lion (AF-M3) in a separate enclosure developed clinical signs on November 8.

Eighteen zookeepers cared for and had close (within ≈1 m) but not direct contact with the lions. Six zookeepers tested COVID-19–positive beginning November 1, 2021, and 4 experienced mild respiratory symptoms starting on November 2.

To minimize stress on the animals, only 2 lions that had more severe signs, AS-M1 and AS-F1, were anesthetized for nasal and oropharyngeal sample collection on November 8. On November 9, we confirmed SARS-CoV-2 infection in the lions by real-time reverse transcription PCR (rRT-PCR); cycle quantitation (Cq) values were <40. Nasal swab samples from AS-M1 and AS-F1 had the highest viral loads (Cq 23.05 for AS-M1, 24.47 AS-F1). We conducted noninvasive infection monitoring for 3 weeks by collecting

and testing individual and pooled fecal samples from both lion enclosures. AF-M3 had the highest fecal sample viral load, Cq 36.02.

Within 5 days of the index case, 10 lions (all 9 Asiatic and 1 African) were infected. Most (8/10) clinically recovered from respiratory signs within 2 weeks; 2 lions took longer to recover, but all animals had recovered by December 3, 2021. Full recovery in the lions was determined by low viral RNA loads (Cq >40), absence of clinical signs, and resumption of normal behavior.

We sequenced RNA from nasal swab samples of AS-M1 and AS-F1 and 1 fecal sample from AF-M3 on the MinION R9.4.1 (Oxford Nanopore Technology, https://nanoporetech.com) platform using ARCTIC-CoV V1/V3 protocols (J.R. Tyson et al., unpub. data, https://doi.org/10.1101/2020.09.04.283077). The 3GS analysis pipeline from Genome Detective (3) generated preliminary contigs, which we stitched together by using sequence alignment information from a zookeeper's publicly available SARS-CoV-2 sequence (GISAID accession no. EPI_ISL_6600690; https://www.gisaid.org). We assessed the assembled sequences by using NextClade (4) to identify mutations and frameshifts compared with a wild-type reference sequence (GenBank accession no. NC_045512.2) and subsequently corrected alignment artifacts in the bam file (Appendix 2, https://wwwnc.cdc.gov/EID/article/29/12/22-1916-App2.xlsx).

We assembled 2 complete SARS-CoV-2 genomes (GenBank accession nos. OP393893.1 and OL677176.2) from nasal swab samples collected from AS-M1 and AS-F1. We obtained a partial genome assembly from a fecal sample from AF-M3 but did not analyze it further.

We conducted a phylogenomic analysis on 39 complete viral genomes, comprising 2 genomes from lions in this study and 37 sequences from GISAID, including the zookeeper's sequence. We built a maximum-likelihood tree by using RAxML-ng version 1.1.0 (https://github.com/stamatak/standard-RAxML) with 2,000 bootstrap replicates and used the wild-type reference sequence as the outgroup. The tree revealed that sequences from the zookeeper and Asiatic lions nested within the same subclade (Figure). Those findings and the high (99.98%) viral genetic similarity between the lions and zookeeper strongly suggest that SARS-CoV-2 infection in the

