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SARS-CoV-2 delta variant in African lions (*Panthera leo*) and humans at Utah's Hogle Zoo, USA, 2021–22

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Abstract

Aims: We conducted a One Health investigation to assess the source and transmission dynamics of SARS-CoV-2 infection in African lions (*Panthera leo*) at Utah's Hogle Zoo in Salt Lake City from October 2021 to February 2022.

Methods and Results: Following observation of respiratory illness in the lions, zoo staff collected pooled faecal samples and individual nasal swabs from four lions. All specimens tested positive for SARS-CoV-2 by reverse transcription-polymerase chain reaction (RT-PCR). The resulting investigation included: lion observation; RT-PCR testing of lion faeces every 1–7 days; RT-PCR testing of lion respiratory specimens every 2–3 weeks; staff interviews and RT-PCR testing; whole-genome sequencing of viruses from lions and staff; and comparison with existing SARS-CoV-2 human community surveillance sequences. In addition to all five lions, three staff displayed respiratory symptoms. All lions recovered and no hospitalizations or deaths were

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CONFLICT OF INTEREST STATEMENT

The authors have declared that no competing interests exist.

ETHICS STATEMENT

This activity was reviewed by CDC and was determined to be public health activity and was conducted consistent with applicable federal laws and CDC policy (See, e.g. 45 C.F.R. part 46.102(l) (2), 21 C.F.R. part 56; 42 U.S.C. §241(d); 5 U.S.C. §552a; 44 U.S.C. §3501 et seq.).

DISCLAIMERS

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention (CDC). The findings and conclusions in this publication are those of the authors and should not be construed to represent any official USDA or U.S. government determination or policy.

reported among staff. Three staff reported close contact with the lions in the 10 days before lion illness onset, one of whom developed symptoms and tested positive for SARS-CoV-2 on days 3 and 4, respectively, after lion illness onset. The other two did not report symptoms or test positive. Two staff who did not have close contact with the lions were symptomatic and tested positive on days 5 and 8, respectively, after lion illness onset. We detected SARS-CoV-2 RNA in lion faeces for 33 days and in lion respiratory specimens for 14 weeks after illness onset. The viruses from lions were genetically highly related to those from staff and two contemporaneous surveillance specimens from Salt Lake County; all were delta variants (AY.44).

Conclusions: We did not determine the sources of these infections, although human-to-lion transmission likely occurred. The observed period of respiratory shedding was longer than in previously documented SARS-CoV-2 infections in large felids, indicating the need to further assess duration and potential implications of shedding.

Keywords

lions; One Health; *Panthera leo* ; SARS-CoV-2 delta variant; viral zoonotic disease

1 | INTRODUCTION

Many animal species have had documented infections with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) during the COVID-19 pandemic, including companion animals, farmed mink, free-ranging wildlife and captive wildlife (USDA APHIS, 2023). Felids have been shown to be particularly susceptible to SARS-CoV-2 and to have the capacity to transmit virus to other cats (Gaudreault et al., 2020; Krüger et al., 2021; Shi et al., 2020). In March 2020, tigers (*Panthera tigris*) and lions (*P. leo*) in Brooklyn, New York, were the first reported zoo animals to be naturally infected with SARS-CoV-2 (Bartlett et al., 2021; McAloose et al., 2020). Lions and tigers are the most common wildlife species under human care reported to be infected with SARS-CoV-2 in the United States (USDA APHIS, 2023).

While animal infections are known to occur, there is no evidence to suggest that transmission of SARS-CoV-2 between humans and animals has played a substantial role in the COVID-19 pandemic. Despite prolonged periods of high transmission among human populations, reports of infections among animals are relatively infrequent (Barua et al., 2021; Cossaboom et al., 2021; Dileepan et al., 2021; Pomorska-Mól et al., 2021; Stevanovic et al., 2021), with the exception of farmed mink (Badiola et al., 2022; Cossaboom et al., 2022; Oude Munnink et al., 2021). Animal-to-human transmission has been documented only rarely, from farmed mink, hamsters, a domestic cat and white-tailed deer (Oude Munnink et al., 2021; Pickering et al., 2022; Sila et al., 2022; Yen et al., 2022). Still, there is much that remains unknown about the epidemiology of SARS-CoV-2 in animals and their potential to contribute to the emergence of new SARS-CoV-2 variants as viral reservoirs. A One Health approach with expanded surveillance for SARS-CoV-2 among animals is vital to allow early detection of circulating strains in animals which may further threaten public health (Sharun et al., 2021).

Zoos are unique settings that bring a diverse array of animal species in close proximity to each other and humans. This provides a variety of benefits, including wildlife conservation and public education. However, these facilities also create a complex human–animal–environment interface with opportunities for disease transmission. Outbreaks of SARS-CoV-2 infection among zoos have impacted vulnerable or endangered species and provided insight into the host range of this virus (USDA APHIS, 2023). Therefore, it is important to carefully monitor and study SARS-CoV-2 in zoos and other wildlife facilities.

On 22 October 2021, the Utah Department of Health and Human Services (DHHS) was notified of SARS-CoV-2 infection in African lions and zoo staff at Utah's Hogle Zoo in Salt Lake City, which prompted a collaborative outbreak investigation to determine transmission dynamics at the zoo. In this report, we present the findings of the investigation and results of post-outbreak SARS-CoV-2 monitoring in lion faecal and nasal swab specimens.

2 | METHODS

2.1 | Animal investigation

Utah's Hogle Zoo is located in Salt Lake City, UT. It is a 42-acre zoo housing 629 species, ranging from invertebrates to megavertebrates. The zoo is accredited by the Association of Zoos and Aquariums and hosts over 900,000 visitors annually. Zoo staff includes approximately 40 animal keepers, 2 veterinarians and 2 veterinary technicians.

In October 2021, Utah's Hogle Zoo housed five lions who lived in two different prides. One pride was comprised of three females (two littermates and an offspring) and the second pride was comprised of two males (littermates). The lions were 5–10 years old with no known underlying health conditions. At this time, seven zoo staff were trained to work with the lions, and one to two of these staff members cared for the lions each day. The zoo was open to the public and visitors were encouraged to wear masks.

Prior to this investigation, Utah's Hogle Zoo did not perform routine surveillance for SARS-CoV-2 among zoo animals. Zoo staff identified clinical signs in the lions through observation and began collecting lion faecal specimens the day after the first signs of illness. Some specimens were from individual lions and other specimens were pooled by sex from the female and male groups. The sampling frequency ranged from daily to weekly for the next 3.5 months. Zoo staff also collected nasal swabs from the lions, beginning 1 week after onset of signs and continuing thereafter every 2–3 weeks for 4 months. The demeanour of one of the female lions (Lion5) did not allow zoo staff to collect nasal swabs from her. The other four lions allowed bilateral nasal swabbing by using protective contact methods while the lions ate meat from a food stick. One veterinarian, one veterinary technician and one animal care supervisor collected nasal swabs during the time that the lions displayed clinical signs. After the lions fully recovered, the animal care supervisor collected the remainder of the nasal swabs with assistance from an animal keeper. All staff who were present during nasal swabbing wore a N-95 mask, gloves and a face shield.

