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Dogs

Surveillance of SARS-CoV-2 in Pets of Harris County, Texas, Revealed More Common Pet Infections in Households With Human COVID-19 Cases

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ABSTRACT

Local health departments can play a critical role in zoonoses surveillance at the human–domestic animal interface, especially when existing public health services and close relationships with community groups can be leveraged. Investigators at Harris County Veterinary Public Health employed a community-based surveillance tool for identifying severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections in dogs and cats in June-–December 2021. Diagnosis was made using both RT-qPCR testing of oral and nasal swabs and plaque reduction neutralization testing of serum samples. Recruitment for this free companion animal surveillance program occurred through the following two streams: case-based and event-based. The case-based stream recruited companion animals of confirmed human COVID-19 cases through the Harris County Public Health case investigations platform and used the information from epidemiological investigations of the owners to conduct further investigations of their pet(s). The event-based stream recruited companion animals participating in free or low-cost spay/neuter events at Harris County Pets Resource Center (HCPRC). A total of 97 animals were tested, with the case-based and event-based streams accounting for 36 and 61, respectively. A total of 13 animals (13.4%) tested seropositive including one that also had positive RT-qPCR swabs. Of the positives, 11 (84.6%) were associated with a confirmed human case of SARS-CoV-2 living in the same household including one household with four out of the seven animals positive for SARS-CoV-2 neutralizing antibodies. These two surveillance methods employed at the local level emphasize the importance of the One Health approach and provide a model for future zoonoses surveillance systems.

1 | Introduction

Since late 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has shaped almost all aspects of modern civilization. Evidence suggests that SARS-CoV-2 originated from an animal source, most likely horseshoe bats (*Rhinolophus* spp), and spilled over into humans through an intermediary animal host (Zhou et al. 2020a). Cases of SARS-CoV-2 have been documented in companion animals globally, and human-to-animal transmission has been widely recognized (Liew et al. 2023). In

