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DETECTION OF SARS-COV-2 IN A SQUIRREL MONKEY (*SAIMIRI SCIUREUS*): A ONE HEALTH INVESTIGATION AND RESPONSE

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Abstract: Through collaborative efforts, One Health partners have responded to outbreaks of COVID-19 among animals, including those in human care at zoos. Zoos have been faced with numerous challenges, including the susceptibility of many mammalian species, and therefore the need to heighten biosecurity measures rapidly. Robust One Health collaborations already exist in Arizona to address endemic and emerging zoonoses, but these have rarely included zoos. The pandemic shed light on this, and Arizona subsequently expanded its SARS-CoV-2 surveillance efforts to include zoo animals. Testing and epidemiologic support was provided to expedite the detection of and response to zoonotic SARS-CoV-2 infection in zoo animals, as well as to understand possible transmission events. Resulting from this program, SARS-CoV-2 was detected from a rectal swab collected from an 8-yr-old squirrel monkey (*Saimiri sciureus*) from a zoo in Southern Arizona. The animal had rapidly become ill with nonrespiratory symptoms and died in July 2022. Genomic sequencing from the swab revealed mutations consistent with the Omicron (BA.2) lineage. An epidemiologic investigation identified an animal caretaker in close proximity to the affected squirrel monkey who tested positive for COVID-19 the same day the squirrel monkey died. Critical One Health partners provided support to the zoo through engagement of local, state, and federal agencies. Necropsy and pathologic evaluation showed significant necrotizing colitis; the overall clinical and histopathological findings did not implicate SARS-CoV-2 infection alone as a causal or contributing factor in the squirrel monkey's illness and death. This report documents the first identification of SARS-CoV-2 in a squirrel monkey and highlights a successful and timely One Health investigation conducted through multisectoral collaboration.

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INTRODUCTION

The necessity of a One Health approach to surveillance, investigation, response, and prevention of emerging zoonotic diseases is well recognized.[3,7,19,26] Many United States (US) agencies define One Health as "a collaborative, multisectoral, and transdisciplinary approach with the goal of achieving optimal health outcomes recognizing the interconnection between people, animals, plants, and their shared environment."[7] On a global scale, the COVID-19 pandemic has greatly impacted animal populations and subsequent management,[8,11,24] thereby providing an opportunity to operationalize One Health collaborations.[17] Early coordination and communication between local, state, and federal animal and public health partners are critical to address zoonotic SARS-CoV-2 events, where transmission occurs between people and animals, including animals in human care in zoos, sanctuaries, and aquariums.[2,10,11,15,17,24,28,31,32]

In the US, zoo animals with respiratory symptoms or suspected SARS-CoV-2 infection are tested following the onset of clinical signs or known exposure to a person with COVID-19.[2,8,10,15,29,31,34] The

majority of SARS-CoV-2–positive zoo animals experienced mild, self-limiting illness and have recovered, although a small proportion have died. More information is needed to understand the role of SARS-CoV-2 infection in fatal outcomes.[2,10,15,22,28,31,33,34] As of 09 January 2024, 42% ($n = 171$) of all individual confirmed positive animal cases have occurred in zoo animals, primarily tigers, lions, snow leopards, otters, and gorillas.[33] In addition to heightening measures to mitigate viral spread, many US zoos have initiated One Health investigations with local, state, and federal governmental partners, as well as with academic and nonprofit entities. These partnerships have been essential for investigation and response to SARS-CoV-2 outbreaks in zoos.[28]

Beginning in 2021, the Arizona Department of Health Services (ADHS) and the Translational Genomics Research Institute (TGen) launched several One Health studies to understand better the impacts of SARS-CoV-2 on animals in the state, including companion animals, zoo animals, and free-ranging wildlife.[37] In partnership with local wildlife agencies, zoos, wildlife rehabilitation centers, and state and federal governments, the program provides testing and epidemiologic support to expedite detection of and response to zoonotic SARS-CoV-2 infection in animals.