2.2 | Human epidemiologic investigation and environmental assessment

In accordance with zoo policy that preceded this outbreak investigation, zoo staff (paid employees and volunteers) self-reported positive SARS-CoV-2 test results to zoo management, who then informed DHHS. Staff 1 and Staff 2 tested positive by reverse transcription-polymerase chain reaction (RT-PCR) and Staff 3 tested positive by antigen test; all tests were performed by different private laboratories. We verified each positive test result in Utah's disease surveillance system. We considered the infectious period to begin 48 h before symptom onset.

On 27 October, we held a voluntary event at the zoo for SARS-CoV-2 sampling and interviews of staff. We collected nasopharyngeal swabs from all zoo staff who might have had close contact with the lions and who had not already reported a positive SARS-CoV-2 test result. Health department employees conducted structured, open-ended interviews with each tested staff member, as well as each SARS-CoV-2-positive zoo staff member reported by zoo management. We asked about work schedule, animal interactions while at work, recent SARS-CoV-2 test results, recent COVID-19-like symptoms, interactions with known SARS-CoV-2-positive persons both inside and outside of work and COVID-19 vaccination status. We defined close contact between humans and lions as being within a distance of 6 feet for any length of time. Additionally, we used a work schedule provided by zoo management to determine the timing and location of staff assignments.

In 2021, approximately 5% of positive SARS-CoV-2 RT-PCR tests performed at the Utah Public Health Laboratory (UPHL) were sequenced for routine surveillance. We reviewed the Utah database of surveillance SARS-CoV-2 whole-genome sequences to identify Utah residents who tested positive for SARS-CoV-2 in October and whose viral sequences were genetically highly related to those from zoo staff. We interviewed these residents to assess any connection to the zoo or zoo staff.

While at the zoo for the sampling event, health department employees observed the indoor and outdoor lion habitat and surrounding areas. The goal of this observation was to evaluate lion housing and management practices, infection prevention measures, the potential for lion interaction with zoo visitors and the potential for lion interaction with other animal species. We also conducted informal, unstructured interviews with zoo managers to assess potential routes of SARS-CoV-2 transmission within the zoo and review practices for staff assignments, biosecurity and animal management.

2.3 | Specimen collection and SARS-CoV-2 RT-PCR testing

Zoo staff stored lion faecal specimens in a standard freezer in the lion building for 1–3 weeks after collection, and then at −80°C in the veterinary hospital for approximately 3 months until they were shipped for testing. Staff placed lion nasal swabs in 0.5 mL 0.9% sterile saline immediately following collection, refrigerated these specimens and shipped them for testing the same day. Staff shipped all lion test specimens on ice.

Lion faecal specimens were collected during the first week of lion illness and all lion nasal swabs were submitted to the National Veterinary Services Laboratories (NVSL) for RT-PCR to detect SARS-CoV-2 RNA. We extracted total RNA from specimens using

the MagMAX CORE kit (Thermo Fisher, Waltham, Massachusetts) per manufacturer's instruction (workflow) using the KingFisher Flex magnetic particle processor (Thermo Fisher, Waltham, Massachusetts). We tested nucleic acid for SARS-CoV-2 using the 2019-nCoV Kit N1 and N2 assays (Integrated DNA Technologies, Inc., Coralville, Iowa). We used Xeno RNA (Thermo Fisher, Waltham, Massachusetts) as an extraction and exogenous control and β-actin as an endogenous control. We prepared reactions using the TaqPath 1-Step RT-qPCR Master Mix, CG Kit (Thermo Fisher, Waltham, Massachusetts). Thermal cycling consisted of UNG incubation at 25°C for 2 min, reverse transcription at 50°C for 15 min and enzyme activation and 95°C for 2 min followed by 45 cycles of 95°C for 3 s and 55°C for 30 s. A positive result required detection of both N1 and N2 targets with a cycle threshold (Ct) ≤45.

Lion faecal specimens collected after the first week of lion illness were submitted to the Utah Veterinary Diagnostic Laboratory (UVDL) for RT-PCR. We detected viral RNA using the SARS-CoV-2 RNA-dependent RNA polymerase gene (RdRp) and E gene primers (Corman et al., 2020). The RdRp gene primers and probes are specific for SARS-CoV-2. The E gene primers and probes are specific for bat-related betacoronaviruses, including SARS-CoV-1 and −2. We suspended faecal specimens in Trizol (Thermo Fisher, Waltham, Massachusetts) and extracted RNA from faeces using the MagMAX CORE Nucleic Acid Purification kit (Thermo Fisher, Waltham, Massachusetts) per manufacturer instructions. We amplified RNA using the TaqMan Fast Virus 1-Step Master Mix Kit (Thermo Fisher, Waltham, Massachusetts) and an ABI 7500 Fast (Thermo Fisher, Waltham, Massachusetts). Controls included a positive extraction control (RdRp gBlock fragment), negative extraction control (nucleic acid-free water), positive amplification control (SARS-CoV-2 whole-genome RNA) and negative amplification control (no template control). Thermal cycling consisted of reverse transcription at 55°C for 10 min, followed by 95°C for 3 min and then 45 cycles of 95°C for 15 s and 58°C for 30 s. A positive result required detection of both RdRp and E gene targets with a Ct ≤45.

The human nasopharyngeal swabs collected during the SARS-CoV-2 sampling event held at the zoo were submitted to UPHL for RT-PCR using Applied Biosystems TaqPath COVID-19 Combo Kit (Thermo Fisher, Waltham, Massachusetts) per manufacturer instructions. We refrigerated specimens after collection and submitted them to UPHL the same day.

2.4 | SARS-CoV-2 whole-genome sequencing and phylogenetic analysis

We performed whole-genome sequencing (WGS) directly from lion and staff respiratory specimens that tested positive by RT-PCR. At NVSL, we prepared cDNA libraries using the Nextera XT DNA Library Preparation Kit (Illumina) according to manufacturer instructions and sequenced lion specimens using the 500-cycle MiSeq Reagent Kit v2 as previously described (Hale et al., 2022). We assembled lion sequences using IRMA v0.6.7 and DNAStar SeqMan NGen v14.0.1. We sequenced human specimens at UPHL using Illumina's COVIDSeq library preparation kits and an Illumina NovaSeq 6000 System for 100 cycles with single-end 75 base pair reads (NovaSeq 6000 S2 Reagent Kit v1.5).

