Article

Not peer-reviewed version

SARS-CoV-2 Surveillance in Free-Ranging Wildlife in the Northeastern United States, 2022–2025

Idrissa Nonmon Sanogo *, Wendy B. Puryear, Alexa F. Simulynas, Elena Cox, Maureen Murray, Zain Khalil, Harm van Bakel, Martin J. R. Feehan, Zak Mertz, Priya Patel, Jennifer Riley, Blaine Hymel, Jonathan A. Runstadler

Posted Date: 19 May 2026

doi: 10.20944/preprints202605.1170.v1

Keywords: SARS-CoV-2; wildlife; surveillance; spillover; New England; United States

 doi:10.20944/preprints202605.1170.v1

Article

SARS-CoV-2 Surveillance in Free-Ranging Wildlife in the Northeastern United States, 2022–2025

Idrissa Nonmon Sanogo [1,*], Wendy B. Puryear [1], Alexa F. Simulynas [1], Elena Cox [1], Maureen Murray [1], Zain Khalil [2], Harm van Bakel [2,3,4], Martin J. R. Feehan [5], Zak Mertz [6], Priya Patel [6], Jennifer Riley [7], Blaine Hymel [8] and Jonathan A. Runstadler [1]

[1] Department of Infectious Disease and Global Health, Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA, 01536, USA
[2] Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
[3] Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
[4] Department of Artificial Intelligence and Human Health, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
[5] Massachusetts Division of Fisheries and Wildlife, Westborough, MA, 01581, USA
[6] New England Wildlife Center, Weymouth, MA, 02190 USA
[7] Blue Ridge Wildlife Center, Boyce, VA 22620, USA
[8] Wildlife Clinic of Rhode Island, Saunderstown, RI 02874, USA
* Correspondence: idrissa_nonmon.sanogo@tufts.edu

Abstract

Since its emergence in 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected a wide range of animal species, including wildlife. Although SARS-CoV-2 infection has been widely reported in wildlife, particularly in white-tailed deer (WTD; Odocoileus virginianus) across the United States, data on viral circulation in New England wildlife remain limited. Here, we evaluated SARS-CoV-2 infection and serological evidence of previous exposure in free-ranging wildlife from the northeastern United States. We examined samples from 1,646 animals representing 28 wildlife species, collected through wildlife rehabilitation centers, clinics, and hunter harvests in New England and Virginia between 2022 and 2025. SARS-CoV-2 RNA was detected in three WTD from Massachusetts and Vermont. Phylogeographic analysis of Vermont WTD sequences indicated a human SARS-CoV-2 lineage as the most likely source, consistent with a single human-to-deer spillover event followed by subsequent circulation within deer. Serological screening using ELISA detected SARS-CoV-2 antibodies in 12 individuals from three species, including Eastern cottontail (Sylvilagus floridanus), Eastern coyote (Canis latrans), and raccoon (Procyon lotor), although neutralizing antibodies were found in only one Eastern cottontail. Overall, these findings reveal ongoing but limited SARS-CoV-2 circulation in northeastern wildlife and highlight the importance of continued surveillance to detect spillover events, monitor viral evolution, and evaluate potential risks posed by wildlife.

Keywords: SARS-CoV-2; wildlife; surveillance; spillover; New England; United States

1. Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the causative agent of the COVID-19 pandemic, has been spread through human-to-human transmission since it emerged in late 2019. However, besides humans, many domestic and wild animal species have been reported to be either naturally infected with SARS-CoV-2 [1–6] or susceptible to the virus through experimental infection [1,7–10]. As of September 2025, SARS-CoV-2 has been detected in 68 animal species across

 doi:10.20944/preprints202605.1170.v1

48 countries, highlighting the broad spectrum of the virus and its remarkable ability to infect new animal species [11].

In the United States, SARS-CoV-2 infection in free-ranging wildlife has been sporadic and confirmed in only nine species, including white-tailed deer (WTD), Eastern cottontail, Virginia opossum, deer mice, Eastern red bat, raccoon, groundhog, wild American mink, and mule deer [2,12]. Of note, aside from a single reported infection in an Eastern red bat [2], SARS-CoV-2 has not been detected in wild North American bats, and both little brown bats and big brown bats appeared to be resistant to infection [13–15]. In addition to SARS-CoV-2 infection, serological evidence of SARS-CoV-2 exposure has been reported in coyote, muskrat, Eastern gray squirrel, and white-footed mouse [2,16]. However, to date, WTD remain the only free-ranging wildlife species with widespread exposure to SARS-CoV-2 and are considered capable of sustaining within-species transmission with the potential for spillback to humans [6,12,17–24].

Since its emergence, numerous SARS-CoV-2 variants have been detected in humans and an increasing number of animals, raising concerns about whether free-ranging wildlife species, especially those living in urban and peri-urban areas, may also be infected through spillover from humans [2,18,19,22]. The establishment of wildlife species as reservoir hosts, which could potentially propagate new SARS-CoV-2 variants and facilitate onward transmission to humans and other animals, remains a concern [2,18]. Consequently, understanding the epidemiology and evolutionary dynamics of SARS-CoV-2 in wildlife is important for assessing potential human-animal transmission and monitoring the emergence of novel variants that may spill back into human populations [21].

Multiple studies across the United States examining the circulation of SARS-CoV-2 in free-ranging wildlife have shown that transmission dynamics and prevalence vary significantly across regions, reflecting differences in local epidemiological and ecological conditions [2,25–30]. However, data on SARS-CoV-2 circulation in New England wildlife are limited, and the impact of the virus on local wildlife health is unclear. These findings collectively emphasize the need for ongoing surveillance to identify potential wildlife hosts that could harbor and spread the virus, support viral evolution, and facilitate possible spillback to humans.

Wildlife rehabilitation centers (WRCs) and wildlife clinics provide access to a diverse range of understudied species that can be challenging to sample in the field. They also provide opportunities for frequent human–animal interactions, potentially increasing the risk of spillover (Yabsley, 2019), making WRCs particularly valuable for pathogen surveillance, including SARS-CoV-2 [31].

In this study, we investigated SARS-CoV-2 infection and serological evidence of past exposure in 28 wildlife species, either admitted to rehabilitation centers and wildlife clinics or obtained through hunter harvests in New England and Virginia between 2022 and 2025, to better understand the prevalence of SARS-CoV-2 in free-ranging wildlife.

