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Source: Journal of Zoo and Wildlife Medicine, 54(3) : 607-616

Published By: American Association of Zoo Veterinarians

URL: https://doi.org/10.1638/2022-0141

DOI:10.1638/2022-0141 *Journal of Zoo and Wildlife Medicine* 54(3): 607–616, 2023

GLOBAL RETROSPECTIVE REVIEW OF SEVERE ACUTE RESPIRATORY SYNDROME SARS COV-2 INFECTIONS IN NONDOMESTIC FELIDS: MARCH 2020–FEBRUARY 2021

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Abstract: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections in nondomestic felids have been documented in North America, South America, Africa, Europe, and Asia. Between March 2020 and February 2021, at nine institutions across three continents, infection was confirmed in 16 tigers (*Panthera tigris*), 14 lions (*Panthera leo*), three snow leopards (*Panthera uncia*), one cougar (*Puma concolor*), and one Amur leopard cat (*Prionailurus bengalensis euptilurus*) ranging from 2 to 21 yr old (average, 10 yr). Infection was suspected in an additional 12 tigers, 4 lions, and 9 cougars. Clinical signs (in order of most to least common) included coughing, ocular and/or nasal discharge, wheezing, sneezing, decreased appetite, lethargy, diarrhea, and vomiting. Most felids recovered uneventfully, but one geriatric tiger with comorbidities developed severe dyspnea and neurologic signs necessitating euthanasia. Clinical signs lasted 1–19 d (average, 8 d); one tiger was asymptomatic. Infection was confirmed by various methods, including antigen tests and/or polymerase chain reaction (PCR) of nasal or oral swabs, tracheal wash, and feces, or virus isolation from feces or tracheal wash. Infection status and resolution were determined by testing nasal swabs from awake animals, fecal PCR, and observation of clinical signs. Shedding of fecal viral RNA was significantly longer than duration of clinical signs. Postinfection seropositivity was confirmed by four institutions including 11 felids (5 lions, 6 tigers). In most instances, asymptomatic or presymptomatic keepers were the presumed or confirmed source of infection, although in some instances the infection source remains uncertain. Almost all infections occurred despite using cloth facemasks and disposable gloves when in proximity to the felids and during food preparation. Although transmission may have occurred during momentary lapses in personal protective equipment compliance, it seems probable that cloth masks are insufficient at preventing transmission of SARS-CoV-2 from humans to nondomestic felids. Surgical or higher grade masks may be warranted when working with nondomestic felids.

INTRODUCTION

A novel coronavirus known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in 2019. The virus quickly spread globally, and the human coronavirus infectious disease 2019 (COVID-19) pandemic ensued. In March 2020, the first occurrence of SARS-CoV-2 infection in nondomestic felids was documented in tigers (*Panthera tigris*) and African lions (*Panthera leo*) housed at the Bronx Zoo in New York, USA.[1,7,13] Since that time, multiple institutions worldwide have reported SARS-CoV-2 infections in nondomestic felids, oftentimes despite the use of precautionary measures and personal protective equipment (PPE).[3,4,6,8–12,14] The following case series summarizes incidents between March 2020 and February 2021. A review of clinical presentation, diagnostic techniques, duration of shedding, and personal preventive measures of these cases is vital to understanding options for diagnosis, treatment, and prevention of the disease in nondomestic felids, as well as how best to protect the health and safety of staff members working with infected nondomestic felids.

CASE REPORTS

Between March 2020 and February 2021, confirmed infections of 35 nondomestic felids were

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Table 1. Chronological occurance, sex (M, male; F, female), and age range (yr) summary of nondomestic felids infected with SARS-CoV-2 between March 2020 and February 2021.[1,3,4,6,7,10,12]

		Tiger		Lion		Cougar		Snow leopard		Amur leopard cat	
Date	Location	M.F	Age Range	M.F	Age Range	M.F	Age Range	M.F	Age Range	M.F	Age Range
Mar 2020	New York, USA	2.3	4–15.5	3.0	6.5						
Jul 2020	Midrand, South Africa[a]					0.1	15				
Oct 2020	Tennessee, USA	2.1	7–11								
Nov 2020	Barcelona, Spain			1.3	4–16						
Nov 2020	Kentucky, USA							2.1	3–9		
Jan 2021	Minnesota, USA[b]	0.1	21								
Jan 2021	Borås, Sweden	1.1	14–17	1.4	3–16						
Feb 2021	Indiana, USA	1.1	9.5								
Feb 2021	Prague, Czech Republic	2.1	13.5–15	1.1	9–11					1.0	2
Average age (yr)			11.5		9.3		15		5.7		2
Total (M.F)		8.8		6.8		0.1		2.1		1.0	
Total animals		16		14		1		3		1	

