SARS-CoV-2 serosurvey of healthy, privately owned cats presenting to a New York City animal hospital in the early phase of the COVID-19 pandemic (2020-2021)

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Abstract: Both domestic and non-domestic cats are now established to be susceptible to infection by SARS-CoV-2, the cause of the ongoing COVID-19 pandemic. While serious disease in cats may occur in some instances, the majority of infections appear to be subclinical. Differing prevalence data for SARS-CoV-2 infection of cats have been reported, and are highly context-dependent. Here, we report a retrospective serological survey of cats presented to an animal practice in New York City, located in close proximity to a large medical center that treated the first wave of COVID-19 patients in the US in the Spring of 2020. We sampled 79, mostly indoor, cats between June 2020 to May 2021, the early part of which time the community was under a strict public health "lock-down". Using a highly sensitive and specific fluorescent bead-based multiplex assay, we found an overall prevalence of 13/79 (16%) serologically-positive animals for the study period; however, cats sampled in the Fall of 2020 had a confirmed positive prevalence of 44%. For SARS-CoV-2 seropositive cats, we performed viral neutralization test with live SARS-CoV-2 to additionally confirm presence of SARS-CoV-2 specific antibodies. Of the thirteen seropositive cats, 7/13 (54%) were also positive by virus neutralization, and 2 of seropositive cats had previously documented respiratory signs, with high neutralization titers of 1:1024 and 1:4096; overall however, there was no statistically significant association of SARS-CoV-2 seropositivity with respiratory signs, or with breed, sex or age of the animals. Follow up sampling of cats, while limited in scope, showed that positive serological titers were maintained over time. In comparison, we found an overall confirmed positive prevalence of 51% for feline coronavirus (FCoV), an endemic virus of cats, with 30% confirmed negative for FCoV. We demonstrate the impact of SARS-CoV in a defined feline population during the first wave of SARS-CoV-2 infection of humans, and suggest that human-cat transmission was substantial in our study group. Our data provide a new context for SARS-CoV-2 transmission events across species.

Introduction

SARS-CoV-2, the causative agent of the COVID-19 pandemic, is a Betacoronavirus that has caused significant morbidity and mortality in the human population since its emergence in December 2019 (1,2). SARS-CoV-2 has relatively broad cellular tropism and utilizes angiotensin converting enzyme 2 (ACE2) as a primary receptor to enter host cells. ACE2 is highly conserved across many mammals—which, in part, may explain why SARS-CoV-2 is also able to infect a range of animal species.

Following a first report in dogs (3), it became apparent that additional species were becoming infected with SARS-CoV-2, including tigers and lions at the Bronx Zoo in New York City in early April 2020 (4). Because of this, and the controversy in testing these animals at the start of the pandemic, cats became a risk as a reservoir of SARS-CoV-2. As the pandemic has proceeded most cases in cats have been found to be subclinical; the virus may circulate between cats (5) , but the public health risk from cats is now considered low— and while transmission back to humans can occur, this is rare. Other examples of cross-species infection with SARS-CoV-2 have now been widely documented, with natural infections also reported in farm-raised American mink, otters, hamsters, and white-tailed deer, among other species (6–9).

Cats, in particular, are of special interest due to their close contact with humans and predicted receptor-based susceptibility to SARS-CoV-2. Feline ACE2 shares significant homology with its human ACE2, especially at the spike receptor binding interface, or RBD(10). Despite initial in vitro studies, the overall impact of spike RBD/ACE-2 interactions on susceptibility to infection may ultimately be quite low. Experimental studies have, however, demonstrated cats' high susceptibility to SARS-CoV-2 infections and their potential to transmit the virus through close contact (11). Numerous studies have now reported seroprevalence of SARS-CoV-2 in domestic, shelter, and stray cats, yielding variable prevalence (12–27). Despite now extensive global documentation, data on the seroprevalence of SARS-CoV-2 in U.S. domestic cats remains relatively scarce (23–27), especially during the early stages of the pandemic.

Here, we present a retrospective serological survey conducted in a small animal veterinary clinic located on the Upper East Side of New York City, in close proximity to a large medical center (New York Presbyterian/Weill Cornell) that was treating the initial US wave of severe COVID-19 cases in humans. Our sampling period spanned June 2020 to May 2021, a period that included an early epicenter of the COVID-19 pandemic in the United States, with numerous human cases identified spanning the New York City boroughs by early March of 2020 (28,29). Given the dense population of both humans and pets in this area, our findings provide key insights into the prevalence and characteristics of SARS-CoV-2 infection in domestic cats during the initial outbreak phase.