2. Materials and Methods

2.1. Sample Collection

From 2022 to 2025, we leveraged our ongoing surveillance efforts through a network of wildlife clinics, rehabilitation facilities, and State Fish and Wildlife Agencies across New England and Virginia to collect 2,053 nasal, oropharyngeal, and rectal swab samples, as well as 502 serum samples from 1,646 animals representing 28 peri-urban wildlife species (Table 1). A subset of the samples (314 nasal and oropharyngeal swabs and 40 serum samples) was collected from hunter-harvested white-tailed deer at check stations in Vermont and Massachusetts in 2024. 80.2% (n=1,647) of the swabs and 88.8% (n=446) of the serum samples were collected in Massachusetts, and the remaining samples were collected in Connecticut, Maine, Rhode Island, Vermont, and Virginia (Figure 1). Each center received sampling supplies along with standard operating procedures for collecting, storing, and shipping samples to the laboratory.

Swabs were placed in individually labeled sterile vials containing 700 µL of viral transport media [32] and stored at -20°C in the rehabilitation facilities until shipped to the laboratory. In the

 doi:10.20944/preprints202605.1170.v1

laboratory, all swab specimens were stored at -80°C, and serum samples were maintained at -20°C until testing. Metadata for each animal tested for SARS-CoV-2, including species name, animal ID, sample type, collection date, location, and the facility that collected the samples, are provided in Supplementary Table 1 (Table S1).

Table 1. Number and type of samples collected from wildlife.

Species	Scientific name	# Unique animals	# Swabs	# Serum samples
Big brown bat	*Eptesicus fuscus*	219	342	4
Black Bear	*Neovison vison*	6	6	1
Bobcat	*Lynx rufus*	22	26	11
Eastern chipmunk	*Tamias striatus*	18	25	4
Eastern cottontail	*Sylvilagus floridanus*	413	437	193
Eastern coyote	*Canis latrans*	20	23	11
Eastern gray squirrel	*Sciurus carolinensis*	166	194	51
Eastern red bat	*Lasiurus borealis*	3	4	-
Eastern small-footed bat	*Myotis leibii*	3	5	-
Evening Bat	*Nycticeius humeralis*	3	3	-
Fisher	*Pekania pennanti*	14	16	1
Gray fox	*Urocyon cinereoargenteus*	19	20	4
Groundhog	*Marmota monax*	42	44	10
Little brown bat	*Myotis lucifugus*	2	2	-
Long-tailed Weasel	*Mustela frenata*	4	4	3
Muskrat	*Ondatra zibethicus*	13	13	2
North American beaver	*Castor canadensis*	10	11	5
North American porcupine	*Erethizon dorsatum*	46	46	17
Norway rat	*Rattus norvegicus*	17	17	8
Raccoon	*Procyon lotor*	165	168	52
Red fox	*Vulpes vulpes*	57	72	13
Red squirrel	*Sciurus vulgaris*	11	11	4
River otter	*Lontra canadensis*	5	6	-
Silver-haired bat	*Lasionycteris noctivagans*	2	3	-
Southern Flying Squirrel	*Glaucomys volans*	6	12	-
Striped skunk	*Mephitis mephitis*	64	65	22
Virginia opossum	*Didelphis virginiana*	132	139	34
White-footed mouse	*Peromyscus leucopus*	5	5	-
White-tailed deer (WTD)*	*Odocoileus virginianus*	159	334	51
Total		**1,646**	**2,053**	**502**

**314 swabs and 40 serum samples were collected from hunter-harvested WTD.*

 doi:10.20944/preprints202605.1170.v1

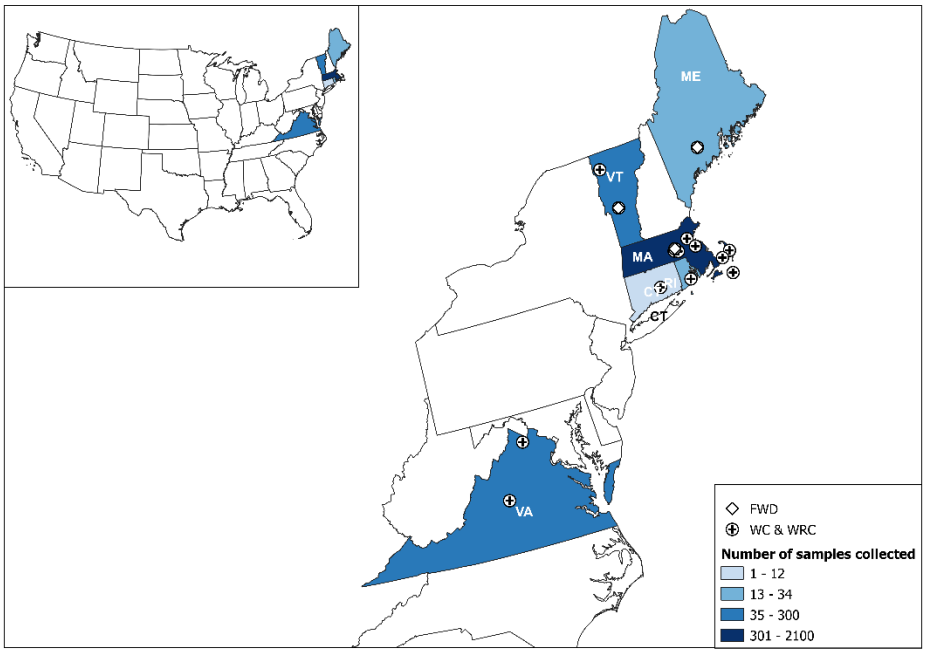

Figure 1. Map showing sample collection sites for SARS-CoV-2 surveillance in the Northeastern United States from 2022 to 2025. *Color intensity indicates the relative number of samples collected per state (CT: Connecticut, ME: Maine, MA: Massachusetts, RI: Rhode Island, VT: Vermont, VA: Virginia). Symbols denote the locations of State Fish and Wildlife Agencies (FWD), wildlife clinics (WC), and wildlife rehabilitation centers (WRC) that contributed samples.*

2.2. RNA Extraction and SARS-CoV-2 RT-qPCR

RNA was extracted from swabs using the Mag-Bind Viral DNA/RNA Extraction Kit (Omega Bio-Tek, Norcross, GA, USA) on a KingFisher Flex platform, following the manufacturer's protocol. All samples were tested for SARS-CoV-2 viral RNA by semi-quantitative real-time reverse transcription PCR (rRT-PCR) on a StepOnePlus platform (ABI). The assay was performed as previously described [33] using qScript XLT 1-Step RT-PCR ToughMix (VWR Cat. No. 89236-672) with two primer sets: one targeting the SARS-CoV-2 ORF1b gene and another for the internal control (β-actin). The positive Cq cutoff value was set at 40 cycles. Each plate included viral transport medium (VTM) as a negative control and a positive control (NR-52285: Genomic RNA from SARS-CoV-2, isolate USA-WA1/2020, BEI Resources). Following initial SARS-CoV-2 RNA detection, positive samples were submitted to the National Veterinary Services Laboratories (NVSL) for confirmation.

