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Convenience Sampling Yields No Evidence of SARS-CoV-2 Infection in Free-Ranging Mammalian Wildlife in Arizona, USA, 2021–23

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ABSTRACT: Susceptibility of free-ranging US wildlife to SARS-CoV-2 infection has been documented. Nasal or oral swabs and blood from 337 wild mammals (31 species) in Arizona USA, tested for antibodies and by reverse-transcription PCR, did not reveal evidence of SARS-CoV-2. Broader surveillance efforts are necessary to understand the role of wildlife.

The emergence and establishment of zoonotic diseases is often driven by complexities across the human-wildlife interface. As an example, the 2002 SARS-CoV-1 outbreak was shown to have originated in bats (Li et al. 2005), followed by spillover to animals sold at wet markets in Guangdong, China, eventually infecting humans and causing a global outbreak (Li et al. 2006); a similar process has been hypothesized for SARS-CoV-2 (Worobey et al. 2022). Although the origin of the COVID-19 pandemic has not been established, evidence points to the precursor virus as a member of the SARS-like bat coronaviruses, which are known to circulate in bats in southeast Asia and occasionally spill over to other mammals (Lytras et al. 2022).

Since the pandemic began, numerous animal species, including companion animals, animals in zoologic or aquaria settings, and free-ranging wildlife, have been documented to be susceptible to SARS-CoV-2 (Murphy and Ly 2021). Animal susceptibility to infection is only one of many One Health concerns associated with SARS-CoV-2. The establishment of these animals as reservoir hosts, potentially propagating new viral variants, and their ability to facilitate continued transmission back to people and other animals pose the greatest risk (WHO 2022). Well-documented incidents in farmed mink (*Neovison vison*; Oude Munnin et al. 2021; Cossaboom et al. 2022) and white-tailed deer (*Odocoileus virginianus*; Pickering et al. 2022; Caserta et al. 2023; Feng et al. 2023) establish that this is not a theoretical concern.

Ongoing studies are in place across the US to evaluate the extent to which SARS-CoV-2 is circulating in wildlife; however, many of these are focused on white-tailed deer and other cervids. Research outside of zoo or aquaria settings investigating potential natural infection in non-cervid wildlife species has been more limited. The objective of our study was to address knowledge gaps regarding the exposure to, and susceptibility of, free-ranging mammalian wildlife in Arizona, US, to circulating SARS-CoV-2 variants.

Convenience sampling of free-ranging wildlife species was conducted in collaboration with wildlife biologists and veterinarians from the Arizona Game and Fish Department and the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services (USDA-APHIS-WS) working locally in Arizona. This effort was part of Arizona's One Health and SARS-CoV-2 surveillance program (Yaglom et al. 2024), aiming to assess the impacts of SARS-CoV-2 on domestic (dogs and cats) and wildlife animal species (including those in zoos) in the state. All samples in this study were obtained from mammalian species during routine wildlife management programs (e.g., captures) or through established disease surveillance activities (e.g., dead animals submitted for disease testing). Corresponding geographic region was recorded relative to where wildlife was collected or captured. Nasal or oral swabs were collected in 2 mL of transport and storage

TABLE 1. Number and species of wildlife sampled in Arizona, USA, 2021–23 and tested for the presence of antibodies to and viral nucleic acid of SARS-COV-2. A total of 337 individuals were sampled.

