DOI: 10.7589/JWD-D-23-00114

Journal of Wildlife Diseases, 60(4), 2024, pp. 924–930

Little Brown Bats (*Myotis lucifugus*) Are Resistant to SARS-CoV-2 Infection

Jeffrey S. Hall,[1,3] Sean Nashold,[1] Erik Hofmeister,[1] Ariel E. Leon,[1] Elizabeth A. Falendysz,[1] Hon S. Ip,[1] Carly M. Malavé,[1] Tonie E. Rocke,[1] Mariano Carossino,[2] Udeni Balasuriya,[2] and Susan Knowles[1]

[1] US Geological Survey National Wildlife Health Center, 6006 Schroeder Rd., Madison, Wisconsin 53711, USA
[2] Louisiana Animal Disease Diagnostic Laboratory, Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Skip Bertman Dr., Baton Rouge, Louisiana 70803, USA
[3] Corresponding author (email: jshall@usgs.go)

ABSTRACT: It has been proposed that the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus that spread through human populations as a pandemic originated in Asian bats. There is concern that infected humans could transmit the virus to native North American bats; therefore, the susceptibility of several North American bat species to the pandemic virus has been experimentally assessed. Big brown bats (*Eptesicus fuscus*) were shown to be resistant to infection by SARS-CoV-2, whereas Mexican free-tailed bats (*Tadarida brasiliensis*) became infected and orally excreted moderate amounts of virus for up to 18 d postinoculation. Little brown bats (*Myotis lucifugus*) frequently contact humans, and their populations are threatened over much of their range due to white-nose syndrome, a fungal disease that is continuing to spread across North America. We experimentally challenged little brown bats with SARS-CoV-2 to determine their susceptibility and host potential and whether the virus presents an additional risk to this species. We found that this species was resistant to infection by SARS-CoV-2. These findings provide reassurance to wildlife rehabilitators, biologists, conservation scientists, and the public at large who are concerned with possible transmission of this virus to threatened bat populations.

Key words: Challenge, infection, little brown bat, *Myotis lucifugus*, resistance, SARS-CoV-2, susceptibility.

INTRODUCTION

Because the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus possibly originated in horseshoe bats (*Rhinolophus* spp.) in Asia (Zhou et al. 2020, 2021; Wacharapluesadee et al. 2021), concerns have been raised that the virus might infect North American bat species and pose additional pressure on already threatened bat populations. Introduction of the fungus *Pseudogymnoascus destructans* (Pd), the causative agent of white-nose syndrome, into North America has had dramatic impacts on many bat species' populations. As a result, several species have become threatened and have been extirpated from many parts of their historic ranges. Little brown bat (*Myotis lucifugus*) populations where Pd has become established have declined by >90% from preintroduction levels (Cheng et al. 2021). As the fungus continues to spread across the continent, more populations will be in peril and potential additional adverse impacts to populations of little brown bats from infection by SARS-CoV-2 are not known.

SARS-CoV-2 has infected populations of other wild mammals including American mink (*Neovison vison*) and white-tailed deer (*Odocoileus virginianus*), presumably by contact with infected humans (Oude Munnink et al. 2020; Chandler et al. 2021). Reciprocally, these species have also transmitted the virus to humans (Oude Munnink et al. 2020; Chandler et al. 2021). Thus, in addition to potential risks to bat populations, there is concern that bats might be hosts that amplify, maintain, or provide environments for rapid adaptation and evolution of SARS-CoV-2, with additional risks to human or other mammalian populations.

We have previously shown big brown bats (*Eptesicus fuscus*) to be resistant to infection by SARS-CoV-2 (Hall et al. 2021); however, Mexican free-tailed bats (*Tadarida brasiliensis*) have been shown to be susceptible to the virus and orally excreted moderate amounts of the virus for up to 18 d postinoculation (DPI; Hall et al. 2023). Little brown bats frequently reside in artificial structures and contact humans, including

wildlife biologists, in caves or other roosts, thus they have potential exposure to SARS-CoV-2 by aerosol transmission from infected humans, contaminated water sources, or other infected wild species. To investigate the potential impact of the virus on little brown bat populations, we experimentally challenged wild little brown bats with SARS-CoV-2 to determine infection rates, virus excretion, morbidity, and pathology in this species.