SARS-CoV-2 genome sequencing and phylogeographic analyses

RNA extracted from positive specimens was subjected to cDNA synthesis using ProtoScript II (New England Biolabs, cat. E6560), followed by whole-genome amplification with two custom and interleaved primer panel sets targeting 1.5- and 2-kb overlapping regions across the SARS-CoV-2 genome, as previously described [34]. PCR amplification was done with Q5 Hot Start High-Fidelity DNA polymerase (New England Biolabs, cat. M0493) in a 25 µL reaction volume. Amplicons were then purified using 1.8X ratio of Ampure XT beads (Beckman Coulter, A63882) and verified by gel electrophoresis. Next, we performed library preparation using the Nextera XT kit (Illumina, cat. FC-131-1096) followed by paired-end sequencing (2x150 nt) on the Illumina MiSeq platform. SARS-CoV-2 genomes were assembled and subjected to quality control using the vRAPID pipeline [35]. Genotypic analysis and clade/lineage assignment of complete genomes were performed using Nextclade CLI (v3.12.0) and pangolin (v4.3) [36,37].

For the phylogeographic analysis, we compiled a dataset of SARS-CoV-2 sequences from 2023–2024, including all publicly available deer sequences from GISAID and BV-BRC, human sequences from Vermont and neighboring states (Massachusetts, New Hampshire, and New York), and the 50 most similar sequences identified through a BLAST search in GenBank. Whole-genome sequences

 doi:10.20944/preprints202605.1170.v1

were aligned using MAFFT v7.526 [38], redundant sequences were removed with CD-HIT v4.8.1 [39], and alignments were manually inspected in BioEdit 7.2.5 [40]. We inferred a maximum-likelihood phylogeny with IQ-TREE v2.4 [41], using ModelFinder [42] to choose the best-fitting nucleotide substitution model, and to identify clades corresponding to independent introductions of SARS-CoV-2 lineages into Vermont white-tailed deer. The resulting phylogenetic tree was used to downsample the dataset for subsequent analyses by excluding outgroup sequences.

A total of 105 sequences were used for subsequent phylogenetic and evolutionary analyses. To investigate the evolutionary history and estimate the time to the most recent common ancestor of the SARS-CoV-2 sequences generated in this study, we performed a discrete phylogeographic reconstruction using a Bayesian Markov Chain Monte Carlo (MCMC) approach in BEAST 1.10.4 [43]. We used the GTR substitution model with gamma-distributed rate variation among sites with a strict molecular clock model. MCMC chains were run until convergence was reached for all parameters (effective sample size (ESS) > 200). After discarding 10% of the sampled trees as burn-in, a maximum clade credibility (MCC) tree was summarized using TreeAnnotator v1.10.4 and visualized using the ggtree (v3.8.2) package in R.

2.3. SARS-CoV-2 Antibody Detection by ELISA

Serum samples were tested for antibodies against SARS-CoV-2 RBD using an in-house ELISA with minor modifications [33]. Briefly, Immulon 4 HB plates were coated with 2 µg/mL purified SARS-CoV-2 RBD (NR-52366, BEI Resources) and incubated for 24 hours at 4 °C. Following incubation, plates were washed three times with phosphate-buffered saline supplemented with 0.1% Tween-20 (PBS-T) and blocked with Pierce Protein-Free Blocking Buffer (Thermo Fisher catalog no. PI37573) at room temperature for 1 hour. Serum samples were diluted 1:5 in PBS in different plates and then transferred to the ELISA plates with a final dilution of 1:50 in PBS-T and 1% milk. The plates were then incubated at room temperature for 2 hours. Positive controls consisted of serum from spike protein-immunized alpacas. After incubation, plates were washed three times, and 50 µL of Pierce Recombinant Peroxidase Conjugated Protein A/G (Thermo Fisher catalog no. 32490) was added at 1:10,000 in PBS-T containing 1% milk and incubated for 1 hour at room temperature. Plates were then washed and developed with SigmaFast o-phenylenediamine dihydrochloride tablets (Sigma-Aldrich catalog no. P9187) for 10 minutes, stopped with 50 µL 3M HCl, and read at 490 nm on a BioTek Synergy 4 Multidetection Plate Reader. The positivity cutoff was set as $\mu + 3\sigma$ of the negative controls ($n = 8$), derived from pre-pandemic wildlife serum samples.

Positive samples from the initial ELISA-RBD screening were validated by a confirmatory ELISA using 3-fold serial dilutions starting at 1:100 against the full-length SARS-CoV-2 spike protein (BEI Resources, NR-52308) [44]. True positives were defined as samples showing a signal greater than the mean plus three standard deviations ($\mu + 3\sigma$) of the negative controls in at least the first two consecutive dilutions.

SARS-CoV-2 neutralization assay

We performed a SARS-CoV-2 virus neutralization test (VNT) in our BSL-3 facility at the New England Regional Biosafety Laboratory (NERBL) to assess neutralizing antibodies in ELISA-positive sera. Serum samples were heat-inactivated (56 °C, 30 min) and serially two-fold diluted starting at 1:4, in duplicate. Dilutions were incubated with 200 $TCID_{50}$ of SARS-CoV-2 Omicron (isolate HCoV-19/USA/MDHP20874/2021; BEI Resources NR-56461) for 1 h at room temperature. The virus–serum mixtures were added to confluent Vero E6 cells (ATCC CRL-1586) in 96-well plates and incubated at 37 °C with 5% CO_2. Cytopathic effect was assessed at 5 days post-infection, and the neutralization titer was defined as the highest serum dilution that achieved complete (100%) inhibition of CPE in both replicate wells. Neutralization titers ≥16 were considered seropositive.

 doi:10.20944/preprints202605.1170.v1

3. Results

Prevalence of SARS-CoV-2 infection in free-ranging wildlife by RT-PCR

We tested a total of 2,053 swabs from 1,646 individual animals representing 28 wildlife species by RT-PCR. 81.3% of the swabs (n= 1673) tested positive for β-actin, indicating the presence of viable RNA. SARS-CoV-2 RNA was detected in only three white-tailed deer (WTD) (1.9%) out of 159 WTD tested (1 out of 56 in MA and 2 out of 103 in Vermont). The SARS-CoV-2–positive deer in Massachusetts was a young fawn with skull fractures recovered from a trail in 2022 and brought to Tufts Wildlife Clinic at Cummings School of Veterinary Medicine at Tufts University (RT-PCR Cq = 35). The two additional positive white-tailed deer were hunter-harvested in Vermont in November 2024, with both nasal and oral swabs testing positive. Whole-genome sequences were successfully obtained only from the two WTD sampled in Vermont (Table 2). We found no detectable SARS-CoV-2 RNA within any swab samples collected from the other wildlife species.