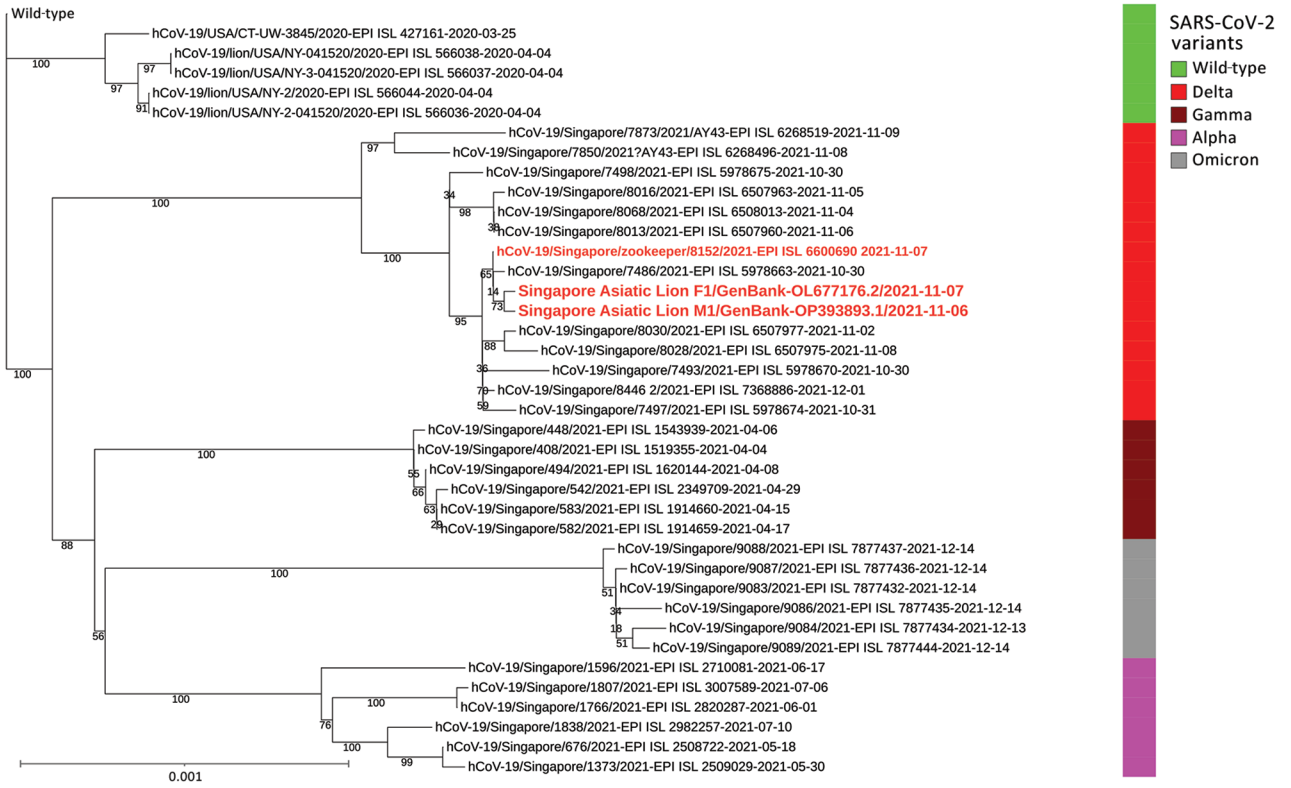

Figure. Maximum-likelihood phylogenomic tree from a case of anthropogenic transmission of SARS-CoV-2 from humans to lions, Singapore, 2021. Tree reconstructed from sequences of 2 lions and 1 zookeeper (red bold text), along with 36 other publicly available sequences representing 4 variants of concern from Singapore, cases of infected lions from the Bronx Zoo, and the wild-type reference genome (GenBank accession no. NC_045512.2) as the outgroup. Scale bar indicates nucleotide substitutions per site. EPI, GISAID (https://www.gisaid.com) EpiFlu database.

lions occurred through a human-to-animal (anthropogenic) transmission route.

We used Pangolin (https://github.com/cov-lineages/pangolin) to identify the subclade as Delta lineage AY.23.1, consistent with the predominant circulating strain in Singapore at that time. Both lions' sequences had 10 key Delta variant spike protein mutations and 2 open reading frame 8 amino acid deletions at positions D119- and F120-, compared with sequences from cases in Singapore (Appendix 1) (*5*). The lions, zookeeper, and 1 community case shared a unique spike protein mutation (S:A1016V), suggesting a potential founder's effect from this anthroponotic transmission event. Our investigation determined that the zookeepers were likely infected 6 days before the lion index case. The lions were not vaccinated against SARS-CoV-2, but 94% of the population of Singapore was fully vaccinated by November 2021.

This study highlights the vulnerability of captive and endangered animal populations to SARS-CoV-2 transmission from humans (*5*–*8*). Close contact between zookeepers and the lions likely led to the transmission, emphasizing the crucial need for strict infection control measures in captive animal facilities, especially during periods of increased community transmission of viruses (*7*).