Materials and Methods

Participant recruitment and sampling

Feline participants from NYC presenting to a private practitioner veterinarian were enrolled between June 2020 and May 2021. From each cat, approximately 2ml of whole blood was collected and allowed to clot. Serum was removed, stored at 4°C and shipped to Cornell on ice. Sampling from cats was approved by the Cornell IACUC 2012-0116, and owners gave their written consent for this procedure. All sampling was performed by licensed veterinary professionals, employed by the collaborating veterinary practice. Basic signalment was collected from each cat. Positive samples identified in this study were reported to New York State Department of Agriculture and Markets, Division of Animal Industry and United States Department of Agriculture, Veterinary Services, and were submitted to the National Veterinary Services Laboratory in Ames, Iowa for follow up analysis.

Multiplex test against SARS-CoV-2 epitopes

Serological status was evaluated using a proprietary, fluorescent bead-based multiplex assay, developed through the Cornell Animal Health Diagnostic Center, Serology Laboratory. The assay was developed and validated for detection of human antibodies (30) and was modified for antibody measurement in cats by using an anti-cat detection reagent. The feline assay evaluates antibody reactivity against three SARS-CoV-2 domains (NP, RBD, and S1). Based on validation with human serum, reactivity against NP or RBD is considered positive, while reactivity against S1 is considered cross-reactivity and not specific for previous SARS-CoV-2 exposure.

Viral neutralization

Vero E6 cells, obtained originally from the American Type Culture Collection (ATCC) were seeded 24-hours prior to infection, under BSL-2 conditions, at a density of $2x10^4$ cells/well of a flat-bottom 96-well plate in 100uL of Dulbecco's modified Eagle medium (DMEM) (Cellgro) supplemented with 25mM HEPES (Cellgro) and 10% HyClone FetalCloneII (GE) at 37°C and 5% CO2. For neutralization assays, under BSL-3 conditions, each heat inactivated sample was 4-fold serially diluted in Eagle's Minimum Essential Medium (EMEM) (Cellgro) supplemented with 4% heat inactivated fetal bovine serum (FBS) (Gibco) and mixed with an equal volume of SARS-CoV-2 isolate USA-WA1/2020 from the Biological and Emerging Infections Resources Program (BEI Resources) for a final virus concentration of 100 TCID50. Dilutions were performed in triplicate. Following a one-hour incubation at 37°C, media was removed from the Vero E6 cells and replaced with the serum/virus mixture. Plates were incubated for 72 hours at 37°C and 5% CO_2. After incubation, serum/virus was removed, and plates were fixed with

4% paraformaldehyde for 30 minutes and then stained with crystal violet for 10 minutes. The plates were washed with water and then allowed to dry. The neutralization titer was determined as the highest dilution in which at two or three of the wells were not-infected, based on visualization of the crystal violet staining. Samples were considered negative if there was no obvious viral neutralization at the 1:4 dilution.

FCoV Serology

FCoV serology was performed through the Animal Health Diagnostic Center at Cornell University, utilizing a commercial ELISA for antibody detection, reported as a signal to noise (S/N) ratio and then classified as positive, negative, or inconclusive.

Serology data mapping

Location data was analyzed and mapped using the Free and Open Source QGIS 3.34.12

Statistical analysis

Unpaired student's T tests and Fisher exact tests were performed in GraphPad Prism 9 (San Diego, CA). A p-value of 0.05 was considered significant.

Results

Serological assay

Our objective was to detect SARS-CoV-2 specific antibodies, and for this purpose, we utilized fluorescent bead-based multiplex testing of the serum samples using SARS-CoV-2 RBD, NP, and S1 proteins.