Whole genome sequences and phylogeographic analysis of SARS-CoV-2 in WTD

Pairwise comparison of the two SARS-CoV-2 genomes obtained from WTD in Vermont revealed 98% nucleotide identity, indicating a high degree of similarity between the sequences. The two sequences were classified within Nextstrain clade 24C and Pango lineage KP.3.2.8, and exhibited the following spike protein substitutions relative to the BA.2.86 reference strain: S12F, A27S, L212I, F456L, and Q493E (Table 2). The KP.3.2.8 lineage was among the dominant SARS-CoV-2 variants circulating among humans in many U.S. states during the time of collection in November 2024.

Table 2. SARS-CoV-2 whole genome sequences from White-tailed deer.

Animal ID	Specimen	RT-qPCR Cq value	Accession number	Clade	Pango lineage	Spike mutations
ZKOT6992	Oropharyngeal swab	25.2	EPI_ISL_19818736	24C	KP.3.2.8	S12F, A27S, L212I, F456L, Q493E
OJLN7737	Oropharyngeal swab	15.0	EPI_ISL_19818737	24C	KP.3.2.8	

We performed a time-calibrated Bayesian phylogenetic analysis to investigate the origin of SARS-CoV-2 in WTD in Vermont. The maximum clade credibility (MCC) tree showed that the two Vermont WTD sequences clustered closely with contemporaneous human sequences from Vermont and New York (Figure 2). The estimated time to the most recent common ancestor for the WTD sequences was March 2024 (95% highest posterior density [HPD]: February – April 2024). The MCC tree identified a human SARS-CoV-2 genomic sequence from Vermont as the most likely precursor of the two WTD sequences, indicating a single spillover event from human to WTD followed by circulation within deer (Figure 2). Notably, our phylogenetic analyses revealed no evidence of spillback from white-tailed deer to humans, as no human-derived genomes clustered with or were directly derived from the Vermont white-tailed deer sequences.

 doi:10.20944/preprints202605.1170.v1

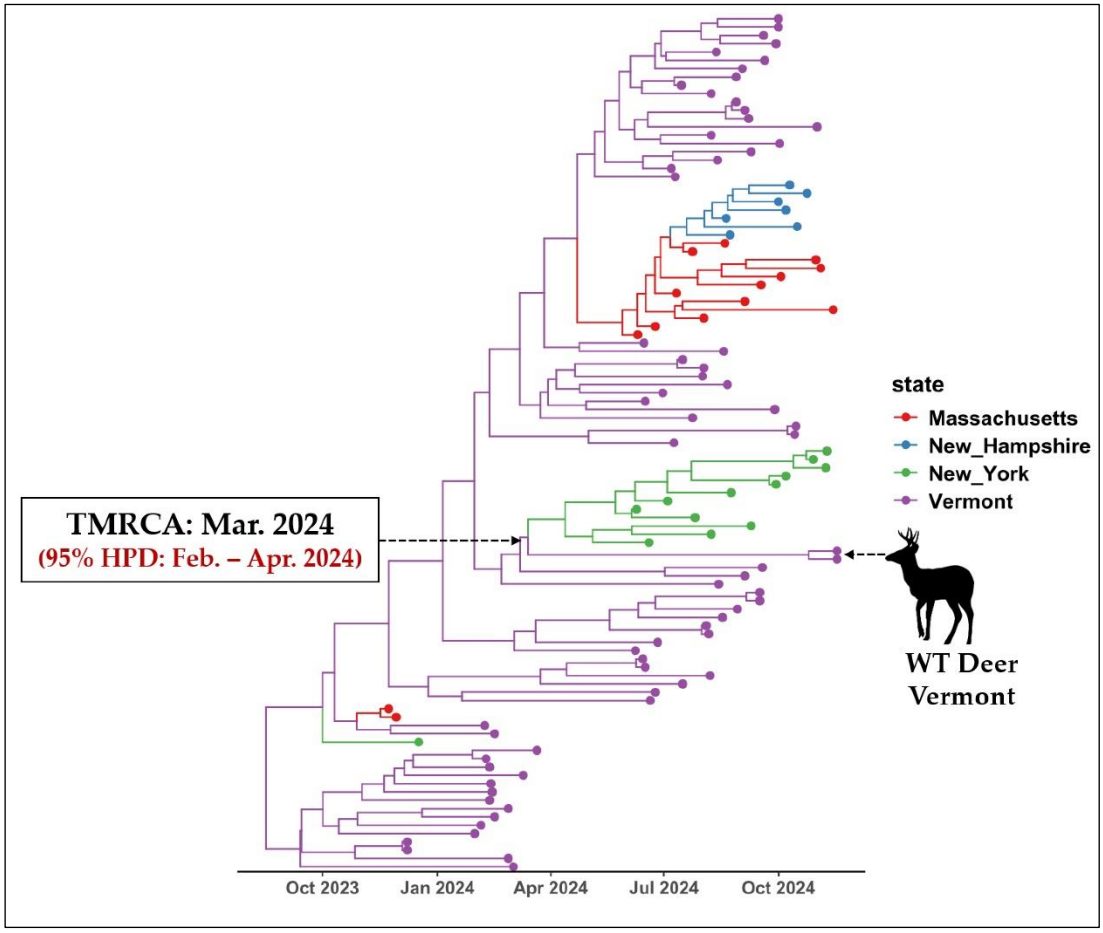

Figure 2. Maximum clade credibility (MCC) tree of Vermont WTD SARS-CoV-2 sequences. *Branches are colored by state, with the x-axis representing the time scale. Vermont deer sequences clustered with human-derived sequences from Vermont (purple) and New York (green). The box with TMRCA indicates the time to the most recent common ancestor for the Vermont WTD sequences with its 95% highest posterior density.*

Serological evidence of SARS-CoV-2 infection in wildlife

Among the 502 serum samples collected, 32 tested positive in the initial SARS-CoV-2 RBD ELISA screening. However, confirmatory testing with the SARS-CoV-2 full-spike ELISA detected antibodies in only 9 samples from three wildlife species: the Eastern cottontail (2%, n=4/193), Eastern coyote (18%, n=2/11), and raccoon (5.7%, n=3/52). All ELISA-positive samples were collected in Massachusetts between 2023 and 2024. Among the ELISA-positive samples, SARS-CoV-2–neutralizing antibodies were detected in a single Eastern Cottontail sampled in 2024 (Table 3).