The implications of SARS-CoV-2 infection in captive lions extend beyond animal health and welfare and can have consequences for the conservation of protected species. Insights from studies on minks and hamsters shed light on the potential for animal-to-human transmission (*6*,*8*). However, mass culling, as noted in those studies of small mammals, is an impractical approach for large or endangered animal species.

Lions already face numerous threats, including habitat loss, poaching, and disease; introduction of a novel virus like SARS-CoV-2 could have devastating consequences for their populations (*7*). Therefore, strengthening biosecurity measures in wildlife conservation centers and promoting vaccination of susceptible animal species whenever feasible and safe are crucial for mitigating viral transmission and protecting vulnerable wildlife populations (*1*,*9*).

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All data are available in the main text or the Appendix materials and can be found on the Zenodo repository at https://doi.org/10.5281/zenodo.8362203. Raw sequence reads generated in this study have been uploaded to the National Center for Biotechnology Information Sequence Read Archive under BioProject no. PRJNA1021696.

The ethical handling of animal subjects during the course of this research has been supervised and approved by National Parks Board, Singapore.

Author contributions: C.L., J.O., and W.K.W. conducted the molecular diagnostic tests; Y.C.A.I. performed the nanopore sequencing for viral genome assembly. Y.C.A.I and A.T. performed data analyses. The figures and the manuscript draft were prepared by Y.C.A.I. with input from C.J.F. Study initiation and guidance was by K.B.H.E, and supervised by C.J.F., S.F.C., Y.H.H., and K.B.H.E. Y.C.A.I. wrote the manuscript and all authors contributed to the manuscript editing process, approved the final version for publication, and declared that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Mass Mortality of Sea Lions Caused by Highly Pathogenic Avian Influenza A(H5N1) Virus

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We report a massive mortality of 5,224 sea lions (*Otaria flavescens*) in Peru that seemed to be associated with highly pathogenic avian influenza A(H5N1) virus infection. The transmission pathway may have been through the close contact of sea lions with infected wild birds. We recommend evaluating potential virus transmission among sea lions.

The panzootic (2020–2023) caused by the highly pathogenic avian influenza (HPAI) A(H5N1) caused numerous global outbreaks in 2022 (*1*). At the end of the year, the H5N1 virus reached South America, causing alarming bird mortalities in Peru (*2*). Comprehensive surveys suggest the virus killed >100,000 wild birds by the end of March 2023 only in protected areas (and >200,000 birds including other areas); particularly affected were Peruvian boobies (*Sula variegata*), guanay cormorants (*Leucocarbo bougainvilliorum*), and Peruvian pelicans (*Pelecanus thagus*) (*3*). The large biomass of infected wild birds may have led to a spillover event affecting marine mammals cohabiting with them, as reported in other parts of the world (*4*). Here, we report the death of several thousand sea lions (*Otaria flavescens*) on the coast of Peru within a few months; the sea lions manifested neurologic and respiratory signs. Clinical signs we observed suggest they were affected by HPAI H5N1, which was later confirmed by government and scientific reports (*5*,*6*).

During January–April 2023, we performed detailed surveillance of dead and agonal sea lions in protected marine areas of Peru (Figure). We found 5,224 animals dead or dying on beaches (Table). The synchronized high mortality rate we observed was concerning; up to 100 dead animals were found floating together in the sea, and 1,112 animals died on 1 island that has one of highest populations of sea lions in Peru (San Gallan, Ica, Reserva Nacional Paracas; Table). Those unprecedented massive mortalities for this region and even the entire world killed ≈5% of Peru's population of this species in a few months (Figure, panels A, B; Appendix Figure, https://wwwnc.cdc.gov/EID/article/29/12/23-0192-App1.pdf) (*7*).

National health authorities implemented restrictions regarding the manipulation of sick animals; for this reason, we were able to perform 1 necropsy, and the other observations were made by veterinarians at prudent distance. The clinical signs of agonal individuals were mainly neurologic, such as tremors, convulsions, and paralysis URLs (Video 1, https://wwwnc.cdc.gov/EID/article/29/12/23-0192-vid1; Video 2, https://wwwnc.cdc.gov/EID/