Seropositivity

During the period of June 2020 to May 2021, we conducted an analysis of a total of 79 domestic cat serum samples, consisting of 33 males and 46 females. The average age of the cats included in the study was 7.3 years old. Out of the 79 samples, 13 cats (16.46%), tested positive for SARS-CoV-2 (Table 1, Figure 1). To further confirm these positive cases, we conducted live virus neutralization tests. Of the thirteen seropositive samples, seven cats (S115, S123, S117, S149, S151, S166, and S167)

exhibited neutralizing antibodies with titers ranging from 16 to 4096 (Table 1). Cats S115 and S123, sampled on 7/11/2020 and 7/18/2020 respectively, had shown respiratory signs in early March 2020. These two, exhibiting the highest viral neutralization test (VNT) titers of 1024 and 4096, were co-housed and owned by an individual who tested positive serologically for SARS-CoV-2 in April 2020. Cats S166 and S167, also sharing the owner and living environment, were part of a household where other human housemates tested positive for SARS-CoV-2. Cats S149 and S151 were co-housed, but there was no reported information regarding their potential exposure to SARS-CoV-2. All these cats were kept indoors, except for S117, which was partially outdoor and inhabited in a community grocery store (Table 2). As for the remaining 6 seropositive cats (S113, S152, S153, S155, S157, and S158), although seropositive in the multiplex test, they tested negative for the viral neutralization test. In addition, we also performed viral neutralization with the 19 seronegative samples via multiplex. All the 19 seronegative cats were also negative by viral neutralization test.

Association with sex, age, and breed

Due to the limited number of seropositive samples in our study, it is difficult to establish a clear association between sex, age, and breed. There was no observed association between cat age and gender with seropositivity in our analysis. Specifically, 84.8% of female cats tested negative, while 15.2% were positive. Among male cats, 81.8% were negative, and 18.2% tested positive. Most of the cats we sampled were domestic long/short hair therefore most of the seropositive cats were domestic long/short hair (Figure 2).

Association with time of sample collection

The collection of samples was conducted over different months, and the seropositivity rates varied accordingly. The highest seroprevalence was observed during the fall of 2020, with 7 out of 16 cats testing positive (43.75%). In the summer of 2020, 4 out of 43 (9.3%) cats tested positive. During the winter of 2021, 2 out of 9 cats tested positive (22.2%). However, all samples collected during the spring of 2021 and summer of 2021 yielded negative results. (Figure 3).

Longitudinal samples

There were 12 longitudinal samples. Sera were collected from the same cat two to three times over a period. Three of these cats were serologically positive by multiplex ELISA and viral neutralization test. For all three samples, the viral neutralization titer decreased over time, but even a year after initial detection, it maintained a relatively high level (Table 3).

FCoV serology

Among the 67 samples tested for FCoV, results showed that 34 (50.74%) tested positive, 20 (29.85%) negative, and 13 (19.4%) were inconclusive. The high prevalence of FCoV among these samples can be due to the fact that most of the cats were adopted from breeders or shelters, typically environments with multiple cats (Figure 4A). Among the 13 cats that tested positive for SARS-CoV-2, six were also found to be positive for FCoV (Figure 4B).

Discussion

Our findings shed light on the seroprevalence of SARS-CoV-2 in domestic cats during the early stages of the COVID-19 pandemic, highlighting the virus's impact on this specific animal population. Although this study did not address aspects of viral shedding, our results suggest that cats were highly susceptible to SARS-CoV-2 infection during the early pandemic, and capable of developing significant and sustained immune responses.

Using a highly sensitive and specific fluorescent bead-based multiplex assay, we found an overall prevalence of 13/79 (16.46%) seropositive animals for the study period. In comparison to other serosurveillance studies conducted on cats in the United States during the early phase of the pandemic (24–26), our study reports the highest seropositivity, which may be due to the geographic location and timing of our sampling—conducted while New York City was the epicenter of the COVID-19 pandemic in the United States.

Only 7/13 serologically positive cats were VNT positive, and no cross-reactive or negative cats were VNT positive. Of these cats, S115 and S123, which were co-housed at the same location, are notable as they had respiratory signs. The owner of these cats reported to have tested positive for SARS-CoV-2. Cat S166 and S167, were also co housed, and the owner reported that the cats lived with SARS-CoV-2 positive housemates. This presents strong circumstantial evidence suggesting that these cats were exposed to SARS-CoV-2 by their owners.