Table 3. Positive samples to confirmatory SARS-CoV-2 spike ELISA

Animal ID	Common name	Scientific name	Collection date	City	VNT titers
24-1872	Eastern cottontail	Sylvilagus floridanus	2024-06-19	Sutton, MA	1:32
24-0940	Eastern cottontail	Sylvilagus floridanus	2024-05-16	Auburn, MA	<1:16
24-1313	Eastern cottontail	Sylvilagus floridanus	2024-05-31	Wrentham, MA	<1:16
24-4074	Eastern cottontail	Sylvilagus floridanus	2024-10-26	Milford, MA	<1:16
24-3041	Eastern coyote	Canis latrans	2024-08-08	Lexington, MA	<1:16
24-3142	Eastern coyote	Canis latrans	2024-08-13	Framingham, MA	<1:16
23-3009	Raccoon	Procyon lotor	2023-08-28	West Warren, MA	<1:16
23-0738	Raccoon	Procyon lotor	2023-05-08	Westford, MA	<1:16
24-4157	Raccoon	Procyon lotor	2024-01-13	Charlton, MA	<1:16

 doi:10.20944/preprints202605.1170.v1

4. Discussion

In this study, we examined the circulation of SARS-CoV-2 in 1,646 free-ranging terrestrial mammals in New England and Virginia between 2022 and 2025.

We detected SARS-CoV-2 RNA in 1.9% (n = 3/159) of white-tailed deer (WTD) sampled in New England, which is a lower prevalence compared to other states, including Ohio (35.8%), Iowa (33.2%), New York (21.1%), Pennsylvania (16.3%), and Nebraska (16.3%) [12,19,22,24,30]. However, our findings align with previous studies of SARS-CoV-2 in WTD, including surveys in Vermont that detected no infection in white-tailed deer during the 2021 and 2022 harvest seasons [25], and only 0.76% of free-ranging deer in Florida tested positive [45].

White-tailed deer are susceptible to SARS-CoV-2 infection and can transmit the virus, as demonstrated in multiple reports of natural infection [12,20,22,24] and experimental studies [8,17,23]. Our phylogeographic analyses showed that SARS-CoV-2 was transmitted into WTD in Vermont via a single spillover event from an infected human. To date, natural SARS-CoV-2 infections detected in white-tailed deer have primarily originated from spillovers from humans, with subsequent instances of deer-to-deer transmission [12,19]. The limited prevalence of SARS-CoV-2 in Massachusetts and Vermont deer may reflect reduced human–deer contact and lower viral pressure [25]. Vermont is sparsely populated, including mostly hunting areas, which likely limits direct or indirect exposure of deer to humans. Moreover, human COVID-19 incidence in Vermont was markedly lower than in neighboring states during our surveillance period, further reducing opportunities for human-to-deer spillover [46].

Compared with other U.S. states where SARS-CoV-2 has been widespread among free-ranging wildlife, including reports from Virginia [2], we found active infections only in white-tailed deer, not in any other sampled species. Our results are consistent with earlier reports from Vermont and various other U.S states, where active infections were either limited to white-tailed deer or not present in other wildlife species [25–28,30]. Collectively, these results suggest that SARS-CoV-2 spillover and subsequent animal-to-animal transmission likely depend on a complex interplay of ecological conditions, human infection dynamics, and species-specific susceptibility, which may limit sustained circulation in some regions such as New England.

Bats are recognized as reservoirs for numerous zoonotic viruses, including members of the Coronaviridae family [47]. In this study, we tested 359 swabs from six bat species, with 95% of the swabs from big brown bats, to assess their potential susceptibility to SARS-CoV-2. No SARS-CoV-2 RNA was detected in any of the samples. These findings are consistent with previous experimental challenge studies, which concluded that North American bat species, including big brown and little brown bats, are resistant to SARS-CoV-2 infection [15,48]. Nevertheless, it is worth noting that SARS-CoV-2 RNA was detected in one Eastern red bat, although the context of exposure remains unclear, and this species has not been evaluated in laboratory experiments [2].

Although no active SARS-CoV-2 infection was found in wildlife except in WTD, we detected evidence of exposure in eastern cottontails, coyotes, and raccoons, with neutralizing antibodies detected in only one eastern cottontail. While the exact route of exposure remains uncertain, the presence of eastern cottontails in urban or peri-urban areas and their frequent interactions with humans or human-contaminated environments may have allowed for potential spillover [49]. Despite experimental evidence indicating limited susceptibility of eastern cottontails to earlier SARS-CoV-2 strains [50], SARS-CoV-2 RNA has been detected in eastern cottontails in Virginia [2], supporting the possibility of field exposure and suggesting that susceptibility may vary with circulating viral strains.

None of the ELISA-positive samples from coyotes and raccoons demonstrated neutralizing activity against SARS-CoV-2, which may indicate previous exposure but likely no strong immune response. This aligns with reports suggesting that both species have been exposed to the virus [2,16]. Notably, prior experimental challenge studies have found that coyotes and raccoons are unlikely to serve as reservoir hosts for SARS-CoV-2 [1,9].

 doi:10.20944/preprints202605.1170.v1

While our study provides valuable insights into the prevalence of SARS-CoV-2 in free-ranging wildlife in the northeastern United States, a few limitations should be considered. First, samples were collected opportunistically, and sample sizes were limited for some species (fewer than five individuals). **Additionally, most of our samples (>80%) were collected in Massachusetts,** which may not reflect the full ecological diversity of wildlife in the region and may bias prevalence estimates toward species or locations that were more accessible during sampling. Second, although we collected 2,053 swab samples, serum, which is more likely to give information on prior exposure, was available for only a subset of the animals tested (n = 502), limiting our ability to assess prior exposure for certain species.

Despite these limitations, our findings provide important baseline data on SARS-CoV-2 exposure in wildlife in the northeastern United States, highlighting the need for continuous surveillance and experimental studies to clarify species susceptibility and spillover risk.

Supplementary Materials: The following supporting information can be downloaded at: Preprints.org, Table S1. List of free-ranging wildlife specimens collected between 2022 and 2025 in New England and Virginia and tested for SARS-CoV-2 RNA and antibodies.

Author Contributions: Conceptualization, I.N.S., W.B.P. and J.A.R.; methodology, I.N.S., W.B.P. and J.A.R.; validation, W.B.P. and J.A.R.; formal analysis, I.N.S., W.B.P., Z.K. and H.B.; investigation, I.N.S., W.B.P., A.F.S., E.C., M.M., Z.K., H.B., M.J.R.F., Z.K., P.P., J.R. and B.H.; resources, I.N.S., W.B.P., A.F.S., J.A.R., E.C., M.M., M.J.R.F., Z.K., P.P., J.R. and B.H.; data curation, I.N.S., W.B.P. and E.C.; writing—original draft preparation, I.N.S.; writing—review and editing, I.N.S., W.B.P., A.F.S., E.C., M.M., Z.K., H.B., M.J.R.F., Z.K., P.P., J.R., B.H. and J.A.R.; visualization, I.N.S.; supervision, W.B.P. and J.A.R.; project administration, J.A.R.; funding acquisition, J.A.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by USDA contract AP23OA000000C022, NIH/NIAID Center of Excellence for Influenza Research and Response (CEIRR) contract 75N93021C00014, and NIH UC7AI180310 Biocontainment Laboratory Operation Cooperative Agreement. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Institutional Review Board Statement: This study was approved by Tufts University Institutional Animal Care and Use Committee (Number: G2023-02).