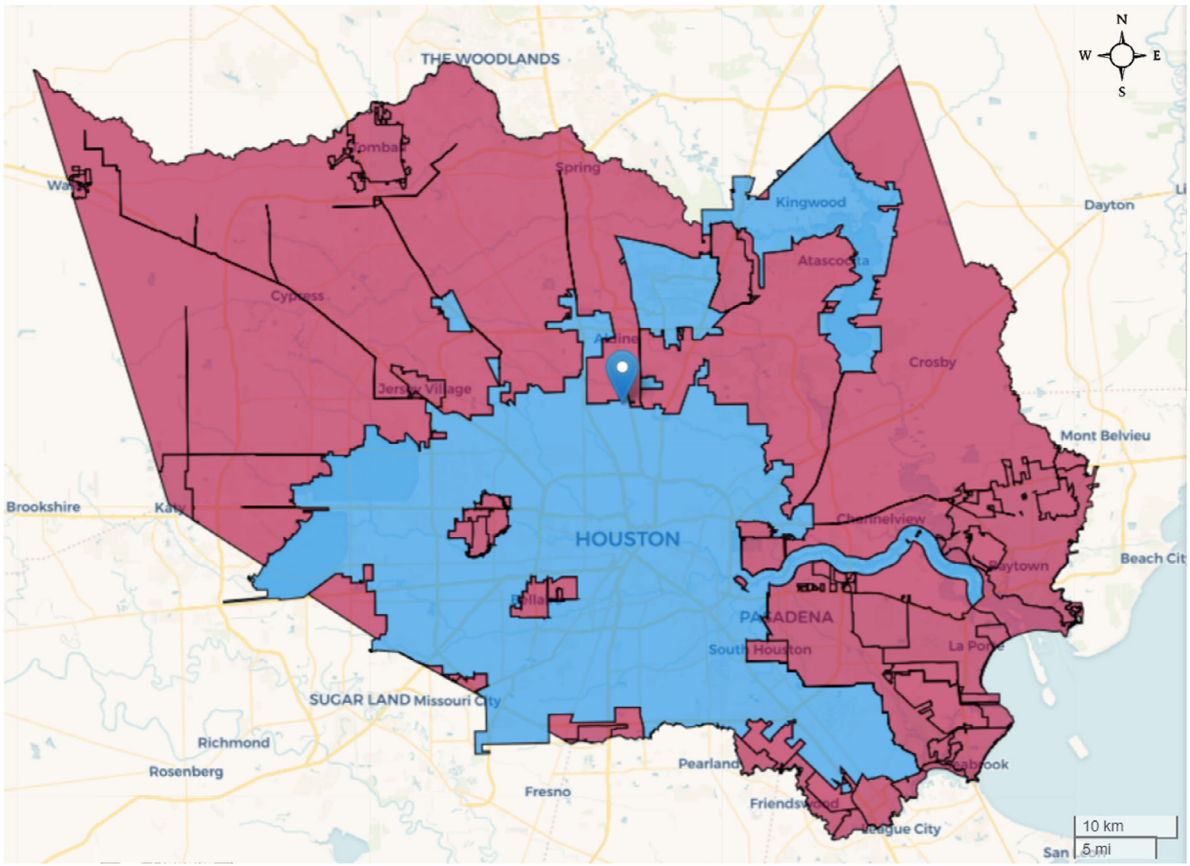

FIGURE 1 | Harris County Public Health provides services to the areas shaded in red, while the Houston Health Department covers the areas in blue. The Harris County Pet Resource Center facility is marked by the blue pin.

a recent review of cases of confirmed SARS-CoV-2 infections in companion animals, clinical signs of illness were present in 48% of infected pets, with respiratory signs most frequently reported, followed by non-specific and gastrointestinal signs (Liew et al. 2023). Animal-to-animal transmission has occurred in captive mink (*Neovison vison*; Enserink 2020) and is suggested in white-tailed deer (*Odocoileus virginianus*; Hale et al. 2021). Currently, animal-to-human transmission has been documented in limited capacity in minks, Syrian hamsters (*Mesocricetus auratus*; Yen et al. 2022), white-tailed deer (Pickering et al. 2022) and cats (*Felis catus*; Sila et al. 2022). Although cats may transmit the virus directly to other cats (Gerhards et al. 2023), there is limited evidence that suggests domestic cats or dogs play a significant role in transmission of SARS-CoV-2 to humans.

Given the unique insight that local health authorities have regarding their jurisdiction and their ability to mobilize and respond quickly to disease outbreaks, we piloted a novel surveillance program to engage a local health department in surveillance for companion animal SARS-CoV-2 infections. Although most infections in pets likely originate from humans, neither risk factors for transmission from humans to pets nor the frequency and characteristics of clinical illness in pets are well defined; this study aimed to fill these knowedge gaps.

2 | Materials and Methods

2.1 | Surveillance Methods

Harris County is the third most populous country in the United States with over 4.7 million people spread over 1778 square miles. Harris County Public Health services about half of this area (Figure 1). Sampling took place between June and December 2021 (Figure 2). Two recruitment streams were used to recruit for the surveillance. The case-based stream recruited from households with positive human COVID-19 cases, and the event-based stream recruited from spay/neuter events. The case-based stream utilized Harris County's COVID-19 human case investigation platform, 'COVID-19 Response Program', by adding screening questions to the standard case investigation form to identify potential pet-owning participants in the research study after which a secondary screening was initiated. This set of secondary questions screened recorded pet demographic information, outdoor access and symptoms. Disqualifying criteria included age (cats needed to be at least 16 weeks and dogs needed to be at least 12 weeks) and weight (cats needed to be at least 4 pounds and dogs needed to be at least 5 pounds). Animals with reported aggression towards strangers were also disqualified. After this, investigators scheduled an appointment for owners to bring their pet to the Harris County Pets Resource Center (HCPRC) for sample collection.