[a] Note that an additional cougar developed clinical signs consistent with SARS-CoV-2 at this location, but confirmatory testing was not performed.
[b] Note that an additional 12 tigers, 4 lions, and 8 cougars developed clinical signs consistent with SARS-CoV-2 at this location, but confirmatory testing was not performed.

documented at nine institutions across three continents (Table 1). These cases are likely an underestimate of the total number of nondomestic felid infections in these and other locations. There was a near-equal distribution of males and females affected (17 males, 18 females). The age of affected felids ranged from 2 to 21 yr (average, 10 yr). None of the felids in this case series were vaccinated against SARS-CoV-2. Most institutions treated clinically ill felids with nonsteroidal anti-inflammatories and/or a broad spectrum antibiotic. Occasionally, maropitant was used for felids with decreased appetite. Two of 35 felids received a steroid injection due to severity of signs. One felid was immobilized in the second week of clinical symptoms for additional supportive care including subcutaneous fluid therapy.

Incident 1

At the Bronx Zoo in New York, USA, in late March 2020, two Malayan tigers (*Panthera tigris jacksoni*), two Amur tigers (*Panthera tigris altaica*), and three Transvaal African lions (*Panthera leo krugeri*) developed clinical signs including coughing and wheezing.[1,7] One tiger and one lion had periods of decreased appetite, and the lion had vomiting that lasted for 3 d (Table 2). Respiratory signs lasted an average of 4.5 d (range, 1–16 d). Signs were considered mild to moderate. One Malayan tiger with more prolonged clinical signs progressing to anorexia was anesthetized for examination, treatment, and sample collection for diagnostics. Polymerase chain reaction (PCR) testing was positive for SARS-CoV-2 on nasal swabs, oral swabs, tracheal wash fluid, and feces. Virus was isolated from tracheal wash fluid, and serum was positive for antibodies on virus neutralization (titer = 64). Virus neutralization opportunistically performed on the other Malayan tiger 7 mon after infection was positive (titer = 32). Virus neutralization performed on one lion 18 mon after infection was also positive (titer = 32). All felids, as

Table 2. Summary of clinical signs in nondomestic felids infected with SARS-CoV-2 between March 2020 and February 2021.[1,3,4,6,7,10,12]

Species	Cough	Discharge[a]	Sneezing	Wheeze	Decreased appetite	Lethargy	Diarrhea	Vomiting
Tiger ($n = 16$)	14	5	3	9	8	7		
Lion ($n = 14$)	12	7	10	3	3	3		1
Cougar ($n = 1$)	1	1			1		1	
Snow leopard ($n = 3$)	2			2				
Amur leopard cat ($n = 1$)		1	1					
Total	29	14	14	14	12	10	1	1

[a] Refers to ocular and/or nasal discharge.

well as a third asymptomatic Amur tiger housed in the same building as the other tigers, were confirmed positive by fecal PCR. Virus was isolated from fecal samples of one Amur tiger and one lion. Prolonged fecal shedding of viral RNA was confirmed. Average duration of shedding (from onset of clinical signs to last positive sample) was 23 d (range, 12–39 d; Table 3). The lions were housed in a separate building from the tigers. Viral genotyping was performed and confirmed that tigers and lions had different strains of the virus (Fig. 1). The zoo was closed to the public at the time, and no staff were allowed to report to work if ill with clinical signs consistent with COVID-19. Health department investigations confirmed that tiger keepers had the same strain of the virus as the tigers and likely infected the animals during a phase of asymptomatic or presymptomatic shedding. Although lion keepers tested serologically positive for SARS-CoV-2, none were PCR positive at the time of testing, so it could not be confirmed whether lions and the keepers had the same strain of the virus. No PPE was used by staff working around the animals before the animals developing clinical signs, because transmission of SARS-CoV-2 from humans to nondomestic felids had never previously been documented, and there were shortages of PPE for use by human medical professionals in New York City at that time. After infection was confirmed, personnel access to the tigers and lions was restricted to those necessary to care for them. In addition, staff used surgical facemasks, face shields, coveralls, and disposable gloves when working around the felids as well as during food preparation. Attempts were made to maintain a minimum of 6 ft (1.8 m) between animals and staff, and voluntary training was suspended. To the extent possible, dry cleaning of enclosures after spraying with a hydrogen peroxide product was performed. To avoid aerosolizing potentially infectious particles, pressure hoses were not used when cleaning enclosures. Note that the keepers that cared for the infected tigers also cared for nine snow leopards (*Panthera uncia*). However, the snow leopards were not housed in the same building; instead, they were housed in outdoor enclosures. One snow leopard with a chronic recurring cough tested negative by fecal PCR 3 mon after the confirmed infection in the tigers; the other snow leopards were not tested.

