

1 **Surveillance for SARS-CoV-2 in Ohio's wildlife, companion, and agricultural animals**

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23 **Abstract**

24 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in humans in late 2019 and
25 spread rapidly to become a global pandemic. A zoonotic spillover event from animal to human was
26 identified as the presumed origin. Subsequently, reports began emerging regarding spillback events
27 resulting in SARS-CoV-2 infections in multiple animal species. These events highlighted critical links
28 between animal and human health while also raising concerns about the development of new reservoir
29 hosts and potential viral mutations that could alter virulence and transmission or evade immune
30 responses. Characterizing susceptibility, prevalence, and transmission between animal species became a
31 priority to help protect animal and human health. In this study, we coalesced a large team of
32 investigators and community partners to surveil for SARS-CoV-2 in domestic and free-ranging animals
33 around Ohio between May 2020 and August 2021. We focused on species with known or predicted
34 susceptibility to SARS-CoV-2 infection, highly congregated or medically compromised animals (e.g.

35 shelters, barns, veterinary hospitals), and animals that had frequent contact with humans (e.g. pets,
36 agricultural animals, zoo animals, or animals in wildlife hospitals). This included free-ranging deer
37 (n=76), mink (n=57), multiple species of bats (n=65), and other wildlife in addition to domestic cats
38 (n=275) and pigs (n= 184). In total, we tested 800 animals (34 species) via rRT-PCR for SARS-CoV-2 RNA.
39 SARS-CoV-2 viral RNA was not detected in any of the tested animals despite a major peak in human
40 SARS-CoV-2 cases that occurred in Ohio subsequent to the peak of animal samplings. Importantly, due
41 to lack of validated tests for animals, we did not test for SARS-CoV-2 antibodies in this study, which
42 limited our ability to assess exposure. While the results of this study were negative, the surveillance
43 effort was critical and remains key to understanding, predicting, and preventing re-emergence of SARS-
44 CoV-2 in humans or animals.

45 **Main text**

46 As of December 2022, there have been over 649 million confirmed cases of COVID-19 worldwide
47 [1]. SARS-CoV-2, the virus which causes COVID-19, infects a broad range of hosts species (n=25) [2].
48 Susceptibility to and transmission of SARS-CoV-2 within and between a growing list of species raises
49 concerns regarding the development of new reservoir hosts, the re-emergence of COVID-19 from these
50 species, and the increased potential for viral mutations that evade immune responses. Experimental
51 animal work and ACE2 models also show the potential for broad host-susceptibility [3,4]. Early in the
52 pandemic, along with rising SARS-CoV-2 prevalence in humans, our goal was to assess prevalence and
53 transmission risk across a wide range of animal species, both under human care and free-ranging. We
54 focused on species with known or predicted susceptibility to SARS-CoV-2; animals in high-risk situations
55 (e.g., densely congregated, medically compromised); and animals that had frequent contact with
56 humans or human environments - pets, agricultural animals, wildlife, animals in zoos or hospitals. A total
57 of 800 animals were sampled from May 2020–August 2021. Sampling locations included: Ohio Wildlife
58 Center, Columbus Zoo & Aquarium, Ohio State University Veterinary Medical Center, MedVet Hilliard,
59 Columbus Humane, Shelter Outreach Services of Ohio, and Ohio county fairs and metroparks. Private
60 citizens—hunters / trappers—also collected samples for this study.

61 Depending on species and sampling conditions, nasal, oropharyngeal, choanal, conjunctival,
62 and/or rectal swabs (Fisherbrand™ Synthetic-Tipped Applicators) were collected from each animal. For
63 species identified as high risk for SARS-CoV-2 (e.g., cats, ferrets), oropharyngeal, conjunctival, nasal, and
64 rectal swabs were collected, as feasible; for pigs and deer - nasal swabs. Swabs were placed in brain
65 heart infusion broth, viral transport media, or RNAlater™ and frozen at -80°C where they remained until

66 extraction. When freezers were not immediately available, samples were placed on ice for up to 12
67 hours or into liquid nitrogen. A few animals (n=8) were tested on multiple dates, and counted as
68 separate individuals as infection could have occurred between testing dates. SARS-CoV-2 rRT-PCR
69 testing was conducted as described previously [5].

70 We sampled 34 species over 16 months, and SARS-CoV-2 viral RNA was not detected in any
71 sample (**Table 1**). Human infection rates in Ohio varied widely during this time, peaking in December
72 2020 at 255,965 cases (2.17% prevalence) [6]. Despite the human caseload of SARS-CoV-2 and although
73 many of the species tested demonstrated moderate to high susceptibility to SARS-CoV-2, none of the
74 800 animals tested positive.

75 **Discussion**

76 Although case reports of animal infections emerged throughout the pandemic, our early active
77 surveillance efforts did not yield any SARS-CoV-2 detections. While it is possible that we missed viral
78 shedding windows, infection dynamics can also change over time. For example, the 78 free-ranging deer
79 tested in this study were sampled October-November 2020 and all were negative. In a related study
80 conducted January-March 2021, 35% (129 of 360) of the free-ranging Ohio deer tested positive for
81 SARS-CoV-2 [5]. The 2021 study occurred after the human COVID-19 peak and after deer gun hunting
82 season in Ohio, both of which would have created additional opportunities for direct and indirect
83 contact (e.g., environmental contamination) between humans and deer. Additional reports nationwide
84 confirmed widespread infection in deer [7,8]. Notably, it took time to detect natural infections in deer.
85 Negative results in early surveillance efforts did not preclude a potential public health threat associated
86 with animal reservoir establishment. In fact, the threat of re-emergence from animal hosts has already
87 been realized through mink-, hamster-, cat-, and probable deer-to-human transmission events [9-13].
88 These cases highlight the critical need for continued surveillance.