MATERIALS AND METHODS

Virus acquisition and propagation

We obtained the SARS-CoV-2 virus (2019-nCoV/USA-WA1/2020) from BEI Resources, National Institute of Allergy and Infectious Diseases (NIAID), National Institute of Health (NIH), Manassas, Virginia. The virus was isolated from the first confirmed human patient with coronavirus disease 2019 in the United States (Harcourt et al. 2020). We propagated and quantified the virus in Vero E6 cell culture using standard techniques (Jureka et al. 2020).

Animal acquisition and husbandry

Wild little brown bats were captured in Pierce County, northern Wisconsin, in October 2021. Both adult and juvenile male bats were collected and immediately placed into a temperature-controlled chamber maintained at approximately 20 C during transport to the US Geological Survey National Wildlife Health Center (NWHC), Madison, Wisconsin.

On arrival at the NWHC, the bats were examined by a veterinarian and treated topically with selamectin for external parasites (Zoetis, Florham Park, New Jersey, USA). The bats were hand fed mealworms (*Tenebrio molito*), and water was provided ad libitum. Bats underwent a quarantine and acclimatization period of 30 d before commencement of the study, during which time the bats learned to feed themselves. All husbandry and experimental protocols were approved by the National Wildlife Health Center Institutional Animal Care and Use Committee (protocol EP200219.A3).

Preinoculation fecal sampling and coronavirus analysis

During the acclimatization period, we collected fecal samples from six bats to determine the presence of other coronaviruses in these subjects. Each fecal sample was suspended (10% w/v) in viral transport medium (VTM; Hank's balanced salt solution, 0.05% gelatin, 5% glycerin, 1,500 units/mL penicillin, 1,500 mg/mL streptomycin, 0.1 mg/mL gentamicin,1 mg/mL fungizone). Viral RNA was extracted using the MagMax Pathogen RNA/DNA kit (Applied Biosystems, Foster City, California, USA) on a magnetic particle processor (KingFisher Flex, Thermo Fisher Scientific, Waltham, Massachusetts, USA) according to the manufacturers' instructions. The presence of coronaviruses was determined using methods described previously (Decaro and Larusso 2020).

Virus inoculation

Experimental inoculations were performed under Biosafety Level 3 conditions at the NWHC by using a technique previously used to inoculate other species with SARS-CoV-2 (Munster et al. 2020; Schlottau et al. 2020; Shi et al. 2020; Hall et al. 2021, 2023). The SARS-CoV-2 inoculum dose of 10^5 50% tissue culture infectious dose ($TCID_{50}$)/bat in 10 µL of brain heart infusion, was administered nasally (4 µL) and orally (6 µL) using a micropipette. The inoculum titer was verified by quantitative real-time PCR (qRT-PCR) as described below for swabs, and virus viability was confirmed in cell culture using Vero E6 cells. After the acclimatization period, in one cage, 7 of 10 bats were inoculated with SARS-CoV-2; the remaining 3 bats were left uninoculated to determine transmission between bats. In a second cage, 7 of 12 little brown bats were housed, with 5 bats left uninoculated. Three bats, housed in a separate cage, were sham inoculated with the same volume of VTM to serve as negative control subjects.

Animal monitoring and sampling

Bats were observed at least twice daily to document development of clinical signs and to monitor health status. Before inoculation, and every second day thereafter, each bat was weighed and oropharyngeal and rectal swabs (Puritan Medical Products, Guilford, Maine, USA) were collected and placed in 0.5 mL of VTM. On each of DPIs 7 and 14, one inoculated bat and one uninoculated bat from one cage were euthanized, a necropsy was conducted, and tissues and blood were collected. At the end of the study (DPI 20), all remaining bats were euthanized. All euthanized bats had blood collected for serologic analyses. Necropsy was carried out on the

control bats and on one inoculated and one uninoculated bat. Brain tissue was taken from every bat for rabies testing. All carcasses were saved frozen at –80 C.