Incident 2

At the Lory Park Zoo and Owl Sanctuary in Midrand, South Africa, in July 2020, two cohoused cougars (*Puma concolor*) developed clinical signs including anorexia, diarrhea, and oculonasal discharge. One cougar had a persistent dry cough. Clinical signs lasted between 8 and 13 d. The cougar with the persistent cough was immobilized for examination and diagnostic testing. SARS-CoV-2 infection was confirmed by PCR testing of a nasal swab collected during the examination. Additional PCR tests were performed on nasal swabs collected from the positive cougar at 1, 3, 5, and 7 wk and fecal swabs at 3, 5, and 7 wk after the initial presentation. At week 5, the nasal swab was still positive, but the fecal swab only showed a weak positive response. The cougar tested negative on both nasal and fecal swabs 7 wk postinfection.[6] The zoo was closed to the public at the time of infection; the cougars were housed in an off-display area, and only staff members could approach within 6 ft of the animals. Staff members with possible signs of COVID-19 were prohibited from working, and all staff were screened daily with health questionnaires and temperature checks. However, presymptomatic staff members working with the cougars 10 d before the cougars developing clinical signs eventually became ill and tested positive for SARS-CoV-2. Prior to the cougars developing signs, PPE included cloth masks that were worn around the cougars and during food preparation. Quaternary ammonium and biguanide footbaths were used. After SARS-CoV-2 infections were confirmed, cougars were serviced by one keeper who wore a disposable surgical mask, disposable surgical gloves, rubber boots, and protective clothing that could be removed and disinfected. Footbaths were changed to a potassium peroxymonosulfate product. Because of a chronic medical condition in one of the cougars necessitating close contact with the keeper, it was elected to continue working within 6 ft of the cat. Disposable enrichment items were used. Note that two tigers that were housed in the same building as the cougars never developed clinical signs.

Incident 3

At the Knoxville Zoo in Tennessee, USA, in October 2020, three Malayan tigers housed individually but within the same building developed clinical signs including coughing, lethargy, and anorexia.[3] One tiger also developed mucoid ocular discharge. Clinical signs ranged from mild to severe and lasted for an average of 8 d (range, 5–11 d). Each tiger was sedated for examination and diagnostics. Two of the tigers tested positive for SARS-CoV-2 by PCR on oral and/or nasal swabs on the day of examinations; the other tiger was not

Table 3. Summary of duration of clinical signs versus duration of shedding of SARS-CoV-2 RNA in nondomestic felids.[1,3,4,6,7,10,12]

Animal	Location	Duration of clinical signs (d)	Assay	Frequency of testing	Duration of shedding (d)[a]
Tiger 1	New York, USA	16	Fecal PCR	Daily	22
Tiger 2	New York, USA	5	Fecal PCR	Daily	14
Tiger 3	New York, USA	1	Fecal PCR	Daily	26
Tiger 4	New York, USA	2	Fecal PCR	Daily	12
Tiger 5	New York, USA	NA[b]	Fecal PCR	Daily	NA
Lion 1	New York, USA	3	Fecal PCR	Daily	34
Lion 2	New York, USA	3	Fecal PCR	Daily	39
Lion 3	New York, USA	2	Fecal PCR	Daily	16
Cougar 1	Midrand, South Africa	13	Fecal PCR	Every 2 wk	49
			Nasal PCR	Every 2 wk	49
Tiger 6	Tennessee, USA	11	Fecal PCR	Every 2–4 d	26
			Nasal PCR	Every 2–4 d	4
Tiger 7	Tennessee, USA	5	Fecal PCR	Every 2–4 d	14
			Nasal PCR	Every 2–4 d	ND[c]
Tiger 8	Tennessee, USA	8	Fecal PCR	Every 2–4 d	ND
			Nasal PCR	Once	7
Lion 4	Barcelona, Spain	14	Fecal PCR[d]	Daily	18
			Nasal PCR	Every 1–3 d	17
			Nasal antigen	Every 1–3 d	13
Lion 5	Barcelona, Spain	18	Fecal PCR	Daily	19
			Nasal PCR	Every 1–3 d	18
			Nasal antigen	Every 1–3 d	14
Lion 6	Barcelona, Spain	19	Fecal PCR	Daily	18
			Nasal PCR	Every 1–3 d	26
			Nasal antigen	Every 1–3 d	16
Lion 7	Barcelona, Spain	16	Fecal PCR	Daily	17
			Nasal PCR	Every 1–3 d	16
			Nasal antigen	Every 1–3 d	8
Snow leopard 1	Kentucky, USA	2	Fecal PCR	Daily	33
Snow leopard 2	Kentucky, USA	16	Fecal PCR	Daily	30
Snow leopard 3	Kentucky, USA	4	Fecal PCR	Daily	32
Tiger 9	Minnesota, USA	8	Nasal PCR	Once	Unknown
Tiger 10	Borås, Sweden	3	Fecal PCR	Monthly	Unknown
Tiger 11	Borås, Sweden	3[e]	PCR, virus isolation	Once	Unknown
Lion 8	Borås, Sweden	7.5[f]	Fecal PCR	Monthly	Unknown
Lion 9	Borås, Sweden	7.5	Fecal PCR	Monthly	Unknown
Lion 10	Borås, Sweden	7.5	Fecal PCR	Monthly	Unknown
Lion 11	Borås, Sweden	7.5	Fecal PCR	Monthly	Unknown
Lion 12	Borås, Sweden	7.5	Fecal PCR	Monthly	Unknown
Tiger 12	Indiana, USA	5	Fecal PCR	Daily	22
Tiger 13	Indiana, USA	6	Fecal PCR	Daily	26
Tiger 14	Prague, Czech Republic	10	Fecal PCR	Every 2 wk	Unknown
Tiger 15	Prague, Czech Republic	11	Fecal PCR	Every 2 wk	Unknown
Tiger 16	Prague, Czech Republic	10	Fecal PCR	Every 2 wk	Unknown
Lion 13	Prague, Czech Republic	9	Fecal PCR	Every 2 wk	Unknown
Lion 14	Prague, Czech Republic	9	Fecal PCR	Every 2 wk	Unknown
Amur leopard cat	Prague, Czech Republic	2	Fecal PCR	Every 2 wk	Unknown