The 6/13 cats which tested positive on the fluorescent bead-based multiplex assay but negative on the VNT, cats S155 and S158 exhibited decreased appetites possibly indicative of a loss of sense of smell, a known symptom in SARS-CoV-2 infections in humans. Additionally, cat S153 was admitted to the local veterinary teaching hospital in May 2020, an environment with potential exposure to SARS-CoV-2 positive staff, raising the possibility of exposure during the hospital stay. The observed discrepancy between the multiplex assay and VNT results could likely be a result of the different analytical

sensitivities of the two assays with lower limits of detection in the low pg/ml versus high ng/ml ranges for multiplex and VNT assays, respectively. The inconsistent results could also originate from either a false positive in the multiplex or a false negative in the viral neutralization test, or that the antibodies present were not neutralizing antibodies and thus only detected by the multiplex assay.

One notable finding from this study was that the seroprevalence of SARS-CoV-2 in cats in Fall 2020 was 43.75%. This occurred in mostly indoor cats and at a time when social contact of their owners in the general population was heavily restricted. While we did not collect any information on the occupation of the owners or their health status, we note that the sampling site (Sutton Animal Hospital) is located in close proximity to a major medical complex (New York Presbyterian/Weill Cornell) that handled large numbers of human COVID-19 patients during the early phase of the pandemic; this proximity suggests a high level of asymptomatic spread in this restricted human population, and subsequently to their pets. Notably, our samples were collected in the context of a “lock-down” that was imposed in New York City, at least during the early phase of the sampling period; the exception to more extended human contact was cat S117, who inhabited a community grocery store (a “bodega”). Cats remain an interesting comparative model to study both serological and genomic/evolutionary responses to SARS-CoV-2 (31). As most cats were housed individually, our data are limited with respect to understanding cat-to-cat transmission.

In our study, we observed a high prevalence (50.74%) of feline coronavirus (FCoV) in owned domestic cats; these were predominantly indoor pets, often living alone with their owner or with just one other cat. The lifestyle of these cats suggests that they were likely exposed to FCoV prior to adoption from shelters or purchase from breeders, i.e., environments with multiple cats. Notably, among the 13 cats seropositive for SARS-CoV-2, six also tested positive for FCoV. Considering the early pandemic timing of our sample collection, we infer that these cats were exposed to FCoV before encountering SARS-CoV-2. There is an over-arching question whether pre-exposure to other coronaviruses protects against SARS-CoV-2. The limited sample size in our study does not allow statistical significance to be reached, underscoring the need for additional research to validate this observation The likelihood of cross-reactivity between FCoV and SARS-CoV-2 in serological tests is minimal, as prior studies have demonstrated an absence of cross-reactivity in FCoV antibodies against SARS-CoV-2 (32–34).

As with all studies of SARS-CoV-2 it is important to assess the individual virus lineages (22), especially with the Omicron variant, as substantial virological changes have occurred as the pandemic has proceeded. Based on the timing of the study, we sampled infections caused predominantly by pre-VOC SARS-CoV-2 (from the B.1 lineage, spanning both S:G614G and S:614D). In line with experimental studies of both “early” and “late” SARS-CoV-2 isolates, our study shows that cats can be readily infected

with SARS-CoV-2 in natural populations, even where these populations are indoor cats, housed individually or in small groups.

The capacity of SARS-CoV-2 to infect a broad range of animal species has heightened concerns about reverse zoonotic transmission. The expansion of this viral reservoir could lead to an accumulation of mutations, potentially resulting in spillback events to the human population. Such events could give rise to variants that are either more transmissible or resistant to existing vaccines. Documented cases of reverse zoonotic transmission from animals to humans have been observed in minks, deer, and domestic cats (8,12,35). While our data did not address and understanding cat-to-human transmission. study underscores the critical need for a comprehensive cross-species approach to virus transmission, which includes rigorous and consistent surveillance measures across companion animals, livestock, and wildlife populations.

In conclusion, our study underscores the importance of considering companion animals, particularly cats, in the broader epidemiological landscape of SARS-CoV-2. It highlights the necessity of ongoing research to understand the dynamics of interspecies transmission and the implications for both animal and human health. This research contributes to the growing body of evidence advocating for a One Health approach in managing and understanding the complexities of the COVID-19 pandemic.