Reid Park Zoo COVID policies and partnership with ADHS/TGen

At the beginning of the pandemic (April 2020), the Reid Park Zoo implemented enhanced COVID-19 policies for all animal care and other essential staff. This included recommending vaccination, requiring staff working with susceptible animal species to wear KN95 masks, requiring all staff to wear gloves and masks during food preparation and feeding, and testing all staff experiencing any signs of illness before coming to work. Staff testing positive for SARS-CoV-2 were required to follow CDC guidelines, including staying home until testing negative. Public admission during the height of the pandemic (2020–2021) was limited; animal encounters were paused, and the public was kept at a greater distance from a number of animal enclosures. Although the zoo has lifted many of these policies since the public health emergency declaration ended, enhanced biosecurity practices and PPE use are still in place. In the spring of 2022, the zoo also began vaccinating known SARS-CoV-2 susceptible species with the experimental COVID-19 vaccine manufactured by Zoetis (Zoetis LLC, Kalamazoo, MI 49007, USA) made available to zoos for use in animals.[29,38]

On 06 April 2022, the zoo was enrolled into the One Health and SARS-CoV-2 surveillance program.[37] ADHS and TGen conducted a site visit at the zoo, delivered sample collection supplies, and reviewed zoo biosecurity and COVID-19 policies. Swab (nasal and rectal) and blood samples were collected by zoo veterinary staff during routine examinations or animal training. Viral and serologic testing was performed on submitted samples. TGen and ADHS maintained regular communications with the zoo staff and were available to assist with any epidemiologic investigations as needed. The following narrative details the identification and One Health investigation of SARS-CoV-2 in a deceased squirrel monkey (*Saimiri sciureus*) at the zoo.

CASE REPORT

Case presentation

An 8-yr-old female squirrel monkey (*Saimiri sciureus*) was observed on 19 June 2022 to be obtunded, experiencing severe weakness, diarrhea, tenesmus, and head tremors, without signs of respiratory illness. Video monitoring from the prior evening showed that she appeared lethargic and reclusive. This monkey was clinically healthy prior to the sudden onset of illness, though she previously suffered from chronic ulcerative gastrointestinal disease. Results of initial examination, bloodwork, and radiographs were consistent with severe sepsis, including severe hypoglycemia, neutropenia, and hypothermia. Signs worsened despite medical intervention, and after several hours in the veterinary intensive care unit, the squirrel monkey died.

Initial histopathological and viral findings

Necropsy and initial histopathology were conducted 2 days after death on 21 Juneat Midwestern University's Veterinary Diagnostic Pathology Center. Significant gross and microscopic findings were limited to the colon, in which there was widespread mucosal ulceration and necrosis with hemorrhage, fibrin, and karyorrhectic debris. The submucosa was thickened by edema and mixed inflammation with prominent neutrophila, which occasionally extended transmurally. Multifocal vascular necrosis with fibrin thrombi and necrosis of mucosal associated lymphoid tissue was also seen. Gram stain identified mixed bacteria, including gram-positive bacilli adhered to the necrotic mucosa. Fecal culture identified numerous *Clostridium perfringens* and *Clostridium hiranonsi*. Identification of *C. perfringens* toxin genes further classified this isolate as type A, with alpha

and beta-2 toxin genes detected. No organisms were isolated from aerobic and anaerobic lung tissue cultures.

As participants of Arizona's One Health and SARS-CoV-2 surveillance program, the zoo's veterinary team notified ADHS and TGen of the squirrel monkey's death on 23 June. A rectal swab collected antemortem tested positive for SARS-CoV-2 by rRT-PCR (Ct values of 32 for both N2 and S4 targets) on 27 June. Confirmatory testing at the National Veterinary Services Laboratories (NVSL) on 29 June was also positive on N1 and N2 targets, with Ct values of 32.8 and 39.0, respectively. The confirmed case was reported to the World Organisation for Animal Health on 08 July 2022.[36] Genomic sequencing[14] (EPI_ISL_17322932) identified mutations consistent with the Omicron (BA.2) variant. Unfortunately, a blood sample was not collected for viral neutralizing antibody testing bcause of the prognosis and deteriorating condition of the animal. Details of the SARS-CoV-2 viral, serologic, and genomic testing can be found in the supplemental material.[4,14,21]

Additional testing and histopathologic analysis

Based on initial investigation findings, formalin-fixed paraffin-embedded (FFPE) tissues were submitted to the Center for Disease Control and Prevention's Infectious Disease Pathology Branch (CDC IDPB) for histopathologic review and further testing.[4] Immunohistochemistry (IHC)[16] for *Clostridium* spp. showed positive immunolabeling of surface bacilli in the necrohemorrhagic colonic tissue (Fig. 1). The liver was evaluated for clostridial bacteremia via IHC and deemed negative. However, absence of bacteremia does not exclude the possibility of toxemia. Lung tissues showed mild congestion and hemorrhage, with no evidence of pneumonia. No other tissues (trachea, kidney, urinary bladder, liver, gallbladder, stomach, adrenals, pancreas, and reproductive tract) yielded remarkable histopathologic findings. Fresh lung tissue tested negative for SARS-CoV-2 by conventional RT-PCR at Midwestern University Veterinary Diagnostic Pathology Center. Conventional RT-PCR was performed on RNA extracts from the FFPE trachea, lung, and colon, and was also negative for SARS-CoV-2.