Family or order[a]	Scientific name	Common name	Count
Bovidae	*Bos taurus*	Cow (feral)	1
Canidae	*Canis latrans*	Coyote[b]	59
	Urocyon cinereoargenteus	Gray fox	6
	Vulpes vulpes	Red fox	1
Cervidae	*Cervus canadensis*	Elk	4
	Odocoileus hemionus	Mule deer	16
	Odocoileus virginianus	White-tailed deer	1
Chiroptera	*Eptesicus fuscus*	Big brown bat	1
	Tadarida brasiliensis	Brazilian free-tailed bat	1
	Myotis californicus	California myotis bat	2
	Myotis velifer	Cave myotis bat	1
	Leptonycteris yerbabuenae	Lesser long-nosed bat	1
	Myotis sp.	Myotis, other	1
	Parastrellus hesperus	Canyon bat	1
	Lasiurus xanthinus	Western yellow bat	1
Felidae	*Lynx rufus*	Bobcat	72
	Puma concolor	Mountain lion	20
Leporidae	*Lepus alleni*	Antelope jackrabbit	1
	Sylvilagus audubonii	Desert cottontail rabbit	2
Mephitidae	*Conepatus leuconotus*	American hog-nosed skunk	5
	Mephitis macroura	Hooded skunk	21
	Spilogale gracilis	Western spotted skunk	3
	Mephitis mephitis	Striped skunk	64
	Mephitidae species	Skunk, type unknown	12
Mustelidae	*Meles meles*	Badger	1
Procyonidae	*Nasua nasua*	Coatimundi	1
	Bassariscus astutus	Ringtail cat	2
	Procyon lotor	Raccoon	13
Rodentia	*Ammospermophilus harrisii*	Harris' antelope squirrel	1
	Cynomys gunnisoni	Gunnison's prairie dog	18
Tayassuidae	*Dicotyles tajacu*	Collared peccary	4

[a] Order name used for bats (Chiroptera) and rodents (Rodentia), otherwise by family.
[b] Two coyotes (*Canis latrans*) were sampled in McKinley and San Juan counties in New Mexico, USA.

medium from all animals for SARS-CoV-2 viral testing. When possible, blood samples were collected via venipuncture or using Nobuto blood strips for antibody testing. This work was approved by the Translational Genomics Research Institute's Animal Care and Use Committee (no. 20163).

Samples were maintained at 4 C and analyzed in the laboratory within 14 d. We extracted RNA from nasal and oral swab samples and performed reverse-transcription PCR (RT-PCR) using SARS-CoV-2 N2 and S4 primers for the nucleocapsid and spike proteins to test for the presence of viral RNA (Yaglom et al. 2024). Negative (distilled water) and positive (RNase P, as published by the Centers for Disease Control and Prevention) controls were included. We tested for viral neutralizing antibodies using the GenScript SARS-CoV-2 Surrogate Virus Neutralization Test (GenScript, Piscataway, New Jersey, USA) an ELISA-based kit used widely for SARS-CoV-2 screening in animals (Meyer et al. 2020).

Samples were collected from 337 individuals of 31 mammalian species (Table 1) from November 2021 through August 2023, a period