89 Mink, bats, and deer have been identified as potential reservoirs for SARS-CoV-2. However,
90 most SARS-CoV-2 sampling efforts have focused on farmed rather than free-ranging mink [13]. Assessing
91 viral prevalence in free-ranging animals is critical to determining the SARS-CoV-2 reservoir and
92 transmission potential of species like mink. This Ohio study is the largest sampling effort, to date, in
93 free-ranging mink (n=57) and in native bats (n=65, 4 species).

94 Deer mice are widespread across Ohio and North America and also have the potential to act a
95 host for SARS-CoV-2 [14]. Limited surveillance in these free-ranging species could mean undetected

96 virus circulation and maintenance in the environment. Although our study included 105 samples from
97 other rodent species, no samples from deer mice were obtained, highlighting key gaps in our
98 surveillance efforts. Targeted efforts to surveil free-ranging deer mice will be necessary to evaluate
99 infection and transmission in these species as well as their potential to serve as an intermediate host for
100 other animals like white-tailed deer. One limitation of this study: We only tested for SARS-CoV-2 virus,
101 not antibodies, which would reflect exposures that may have occurred weeks to months earlier [15]. The
102 window to detect virus in infected animals is relatively short, and rRT-PCR testing outside the viral
103 shedding window yields negative results.

104 SARS-CoV-2 presumably originated in an animal and spilled over into humans with subsequent
105 spillback from humans into many other animal species. While SARS-CoV-2 remains primarily a human
106 pathogen, known transmission to and from multiple species raises critical public health concerns and
107 the need to identify potential amplifying or reservoir hosts, and routes of transmission. From humans, to
108 endangered wildlife, to pets and agricultural animals, ongoing surveillance is essential as SARS-CoV-2
109 variants continue to emerge, creating a dynamic landscape of susceptibility and transmission risks within
110 and between species that could have far-reaching implications on conservation, ecosystem health, and
111 food production, in addition to economics and public health.

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125 **Declaration of interest statement:**

126 The authors report there are no competing interests to declare.

127 **Author Contributions:**

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131 Investigation (Analysis): ME, VLH, ASB, JMN

132 Visualization: ME

133 Funding acquisition: VLH, SAF, ASB

134 Project administration: VLH, SAF, ASB

135 Supervision / Consultation: VLH, PD, DD-C, ASB, JW, PY, AV, SK, QW, LJS

136 Writing – original draft: ME, VLH

137 Writing – review & editing: All

138 **Animal Subjects:**

139 All animal sampling conducted in this study was IACUC approved (live animal sampling) or exempt.

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177 **Table 1. Species in Ohio tested for SARS-CoV-2 (May 2020-August 2021).**

178

Scientific Name	Common Name	Number Individuals Tested	Provenance (HC = under human care; FR = free-ranging)
<i>Acinonyx jubatus</i>	Cheetah	2	HC
<i>Branta canadensis</i>	Canada Goose	1	HC
<i>Canis latrans</i>	Coyote	1	FR
<i>Castor canadensis</i>	American Beaver	4	FR
<i>Colobus guereza</i>	Guereza Colobus Monkey	1	HC
<i>Didelphis virginiana</i>	Virginia Opossum	7	HC
<i>Eptesicus fuscus</i>	Big Brown Bat	61	HC
<i>Felis catus</i>	Domestic Cat*	275	pets: n=84; shelter: n=191
<i>Hydrochoerus hydrochaeris</i>	Capybara	1	HC
<i>Lasionycteris noctivagans</i>	Silver-haired bat	1	HC
<i>Lasiurus borealis</i>	Eastern Red Bat	2	HC
<i>Lemur catta</i>	Ringtailed lemur	1	HC
<i>Lontra canadensis</i>	North American River Otter	1	HC
<i>Mandrillus sphinx</i>	Mandrill	1	HC
<i>Marmota monax</i>	Groundhog	3	HC
<i>Mephitis mephitis</i>	Striped Skunk	25	HC
<i>Mustela furo</i>	Domestic Ferret	6	pets
<i>Neogale vison</i>	American Mink	58	FR: n=57; HC: n=1
<i>Odocoileus virginianus</i>	White-tailed deer**	76	FR

<i>Ondatra zibethicus</i>	Common Muskrat	36	FR: n=57; HC: n=1
<i>Pan paniscus</i>	Bonobo	1	HC
<i>Perimyotis subflavus</i>	Tricolored Bat	1	HC
<i>Pongo pygmaeus</i>	Bornean Orangutan	1	HC
<i>Potos flavus</i>	Kinkajou	1	HC
<i>Procyon lotor</i>	Raccoon	15	FR: n=57; HC: n=1
<i>Sciurus carolinensis</i>	Eastern Grey Squirrel	11	HC
<i>Sciurus niger</i>	Eastern Fox Squirrel	6	HC
<i>Sus scrofa domesticus</i>	Domestic Pig	184	agricultural
<i>Sylvilagus floridanus</i>	Eastern Cottontail Rabbit	12	HC
<i>Tamias striatus</i>	Eastern Chipmunk	1	HC
<i>Tamiasciurus hudsonicus</i>	American Red Squirrel	1	HC
<i>Trachypithecus cristatus</i>	Silvered Leaf Langur	1	HC
<i>Turdus migratorius</i>	American Robin	1	HC
<i>Varecia rubra</i>	Red ruffed lemur	1	HC

179

180 *A subset of these cats are described in more detail (J. Winston, in preparation)

181 **Does not include Ohio deer described in [5].