EPIDEMIOLOGIC INVESTIGATION

Reid Park Zoo staff consulted with TGen and ADHS regarding an epidemiologic investigation to inform public health measures. The investigation included contacting all staff in close proximity to the squirrel monkey and reviewing all recent reports of staff illness. Engagement of critical One Health partners occurred 28–30 June. Figure 2 depicts the timeline of key events and actions carried out by the One Health partner agencies involved in this investigation.

One of the animal caretakers reported development of mild respiratory symptoms, including sneezing, coughing, and sinus drainage, beginning around 04 June. Multiple at-home COVID-19 antigen tests were negative in subsequent days. Upon returning to work on 08 June (11 days before the squirrel monkey's death), the animal caretaker continued to follow infection-prevention measures outlined in zoo policy, including wearing a KN95 mask and gloves and using a foot bath prior to entering the squirrel monkey enclosure. The animal caretaker visited urgent care on 16 June for continued intermittent mild symptoms and again tested negative by a COVID-19 antigen test. The caretaker returned to work on 17 June (2 days prior to the squirrel monkey's death). On the morning of 19 June, the same day of the animal's death, the animal caretaker yielded a positive result for COVID-19 concurrent with another positive, symptomatic adult in the household. Samples from the caretaker and family member were discarded and not available for genomic sequencing.

Two asymptomatic adult squirrel monkeys housed in the same enclosure tested negative for SARS-CoV-2 by rRT-PCR on serial fecal samples. The three squirrel monkeys were not included in the first round of Zoetisvaccinations[29,38] because of routine health monitoring concerns (e.g., preexisting health conditions in one female, and a suspected pregnancy in the other female). Serum samples collected 3 mon later (October 2022) from the other two squirrel monkeys tested negative for SARS-CoV-2 neutralizing antibodies; these monkeys have since been immunized. There were no other animals housed in the squirrel monkey enclosure. Other nearby exhibits were greater than 6 ft away and tended to by different caretakers.

Viral and serologic testing as part of the One Health and SARS-CoV-2 surveillance program was conducted on samples from 55 other animals at the Reid Park Zoo, including African lions, ring-tailed lemurs, spotted necked otters, macaques, and goats. All samples were negative for SARS-CoV-2.

DISCUSSION

Over the past decade, numerous successful multi-institutional One Health collaborations have been formed in Arizona to understand the circulation of endemic diseases better, to respond to zoonotic