We assessed viral relatedness via single-nucleotide polymorphism (SNP) differences. We defined a close genetic relationship to be sequenced with a difference of 0–1 SNP, supported

by previous literature which suggests that direct viral transmission most often results in ≤1 SNP (Hare et al., 2022; Lumley et al., 2021; Meredith et al., 2020; Snell et al., 2022; Stirrup et al., 2021). We compared SARS-CoV-2 genome sequences from the lions and zoo staff, as well as the original Wuhan reference strain (MN908947), and Utah residents previously sequenced for surveillance purposes. We assigned PANGO lineages to consensus sequences for viruses detected from both humans and lions with pangolin v4.0.5 (Rambaut et al., 2020) and aligned sequences with MAFFT (Katoh et al., 2002). We constructed a phylogenetic tree with IQ-TREE2 (Minh et al., 2020), assessed SNP differences with SNP-dists (Seeman et al., 2017) as part of the Cecret nextflow workflow (Utah Public Health Laboratory, Utah Department of Health and Human Services, 2020) and visualized with ggtree (Yu et al., 2017).

3 | RESULTS

3.1 | Outbreak description and timeline

Zoo staff first noticed signs of illness in the lions on 13 October, when Lion 1, a female, was slow to eat. During 13–20 October, all five lions began showing signs of mild illness (Figure 1). The primary observed clinical signs were sneezing and coughing, with nasal congestion, wheezing and nasal discharge also occasionally noted (Table 1). The duration of clinical signs in the lions ranged between 2 and 21 days. All five lions recovered fully. No other animals at the zoo were reported to have respiratory symptoms of concern during the month before and several months after the lions were ill.

On 22 October, zoo management reported three zoo staff had tested positive for SARS-CoV-2 during October 2021: Staff 1, Staff 2 and Staff 3 (Figure 1). No staff reported testing positive in the 10 days before Lion 1 became ill. Staff 1 first experienced COVID-19-like symptoms on 16 October and tested positive by RT-PCR on 17 October. Staff 2 first experienced symptoms on 17 October and tested positive by RT-PCR on 18 October. Staff 3 developed symptoms on 21 October and tested positive with a rapid antigen test the same day. To obtain a specimen for WGS, Staff 3 was tested by RT-PCR on 26 October, but the test was negative. No hospitalizations or deaths among these human cases were reported. Ten other zoo staff members attended the interview and sampling event. All were negative for SARS-CoV-2 via RT-PCR and none reported experiencing COVID-19-like symptoms in the previous month.

3.2 | Staff epidemiologic investigation findings and zoo environmental assessment

3.2.1 | Staff interaction with lions—According to zoo management, the 13 zoo staff we interviewed included all staff who could have had close contact with the lions from 3 to 12 October (the 10 days before the earliest illness onset in the lions). Based on interviews and a staff work schedule, we identified 3 zoo staff among these 13 who had close contact with the lions during this time: Staff 2, Staff 4 and Staff 5. Staff 2, who self-reported testing positive for SARS-CoV-2 on 18 October, was a primary caretaker for the lions and cared for them for 11 days in October (3–7, 10–11, 13–14 and 17–18 October). Staff 4 and Staff 5 tested negative at our sampling event and did not report recent COVID-19-like symptoms. Staff 4 cared for the lions on 8 and 9 October. Staff 5 cared for the lions on 12 October.

3.2.2 | Zoo staff exposures—None of the 13 interviewed zoo staff members reported a known exposure to COVID-19 outside of work; however, reported COVID-19 incidence in the community was high during this time. Only one of the known interactions among Staff 1, Staff 2 and Staff 3 in October could have resulted in viral transmission between staff. This is when Staff 1 worked with Staff 2 in the kitchen area of the lion building on 14 October, 2 days before Staff 1 developed symptoms.

3.2.3 | Environmental assessment findings—During this assessment, the female lion group was housed separately from the male lion group. When in the outdoor habitats (in public view), the female and male groups separately occupied a larger or a smaller enclosure in rotation. When indoors (not in public view), the female and male groups shared airspace within the same holding room. The groups alternated between two different sides of the room separated by an approximately 10-foot-wide alleyway used by zoo staff. No other animal species were housed in the outdoor or indoor lion exhibits. Zoo staff who cared for the lions also worked with other animal species during the same shift, including meerkats, warthogs, giraffes, zebras and guinea fowl. No other animal species were tested for SARS-CoV-2 during this investigation.

All public viewing areas for the lions were outdoors. The main viewing areas were behind impermeable transparent barriers. All viewing areas which contained air-permeable fencing required visitors to be at least 6 feet away from the lions; per zoo staff, the lions rarely spent time in these areas.

3.2.4 | Preventive measures—All 13 of the zoo staff whom we investigated reported completing the primary vaccination series for COVID-19 before October 2021. In October 2021, zoo policy on personal protective equipment (PPE) required zoo staff to wear masks at all times while working indoors; the type of mask was not specified. Lion habitat protocol called for disinfection of the lion stalls twice per month with quaternary ammonia and the feed pans once per month with bleach. Following the onset of clinical signs in the lions, the zoo required enhanced PPE while in the lion building (KN95 or N95 mask, dedicated boots, face shield and gloves); discontinued all training and other activities that put staff in close proximity to the lions with the exception of veterinary procedures; discontinued high-pressure washing of lion spaces; added quaternary ammonia foot baths; and increased disinfection frequency of lion stalls and feed pans. At the time of this study, the lions were not vaccinated for SARS-CoV-2.

3.3 | SARS-CoV-2 detection in the lions by RT-PCR

Seven faecal specimens were collected from the lions during 14–20 October. Six nasal swabs were collected from four lions on 20 Oct. All 13 specimens tested positive for SARS-CoV-2 by RT-PCR. Ct value ranges from the first faecal (14 October) and nasal swab (20 October) RT-PCR specimens from the lions were 16–19 and 25–26, respectively (Figure 2).

Faecal specimens from the lions remained positive for SARS-CoV-2 RNA for 33 days after the earliest detected clinical signs in Lion 1 (13 October). Specimens from male lions were consistently positive during this time, and specimens from female lions were intermittently

positive. After 33 days, all lion faecal specimens tested negative (Figure 2). Nasal swabs from all four lions remained positive for SARS-CoV-2 RNA for 14 weeks after the earliest detected clinical signs in Lion 1. After initial detection, Ct values in nasal swabs increased to a plateau of around 34 for about 3 months until all lions tested negative (Figure 2).

3.4 | SARS-CoV-2 genome sequencing and phylogenetic analysis

For sequence comparison between specimens from lions and staff, we used: for lions, the index respiratory specimens (collected on 20 October); for Staff 1, a specimen collected on 26 October (the RT-PCR-positive specimen collected from Staff 1 on 17 October was not available for sequencing); and, for Staff 2, the specimen collected on 18 October. All six lion and human SARS-CoV-2 genomes used for comparison were delta variants of the AY.44 PANGO lineage and were genetically closely related to each other (Figure 3). Four of the six viruses (three lions and Staff 1) were identical based on WGS (0 SNPs different from each other). The two other viruses (Lion 3 and Staff 2) were each 1 SNP different from the group of four identical sequences, and 2 SNPs different from each other. Viral sequences generated from lion respiratory specimens collected >21 days after their onset of illness yielded low sequence coverage and were excluded from phylogenetic analysis.