Data Availability Statement: The data that support the findings of this study are available as supplemental material.

Acknowledgments: We sincerely thank the wildlife rehabilitation centers, wildlife clinics, and the many staff members and volunteers who contributed to sample collection for this study. We are especially grateful to Beth Murthy of Return 2 Wild; Ceacy Henderson of the Colrain Center for Conservation and Wildlife; Maureen Heidtman and Kelle McDougal of Winghand Bat Rehabilitation & Education; Barry Genzlinger of Vermont Bat Center; Elka Hutcheson of Rockfish Wildlife Sanctuary; Stephanie Ellis and Jennifer Taylor of Wild Care; and Tegwin Taylor and Danielle D'Auria of Maine Department of Inland Fisheries and Wildlife for their assistance in providing biological samples. We thank Nick Fortin and Noel Dodge of the Fish and Wildlife Department, Vermont Agency of Natural Resources, for their assistance in obtaining the white-tailed deer samples in Vermont. We also thank Drs. Florian Krammer and Charles Shoemaker for generously providing purified SARS-CoV-2 proteins and serum from spike (S) protein–immunized alpacas, respectively. In addition, we thank Dr. Kaitlin Sawatzki for her contributions to the early development of the assays used in this study. The following reagent was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH: SARS-Related Coronavirus 2, isolate HCoV-19/USA/MD-HP20874/2021 (NR-56461). The following reagents were produced under HHSN272201400008C and obtained through BEI Resources, NIAID, NIH: Spike Glycoprotein Receptor Binding Domain (RBD) from SARS-Related Coronavirus 2, Wuhan-Hu-1, with C-terminal histidine tag, recombinant from HEK293F cells (NR-52366); and stabilized Spike Glycoprotein from SARS-Related Coronavirus 2, Wuhan-Hu-1, with C-terminal histidine tag, recombinant from baculovirus (NR-

 doi:10.20944/preprints202605.1170.v1

52308). Biosafety level 3 work was conducted at the New England Regional Biosafety Laboratory with support from the National Institute of Health under Grant AI180310 "Resources and workforce development for the New England Regional Biosafety Laboratory".

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Porter, S.M.; Hartwig, A.E.; Bielefeldt-Ohmann, H.; Bosco-Lauth, A.M.; Root, J.J. Susceptibility of Wild Canids to SARS-CoV-2. *Emerg. Infect. Dis.* **2022**, *28*, 1852–1855.
2. Goldberg, A.R.; Langwig, K.E.; Brown, K.L.; Marano, J.M.; Rai, P.; King, K.M.; Sharp, A.K.; Ceci, A.; Kailing, C.D.; Kailing, M.J.; et al. Widespread Exposure to SARS-CoV-2 in Wildlife Communities. *Nature Communications 2024 15:1* **2024**, *15*, 1–13.
3. Delahay, R.J.; de la Fuente, J.; Smith, G.C.; Sharun, K.; Snary, E.L.; Flores Girón, L.; Nziza, J.; Fooks, A.R.; Brookes, S.M.; Lean, F.Z.X.; et al. Assessing the Risks of SARS-CoV-2 in Wildlife. *One Health Outlook* **2021**, *3*, 1–14.
4. Gaudreault, N.N.; Trujillo, J.D.; Carossino, M.; Meekins, D.A.; Morozov, I.; Madden, D.W.; Indran, S. V.; Bold, D.; Balaraman, V.; Kwon, T.; et al. SARS-CoV-2 Infection, Disease and Transmission in Domestic Cats. *Emerg. Microbes Infect.* **2020**, *9*, 2322–2332.
5. Fang, R.; Yang, X.; Guo, Y.; Peng, B.; Dong, R.; Li, S.; Xu, S. SARS-CoV-2 Infection in Animals: Patterns, Transmission Routes, and Drivers. *Eco-Environment & Health* **2024**, *3*, 45–54.
6. Chandler, J.C.; Bevins, S.N.; Ellis, J.W.; Linder, T.J.; Tell, R.M.; Jenkins-Moore, M.; Root, J.J.; Lenoch, J.B.; Robbe-Austerman, S.; DeLiberto, T.J.; et al. SARS-CoV-2 Exposure in Wild White-Tailed Deer (Odocoileus Virginianus). *Proc. Natl. Acad. Sci. U. S. A.* **2021**, *118*, e2114828118.
7. Adney, D.R.; Lovaglio, J.; Schulz, J.E.; Yinda, C.K.; Avanzato, V.A.; Haddock, E.; Port, J.R.; Holbrook, M.G.; Hanley, P.W.; Saturday, G.; et al. Severe Acute Respiratory Disease in American Mink Experimentally Infected with SARS-CoV-2. *JCI Insight* **2022**, *7*.
8. Palmer, M. V.; Martins, M.; Falkenberg, S.; Buckley, A.; Caserta, L.C.; Mitchell, P.K.; Cassmann, E.D.; Rollins, A.; Zylich, N.C.; Renshaw, R.W.; et al. Susceptibility of White-Tailed Deer (Odocoileus Virginianus) to SARS-CoV-2. *J. Virol.* **2021**, *95*.
9. Francisco, R.; Hernandez, S.M.; Mead, D.G.; Adcock, K.G.; Burke, S.C.; Nemeth, N.M.; Yabsley, M.J. Experimental Susceptibility of North American Raccoons (Procyon Lotor) and Striped Skunks (Mephitis Mephitis) to SARS-CoV-2. *Front. Vet. Sci.* **2022**, *8*, 715307.
10. Porter, S.M.; Hartwig, A.E.; Bielefeldt-Ohmann, H.; Marano, J.M.; Root, J.J.; Bosco-Lauth, A.M. Experimental SARS-CoV-2 Infection of Elk and Mule Deer - Volume 30, Number 2—February 2024 - Emerging Infectious Diseases Journal - CDC. *Emerg. Infect. Dis.* **2024**, *30*, 354–357.
11. Food and Agriculture Organization SARS-CoV-2 in Animals Available online: https://www.fao.org/animal-health/situation-updates/sars-cov-2-in-animals/en (accessed on 29 November 2025).
12. Hale, V.L.; Dennis, P.M.; McBride, D.S.; Nolting, J.M.; Madden, C.; Huey, D.; Ehrlich, M.; Grieser, J.; Winston, J.; Lombardi, D.; et al. SARS-CoV-2 Infection in Free-Ranging White-Tailed Deer. *Nature 2022 602:7897* **2021**, *602*, 481–486.
13. Moran, M.L.; Boyd, W.; De La Cruz, J.L.; Bertke, A.S.; Ford, W.M. Oral Sampling of Little Brown Bat (Myotis Lucifugus) Maternity Colonies for SARS-CoV-2 in the Northeast and Mid-Atlantic, USA. *Animals 2023, Vol. 13, Page 550* **2023**, *13*, 550.
14. Hall, J.S.; Knowles, S.; Nashold, S.W.; Ip, H.S.; Leon, A.E.; Rocke, T.; Keller, S.; Carossino, M.; Balasuriya, U.; Hofmeister, E. Experimental Challenge of a North American Bat Species, Big Brown Bat (Eptesicus Fuscus), with SARS-CoV-2. *Transbound. Emerg. Dis.* **2021**, *68*, 3443–345.
15. Hall, J.S.; Nashold, S.; Hofmeister, E.; Leon, A.E.; Falendysz, E.A.; Ip, H.S.; Malavé, C.M.; Rocke, T.E.; Carossino, M.; Balasuriya, U.; et al. Little Brown Bats (Myotis Lucifugus) Are Resistant to SARS-CoV-2 Infection. *J. Wildl. Dis.* **2024**, *60*, 924–930.