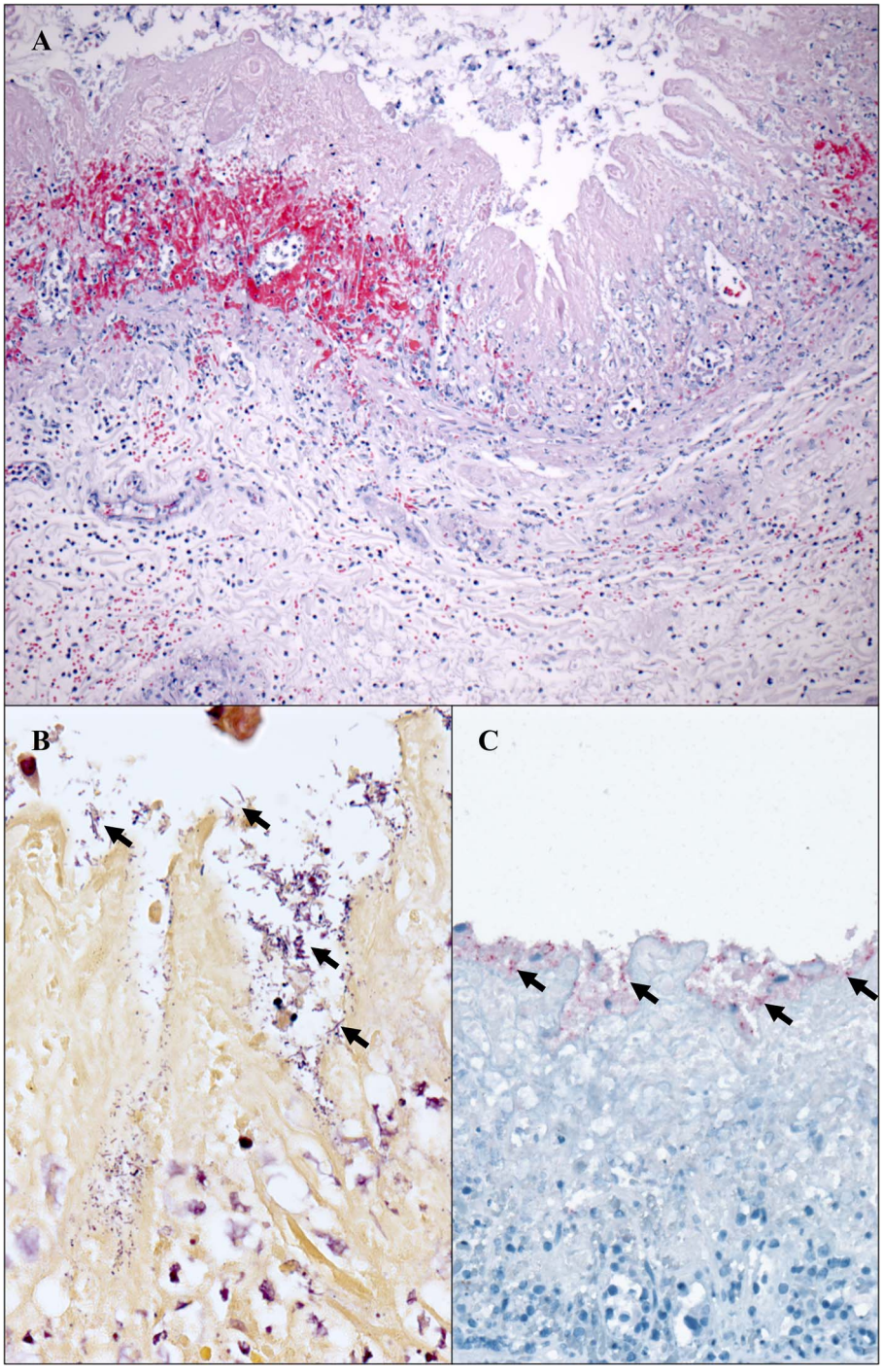

Figure 1. Histopathologic findings in formalin-fixed paraffin-embedded (FFPE) colon tissue from squirrel monkey submitted to the Centers for Disease Control's (CDC) Infectious Disease Pathology Branch (IDPB). **(A)** Top: Colon with mucosal necrosis and hemorrhage, submucosal edema, and mixed acute inflammation. Hematoxylin–eosin stain, 100×. **(B)** Bottom left: Gram stain showing mixed surface bacteria with prominent gram-positive rods (arrows). Gram stain, 400×. **(C)** Bottom right: Immunohistochemistry (IHC) for *Clostridium* spp. showing red immunolabeling (arrows) along the necrotic colonic mucosal surface. *Clostridium* spp. IHC, 400×. Images courtesy of CDC IDBP.