qRT-PCR analyses from swabs

We performed RNA extractions of swab material in 96-well plates by using the Mag Max-96 AI/ND Viral RNA Isolation kit (Applied Biosystems) following the manufacturer's instructions. A positive control sample consisting of a 1:100 dilution of the SARS-CoV-2 inoculum used in the study was included with each extraction series to validate successful RNA extraction. The qRT-PCR analyses were conducted using the Centers for Disease Control 2019-nCoV N1 primers and probe (Mina and Andersen 2021) and AgPath-ID One-Step RT-PCR reagents (Ambion/Thermo Fisher Scientific). We included a standard curve of serial dilutions of RNA extracted from SARS-CoV-2 virus stock (10^7 $TCID_{50}$/mL) in each qRT-PCR assay to quantify viral amounts.

Necropsy and histopathology

Two animals (inoculated and uninoculated cage-mates) were euthanized at DPI 7 (bats 2950 and 5291) and DPI 14 (bats 0777 and 4666) and an additional set of cage-mates at DPI 20 (bats 4033 and 4269), by using an overdose of isoflurane with subsequent decapitation. One uninoculated control animal (6442) was also euthanized at DPI 20. One bat (4838) died at DPI 9 and uninoculated control bat (0964) died at DPI 10 for unknown reasons (see below). Control bat (1037) was euthanized at DPI 12. These subjects were immediately necropsied after euthanasia or within 2 h after bats were found dead, and body condition and gross observations were recorded. Portions of the nares, caudal lung, cranial lung, heart, liver, spleen, kidney, small intestine, colon, and brain were collected and saved frozen at −80 C for virologic analyses. Additional tissue portions were fixed in 10% neutral buffered formalin (NBF) for histologic analysis. Lungs were inflated with 10% NBF by using a tuberculin syringe for all bats except 1037. For histopathologic examination, fixed tissues were processed routinely, sectioned at approximately 5 µm, and stained with H&E at the Wisconsin Veterinary Diagnostic Laboratory (Madison). At DPI 20, all remaining bats were euthanized, serum was collected, and all bat carcasses were saved frozen.

Rabies diagnostics

After euthanasia, brain tissues from all bats were assessed for rabies infection by using the direct fluorescent antibody test. After brain impressions were fixed in acetone, slides were stained with a fluorescein isothiocyanate–labeled monoclonal antibody conjugate (Fujirebio U.S. Inc., Malvern, Pennsylvania, USA) and visualized under a fluorescent microscope (Dean et al. 1996).

Postinoculation SARS-CoV-2–specific IHC and qPCR

Tissues were formalin fixed immediately at necropsy for a maximum of 7 d before immunohistochemistry (IHC). For IHC, 4-µm sections of formalin-fixed, paraffin-embedded tissues (rostral nasal cavity, lung, heart, spleen, liver, pancreas, stomach, small and large intestine, and brain) were mounted on positively charged Superfrost Plus slides (Thermo Fisher Scientific and then subjected to IHC by using an anti-nucleocapsid rabbit monoclonal antibody (HL344, Cell Signaling Technology, Danvers, Massachusetts, USA). The IHC was performed using the automated BOND-RXm platform and the Polymer Refine Red Detection kit (both Leica Biosystems, Wetzlar, Germany). After automated deparaffinization, heat-induced epitope retrieval was performed using a ready-to-use citrate-based solution (pH 6.0; Leica Biosystems) at 100 C for 20 min. Sections were then incubated with the primary antibody (diluted at 1:1,600 in primary antibody diluent [Leica Biosystems]) for 30 min at room temperature, followed by a polymer-labeled goat anti-rabbit immunoglobulin G coupled with alkaline phosphatase (30 min). Fast Red was used as the chromogen (15 min), and counterstaining was performed with hematoxylin for 5 min. Slides were dried in a 60 C oven for 30 min and mounted with a permanent mounting medium (Micromount, Leica Biosystems). Lung sections from a SARS-CoV-2–infected hamster (*Mesocricetus auratus*) were used as positive assay controls.