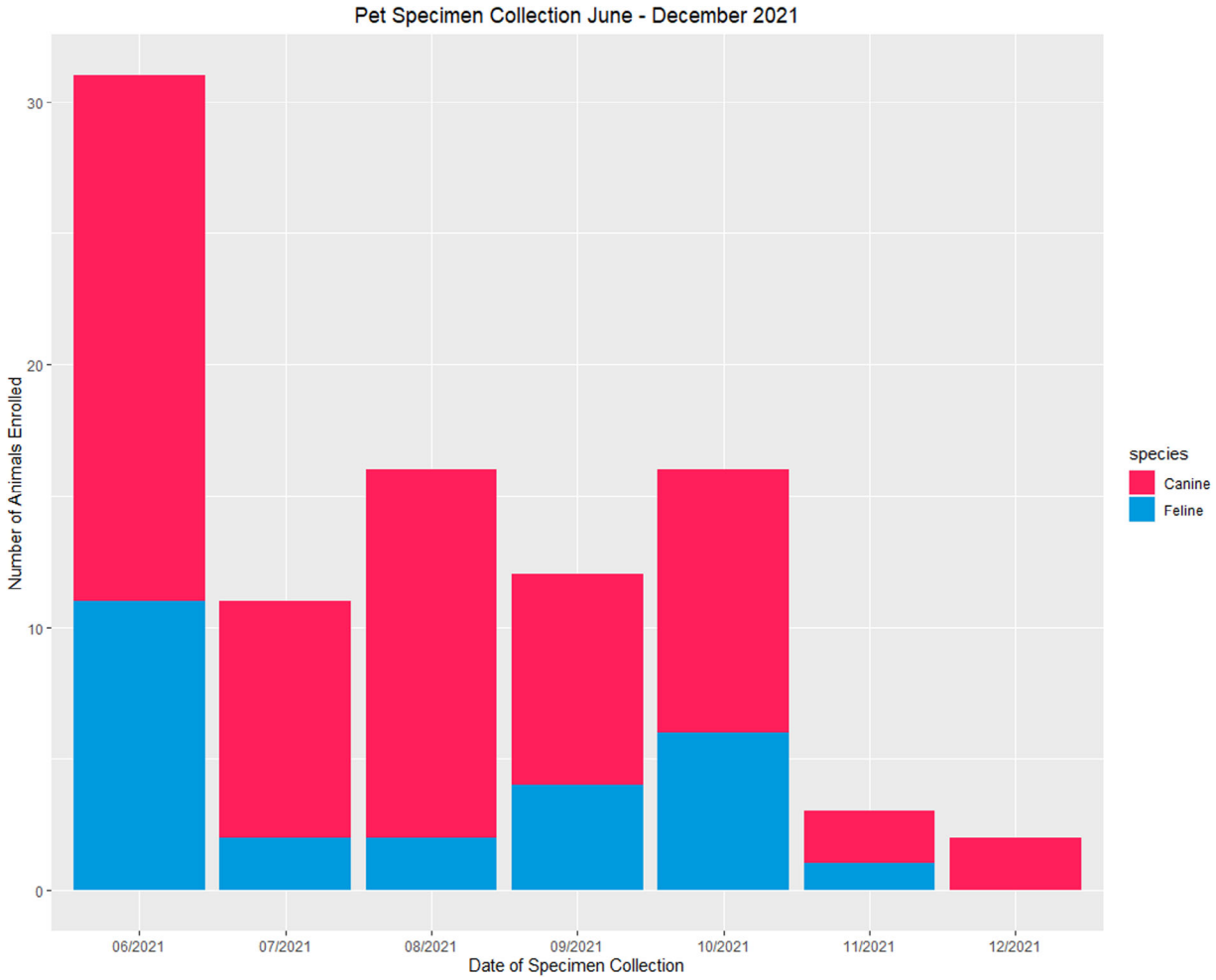

FIGURE 2 | Samples from dogs and cats in Harris County, Texas, were collected for SARS-CoV-2 testing from June to December 2021.

Sample collection took place in the facility's isolated sally port, which included a secure entryway door to the facility with a negative pressure exhaust system to keep potentially contaminated air from escaping the facility. The pet owners were instructed to wear a mask. A staff veterinarian, in full PPE (gown, gloves and mask), took each animal inside the sally port individually while the owners waited in their vehicle. Sample collection included 3–5 mL of whole blood and nasal and oropharyngeal swabs. Both swabs were immediately immersed into a single vial of 4 mL of viral transport media (VTM) and placed in a cooler, followed by freezer storage at −80°C until processing. The whole blood was centrifuged and the serum was separated and frozen.