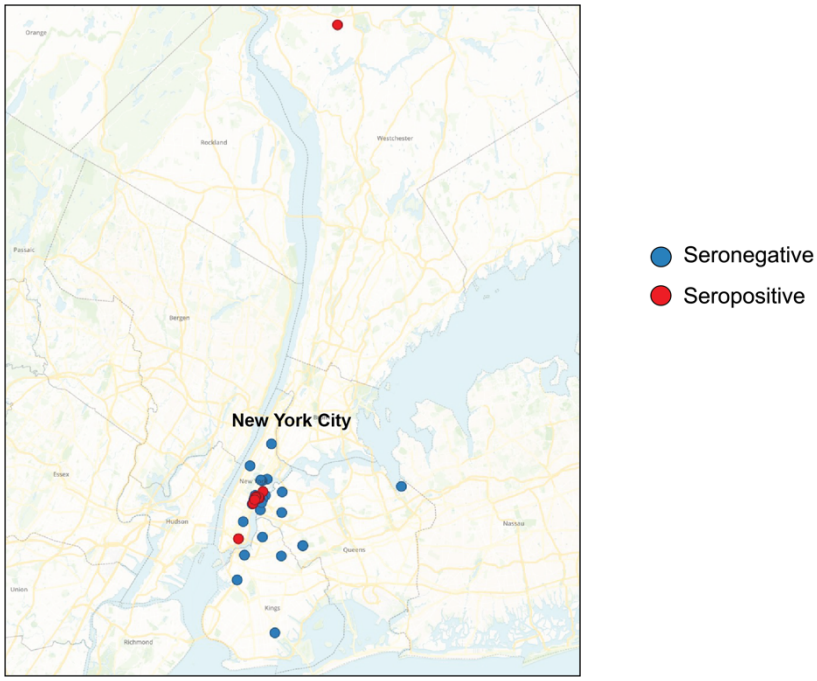
Limitations of this study

In this study, we did not directly collect information of owner exposure for SARS-CoV-2. As a result, we have incomplete data regarding the potential exposure of cats to SARS-CoV-2. It is plausible that some seronegative cats were exposed to SARS-CoV-2 but did not get infected or develop detectable antibodies. Therefore, the extent to which cats exposed to SARS-CoV-2 naturally gets infected or seroconvert remains unclear in our study.

Table 1. Multiplex ELISA and viral neutralization test

cat ID	NP	RBD	S1	multiplex result	VNT result
S167	1889	8684	859	positive	1:16
S115	6294	17400	967	positive	1:1024
S117	714	5294	688	positive	1:256
S149	7247	10862	1828	positive	1:256
S151	8959	10139	1735	positive	1:256
S123	9040	13408	1971	positive	1:4096
S166	7055	3208	628	positive	1:64
S101	487	1585	1759	crossreactive (neg)	Neg
S103	719	1455	1616	crossreactive (neg)	Neg
S105	1709	1583	1786	crossreactive (neg)	Neg
S108	3544	2794	3290	crossreactive (neg)	Neg
S109	4487	6151	7401	crossreactive (neg)	Neg
S113	7044	1787	1783	positive	Neg
S114	2731	699	665	negative	Neg
S116	416	534	483	negative	Neg
S127	892	766	762	negative	Neg
S131	568	559	586	negative	Neg
S132	453	538	576	negative	Neg
S135	2207	754	743	negative	Neg
S141	2391	4861	5135	crossreactive (neg)	Neg
S145	3603	6386	6415	crossreactive (neg)	Neg
S152	4572	2633	2820	positive	Neg
S153	7295	2319	2255	positive	Neg
S155	4806	415	478	positive	Neg
S157	5332	718	982	positive	Neg
S158	5201	901	1035	positive	Neg
S159	2711	829	1000	negative	Neg
S160	4513	1953	1970	crossreactive (neg)	Neg
S163	4216	4241	4485	crossreactive (neg)	Neg
S165	4228	887	1067	crossreactive (neg)	Neg
S170	2860	1129	923	negative	Neg
S172	3888	1377	1276	crossreactive (neg)	Neg
S180	1897	1780	1909	crossreactive (neg)	Neg
S181	2454	958	797	negative	Neg
S102	695	938	1028	negative	
S104	501	1265	1299	negative	
S106	376	942	997	negative	
S107	469	1074	1040	negative	
S110	537	458	707	negative	
S111	568	521	808	negative	
S112	818	902	1288	negative	
S118	1260	911	955	negative	
S119	1451	1151	1252	negative	
S120	404	497	528	negative	
S121	543	590	518	negative	
S122	942	592	566	negative	
S124	389	527	546	negative	
S125	590	771	754	negative	
S126	727	744	778	negative	
S128	561	570	564	negative	
S129	728	428	490	negative	
S130	388	531	561	negative	
S133	411	503	471	negative	
S134	567	547	575	negative	
S136	712	800	936	negative	
S137	448	476	543	negative	
S138	886	865	982	negative	
S139	1060	758	850	negative	
S140	1197	745	836	negative	
S142	820	712	754	negative	
S143	594	731	819	negative	
S144	261	387	380	negative	
S146	989	664	629	negative	
S147	326	264	275	negative	
S148	540	822	747	negative	
S150	3037	873	736	negative	
S154	2369	304	307	negative	
S156	610	727	794	negative	
S169	1406	478	479	negative	
S162	1784	623	670	negative	
S161	3008	560	603	negative	
S164	2777	845	909	negative	
S168	1484	659	750	negative	
S171	1749	965	897	negative	
S173	2427	1277	1360	negative	
S174	1969	1135	1027	negative	
S177	1081	947	785	negative	
S178	2044	1018	895	negative	
S179	1303	1059	859	negative	