Approximately 10 mg of each tissue collected from the 10 necropsied bats was macerated in extraction buffer and extracted using the ZYMO Research Quick DNA/RNA Pathogen Miniprep kit (ZYMO Research, Irvine, California, USA) according to the manufacturer's directions. The qRT-PCR analyses were performed as described for the swabs.

Antibody detection

To detect antibodies to SARS-CoV-2, bat sera were screened at a 1:10 dilution by using a competitive ELISA (SARS-CoV-2 sVNT, GenScript, Piscataway, New Jersey. USA) according to the manufacturer's instructions. As directed, a reduction in optical density (OD) of ≥30% compared with the mean OD of the negative control was considered positive for the presence of neutralizing antibodies. In addition to the positive control provided in the kit, we used positive guinea pig serum obtained through Guinea Pig NR-10361 Polyclonal Anti-SARS Coronavirus antiserum (BEI Resources, NIAID, NIH).

RESULTS

Exogenous virus detection

We did not detect the presence of other coronaviruses, including bat alphacoronavirus in the feces of any of the bats preinoculation. We found no evidence of rabies virus infection in the brain in any of the bats.

Clinical signs and health status

During our twice-daily health observations after inoculation, no overt clinical signs attributed to virus infection were noted in any little brown bat. The bats' body weights were variable, with most bats losing moderate amounts of weight or remaining stable, although several bats gained weight over the course of the study (Supplementary Material Table S1). Three bats (1037, 0964, and 4838) died during the study due to unknown causes not related to SARS-CoV-2 infection. Two of these bats (1037 and 0964) were sham-inoculated controls.

Oral and rectal SARS-CoV-2 excretion

Quantitative RT-PCR analyses of oral swabs taken after little brown bats were inoculated with SARS-CoV-2 revealed no definitive indication of viral infection in any of the bats (Supplementary Material Table S2). Two of the bats (3498 on DPI 4; 612 on DPI 2) not inoculated, but cohoused with inoculated bats, had high cycle threshold (Ct) values from the swabs (39.85 and 39.86, respectively). Repeat extraction and analysis of these samples had no detectable Ct values; therefore, we consider these two samples as false positives. Inoculated bat 7988 had positive Ct values on DPI 2 and 4, and repeat analyses verified these results. It is likely that these results are from residual inoculum in the oral cavity that was picked up on the swabs, not from viral infection, a finding confirmed by lack of seroconversion of this bat to SARS-CoV-2 (see below). Three rectal swab samples had relatively high Ct values; however, repeat analyses of those samples were all negative (Table 1).

Serologic and pathologic findings

Sera taken from all little brown bats (control, inoculated, or uninoculated) at the end of the study were all negative for SARS-CoV-2 antibodies (Table 1). All bats were in good-to-excellent body condition evidenced by moderate-to-abundant fat stores, except one bat that was in fair body condition with lesser fat stores. Gross findings included reddening or mottling of the lungs (n=8), blood clots in the oral cavity (n=1), and tracheal hemorrhage (n=2). Histopathologic findings included pulmonary congestion (n=10), pulmonary hemorrhage (n=7), alveolar collapse (n=1), fibrin in airways (n=2), pulmonary edema (n=1), basophilic intranuclear inclusion bodies (suggestive of adenovirus) in pneumocytes with syncytia (n=1), pulmonary perivascular inflammation (n=1), meningeal hemorrhage (n=2), cerebral hemorrhage (n=1), nasal cavity hemorrhage (n=2), pharyngitis (n=2), exocytosis of neutrophils in nasal turbinate epithelium (n=2), nasal dermatitis (n=8), dermal myositis (n=1), splenic red pulp congestion (n=4), polymorphonuclear cells in hepatic sinusoids (n=3), hepatic perivascular neutrophils (n=1), intestinal trematodiasis (n=10), renal perivascular inflammation (n=1), eosinophilic material in renal tubular lumina (n=1), bacteria on the dermal stratum corneum (n=1), and bacteria in multiple organs (n=1). Autolysis was observed in the two bats that died. There were no significant histologic findings in the heart or colon. Pulmonary findings were not consistent with those observed in other animals experimentally infected with SARS-CoV-2