[a] Duration of shedding defined as the time from the start of clinical signs to the last positive test date.
[b] NA, nonapplicable, because this tiger remained asymptomatic.
[c] ND, not detected.
[d] Fecal PCR data for the lions in Barcelona is based on group fecal submissions.
[e] Tiger died on the third day of clinical signs and was tested postmortem.
[f] All lions at this facility had clinical signs for 5–10 d, averaging 7.5 d.

swabbed. On subsequent swabs collected voluntarily from the nose and/or mouth of two tigers over the next 40 d, none were positive. Fecal PCR was positive in two of three tigers that remained positive for 11 and 23 d, respectively. Serology by enzyme-linked immunosorbent assay (ELISA) and serum neutralization was positive on the two tigers that had blood repeatedly collected

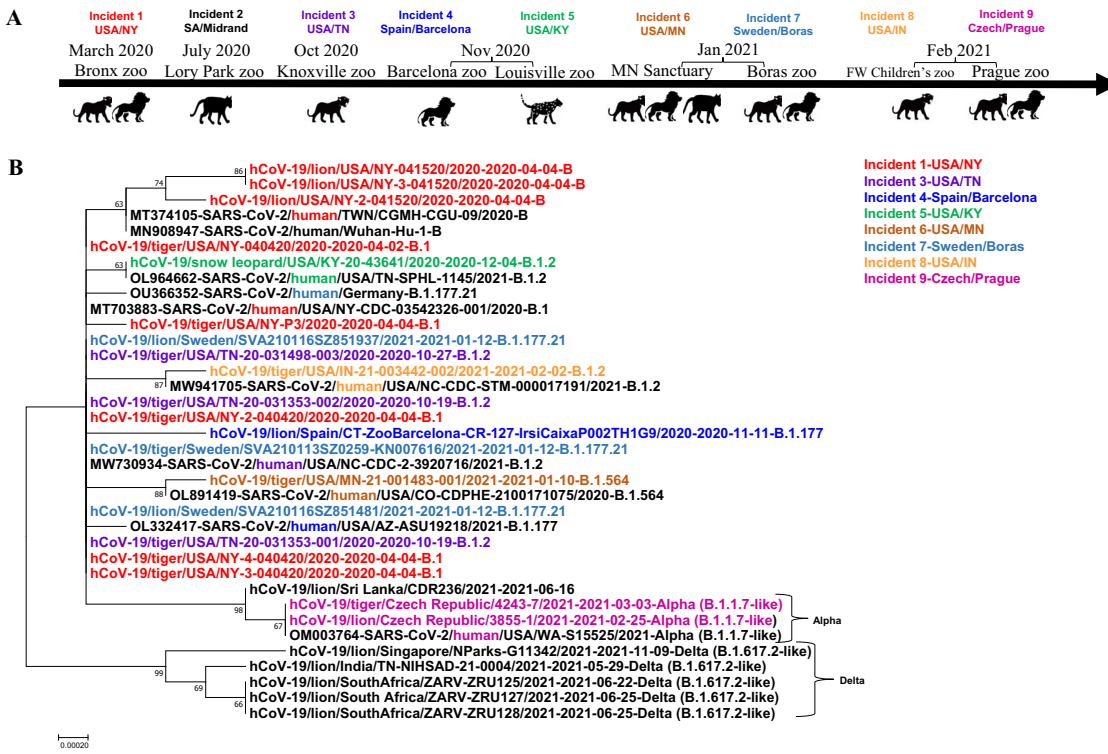

Figure 1. (A) Timeline and location of SARS-CoV-2 infections in nondomestic felids between March 2020 and February 2021. (B) SARS-CoV-2 isolates from nondomestic felids in the case series compared with human isolates and isolates from subsequent infections in nondomestic felids.[1,3,4,6–8,10,12,13]

voluntarily over the course of 2–3 mon. Serum neutralization titers peaked at 181 for one tiger around the 1-mon mark and at >4,096 for the other tiger around the 2-mon mark. Staff members had to complete a daily health questionnaire, and those with possible signs of COVID-19 were prohibited from working. However, no staff members working with the felids were ever confirmed to be infected with SARS-CoV-2 prior to the tigers developing clinical signs. Despite intensive investigations by the Department of Public Health, a source of infection for the Malayan tigers was not identified. The zoo was open to the public at the time of infection, and patrons could traverse a walkway approximately 6 ft over the exhibit. This may have served as the source of infection, but it cannot be confirmed. Keepers working with the tigers developed clinical signs after the tigers did, which may indicate possible transmission from the tigers to the staff, although the evidence was not conclusive.[5] Prior to the felids developing clinical signs, staff wore gloves and cloth facemasks when around the cats and for food preparation. After SARS-CoV-2 was confirmed in the tigers, PPE was switched to Tyvek® (Dupont, Wilmington, DE 19805, USA) suits, N95 masks (minimum filtration percentage, 95%), boot covers, and face shields. Only limited personnel were allowed near the Malayan tigers. Hand feeding and nonmedically necessary training were suspended.