AY.44 was a dominant SARS-CoV-2 variant in Utah in October 2021, accounting for approximately one-third of SARS-CoV-2 specimens sequenced at UPHL during that time. Comparison to Utah community surveillance SARS-CoV-2 sequences revealed two Salt Lake County residents in October 2021 with sequences closely related to those from lions and zoo staff (0–1 SNP different from the group of four identical sequences, as shown in Figure 3). During interviews, these two did not report any association with or recent visits to Utah's Hogle Zoo.

4 | DISCUSSION

This report describes an outbreak of infections with the delta variant of SARS-CoV-2 among African lions and zoo staff at Utah's Hogle Zoo in Salt Lake City. Viral genome sequencing demonstrated the lions and zoo staff were infected with a genetically highly related SARS-CoV-2 strain. The observation of two community SARS-CoV-2 sequences during this timeframe that were highly related to the zoo outbreak sequences, with no reported epidemiologic linkage to the zoo, suggests this outbreak's viral strain was contemporaneously circulating in Salt Lake County.

Despite a multifaceted One Health investigation, we could not definitively determine the source(s) or transmission dynamics of this outbreak, although it is likely that human-to-lion transmission occurred. Similar to other reported outbreaks among zoo animals (Allender et al., 2022; Bartlett et al., 2021; Fernández-Bellon et al., 2021; Grome et al., 2022; Koeppel et al., 2022; McAloose et al., 2020; Wang et al., 2022), SARS-CoV-2-positive zoo staff members were identified around the time of lion illness. One of the positive staff members (Staff2) had repeated close contact with the lions. Based on the transmission dynamics of the delta variant (Kang et al., 2022), however, it is unlikely that any of the three zoo staff with reported COVID-19 was the source of SARS-CoV-2 exposure for the lions. Each of these staff developed symptoms ≥3 days after Lion 1 began displaying clinical signs. It

is possible that an asymptomatic zoo staff member with undetected infection introduced SARS-CoV-2 to the lions. Staff 4 and Staff 5 tested negative on 27 October and did not report recent COVID-19 symptoms; however, they might previously have had asymptomatic SARS-CoV-2 infection and been infectious when they had close contact with the lions in the week prior to Lion 1's illness onset. The lion habitat design prevented close contact between the animals and the public, making viral transmission between zoo visitors and the lions extremely unlikely. Other theoretical routes of transmission, such as via fomites (e.g., prepared feed) or incursion of other animals in the lion habitat (e.g. rodents and birds), could not be ruled out, but are less probable than a human source. Regardless of the source, once at least one lion was infected, transmission between the lions likely occurred readily due to their close contact and shared air space while indoors.

Lion-to-human transmission in this outbreak was also possible. Staff 2 might have become infected during close interaction with the lions on 11, 13 or 14 October. Animal-to-human transmission has been documented (Oude Munnink et al., 2021; Pickering et al., 2022; Sila et al., 2022; Yen et al., 2022), but is thought to be rare and difficult to definitively demonstrate, particularly during periods of high human-to-human transmission. Hence, while lion-to-human transmission cannot be ruled out, the three zoo staff with known COVID-19 were more likely to have acquired their infection from other humans than from the lions.

Infection with the delta variant has been previously reported in lions (Allender et al., 2022; Karikalan et al., 2022; Koeppel et al., 2022; Mishra et al., 2021). All five lions at the zoo displayed respiratory clinical signs similar to the symptoms of COVID-19 in humans and comparable with the clinical findings of other SARS-CoV-2 infections among captive large felids (Allender et al., 2022; Bartlett et al., 2021; Fernández-Bellon et al., 2021; Grome et al., 2022; Koeppel et al., 2022; McAloose et al., 2020; Mitchell et al., 2021; Wang et al., 2022). SARS-CoV-2 RNA was detected in lion faecal and respiratory specimens >4 and 14 weeks, respectively, after the earliest detected clinical signs in the lions. This duration of faecal shedding is comparable to other reported infections of SARS-CoV-2 among large felids (Allender et al., 2022; Bartlett et al., 2021; Cushing et al., 2021; Fernández-Bellon et al., 2021; Koeppel et al., 2022). However, the protracted period of respiratory viral shedding in lions observed in this investigation is longer than previously reported among large felids (Cushing et al., 2021; Fernández-Bellon et al., 2021; Koeppel et al., 2022). Extended presence of SARS-CoV-2 RNA might increase the risk of transmission to other animals and zoo staff, as well as of viral mutation, although we did not perform virus isolation to determine infectivity and later specimens yielded relatively high Ct values. Additional studies of SARS-CoV-2 infections in animals will be needed to understand the duration and importance of viral shedding and guide zoo infection prevention protocols.

Our study has a few limitations. Since we did not perform virus isolation, we do not know if the lions were shedding infectious virus during their prolonged period of SARS-CoV-2 RNA detection. We also did not perform phylogenetic analysis of viral sequences from samples collected >7 days after the lions' onset of illness due to poor sequence coverage in later samples. Therefore, we could not assess for viral mutation.

Zoos provide many benefits to humans and animals, including support for wildlife conservation, educational programmes and research. Unfortunately, zoos also offer opportunities for interspecies transmission of zoonotic diseases, including SARS-CoV-2. Large felids, like the African lions in this study, are among the animal species most susceptible. Prolonged shedding of viral RNA in these animals might increase the risk of further spread to animals and humans in close proximity and could provide more opportunity for genomic mutation. Enhanced infection prevention measures may, therefore, be beneficial for several months after clinical signs resolve following SARS-CoV-2 infections in large felids. Additionally, screening of zoo staff for SARS-CoV-2 before close contact with particularly susceptible animal species may be worthwhile to decrease the risk of transmission from staff with asymptomatic infections, especially during periods of elevated community transmission. While animal infections are not currently believed to play a significant role in human COVID-19 infections, additional studies are needed to better understand SARS-CoV-2 dynamics at the human–animal–environment interface.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study may be available on request from the corresponding author.