A.



B.

Figure 1. Location of sampled cats.
(A) Location of all sampled cats. Blue corresponds to seronegative and red corresponds to seropositive cats. (B) Location of seropositive cats.

Table 2. Association of seropositivity with respiratory signs.

Cat ID	Multi-cat home	Respiratory signs	SARS-CoV-2 positive housemate	Multiplex result	VNT	Lifestyle
S115	Yes (co housed with S123)	Yes	Yes	positive	1:1024	indoor
S123	Yes (co housed with S115)	Yes	Yes	positive	1:4096	indoor
S117	No	No	No	positive	1:256	Indoor/outdoor
S149	Yes (co housed with S151)	No	Unknown	positive	1:256	indoor
S151	Yes (co housed with S149)	No	Unknown	positive	1:256	indoor
S166	Yes (Co housed with S167)	No	Yes	positive	1:64	indoor
S167	Yes (Co housed with S166)	No	Yes	positive	1:16	indoor
S113	Yes	No	No	positive	Neg	indoor
S152	No	No	Unknown	positive	Neg	indoor
S153	Yes	No	Unknown	positive	Neg	indoor
S155	Yes	No	No	positive	Neg	indoor
S157	No	No	Unknown	positive	Neg	indoor
S158	No	No	No	positive	Neg	indoor