Incident 4

At the Barcelona Zoo in Spain in November 2020, four Southwest African lions (*Panthera leo bleyenbergui*) housed together developed clinical signs including coughing, sneezing, and nasal discharge.[4] Two of these felids also showed apathy and decreased appetite. Generally, signs were considered mild, although one lion had a severe cough. Clinical signs lasted for an average of 17 d (range, 14–19 d). Nasal swabs were collected from each awake lion every few days by opportunistically swabbing while the cat targeted on an object. Then lions remained positive by the antigen test (COVID-19 Ag Test reference 243103N-20, 47445 Moers, Germany) for an average of 13 d (range, 8–16 d). PCR testing of nasal swabs remained positive for an average of 19 d

(range, 16–26 d). Fecal PCR testing of combined samples from the group remained positive for approximately 18 d. Virus was isolated from the nasal swabs of two lions, but from none of the fecal samples. Within 2–5 wk of the initial diagnosis, each lion was anesthetized for blood collection for serology, including virus neutralization and ELISA. Seropositivity was confirmed by both tests on both sampling dates. Serum neutralization titers ranged from 32 to 128 on the initial test. Sampling was repeated on three lions 4 mon after clinical presentation; titers were sustained in two of the felids, but decreased in the third felid. The lion with the decreased titer was confirmed seronegative 7 mon after clinical presentation. The other two lions sustained high titers (32–64) 10 mon after infection. The zoo was closed to the public when the lions developed clinical signs. Staff members with possible signs of COVID-19 were prohibited from working. Two lion keepers who worked with the lions 2–4 d before they became ill tested positive for SARS-CoV-2. They were asymptomatic when working with the lions. Before confirming infections in the lions, PPE included cloth masks while working inside the building as well as when preparing food and enrichment items. After confirming SARS-CoV-2 infections, PPE requirements were increased to include a staff changing area with footbaths containing aldehyde-based disinfectant, FFP3 masks (minimum filtration percentage, 99%), face shields, Tyvek coveralls, and rubber boots. No changes were implemented in training routines; however, enrichment items donated by the public were not introduced to the lions for 24 h after donation.