REFERENCES

Allender MC, Adkesson MJ, Langan JN, Delk KW, Meehan T, Aitken-Palmer C, McEntire MM, Killian ML, Torchetti M, Morales SA, Austin C, Fredrickson R, Olmstead C, Ke R, Smith R, Hostnik ET, Terio K, & Wang L (2022). Multi-species outbreak of SARS-CoV-2 Delta variant in a zoological institution, with the detection in two new families of carnivores. Transboundary and Emerging Diseases, 69(5), e3060–e3075. 10.1111/tbed.14662 [PubMed: 35839756]

Badiola JJ, Otero A, Sevilla E, Marín B, García Martínez M, Betancor M, Sola D, Pérez Lázaro S, Lozada J, Velez C, Chiner-Oms Á, Comas I, Cancino-Muñoz I, Monleón E, Acín M, Acin C, Bolea R, & Moreno B (2022). SARS-CoV-2 outbreak on a Spanish mink farm: Epidemiological, molecular, and pathological studies. Frontiers in Veterinary Science, 8, 805004. 10.3389/fvets.2021.805004 [PubMed: 35127883]

Bartlett SL, Diel DG, Wang L, Zee S, Laverack M, Martins M, Caserta LC, Killian ML, Terio K, Olmstead C, Delaney MA, Stokol T, Ivančić M, Jenkins-Moore M, Ingerman K, Teegan T, McCann C, Thomas P, McAloose D, Calle PP (2021). SARS-CoV-2 infection and longitudinal fecal screening in Malayan tigers (*Panthera tigris jacksoni*), Amur tigers (*Panthera tigris altaica*), and African lions (*Panthera leo krugeri*) at the Bronx zoo, New York, USA. Journal of Zoo and Wildlife Medicine, 51(4), 733–744. 10.1638/2020-0171 [PubMed: 33480553]

Barua S, Hoque M, Adekanmbi F, Kelly P, Jenkins-Moore M, Torchetti MK, Chenoweth K, Wood T, & Wang C (2021). Antibodies to SARS-CoV-2 in dogs and cats, USA. Emerging Microbes & Infections, 10(1), 1669–1674. 10.1080/22221751.2021.1967101 [PubMed: 34374631]

Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK , Bleicker T, Brünink S, Schneider J, Schmidt ML, Mulders DG, Haagmans BL, van der Veer B, van den Brink S, Wijsman L , Goderski G, Romette JL, Ellis J, Zambon M, … Drosten C (2020). Detection of

2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance, 25(3), 2000045. 10.2807/1560-7917.ES.2020.25.3.2000045 [PubMed: 31992387]

Cossaboom CM, Medley AM, Spengler JR, Kukielka EA, Goryoka GW, Baird T, Bhavsar S, Campbell S, Campbell TS, Christensen D, Condrey JA, Dawson P, Doty JB, Feldpausch A, Gabel J, Jones D, Lim A, Loiacono CM, Jenkins-Moore M, … Barton Behravesh C (2021). Low SARS-CoV-2 seroprevalence and no active infections among dogs and cats in animal shelters with laboratory-confirmed COVID-19 human cases among employees. Biology, 10(9), 898. 10.3390/biology10090898 [PubMed: 34571775]

Cossaboom CM, Wendling NM, Lewis NM, Rettler H, Harvey RR, Amman BR, Towner JS, Spengler JR, Erickson R, Burnett C, Young EL, Oakeson K, Carpenter A, Kainulainen MH, Chatterjee P, Flint M, Uehara A, Li Y, Zhang J, … Barton Behravesh C (2022). One health investigation of SARS-CoV-2 in people and animals on multiple mink farms in Utah. Viruses, 15(1), 96. 10.3390/v15010096 [PubMed: 36680136]

Cushing AC, Sawatzki K, Grome HN, Puryear WB, Kelly N, & Runstadler J (2021). Duration of antigen shedding and development of antibody titers in Malayan tigers (*Panthera tigris jacksoni*) naturally infected with SARS-CoV-2. Journal of Zoo and Wildlife Medicine, 52(4), 1224–1228. 10.1638/2021-0042 [PubMed: 34998292]

Dileepan M, Di D, Huang Q, Ahmed S, Heinrich D, Ly H, & Liang Y (2021). Seroprevalence of SARS-CoV-2 (COVID-19) exposure in pet cats and dogs in Minnesota, USA. Virulence, 12(1), 1597–1609. 10.1080/21505594.2021.1936433 [PubMed: 34125647]

Fernández-Bellon H, Rodon J, Fernández-Bastit L, Almagro V, Padilla-Solé P, Lorca-Oró C, Valle R, Roca N, Grazioli S, Trogu T, Bensaid A, Carrillo J, Izquierdo-Useros N, Blanco J, Parera M, Noguera-Julián M, Clotet B, Moreno A, Segalés J, & Vergara-Alert J (2021). Monitoring natural SARS-CoV-2 infection in lions (*Panthera leo*) at the Barcelona zoo: Viral dynamics and host responses. Viruses, 13(9), 1683. 10.3390/v13091683 [PubMed: 34578266]

Gaudreault NN, Trujillo JD, Carossino M, Meekins DA, Morozov I, Madden DW, Indran SV, Bold D, Balaraman V, Kwon T, Artiaga BL, Cool K, García-Sastre A, Ma W, Wilson WC, Henningson J, Balasuriya U, & Richt JA (2020). SARS-CoV-2 infection, disease and transmission in domestic cats. Emerging Microbes & Infections, 9(1), 2322–2332. 10.1080/22221751.2020.1833687 [PubMed: 33028154]

Grome HN, Meyer B, Read E, Buchanan M, Cushing A, Sawatzki K, Levinson KJ, Thomas LS, Perry Z, Uehara A, Tao Y, Queen K, Tong S, Ghai R, Fill MM, Jones TF, Schaffner W, & Dunn J (2022). SARS-CoV-2 outbreak among Malayan tigers and humans, Tennessee, USA, 2020. Emerging Infectious Diseases, 28(4), 833–836. 10.3201/eid2804.212219 [PubMed: 35318922]

Hale VL, Dennis PM, McBride DS, Nolting JM, Madden C, Huey D, Ehrlich M, Grieser J, Winston J, Lombardi D, Gibson S, Saif L, Killian ML, Lantz K, Tell RM, Torchetti M, Robbe-Austerman S, Nelson MI, Faith SA, & Bowman AS (2022). SARS-CoV-2 infection in free-ranging white-tailed deer. Nature, 602(7897), 481–486. 10.1038/s41586-021-04353-x [PubMed: 34942632]

Hare D, Meaney C, Powell J, Slevin B, O' Brien B, Power L, O' Connell NH, De Gascun CF, Dunne CP, & Stapleton PJ (2022). Repeated transmission of SARS-CoV-2 in an overcrowded Irish emergency department elucidated by whole-genome sequencing. The Journal of Hospital Infection, 126, 1–9. 10.1016/j.jhin.2022.04.015 [PubMed: 35562074]

Kang M, Xin H, Yuan J, Ali ST, Liang Z, Zhang J, Hu T, Lau EH, Zhang Y, Zhang M, Cowling BJ, Li Y, & Wu P (2022). Transmission dynamics and epidemiological characteristics of SARS-CoV-2 Delta variant infections in Guangdong, China, may to June 2021. Eurosurveillance, 27(10), 2100815. 10.2807/1560-7917.ES.2022.27.10.2100815 [PubMed: 35272744]