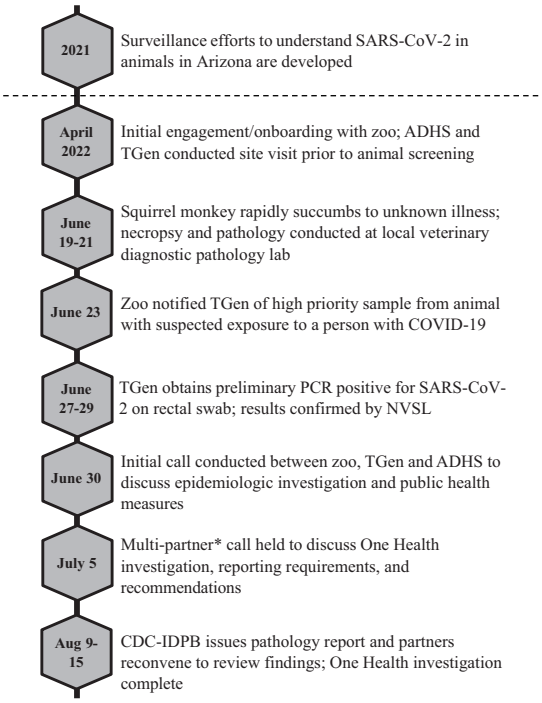

Figure 2. Timeline highlighting actions and involvement of partner agencies in the One Health investigation of SARS-CoV-2 in a squirrel monkey. *Partners included Reid Park Zoo veterinary staff and executive leadership, Translational Genomics Research Institute (TGen), Arizona Department of Health Services (ADHS), the veterinary pathologist from Midwestern University Veterinary Diagnostic Pathology Center, individuals from the Centers for Disease Control One Health Office, Centers for Disease Control Infectious Disease Pathology Branch (IDBP), National Veterinary Services Laboratories (NVSL), and Office of Interagency Coordination within US Department of Agriculture (USDA)–Animal and Plant Health Inspection Service Veterinary Services. Reporting to the World Organization for Animal Health (WOAH) was facilitated by USDA.

disease outbreaks, and to conduct surveillance of emerging pathogens.[5,18,23,37] Despite robust implementation of the One Health approach in Arizona, collaborations have previously overlooked the routine inclusion of zoos and wildlife facilities. Zoonotic transmission of SARS-CoV-2 presents novel concerns and challenges in zoo settings.[2,10,15,28,34] In the beginning of the pandemic, zoos faced challenges in implementing effective biosecurity practices. The emergence of new variants and changing guidance further prompted shifts in policy governing general zoo operations.[13] Close interactions with animal caretakers and the risk imposed by congregate housing continue to be implicated as important factors in SARS-CoV-2 outbreaks at zoos.[2,8,10,15,28,31,34] Taken together, these issues highlight the need to include these partners in surveillance, preparedness, and response.

In this case study, necropsy and pathologic evaluation revealed necrotizing colitis and, together with fecal culture results, suggested toxemia due to *Clostridium perfringens* type A as the driver of the fatal outcome in the squirrel monkey.[30] Pathologic findings did not implicate SARS-CoV-2 as a causal or contributing factor in the animal's illness and death, although its burden on the immune system may have limited the squirrel monkey's ability to respond to the clostridium infection. Using the algorithm established through rigorous clinical, epidemiologic, and pathologic investigation by Carpenter et al.[6] to assess the role of SARS-CoV-2 in an animal's death, infection was deemed incidental to the death of the squirrel monkey. Additionally, given the rapid decline, it is plausible that SARS-CoV-2 infection never established itself systemically in the squirrel monkey. Lastly, although samples for sequencing from the animal caretaker were unavailable, the most plausible hypothesis is that the squirrel monkey was exposed to SARS-CoV-2, likely by a caretaker. This is supported by three lines of evidence: (1) other animals at the zoo, including nonhuman primates and the two cohabitating squirrel monkeys were negative for SARS-CoV-2, suggesting other animals were not the source of exposure; (2) public contact with the squirrel monkey was limited, suggesting a caretaker source; and (3) the animal was found to be infected with the Omicron variant, which was the predominant lineage circulating in the human population during the same timeframe and geographic location. The Omicron variant has also been identified in other animals in human care, including a gorilla and a tiger.[33,35]

Little is known about natural infections of SARS-CoV-2 in nonhuman primates housed in zoos. Most infections in zoo settings to date have been documented in gorillas, with few reported natural infections in monkeys.[28,33] Squirrel monkeys (*Saimiri* spp.) are commonly found in zoological collections throughout the US,[1] and studies have shown that squirrel monkeys appear to have the least severe pathological changes when infected with SARS-CoV-2, in comparison to other nonhuman primates.[9] This may in part be because of genetic differences in cellular receptors mediating the entry of SARS-CoV-2 in New World monkeys.[20] Given these experimental data, limited evidence of SARS-CoV-2 in neotropical nonhuman primates,[27] and the now reported infection in this

particular species, further studies to understand the range of susceptible host and transmission factors are warranted. Moreover, it is also essential to consider the potential conservation impact SARS-CoV-2 and other pathogens transmitted from or between humans to animals can have on primate hosts.[12]

Overall, the establishment of an active One Health surveillance program for SARS-CoV-2 in Arizona enabled the rapid detection and investigation of SARS-CoV-2 in a novel species and prompt response to prevent spread to other people or animals. Establishing trusted partnerships between the zoo, ADHS, and TGen prior to this incident (April 2022) facilitated smooth and effective public health action. By continuing to foster relationships with multisectoral partners, additional collaborations to address critical One Health issues will be formed, enabling the expansion of zoonotic disease surveillance opportunities across the human–animal–environment interface.[17,25,26,32]

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