Incident 5

At the Louisville Zoo in Kentucky, USA, in November 2020, three snow leopards housed individually but within the same building developed mild clinical signs including coughing, wheezing, and sneezing.[12] Clinical signs lasted an average of 7 d (range, 2–16 d). Infection with SARS-CoV-2 was confirmed by fecal PCR. Daily fecal sampling was performed until no viral RNA was detected for at least a week. The snow leopards shed viral RNA intermittently for an average of 31.5 d (range, 30–33 d). A snow leopard keeper tested positive for SARS-CoV-2 approximately 1 wk before the onset of clinical signs in the snow leopards. The strain of virus from the keeper was not sequenced, but it was assumed the virus was transmitted from the staff to the snow leopards. The zoo was open at the time of the snow leopards' infections, but no members of the public were allowed within 6 ft of the animals at any time. No staff members with clinical signs of COVID-19 were allowed to report to work and were expected to monitor themselves for fever before reporting. Before confirming infections in the snow leopards, PPE included facemasks (cloth or surgical) while working near the leopards and when preparing food. Disposable gloves were to be worn during food preparation, but staff compliance was not consistent. After SARS-CoV-2 was documented in felids at the Bronx Zoo, the Louisville Zoo implemented social distancing with susceptible species including the snow leopards, maintaining at least a 6-ft distance from the animals when possible, as well as minimizing any training and feeding by stick. After strongly suspecting SARS-CoV-2 infections in the snow leopards, staff PPE requirements were increased to include face shields, dedicated Tyvek coveralls, plastic shoe coverings, and footbaths containing phenolic-based disinfectant. Stalls were disinfected with a quaternary ammonia product. Note that two tigers were housed in the same building as the snow leopards, and the same keeper staff cared for a jaguar (*Panthera onca*), a cougar, and two Canada lynx (*Lynx canadensis*); only the snow leopards developed clinical signs.

Incident 6

At The Wildcat Sanctuary in Minnesota, USA, in January 2021, 12 Bengal tigers (*Panthera tigris tigris*), one Sumatran × Bengal cross tiger, three African lions, a white lion, and eight cougars developed clinical signs including coughing, wheezing, and deceased appetite. Of the 127 felids housed at the sanctuary, 47 felids spread throughout five buildings were potentially exposed by being housed within the same building, sharing a fenceline with sick felids, and/or by use of shared tools. Twenty-five felids developed clinical signs that lasted an average of 6 d (range, 1–16 d). One tiger was anesthetized and confirmed positive for SARS-CoV-2 by PCR on a nasopharyngeal swab. Infection with SARS-CoV-2 was presumed, but not confirmed, for the other clinically affected felids. Staff members were confirmed to have COVID-19 infections before the felids becoming ill; however, they likely transmitted the virus during an asymptomatic or presymptomatic period. Sanctuary policy prevented staff members with clinical signs of COVID-19 from working. The sanctuary was closed to the public during the time when the felids developed clinical signs. Before

confirming infection in the tiger, PPE requirements included wearing cloth masks around the felids and using gloves during food preparation. After the felids developed clinical signs of SARS-CoV-2, staff wore N95 masks. When working with cats with clinical signs, staff also wore goggles and Tyvek suits or other designated clothing. Staff stopped sharing utensils between cats or, when necessary, disinfected utensils between cats. Bleach footbaths were instituted. Enclosures were spot cleaned instead of cleaned with high-pressure hosing. Voluntary examinations of felids were suspended. Training was limited in an attempt to maintain a minimum of a 6-ft distance from the felids when possible, although feeding by tongs was maintained.

Incident 7

At the Borås Zoo in Sweden in January 2021, two Amur tigers and five African lions housed within the same building developed clinical signs including coughing, sneezing, and dyspnea (severe in the tigers, mild-to-moderate signs in the lions). The male tiger and one lion had nasal discharge. Wheezing was noted in both tigers. Clinical signs lasted 5–10 d in the lions and 3 d in the male tiger. The 17-yr-old female tiger developed a decreased appetite, and 2 d later was found moribund with severe respiratory symptoms and neurologic deficits. Because of a poor prognosis and geriatric status, euthanasia was elected. Necropsy revealed distal tracheobronchitis with intraluminal mucohemorrhagic-purulent exudate, necrotizing bronchiolitis, and mucosal adenitis as well as focal acute pneumonia. Immunohistochemistry confirmed the presence of SARS-CoV-2 antigen associated with these lesions, and the tiger tested positive for SARS-CoV-2 on PCR of oropharyngeal swab and feces. Virus isolation was positive. Neoplasia of the liver and cervix were identified along with pyometra and hemometra as significant comorbidities. Repeated fecal PCR testing for SARS-CoV-2 was performed on the male tiger and pooled fecal samples from the lion group. All tested positive on the first test, but they were negative when retested twice approximately 1 mon after clinical signs were first noted. A keeper tested positive for COVID-19 infection before the felids becoming ill; however, the keeper likely transmitted the virus during an asymptomatic or presymptomatic period. Two additional staff members became ill soon after clinical signs were seen in the felids. The same strain of virus was confirmed in the female tiger and three staff members. Zoo policy prevented staff members with clinical signs of COVID-19 from working. The zoo was closed to the public when the felids developed clinical signs. Before confirming SARS-CoV-2 in the felids, safety precautions and PPE consisted of good hand hygiene with disinfection and common (although not consistent) use of disposable gloves. After infection was confirmed, PPE requirements included consistent use of gloves, three-layer medically approved reusable facemasks, a potassium peroxymonosulfate–based footbath, and coveralls. A reduced number of staff prepared the felids' food and cleaned the enclosures. Efforts were made to maintain a 6-ft distance from the felids, and training sessions were suspended. Enrichment items were no longer shared between felids. Upon receiving two negative fecal PCR tests, the use of footbaths and coveralls was discontinued.