Karikalan M, Chander V, Mahajan S, Deol P, Agrawal RK, Nandi S, Rai SK, Mathur A, Pawde A, Singh KP, & Sharma GK (2022). Natural infection of Delta mutant of SARS-CoV-2 in Asiatic lions of India. Transboundary and Emerging Diseases, 69(5), 3047–3055. 10.1111/tbed.14290 [PubMed: 34404118]

Katoh K, Misawa K, Kuma K, & Miyata T (2002). MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research, 30(14), 3059–3066. 10.1093/nar/gkf436 [PubMed: 12136088]

Koeppel KN, Mendes A, Strydom A, Rotherham L, Mulumba M, & Venter M (2022). SARS-CoV-2 reverse zoonoses to pumas and lions, South Africa. Viruses, 14(1), 120. 10.3390/V14010120 [PubMed: 35062324]

Krüger N, Rocha C, Runft S, Krüger J, Färber I, Armando F, Leitzen E, Brogden G, Gerold G, Pöhlmann S, Hoffmann M, & Baumgärtner W (2021). The upper respiratory tract of felids is highly susceptible to SARS-CoV-2 infection. International Journal of Molecular Sciences, 22(19), 10636. 10.3390/ijms221910636 [PubMed: 34638978]

Lumley SF, Constantinides B, Sanderson N, Rodger G, Street TL, Swann J, Chau KK, O'Donnell D, Warren F, Hoosdally S, OUH Microbiology laboratory, OUH Infection Prevention and Control team, O'Donnell AM, Walker TM, Stoesser NE, Butcher L, Peto TE, Crook DW, Jeffery K, … Eyre DW (2021). Epidemiological data and genome sequencing reveals that nosocomial transmission of SARS-CoV-2 is underestimated and mostly mediated by a small number of highly infectious individuals. The Journal of Infection, 83(4), 473–482. 10.1016/j.jinf.2021.07.034 [PubMed: 34332019]

McAloose D, Laverack M, Wang L, Killian ML, Caserta LC, Yuan F, Mitchell PK, Queen K, Mauldin MR, Cronk BD, Bartlett SL, Sykes JM, Zee S, Stokol T, Ingerman K, Delaney MA, Fredrickson R, Ivančić M, Jenkins-Moore M, … Diel DG (2020). From people to panthera: Natural SARS-CoV-2 infection in tigers and lions at the Bronx zoo. MBio, 11(5), e02220–20. 10.1128/mBio.02220-20 [PubMed: 33051368]

Meredith LW, Hamilton WL, Warne B, Houldcroft CJ, Hosmillo M, Jahun AS, Curran MD, Parmar S, Caller LG, Caddy SL, Khokhar FA, Yakovleva A, Hall G, Feltwell T, Forrest S, Sridhar S, Weekes MP, Baker S, Brown N, … Goodfellow I (2020). Rapid implementation of SARS-CoV-2 sequencing to investigate cases of health-care associated COVID-19: A prospective genomic surveillance study. The Lancet Infectious Diseases, 20(11), 1263–1272. 10.1016/S1473-3099(20)30562-4 [PubMed: 32679081]

Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, & Lanfear R (2020). IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. Molecular Biology and Evolution, 37(5), 1530–1534. 10.1093/molbev/msaa015 [PubMed: 32011700]

Mishra A, Kumar N, Bhatia S, Aasdev A, Kanniappan S, Sekhar AT , Gopinadhan A, Silambarasan R, Sreekumar C, Dubey CK, Tripathi M, Raut AA, & Singh VP (2021). SARS-CoV-2 Delta variant among Asiatic lions, India. Emerging Infectious Diseases, 27(10), 2723–2725. 10.3201/eid2710.211500 [PubMed: 34545805]

Mitchell PK, Martins M, Reilly T, Caserta LC, Anderson RR, Cronk BD, Murphy J, Goodrich EL, & Diel DG (2021). SARS-CoV-2 B.1.1.7 variant infection in Malayan tigers, Virginia, USA. Emerging Infectious Diseases, 27(12), 3171–3173. 10.3201/eid2712.211234 [PubMed: 34808082]

Oude Munnink BB, Sikkema RS, Nieuwenhuijse DF, Molenaar RJ, Munger E, Molenkamp R, van der Spek A, Tolsma P, Rietveld A, Brouwer M, Bouwmeester-Vincken N, Harders F, Hakze-van der Honing R, Wegdam-Blans M, Bouwstra RJ, GeurtsvanKessel C, van der Eijk AA, Velkers FC, Smit L, … Koopmans M (2021). ransmission of SARS-CoV-2 on mink farms between humans and mink and back to humans. Science, 371(6525), 172–177. 10.1126/science.abe5901 [PubMed: 33172935]

Pickering B, Lung O, Maguire F, Kruczkiewicz P, Kotwa JD, Buchanan T, Gagnier M, Guthrie JL, Jardine CM, Marchand-Austin A, Massé A, McClinchey H, Nirmalarajah K, Aftanas P, Blais-Savoie J, Chee HY, Chien E, Yim W, Banete A, … Bowman J (2022). Divergent SARS-CoV-2 variant emerges in white-tailed deer with deer-to-human transmission. Nature Microbiology, 7(12), 2011–2024. 10.1038/s41564-022-01268-9

Pomorska-Mól M, Turlewicz-Podbielska H, Gogulski M, Ruszkowski JJ, Kubiak M, Kuriga A, Barket P, & Postrzech M (2021). A cross-sectional retrospective study of SARS-CoV-2 seroprevalence in domestic cats, dogs and rabbits in Poland. BMC Veterinary Research, 17(1), 322. 10.1186/s12917-021-03033-2 [PubMed: 34620166]

Rambaut A, Holmes EC, O'Toole Á, Hill V, McCrone JT, Ruis C, du Plessis L, & Pybus OG (2020). A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. Nature Microbiology, 5(11), 1403–1407. 10.1038/s41564-020-0770-5

Seeman T, Klötzl F, & Page A (2017). Pairwise SNP distance matrix from a FASTA sequence alignment, https://github.com/tseemann/snp-dists

Sharun K, Tiwari R, Natesan S, & Dhama K (2021). SARS-CoV-2 infection in farmed minks, associated zoonotic concerns, and importance of the one health approach during the ongoing COVID-19 pandemic. The Veterinary Quarterly, 41(1), 50–60. 10.1080/01652176.2020.1867776 [PubMed: 33349165]

Shi J, Wen Z, Zhong G, Yang H, Wang C, Huang B, Liu R, He X, Shuai L, Sun Z, Zhao Y, Liu P, Liang L, Cui P, Wang J, Zhang X, Guan Y,Tan W, Wu G, Bu Z (2020). Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. Science (New York, N.Y.), 368(6494), 1016–1020. 10.1126/science.abb7015 [PubMed: 32269068]

Sila T, Sunghan J, Laochareonsuk W, Surasombatpattana S, Kongkamol C, Ingviya T, Siripaitoon P, Kositpantawong N, Kanchanasuwan S, Hortiwakul T, Charernmak B, Nwabor OF, Silpapojakul K, & Chusri S (2022). Suspected cat-to-human transmission of SARS-CoV-2, Thailand, July-September 2021. Emerging Infectious Diseases, 28(7), 1485–1488. 10.3201/eid2807.212605 [PubMed: 35666777]