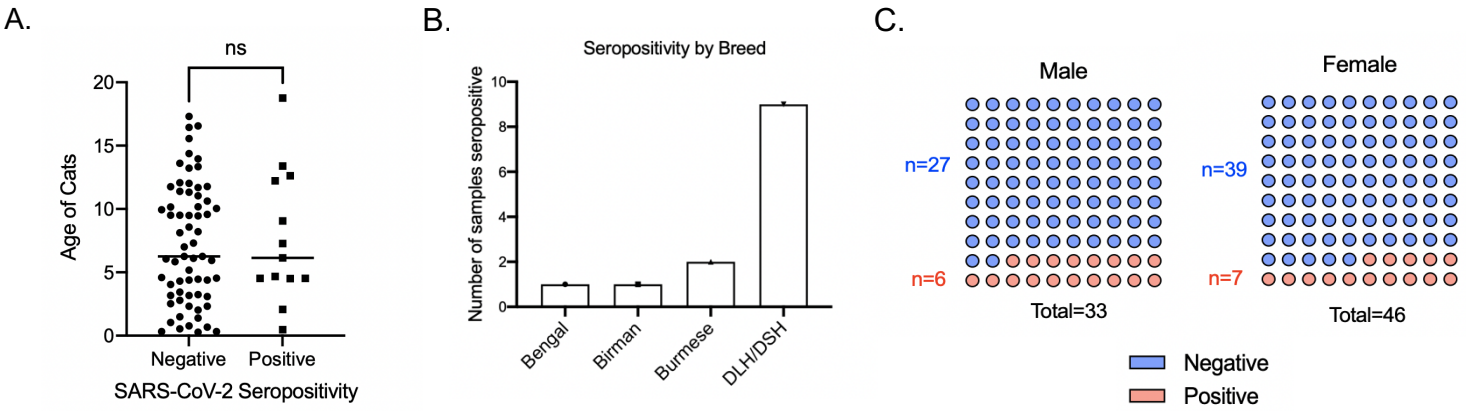


Figure 2. Association of seropositivity with age, breed, and sex.
(A) Association between age of cats and seropositivity of cats. Each individual dot indicates individual cats. Statistical analysis was performed using unpaired student's T test. (B) Seropositivity of different breeds of cats sampled. DLH: Domestic long hair. DSH: Domestic short hair. (C) Number of male and female cats sampled and their seropositivity. Statistical analysis was performed using Fisher exact test.

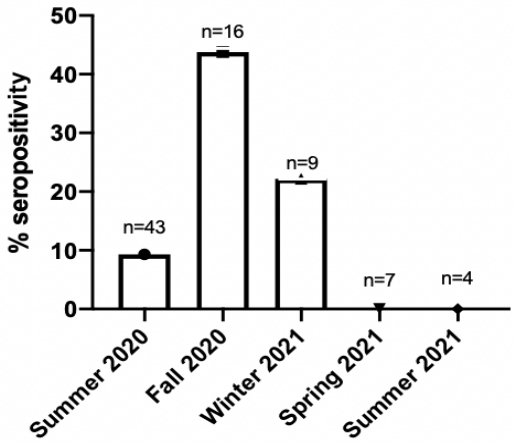
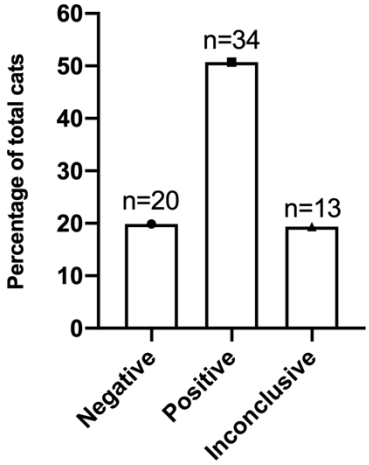


Figure 3. Association of seropositivity with time of sample collection.
Number of seropositive samples based on time of sample collection. Samples were collected seasonally as follows: Summer (June, July, August), Fall (September, October, November), Winter (December, January, February), and Spring (March, April, May).

A.



B.

Cat ID	SARS-CoV-2	FCoV
S117	positive	Inconclusive
S123	positive	Inconclusive
S149	positive	Inconclusive
S115	positive	negative
S151	positive	negative
S152	positive	negative
S167	positive	negative
S113	positive	positive
S153	positive	positive
S155	positive	positive
S157	positive	positive
S158	positive	positive
S166	positive	positive

Figure 4. Prevalence of FCoV in feline sera collected.
(A) Number of cats that tested negative, positive, and inconclusive for FCoV. (B) Table of SARS-CoV-2 seropositive cats and corresponding FCoV serology results.

Table 3. Longitudinal samples

Repeat samples	Multiplex result	VNT result	Date collected
S117	positive	1:256	7/21/20
S117 Repeat	low positive	1:16	7/16/21
S123	positive	1:4096	7/18/20
S123 Repeat 1	positive	1:256	1/9/21
S123 Repeat 2	positive	1:256	7/17/21
S115	positive	1:1024	7/11/20
S115 Repeat	positive	1:256	1/9/21
S121	negative		7/17/20
S121 Repeat	low positive	negative	1/9/21
S142	negative		8/22/20
S142 Repeat	low positive	negative	1/5/21
S153	positive	negative	9/5/20
S153 Repeat	negative		1/9/21
S131	negative	negative	8/11/20
S131 Repeat	negative		2/9/21
S107	negative		6/14/20
S107 Repeat	negative		2/27/21
S102	negative		6/14/20
S102 Repeat	negative		3/13/21
S134	negative		8/25/20
S134 Repeat	negative		7/24/21
S135	negative	negative	8/25/20
S135 Repeat	negative		7/24/21
S137	negative		8/25/20
S137 Repeat	negative		7/24/21

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This study was approved by The Cornell University Institutional Review Board for Human Participant Research (IRB) Office (IRB0009690; Serological study of coronavirus in cats) as an exempt protocol.

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