Incident 8

At the Fort Wayne Children's Zoo in Indiana, USA, in February 2021, two cohoused Sumatran tigers (*Panthera tigris sumatrae*) developed mild clinical signs including coughing, wheezing, deceased appetite, and occasional sneezing. One tiger had a chronic intermittent unilateral nasal discharge that worsened as the other clinical signs ensued. Clinical signs lasted 5.5 d. Infection with SARS-CoV-2 was confirmed by fecal PCR testing. Daily fecal samples were collected from both tigers until both were confirmed negative for seven consecutive tests. The tigers remained positive for 22 and 26 d, respectively, after the onset of clinical signs. One tiger shed intermittently, sometimes going 5 d without shedding viral RNA. The other tiger shed consistently every day until shedding abruptly stopped. Repeated voluntary blood collection was performed for serology with a mix-and-read (fluorescence-based) assay. Seroconversion was confirmed in both tigers. The source of the infection was never confirmed; all tiger keepers were tested soon after the tigers developed signs and were confirmed to be negative. No keepers reported any symptoms. One keeper that worked in the same building as the tigers tested positive for SARS-CoV-2, but did not have close contact with the tigers or the tiger keepers. Zoo policy prevented staff members with clinical signs of COVID-19 from working. Daily health questionnaires had to be completed before arriving at work. The zoo was closed to the public during the time when the felids developed clinical signs. Before confirming SARS-CoV-2 infection in the felids, PPE consisted of cloth facemasks when

around the felids, although compliance was inconsistent. Disposable gloves were used during food preparation. Efforts were made to maintain a 6-ft distance from the felids, but due to the dimensions of the indoor holding space, this was difficult to maintain. Training sessions were limited to 10 min in duration. After infection was suspected PPE included N95 masks, face shield or goggles, scrubs or coveralls over clothing, dedicated footwear or boot covers, two pairs of disposable gloves, quaternary ammonium–based footbaths, and double bagging of fecal waste for disposal. Stalls were generally dry cleaned or, when hosing was needed, the stalls were disinfected with a quaternary ammonium product 10 min before gentle hosing. The outer layer of gloves and mask were disposed after hosing. Voluntary training sessions were minimized, except for blood collection to measure antibody response. Sessions were limited to just one keeper and veterinarian.

Incident 9

At the Prague Zoo in the Czech Republic in February–March 2021, two Asiatic lions (*Panthera leo leo*), two Malayan tigers, one Sumatran tiger, and one Amur leopard cat (*Prionailurus bengalensis euptilurus*) housed in the same building developed clinical signs of illness.[10] Of the six felids, all had nasal discharge, four had coughing, four had lethargy, three had sneezing, and one had a decreased appetite. Duration of clinical signs lasted 2–11 d. Infection with SARS-CoV-2 was confirmed by fecal PCR. Testing was repeated 14 d later, at which time all felids were negative. One to 2 d before the onset of clinical signs in the felids, two keepers were diagnosed with COVID-19. The felids and one keeper were confirmed to have infection with the same variant (Alpha). The zoo was closed to the public at the time of infection. Before confirming SARS-CoV-2 in the felids, PPE consisted of KN95, FFP2/FFP3 masks, gloves, hand disinfectants, and disinfection mats. Despite this precaution, it is suspected that the infection transmitted from the staff to the felids.