The event-based stream utilized monthly low-cost spay/neuter events hosted at HCPRC as well as veterinary appointments at the low-cost clinic at HCPRC as recruitment events. Owners of pets with scheduled surgeries or appointments were contacted prior to the event and offered free SARS-CoV-2 testing for their pet with the survey being filled out via phone interview or they were offered the testing at the event.

At the end of the surveillance period, the investigators reached out to the three households that had pets that tested seropositive with an additional questionnaire. This questionnaire asked the positive human COVID-19 case questions about how often they interacted with each pet, how often each pet slept in the same bed as them, if the pet ever ate tissues or napkins during their illness and how often the pets played, cuddled, drank out of the same water bowl and played with the same toy with each other. Out of the three households, only one agreed to the additional questionnaire.

2.2 | Molecular Diagnostics

Aliquots of VTM supernatant were subjected to total nucleic acid extraction by MagMAX CORE Nucleic Acid Purification Kit (ThermoFisher Scientific, Waltham, MA). An aliquot of purified nucleic acid was tested for SARS-CoV-2 RNA by specific real-time RT-qPCR to amplify the RdRp gene using primers RdRp_SARSr-F, RdRp_SARSr-R and probe RdRp_SARSr-P2 (Corman et al. 2020) using a CFX96 Real-Time System (BIORAD, Hercules, CA, USA). A control plasmid containing a portion of the RdRp gene served as a positive control (Integrated DNA Technologies, Coralville, IA, USA). Using this same protocol, our lab successfully completed the USDA COVID proficiency testing exercise (ICE-2) in the summer of 2021 (Deng et al. 2021).

TABLE 1 | Demographics and descriptive statistics of all pets tested for the SARS-CoV-2 using both case-based recruitment (in households with active human COVID-19 cases) and event-based recruitment (at low/no cost spay/neuter events), Harris County, Texas, 2021. Ab = antibody (anti-SARS-CoV-2 neutralizing antibodies as measured by the $PRNT_{90}$ tests).

	Case-based recruitment ($N = 34$)	Event-based recruitment ($N = 57$)	Overall ($N = 91$)
Animal sex			
Female—intact	6 (17.6%)	34 (59.6%)	40 (44.0%)
Female—spayed	11 (32.4%)	0 (0%)	11 (12.1%)
Male—intact	4 (11.8%)	23 (40.4%)	27 (29.7%)
Male—neutered	13 (38.2%)	0 (0%)	13 (14.3%)
Animal housing			
Multipet house	23 (67.6%)	51 (89.5%)	74 (81.3%)
Single-pet house	11 (32.4%)	6 (10.5%)	17 (18.7%)
Species			
Canine	28 (82.4%)	37 (64.9%)	65 (71.4%)
Feline	6 (17.6%)	20 (35.1%)	26 (28.6%)
Ab			
Ab negative	23 (67.6%)	55 (96.5%)	78 (85.7%)
Ab positive	11 (32.4%)	2 (3.5%)	13 (14.3%)
Age (years)			
< 1	3 (8.8%)	18 (31.6%)	21 (23.1%)
1–3	9 (26.5%)	25 (43.9%)	34 (37.4%)
4–7	11 (32.4%)	14 (24.6%)	25 (27.5%)
8+	11 (32.4%)	0 (0%)	11 (12.1%)

2.3 | Plaque Reduction Neutralization Tests$_{90}$ ($PRNT_{90}$)

Serum samples were tested by $PRNT_{90}$ to quantify antibodies able to neutralize the formation of 90% or more SARS-CoV-2 plaques on Vero CCL-81 cell cultures following standard protocols (Beaty, Calisher, and Shope 1995; Roundy et al. 2022) in a Biosafety Level 3 laboratory. The PRNT serodiagnostic approach may detect both IgM and IgG isotypes (Hu et al. 2020). Serum samples were heat inactivated and screened at a dilution of 1:10, and those that neutralized SARS-CoV-2 viral plaques by at least 90%, when compared to the virus control, were further tested at serial twofold dilutions from 1:10 to 1:320 to determine 90% endpoint titers. Infectious viral stocks used for $PRNT_{90}$ were prepared with SARS-CoV-2 Isolate USAIL1/2020, NR 52381. This isolate was assigned lineage B and GISAID clade O using Phylogenetic Assignment of Named Global Outbreak LINeages (PANGOLIN) tool (BEI Resources, Manassas, VA).