Snell LB, Fisher CL, Taj U, Stirrup O, Merrick B, Alcolea-Medina A, Charalampous T, Signell AW, Wilson HD, Betancor G, Kia Ik MT, Cunningham E, Cliff PR, Pickering S, Galao RP, Batra R, Neil SJD, Malim MH, Doores KJ, … Awan AR (2022). Combined epidemiological and genomic analysis of nosocomial SARS-CoV-2 infection early in the pandemic and the role of unidentified cases in transmission. Clinical Microbiology and Infection, 28(1), 93–100. 10.1016/j.cmi.2021.07.040 [PubMed: 34400345]

Stevanovic V, Vilibic-Cavlek T, Tabain I, Benvin I, Kovac S, Hruskar Z, Mauric M, Milasincic L, Antolasic L, Skrinjaric A, Staresina V, & Barbie L (2021). Seroprevalence of SARS-CoV-2 infection among pet animals in Croatia and potential public health impact. Transboundary and Emerging Diseases, 68(4), 1767–1773. 10.1111/tbed.13924 [PubMed: 33191649]

Stirrup O, Hughes J, Parker M, Partridge DG, Shepherd JG, Blackstone J, Coll F, Keeley A, Lindsey BB, Marek A, Peters C, Singer JB, COVID-19 Genomics UK (COG-UK) consortium, Tamuri A, de Silva TI, Thomson EC, & Breuer J (2021). Rapid feedback on hospital onset SARS-CoV-2 infections combining epidemiological and sequencing data. eLife, 10, e65828. 10.7554/eLife.65828 [PubMed: 34184637]

USDA APHIS. (2023). Confirmed Cases of SARS-COV-2 in Animals in the United States, https://www.aphis.usda.gov/aphis/dashboards/tableau/sars-dashboard

Utah Public Health Laboratory, Utah Department of Health and Human Services. (2020). Nextflow workflow for our analysis of SARS-CoV-2 – Cecret. https://github.com/UPHL-BioNGS/Cecret

Wang L, Gyimesi ZS, Killian ML, Torchetti M, Olmstead C, Fredrickson R, & Terio KA (2022). Detection of SARS-CoV-2 clade B.1.2 in three snow leopards. Transboundary and Emerging Diseases, 69(5), e3346–e3351. 10.1111/tbed.14625 [PubMed: 35698174]

Yen HL, Sit THC, Brackman CJ, Chuk SSY, Gu H, Tam KWS, Law PYT, Leung GM, Peiris M, Poon LLM, & HKU-SPH study team. (2022). Transmission of SARS-CoV-2 delta variant (AY.127) from pet hamsters to humans, leading to onward human-to-human transmission: A case study. Lancet, 399(10329), 1070–1078. 10.1016/S0140-6736(22)00326-9 [PubMed: 35279259]

Yu G, Smith DK, Zhu H, Guan Y, & Lam TT-Y (2017). Ggtree: An r package for visualization and annotation of phylogenetic trees with their covariates and other associated data. Methods in Ecology and Evolution, 8(1), 28–36. 10.1111/2041-210X.12628

Impacts

- African lions and zoo staff members at Utah's Hogle Zoo were infected with the delta variant of SARS-CoV-2 in October of 2021.
- Whole-genome sequencing showed lions and zoo staff were infected with a highly related viral strain. Viral transmission likely occurred between lions and humans, although direction of transmission could not be determined.
- Lions can shed SARS-CoV-2 RNA for prolonged periods in both respiratory (14 weeks) and faecal (33 days) specimens.

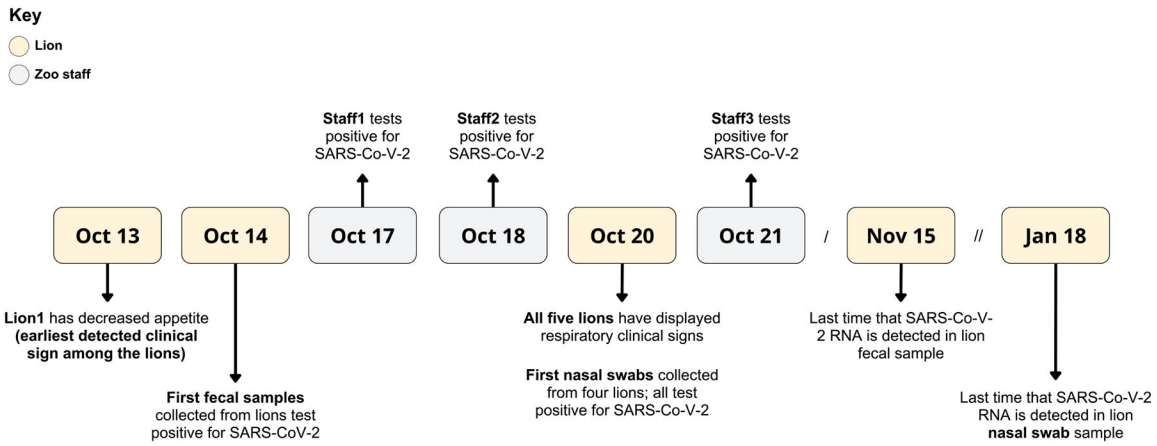

FIGURE 1.

Timeline for SARS-CoV-2 infection in African lions and humans at Utah's Hogle Zoo, October 2021–January 2022.