Viral sequencing

Viral sequencing was attempted, but not successful, for all cases. Generally, the sequence was consistent with the strain that was circulating in the human population at the time (Fig. 1). Phylogenetic tree analysis of the SARS-CoV-2 spike gene showed that isolates from Incidents 1 and 3–9 were closely related to contemporary human isolates and belonged to seven different lineages: B1, B.1, B.1.177, B.1.564, B.1.1.7, B.1.177.21, and B.1.2 (Fig. 1B). These large cat strains shared from 99.8 to 100% identities with corresponding human SARS-CoV-2 strains in the spike gene. All these outbreaks were caused by a single introduction except Incident 1 at the Bronx Zoo, where two different lineages were involved with human-to-felid transmissions. The SARS-CoV-2 zoo animal infections that occurred in the early period of the pandemic including Incidents 1–8 were nonvariant strains, whereas Incident 9 was caused by the Alpha variant (Fig. 1B). None of the felids were positive for the Delta variant.

DISCUSSION

All but one of the nondomestic felids in this retrospective study made a full recovery. The one tiger that was euthanized had clinical signs that were at least in part associated with SARS-CoV-2 infection, but it also had significant comorbidities and was of advanced age (17 yr). None of the felids in this study were diagnosed with the Delta variant, which emerged in late 2020.[15] Although the mortality level in this case series was low, there have been many additional cases of SARS-CoV-2 infection in nondomestic felids since February 2021, and at least eight felids (three lions and five snow leopards) have died after infection with the Delta variant.[8] There is concern that the Delta variant may cause more severe disease in nondomestic felids (Terio, pers. comm.).

The average age of affected felids was 10 yr, and 10 of 35 felids were 15 yr or older. There did not appear to be a correlation with age of the cat and severity of clinical signs or prognosis. Coughing was the most common clinical sign, with nearly 75% of the felids affected. Sneezing, wheezing, ocular and/or nasal discharge were observed in approximately 40% of the cases. Less commonly seen clinical signs included lethargy and decreased appetite, with diarrhea and vomiting rarely occurring. One felid remained asymptomatic.

The duration of viral RNA shedding as detected by PCR testing of feces and nasal swabs frequently outlasted the duration of clinical signs. For the 20 felids that had serial fecal PCR performed, the average duration of shedding (23 d) outlasted the average duration of clinical signs (8 d). The four lions in Barcelona were tested by three different methods: fecal PCR, nasal swab PCR, and nasal swab antigen test. The duration of viral RNA shedding as detected by fecal PCR (18 d) and nasal swab PCR (19 d) slightly outlasted

the duration of the clinical signs (17 d). Note that the duration of shedding as detected by the nasal swab antigen test was actually shorter (13 d) than the duration of clinical signs. For the cougar that was tested by serial nasal swabs for PCR, the duration of shedding (49 d) outlasted the duration of clinical signs (13 d). It is not known whether the viral RNA detected by these tests was infectious, because PCR detects both infectious and noninfectious RNA. In studies on humans with SARS-CoV-2, there is an association with low cycle threshold values and positive viral culture, which could be consistent with increased infectivity.[2]

The source of infection in most cases was confirmed or strongly suspected to be from keepers working closely with the felids. All institutions in the case series had policies in place at the time that ill staff members not report to work; thus, infections occurred when staff were asymptomatically or presymptomatically shedding virus. Although the same viral sequence was not confirmed in the felids and the keepers in every instance, in most cases one or more keepers developed clinical signs a few days before the onset of signs in the felids. For two institutions, the likely source of the infections in the felids was not determined despite extensive investigations and testing.

At several institutions, SARS-CoV-2 infections were confirmed in only a few species of nondomestic felid, despite additional species being cared for by the same keeper staff and/or being housed in the same building and air space as the infected felids. Different felid species or even individuals within a species may have varying susceptibility to particular variants of SARS-CoV-2. Nondomestic felids may develop asymptomatic infection more commonly than is currently appreciated, which would not be detected unless screening is performed. In addition, it is possible that, for those animals managed mostly or entirely outdoors, the abundant ventilation may have prevented transmission.

At the time of these infections, most institutions required staff working with the felids to wear cloth masks when around the cats and for food preparation. Gloves were also frequently used when handling food. Despite this, SARS-CoV-2 was transmitted to the felids. Although transmission may have occurred during momentary lapses in PPE compliance, it seems probable that cloth masks are insufficient at preventing transmission of SARS-CoV-2 from humans to nondomestic felids; surgical or higher grade masks may be warranted.

Acknowledgments: The authors thank the dedicated animal care and veterinary staff for assistance with these cases. The authors sincerely appreciate the researchers worldwide who sequenced and shared the complete genome data of SARS-CoV-2 from Global Initiative on Sharing All Influenza Data (GISAID; https://www.gisaid.org/). Accession IDs of GISAID and GenBank are listed on the Figure 1 file.

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Accepted for publication 10 May 2023