 doi:10.20944/preprints202605.1170.v1

16. Wilson-Henjum, G.; Jeffrey Root, J.; Worgo, A.; Chandler, J.; Dyer, R.; Flores, J.; Morris, J.; Plummer, I.; Seman, J.P.; Van Why, K.; et al. Community-Scale Surveillance of SARS-CoV-2 and Influenza A Viruses in Wild Mammals, United States, 2022–2023. *Emerg. Infect. Dis.* **2025**, *31*, 1625–1629.
17. Martins, M.; Boggiatto, P.M.; Buckley, A.; Cassmann, E.D.; Falkenberg, S.; Caserta, L.C.; Fernandes, M.H.V.; Kanipe, C.; Lager, K.; Palmer, M. V.; et al. From Deer-to-Deer: SARS-CoV-2 Is Efficiently Transmitted and Presents Broad Tissue Tropism and Replication Sites in White-Tailed Deer. *PLoS Pathog.* **2022**, *18*, e1010197.
18. McBride, D.S.; Garushyants, S.K.; Franks, J.; Magee, A.F.; Overend, S.H.; Huey, D.; Williams, A.M.; Faith, S.A.; Kandeil, A.; Trifkovic, S.; et al. Accelerated Evolution of SARS-CoV-2 in Free-Ranging White-Tailed Deer. *Nat. Commun.* **2023**, *14*, 5105.
19. Kuchipudi, S. V.; Surendran-Nair, M.; Ruden, R.M.; Yon, M.; Nissly, R.H.; Vandegrift, K.J.; Nelli, R.K.; Li, L.; Jayarao, B.M.; Maranas, C.D.; et al. Multiple Spillovers from Humans and Onward Transmission of SARS-CoV-2 in White-Tailed Deer. *Proc. Natl. Acad. Sci. U. S. A.* **2022**, *119*, e2121644119.
20. Vandegrift, K.J.; Yon, M.; Surendran Nair, M.; Gontu, A.; Ramasamy, S.; Amirthalingam, S.; Neerukonda, S.; Nissly, R.H.; Chothe, S.K.; Jakka, P.; et al. SARS-CoV-2 Omicron (B.1.1.529) Infection of Wild White-Tailed Deer in New York City. *Viruses* **2022**, *14*, 2770.
21. Feng, A.; Bevins, S.; Chandler, J.; DeLiberto, T.J.; Ghai, R.; Lantz, K.; Lenoch, J.; Retchless, A.; Shriner, S.; Tang, C.Y.; et al. Transmission of SARS-CoV-2 in Free-Ranging White-Tailed Deer in the United States. *Nature Communications 2023 14:1* **2023**, *14*, 1–17.
22. Marques, A.D.; Sherrill-Mix, S.; Everett, J.K.; Adhikari, H.; Reddy, S.; Ellis, J.C.; Zeliff, H.; Greening, S.S.; Cannuscio, C.C.; Strelau, K.M.; et al. Multiple Introductions of SARS-CoV-2 Alpha and Delta Variants into White-Tailed Deer in Pennsylvania. *mBio* **2022**, *13*.
23. Cool, K.; Gaudreault, N.N.; Morozov, I.; Trujillo, J.D.; Meekins, D.A.; Mcdowell, C.; Carossino, M.; Bold, D.; Mitzel, D.; Kwon, T.; et al. Infection and Transmission of Ancestral SARS-CoV-2 and Its Alpha Variant in Pregnant White-Tailed Deer. *Emerg. Microbes Infect.* **2022**, *11*, 95–112.
24. Caserta, L.C.; Martins, M.; Butt, S.L.; Hollingshead, N.A.; Covaleda, L.M.; Ahmed, S.; Everts, M.R.R.; Schuler, K.L.; Diel, D.G. White-Tailed Deer (Odocoileus Virginianus) May Serve as a Wildlife Reservoir for Nearly Extinct SARS-CoV-2 Variants of Concern. *Proc. Natl. Acad. Sci. U. S. A.* **2023**, *120*, e2215067120.
25. Despres, H.W.; Mills, M.G.; Schmidt, M.M.; Gov, J.; Perez, Y.; Jindrich, M.; Crawford, A.M.L.; Kohl, W.T.; Rosenblatt, E.; Kubinski, H.C.; et al. Surveillance of Vermont Wildlife in 2021–2022 Reveals No Detected SARS-CoV-2 Viral RNA. *Scientific Reports 2023 13:1* **2023**, *13*, 1–8.
26. Castañeda, D.; Isaac, E.J.; Schnieders, B.P.; Kautz, T.; Romanski, M.C.; Moore, S.A.; Aliota, M.T. Absence of SARS-CoV-2 in Wildlife of Northeastern Minnesota and Isle Royale National Park. *Zoonoses Public Health* **2024**, *71*, 744–747.
27. Ehrlich, M.; Madden, C.; McBride, D.S.; Nolting, J.M.; Huey, D.; Kenney, S.; Wang, Q.; Saif, L.J.; Vlasova, A.; Dennis, P.; et al. Lack of SARS-CoV-2 Viral RNA Detection among a Convenience Sampling of Ohio Wildlife, Companion, and Agricultural Animals, 2020–2021. *Animals* **2023**, *13*, 2554.
28. Yaglom, H.D.; Van Pelt, L.; Howard, A.L.; Jansen, B.; Smith, P.; Sorensen, R.; Hecht, G.; Venkat, H.; Justice-Allen, A.; Bergman, D.L.; et al. Convenience Sampling Yields No Evidence of SARS-CoV-2 Infection in Free-Ranging Mammalian Wildlife in Arizona, USA, 2021–23. *J. Wildl. Dis.* **2024**, *60*, 1016–1020.
29. Sims, M.; Helal, Z.; Levin, M.; Rittenhouse, T.; Hawley, J.; Risatti, G.R. Suburban Population of Bobcats (Lynx Rufus) in Connecticut, USA, Tested Negative for SARS-CoV-2, November 2021–February 2022. *J. Wildl. Dis.* **2024**, *60*, 193–197.
30. Loy, D.S.; Birn, R.; Poonsuk, K.; Tegomoh, B.; Bartling, A.; Wiley, M.R.; Loy, J.D. SARS-CoV-2 Surveillance and Detection in Wild, Captive, and Domesticated Animals in Nebraska: 2021–2023. *Front. Vet. Sci.* **2024**, *11*, 1496207.
31. Yabsley, M.J. The Role of Wildlife Rehabilitation in Wildlife Disease Research and Surveillance. *Medical Management of Wildlife Species: A Guide for Practitioners* **2019**, 159–165.
32. WHO *Collecting, Preserving and Shipping Specimens for the Diagnosis of Avian Influenza A(H5N1) Virus Infection : Guide for Field Operations*; 2006.