TABLE 1. SARS-CoV-2 presence on rectal swabs of experimentally inoculated little brown bats as determined by quantitative reverse-transcription PCR analyses. Inoculated bats were inoculated with SARS-CoV-2 (10^5 $TCID_{50}$/bat). Transmission bats were cohoused with inoculated bats. Control bats were sham inoculated and housed separately from inoculated bats. Positive Ct values are in bold, with repeat analyses. Serologic analysis of terminal sera samples was performed by competitive ELISA.

Bat ID	Treatment	Day 0	DPI 2	DPI 4	DPI 6	DPI 8	DPI 10	Serology
1037	Control	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Negative
6442	Control	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Negative
964	Control	No Ct	No Ct	No Ct	No Ct	No Ct	Died DPI10	Negative
4033	Inoculated	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Negative
4666	Inoculated	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Negative
4820	Inoculated	No Ct	No Ct	No Ct	No Ct	No Ct	**40.08; No Ct**	Negative
2920	Inoculated	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Negative
4589	Inoculated	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Negative
3319	Inoculated	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Negative
4838	Inoculated	No Ct	No Ct	No Ct	No Ct	No Ct	Died DPI9	Negative
3498	Transmission	NS	No Ct	No Ct	No Ct	No Ct	No Ct	Negative
777	Transmission	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Negative
6637	Transmission	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Negative
4269	Inoculated	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Negative
3298	Inoculated	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Negative
5291	Inoculated	No Ct	No Ct	No Ct	No Ct	X[b]	X	Negative
5765	Inoculated	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Negative
4871	Inoculated	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Negative
7988	Inoculated	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Negative
8491	Inoculated	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Negative
5223	Transmission	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Negative
3474	Transmission	No Ct	No Ct	No Ct	No Ct	No Ct	No Ct	Negative
612	Transmission	No Ct	**39.79; No Ct**	No Ct	No Ct	No Ct	No Ct	Negative
2950	Transmission	No Ct	No Ct	No Ct	No Ct	X	X	Negative
1375	Transmission	No Ct	**40.05; No Ct**	No Ct	No Ct	No Ct	No Ct	Negative

[a] SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; TCID = 50% tissue culture infectious dose; Ct = cycle threshold; DPI = days post inoculation; NS = no sample.
[b] X = animal died or was euthanized and no samples collected.

(Munster et al. 2020; Schlottau et al. 2020; Shi et al. 2020), and we attributed the pulmonary, congestion, hemorrhage and edema as well as the nasal cavity hemorrhage to the effects of euthanasia and subsequent decapitation (Coman et al. 2022). Cerebral and meningeal hemorrhage and splenic congestion were also attributed to the effect of euthanasia. Intestinal trematodes were observed commonly in bats and were considered an incidental finding as most are not known to cause disease (Farina and Lankton 2018). Additional histologic findings were considered incidental and not related to the experimental infection.

SARS-CoV-2 detection in bat tissues by PCR and IHC

The qRT-PCR analyses of tissues collected from little brown bats on DPI 7, 14, and 20, as well as tissues from the bats that died during the study (0964, 4838, and 1037), revealed no detectable SARS-CoV-2 RNA in any bat tissue examined. No viral antigen was detected by IHC in any of the tissues examined.

DISCUSSION

Little brown bat populations are threatened, even extirpated over much of their historic range due to white-nose syndrome, and if susceptible,

additional pressures from SARS-CoV-2 could have potentially catastrophic effects. A recent surveillance study examined oral swabs from wild little brown bats captured in the northeastern United States (Moran et al. 2023) and found no instance of SARS-CoV-2 virus shedding in any bat sampled (n=235). This is consistent with our SARS-CoV-2 experimental challenge study in little brown bats. After inoculation with a moderately high virus dose, we found no evidence of viral shedding, either orally or rectally. No transmission between animals occurred and no individual bat developed antibodies after inoculation. No clinical signs of disease were evident, and we detected no SARS-CoV-2 in any tissue examined by either IHC or qRT-PCR. Thus, it appears that little brown bats are resistant to SARS-CoV-2 infection. This is reassuring news for scientists monitoring bat populations, bat rehabilitators, and others who are concerned that infected humans could transmit the virus to naïve, at-risk colonies of little brown bats.