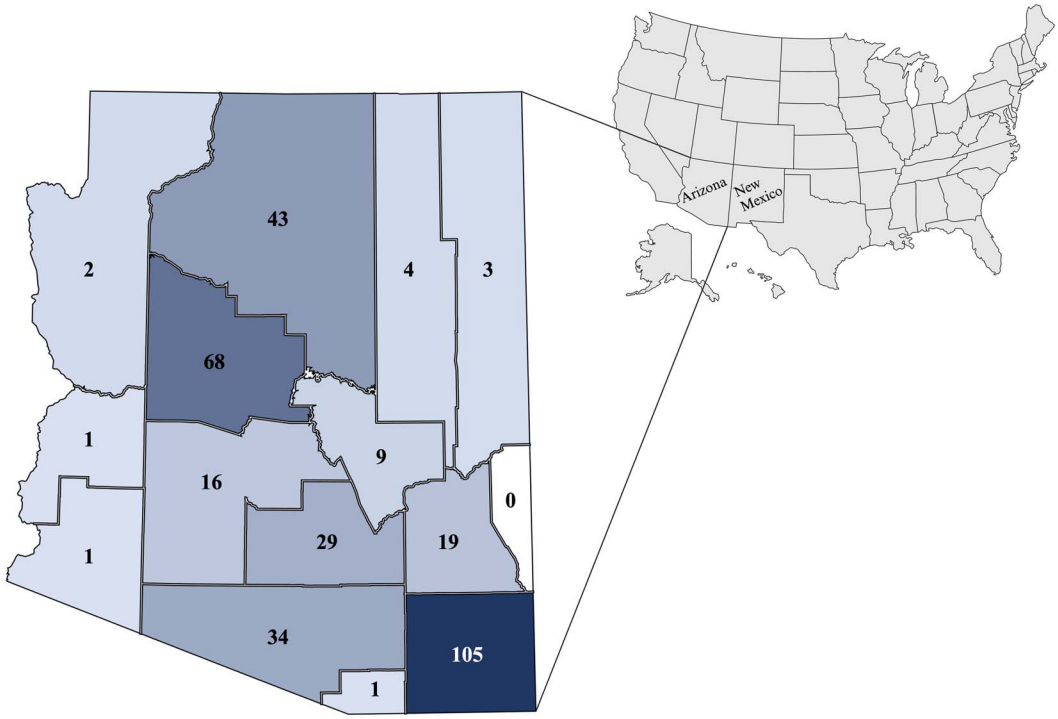

FIGURE 1. Number of animals (n=335) sampled per Arizona, USA, county, 2021–2023 and 23 and tested for the presence of antibodies to and viral nucleic acid of SARS-COV-2. An additional two animals (not shown) were sampled in McKinley and San Juan counties in New Mexico, USA, on the eastern border of Arizona.

that saw dominant circulation of Omicron in human populations with limited Delta overlap (Delta timeframe: November 2021 to February 2022). Samples collected included 305 nasal swabs, 10 oral swabs, and 101 blood samples. Animals were sampled from 14/15 Arizona counties (n=335; Fig. 1), plus two animals from northeastern New Mexico, US, on the eastern Arizona border. We did not detect SARS-CoV-2 on any nasal or oral swab by PCR, and no exposure was detected on antibody assay of the blood samples.

These results add to the current knowledge of SARS-CoV-2 susceptible free-ranging mammalian wildlife species in the US, specifically in the Southwest region. Similar to efforts conducted in Arizona, recent surveillance studies in Vermont and Ohio, US, that included red and gray foxes (*Vulpes vulpes* and *Urocyon cineroargentus*), bobcats (*Lynx rufus*), white-tailed deer, raccoons (*Procyon lotor*), and American mink also revealed no SARS-CoV-2 viral detections (Despres et al. 2023; Ehrlich et al. 2023). Some limitations to our study included 1) this surveillance was conducted as a convenience sampling, 2) paired blood samples were not collected from many animals, and 3) sampling was conducted at only a single time point.

Although cervids made up 6.3% of animals sampled as part of this surveillance effort, they were not the focus, given separate ongoing USDA-APHIS-WS–led collaborative studies in Arizona and nationally to monitor for SARS-CoV-2 in these species. Inclusion of these cervids was nonetheless important, given there has been previous evidence of white-tailed deer being infected with Alpha, Delta, and Omicron variants (Caserta et al. 2023; Feng et al. 2023; USDA 2023). Additionally, mule deer (*Odocoileus hemionus*) have tested positive for SARS-CoV-2 in Arizona, Utah, and California, US, although no other cervids have tested positive in the Southwest (USDA 2023). Finally, interaction between other free-ranging wildlife and cervids is plausible; therefore, should the virus

become established in Arizona wildlife, a risk of interspecies viral transmission exists.

Although none of the Arizona free-ranging wildlife tested in this study showed evidence of SARS-CoV-2 infection or exposure, increased human-wildlife interactions, particularly in urban and suburban environments, pose a risk for viral spillover between humans and animals. Given the vast differences in wildlife populations across the US, the environments in which they live, and the proximity in which they live to humans and other animals, many unanswered questions remain about SARS-CoV-2 viral dynamics at the human-wildlife interface. Perhaps one of the greatest concerns is that emerging SARS-CoV-2 variants may be able to adapt to new host species, causing potential shifts in transmission dynamics and a potential source for novel variant introduction back to humans. Although the public health emergency has ended, continued One Health surveillance efforts in humans and animals remains necessary.

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