 doi:10.20944/preprints202605.1170.v1

33. Sawatzki, K.; Hill, N.J.; Puryear, W.B.; Foss, A.D.; Stone, J.J.; Runstadler, J.A. Host Barriers to SARS-CoV-2 Demonstrated by Ferrets in a High-Exposure Domestic Setting. *Proc. Natl. Acad. Sci. U. S. A.* **2021**, *118*, e2025601118.
34. Gonzalez-Reiche, A.S.; Alshammary, H.; Schaefer, S.; Patel, G.; Polanco, J.; Carreño, J.M.; Amoako, A.A.; Rooker, A.; Cognigni, C.; Floda, D.; et al. Sequential Intrahost Evolution and Onward Transmission of SARS-CoV-2 Variants. *Nature Communications 2023 14:1* **2023**, *14*, 1–13.
35. Khalil, Z.; Sullivan, M.; Gonzalez-Reiche, A.S.; Obla, A.; Bakel, H. van VRAPID: Virus Reference-Based Assembly Pipeline and IDentification. *Zenodo* **2023**.
36. Aksamentov, I.; Roemer, C.; Hodcroft, E.; Neher, R. Nextclade: Clade Assignment, Mutation Calling and Quality Control for Viral Genomes. *J. Open Source Softw.* **2021**, *6*, 3773.
37. O'Toole, Á.; Scher, E.; Underwood, A.; Jackson, B.; Hill, V.; McCrone, J.T.; Colquhoun, R.; Ruis, C.; Abu-Dahab, K.; Taylor, B.; et al. Assignment of Epidemiological Lineages in an Emerging Pandemic Using the Pangolin Tool. *Virus Evol.* **2021**, *7*.
38. Katoh, K.; Standley, D.M. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Mol. Biol. Evol.* **2013**, *30*, 772–780.
39. Fu, L.; Niu, B.; Zhu, Z.; Wu, S.; Li, W. CD-HIT: Accelerated for Clustering the next-Generation Sequencing Data. *Bioinformatics* **2012**, *28*, 3150–3152.
40. Hall, T.A. BioEdit 7.2. 5. BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows. **2013**, *95*, 98.
41. Nguyen, L.T.; Schmidt, H.A.; Von Haeseler, A.; Minh, B.Q. IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. *Mol. Biol. Evol.* **2015**, *32*, 268–274.
42. Kalyaanamoorthy, S.; Minh, B.Q.; Wong, T.K.F.; Von Haeseler, A.; Jermiin, L.S. ModelFinder: Fast Model Selection for Accurate Phylogenetic Estimates. *Nature Methods 2017 14:6* **2017**, *14*, 587–589.
43. Suchard, M.A.; Lemey, P.; Baele, G.; Ayres, D.L.; Drummond, A.J.; Rambaut, A. Bayesian Phylogenetic and Phylodynamic Data Integration Using BEAST 1.10. *Virus Evol.* **2018**, *4*.
44. Stadlbauer, D.; Amanat, F.; Chromikova, V.; Jiang, K.; Strohmeier, S.; Arunkumar, G.A.; Tan, J.; Bhavsar, D.; Capuano, C.; Kirkpatrick, E.; et al. SARS-CoV-2 Seroconversion in Humans: A Detailed Protocol for a Serological Assay, Antigen Production, and Test Setup. *Curr. Protoc. Microbiol.* **2020**, *57*, e100.
45. Grace, S.G.; Wilson, K.N.; Dorleans, R.; White, Z.S.; Pu, R.; Gaudreault, N.N.; Cool, K.; Campos Krauer, J.M.; Franklin, L.E.; Clemons, B.C.; et al. Low Prevalence of SARS-CoV-2 in Farmed and Free-Ranging White-Tailed Deer in Florida. *Viruses* **2024**, *16*, 1886.
46. Vermont Department of Health *Weekly COVID-19 Surveillance Report*; 2024.
47. Wong, A.C.P.; Li, X.; Lau, S.K.P.; Woo, P.C.Y. Global Epidemiology of Bat Coronaviruses. *Viruses 2019, Vol. 11, Page 174* **2019**, *11*, 174.
48. Hall, J.S.; Knowles, S.; Nashold, S.W.; Ip, H.S.; Leon, A.E.; Rocke, T.; Keller, S.; Carossino, M.; Balasuriya, U.; Hofmeister, E. Experimental Challenge of a North American Bat Species, Big Brown Bat (Eptesicus Fuscus), with SARS-CoV-2. *Transbound. Emerg. Dis.* **2021**, *68*, 3443–3452.
49. Hunt, V.M.; Magle, S.B.; Vargas, C.; Brown, A.W.; Lonsdorf, E. V; Sacerdote, A.B.; Sorley, E.J.; Santymire, R.M.; Magle, S.B.; Vargas, C.; et al. Survival, Abundance, and Capture Rate of Eastern Cottontail Rabbits in an Urban Park. *Urban Ecosystems 2013 17:2* **2013**, *17*, 547–560.
50. Bosco-Lauth, A.M.; Root, J.J.; Porter, S.M.; Walker, A.E.; Guilbert, L.; Hawvermale, D.; Pepper, A.; Maison, R.M.; Hartwig, A.E.; Gordy, P.; et al. Peridomestic Mammal Susceptibility to Severe Acute Respiratory Syndrome Coronavirus 2 Infection. *Emerg. Infect. Dis.* **2021**, *27*, 2073–2080.