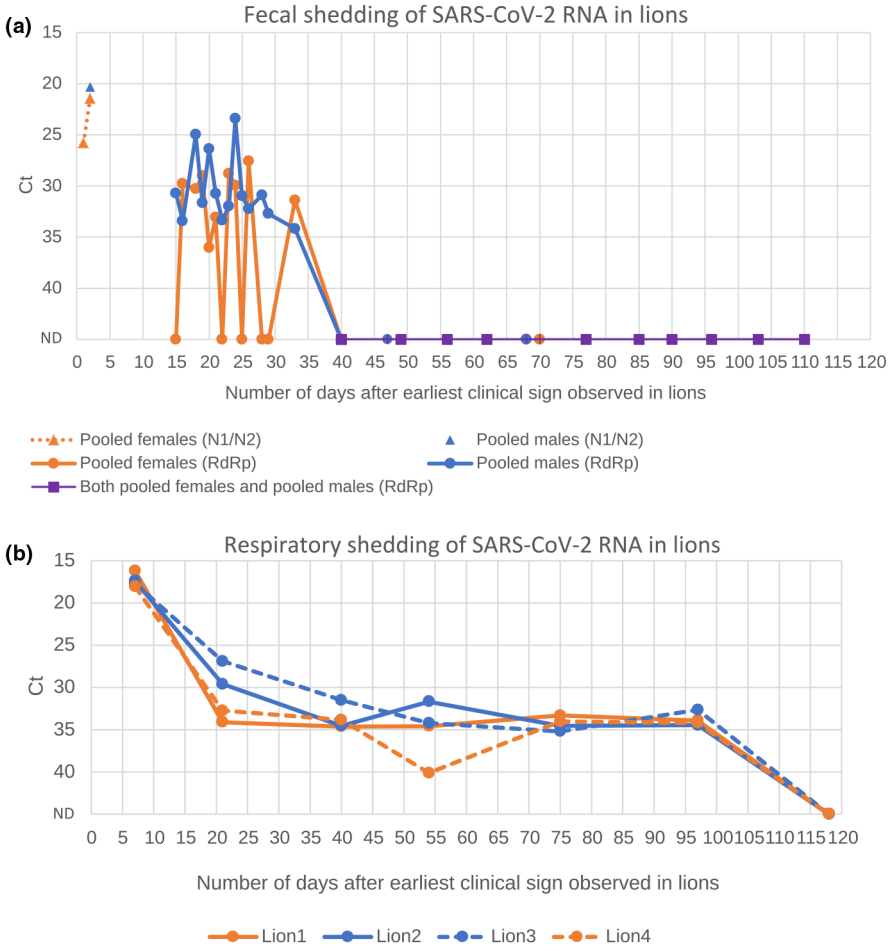

FIGURE 2.

SARS-CoV-2 cycle threshold (Ct) values from African lions from Utah's Hogle Zoo, October 2021–February 2022. Specimens with no detectable RNA (a negative or 'not detected' result) are indicated by 'ND'. (a) Lion faecal specimens, pooled by sex. Specimens collected 1 day and 3 days after the earliest clinical sign in the lions were analysed at the USDA National Veterinary Service Laboratories (NVSL) for N1 and N2 nucleocapsid gene targets. The average Ct for both N targets is shown. Faeces from Lion 1 and Lion 2 were also individually collected 5 days after the earliest clinical sign in the lions and analysed at NVSL, which yielded average Ct values of 23.6 and 23.3, respectively, for N1 and N2 targets (not shown in figure). Specimens collected ≥15 days after the earliest clinical sign in the lions were analysed at the Utah Veterinary Diagnostic Laboratory for the RNA-dependent RNA polymerase (RdRp) gene target. (b) Lion nasal swabs. All respiratory swabs were analysed at NVSL. N1 and N2 Ct values were averaged for each lion on each sampling date (including when multiple specimens were collected from the same lion on the same date).

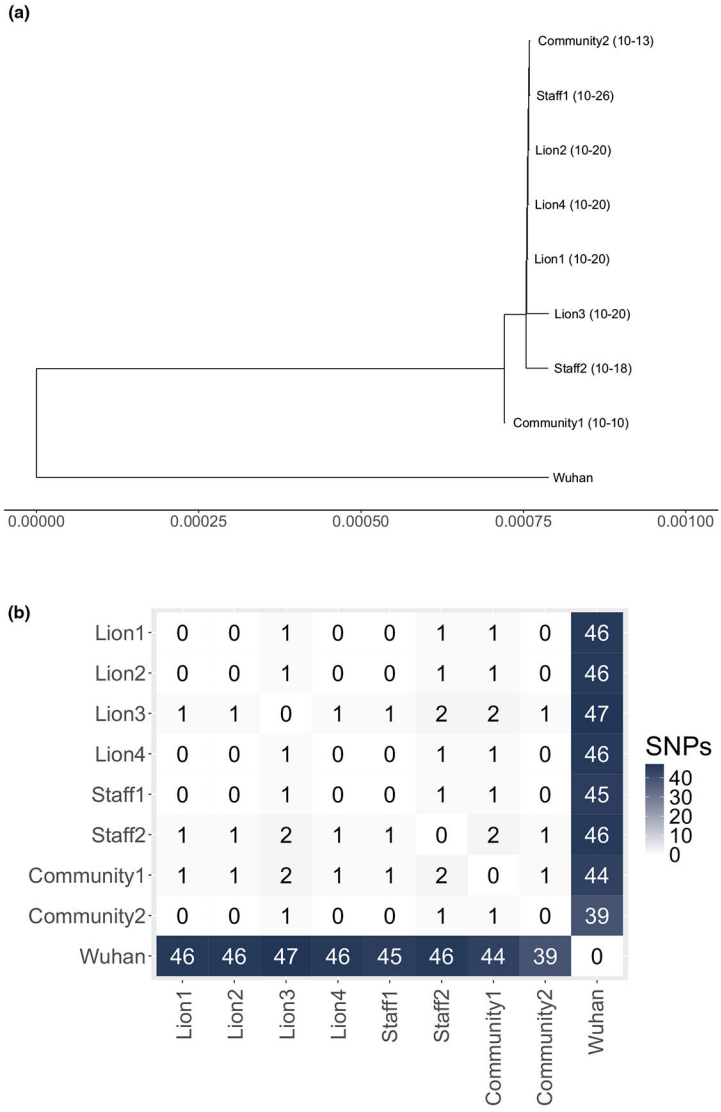

FIGURE 3.

SARS-CoV-2 genomic analysis results from respiratory specimens collected in October 2021 from African lions at Utah's Hogle Zoo, zoo staff and members of the surrounding community (Salt Lake County residents), as well as the Wuhan reference sequence (MN908947). For Staff 1, the sequence from the specimen collected on 26 October was used (the RT-PCR-positive specimen collected from Staff 1 on 17 October was not available for sequencing). (a) Phylogenetic tree with specimen collection dates in parentheses and scale of branch lengths below. (b) Associated pairwise single-nucleotide polymorphism (SNP) matrix derived from multiple sequence alignment.

TABLE 1

Clinical summary of SARS-CoV-2 infections in African lions at Utah's Hogle Zoo, October–November 2021.

Lion	Lion 1	Lion 2	Lion 3	Lion 4	Lion 5[a]
Sex	Female	Male	Male	Female	Female
Symptom start date	13 Oct	15 Oct	19 Oct	20 Oct	17 Oct
Symptom end date	25 Oct	4–5 Nov (heard sneeze from one of the males on 5 Nov; unsure which one)	3–5 Nov (heard sneeze from one of the males on 5 Nov; unsure which one)	22–24 Oct (heard sneeze from one of the females on 24 Oct; unsure which one)	24 Oct
Duration of illness (days)	12	20–21	15–17	2–4	7
Symptoms	Sneezing Cough Slow to eat (1 day only) Laboured breathing (once)	Sneezing Cough Congestion Wheezing (once)	Sneezing Cough	Cough Possible sneezing	Sneezing Cough Nasal discharge Wheezing (once)
Nasal swabs collected?	Yes	Yes	Yes	Yes	No

[a]Lion 5 is listed out of chronological order based on illness start date for ease of reporting, as this lion was not sampled.