2.4 | Criteria for Positivity

Positivity was defined using the USDA presumptive positive and confirmed positive case definitions (released on 6/11/2021). An animal was considered positive if it tested positive via RT-qPCR and/or virus neutralizing antibodies. The USDA case definition for the confirmed positive has the additional requirement of sequence confirmation of virus, obtaining a virus isolate, or detection of SARS-CoV-2 neutralizing antibodies (Animal and Plant Health Inspection Service 2021). Fisher's exact tests were used to compare infection across study groups, given the small sample size of positive animals. Once results were available, owners were notified of their pets' results by email and positive results by phone. At that time, investigators reviewed CDC guidance on SARS-CoV-2 infections in companion animals.

3 | Results

Within the case-based recruitment stream, 303 human cases were identified through the first set of screening questions. Of these 303 individuals, the team was able to make at least one contact attempt to 266 people, with interviews to solicit participation occurring an average of 10 days (1–28) after the humans' positive lab test, and specimens were collected from the pets an average of 15 days (3–38) after the humans' positive lab test.

Between 5 June 5 and 10 December 2021, 97 pets from 70 unique households were sampled (Table 1), with the case-based and event-based streams accounting for 36 and 61, respectively. The average number of days between the positive human specimen collection and animal specimen collection, or sampling interval, was 15.2 days in the case-based stream.

Out of the 97 animals, 13 (13.4%) were confirmed as positive, one was positive via RT-qPCR and neutralizing antibodies and

12 were positive for neutralizing antibodies only (Table 2). The PRNT endpoint titers of these seropositive animals ranged from 10 to 640, with a median of 40 and geometric mean endpoint titer of 42.2. Significantly, more pets tested positive in the case-based surveillance stream versus the event-based surveillance stream ($p < 0.001$); 30.6% of pets recruited through the case-based stream tested positive whereas only 3.3% of pets recruited through the event-based stream tested positive. In total, 11 of the 13 positives (84.6%) were from the case-based surveillance and thus lived in a house with a confirmed human case of SARS-CoV-2. Of the 22 pet-containing homes with a human COVID-19 case, approximately one-third of homes (31.8%) harboured at least one positive pet. Only two animals (one cat and one dog) from the event-based stream tested positive; both were from multipet households but were the only pet from their household at the event. In a risk factor analysis, there were no statistically significant factors for cats, most likely because of the small sample size of 26, with only 2 seropositive cats. Dogs under the age of 1 were least likely to test positive while dogs over 8 years old were most likely to test positive ($p = 0.011$; Table 2). Spayed females were most likely to test positive followed by neutered males ($p = 0.002$; Table 2).

The sampling interval was not significantly different for the negative versus positive pets ($p = 0.079$), with negative pets having samples collected an average of 14.9 days after the positive humans' specimen collection (sd 9.23; range 3–38 days) and positive pets having samples collected an average of 15.8 days after the positive humans' specimen collection (sd 9.27; range 7–34 days). The single RT-qPCR positive pet specimen was collected 12 days after the positive human's specimen.

Of the 20 households for which more than one pet was tested, 17 were associated with all negative pets whereas three had both positive and negative pets. Only one of three multi-pet houses with a positive pet completed the additional questionnaire, which aimed to document the behavioural and social patterns of the pets to determine transmission pathways. This single household had seven pets (five dogs and two cats) and all were tested; four animals (all dogs) were seropositive (Table 3). None of the pets was noted by the owner to show any symptoms. All of the pets shared the same water bowls, and the dogs shared the same toys. Each pet received contact from the owner in the form of pets or scratches approximately 1–3 times throughout the average day. None of the pets was known to eat tissues or napkins. Three of the seven pets (Pets B, E and F) were reported to sleep with the owner at night, of which only Pet B was positive. The owner was asked about the interactions of the pets with each other, which we referred to as the 'play interactions' (Table 4). None of these differences correlated with the test results of the pets.