This is the third North American bat species that has been challenged with SARS-CoV-2, with varying results. Mexican free-tailed bats were found to be susceptible to SARS-CoV-2 infection (Hall et al. 2023), whereas big brown bats (Hall et al. 2021) and now little brown bats have been shown to be resistant to the virus. The reason for the difference between free-tailed bats and the other species is currently unknown; whether it is a physiological, behavioral, or ecological difference needs further examination.

SARS-CoV-2 is essentially a human virus and the mechanisms of transmission to new host species, including wild mammals, are unclear. Proximity of animals to infected human populations and exposure to human wastes and wastewater are factors likely to be involved in the virus' host expansion. The receptor for SARS-CoV-2 spike protein binding and cellular entry is the angiotensin-converting enzyme 2 (ACE2). The expression and virus binding ability of this protein is a major determinant of the virus' host range (Yan et al. 2021). IHC and binding assays have shown that little brown bats express abundant amounts of ACE2 in the trachea and intestine, comparable with expression in humans; therefore, theoretically this species should be a competent host of the virus (Chothe et al. 2023). Our experimental findings indicate that there must be other factors in addition to ACE2 that are involved in SARS-CoV-2 infections. More studies are needed to define the host determinants of productive SARS-CoV-2 infections, including challenge studies with the newer SARS-CoV-2 variants.

ACKNOWLEDGMENTS

The authors thank the Wisconsin Department of Natural Resources for support and assistance collecting bats for this study. This study would not have been possible without the excellent work of the NWHC Animal Care staff and the volunteers who assisted with the acclimatization of the bats. We also acknowledge members of the Histology and Immunohistochemistry sections at the Louisiana Animal Disease Diagnostic Laboratory for assistance. This work was funded by the Ecosystems Mission Area of the US Geological Survey. Funding for this project was also partially provided by the American Rescue Plan Act through US Department of Agriculture (USDA), Animal and Plant Health Inspection Service (USDA National Institute of Food and Agriculture award 2023-70432-39465) to M.C. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US Government.

SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/JWD-D-23-00114.

LITERATURE CITED

Chandler JC, Bevins SN, Ellis JW, Linder TJ, Tell RM, Jenkins-Moore M, Root JJ, Lenoch JB, Robbe-Austerman S, et al. 2021. SARS-CoV-2 exposure in wild white-tailed deer (*Odocoileus virginianus*). *Proc Natl Acad Sci U S A* 118:e2114828118.

Cheng TL, Reichard JD, Coleman JTH, Weller TJ, Thogmartin WE, Reichert BE, Bennett AB, Broders HG, Campbell J, et al. 2021. The scope and severity of white-nose syndrome on hibernating bats in North America. *Conserv Biol* 5:1586–1597.

Chothe SK, Jakka P, Boorla VS, Ramasamy S, Gontu A, Nissly RH, Brown J, Turner G, Sewall BJ, et al. 2023. Little brown bats (*Myotis lucifugus*) support the binding of SARS-CoV-2 spike and are likely susceptible to SARS-CoV-2 infection. *Viruses* 15:1103

Coman C, Ancuta D, Soare T. 2022. Histological evaluation of two euthanasia methods in a toxicity study in laboratory rats. *Sci Works Ser C Vet Med* 68:183–188.

Dean D, Abelseth M, Atanasiu P. 1996. The fluorescent antibody test. In: *Laboratory techniques in rabies*, 4th Ed., Meslin F, Kaplan MM, Koprowski H, editors, World Health Organization, Geneva, Switzerland, pp. 83–93.

Decaro N, Larusso A. 2020. Novel human coronavirus (SARS-CoV-2): A lesson from animal coronaviruses. *Vet Microbiol* 244:108693.

Farina LL, Lankton JS. 2018. Chiroptera. In: *Pathology of wildlife and zoo animals*, Terio KA, McAloose D, St. Leger J, editors. Academic Press, Philadelphia, Pennsylvania, pp. 607–633.

Hall JS, Hofmeister E, Ip HS, Nashold SW, Leon AE, Malavé CM, Falendysz EA, Rocke TE, Carossino M, et al. 2023. Experimental infection of Mexican free-tailed bats (*Tadarida brasiliensis*) with SARS-CoV-2. *mSphere* 8:e00263-22.

Hall JS, Knowles S, Nashold SW, Ip HS, Leon AE, Rocke T, Keller S, Carossini M, Balasuriya U, Hofmeister E. 2021. Experimental challenge of a North American bat species, big brown bat (*Eptesicus fuscus*), with SARS-CoV-2. *Transboundary Emerg Dis* 68:3443–3452.

Harcourt J, Tamin A, Lu X, Kamili S, Sakthivel S, Murray J, Queen K, Tao Y, Paden C, et al. 2020. Severe acute respiratory syndrome coronavirus 2 from patient with coronavirus disease, United States. *Emerg Infect Dis* 26:1266–1273.

Jureka AS, Silvas JA, Basler CF. 2020. Propagation, inactivation, and safety testing of SARS-CoV-2. *Viruses* 12:622.

Mina MJ, Andersen KG. 2021. COVID-19 testing: One size does not fit all. *Science* 371:126–127.

Moran ML, Boyd W, De La Cruz JL, Bertke AS, Ford WM. 2023. Oral sampling of little brown bat (*Myotis lucifugus*) maternity colonies for SARS-CoV-2 in the Northeast and Mid-Atlantic, USA. *Animals (Basel)* 13:550.

Munster V, Feldmann F, Williamson B, van Doremalen N, Pérez-Pérez L, Schulz J, Meade-White K, Okumura A, Callison J, et al. 2020. Respiratory disease and virus shedding in rhesus macaques inoculated with SARS-CoV-2. *Nature* 585:268–272.

Oude Munnink BB, Sikkema RS, Nieuwenhuijse DF, Molenaar RJ, Munger E, Molenkamp R, van der Spek A, Tolsma P, Rietveld A, et al. 2020. Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans. *Science* 371:172–177.

Shi J, Wen Z, Zhong G, Yang H, Wang C, Huang B, Liu R, He X, Shuai L, et al. 2020. Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. *Science* 368:1016–1020.

Schlottau K, Rissmann M, Graaf A, Schön J, Sehl J, Wylezich C, Höper D, Mettenleiter TC, Balkema-Buschmann A, et al. 2020. SARS-CoV-2 in fruit bats, ferrets, pigs, and chickens: an experimental transmission study. *Lancet Microbe* 1:e218–e225.

Wacharapluesadee S, Tan CW, Maneeorn P, Duengkae P, Zhu F, Joyjinda Y, Kaewpom T, Chia WN, Ampoot W, et al. 2021. Evidence for SARS-CoV-2 related coronaviruses circulating in bats and pangolins in Southeast Asia. *Nat Commun* 12:972.

Yan H, Jiao H, Liu Q, Zhang Z, Xiong Q, Wang BJ, Wang X, Gua M, Wang LF, et al. 2021. ACE2 receptor usage reveals variation in susceptibility to SARS-CoV and SARS-CoV-2 infection among bat species. *Nat Ecol Evol* 5:600–608.

Zhou H, Ji J, Chen X, Bi Y, Li J, Wang Q, Hu T, Song H, Zhao R, et al. 2021. Identification of novel bat coronaviruses sheds light on the evolutionary origins of SARS-CoV-2 and related viruses. *Cell* 184:4380–4391.

Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, et al. 2020. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 579:270–273.

Submitted for publication 12 July 2023.
Accepted 8 May 2024.