4 | Discussion

This study supports the growing body of evidence that companion animals can be infected with SARS-CoV-2 in households where infected humans reside (see reviews in Guo et al. 2023; Heydarifard et al. 2024). Our study took place in June–December 2021, when the predominant SARS-CoV-2 variant was Delta, but the end of the sampling occurred during the beginning of the surge of the Omicron variant in November. We found that 13 animals (13.4%) tested seropositive including the one that also had positive RT-qPCR swabs. In contrast, other veterinary studies failed to detect SARS-CoV-2 exposure or infection in pets, including pets with respiratory signs and those in close contact with human COVID-19 patients (e.g., Kadi et al. 2022; Temmam et al. 2020), underscoring that pet infections are likely to reflect temporal and spatial dynamics of the virus and individual-level risk factors.

We found a higher proportion of infected pets recruited through the case-based versus event-based surveillance stream. This is not surprising as the animals in the case-based stream had at least one human confirmed positive COVID-19 case in the household, while the pets in the event-based recruitment may not have had any exposures to SARS-CoV-2 in their lifetime. Also, given that 31.6% of the pets involved in the event-based recruitment were under a year old (arriving for a spay or neuter surgery), these young animals would have had less opportunity for exposure earlier in the pandemic. Similarly, a US-wide review of companion animal cases in which SARS-CoV-2 infection was confirmed also showed that infection was most reported in older animals, with the mean age of infected cats and dogs of 6.64 and 6.89 years, respectively (Liew et al. 2023).

The focal investigation of the seven-pet household provides the opportunity to speculate on transmission within the household. Based on the answers obtained from the owner, no significant risk factors were identified. Although cats were determined to be more susceptible to infection compared to dogs (Dileepan et al. 2021), both cats in this household tested negative for neutralizing antibodies. However, the SARS-CoV-2 viral variant in circulation may impact animal susceptibility, as a similar study done during the same period when the Delta variant was dominant also found higher infection prevalence in dogs (Michelitsch et al. 2023). Only one of the three pets who slept in bed with the owner tested positive for neutralizing antibodies despite this being previously documented as a significant risk factor (Calvet et al. 2021; Bienzle et al. 2022). A different study found that the pets who tested seronegative were less likely to interact with the human case of COVID-19 (Michelitsch et al. 2023). This household raises questions on how susceptibility to SARS-CoV-2 can vary between animals of the same species in the same environment.

The lag time from human case identification to animal sampling spanned weeks in some cases and may have contributed to negative RT-qPCR results due to missing the window of active shedding of the virus from samples collected too late relative to the onset of infection. The single cat that tested RT-qPCR positive had the specimen collected 12 days after the owner's specimen was collected. Some research has even suggested that dogs may not shed the virus at all and cats may shed the virus in 1–6 days (Meekins, Gaudreault, and Richt 2021). Similar household studies have found a short window of acute infection, ranging from 7 to 13 days, in both cats and dogs (Hamer et al. 2021; Barroso et al. 2022). The impact of the viral variant on the duration of shedding in naturally infected dogs and cats remains unknown.

Participation in this study was voluntary leading to selection bias during pet recruitment. In the case-based surveillance, pet owners were only eligible if they took measures on their own to be tested for SARS-CoV-2 and test positive. Further, we noted that the owners' attitude about COVID-19 may have

TABLE 2 | Factors associated with SARS-CoV-2 testing results for dog and cat samples collected in Harris County, Texas, 2021. Statistical significance is noted in bold. Ab = antibody (anti-SARS-CoV-2 neutralizing antibodies as measured by the $PRNT_{90}$ tests).

	Canine			Feline			Table Overall		
Characteristic	**Ab negative *n* = 54[a]**	**Ab positive n = 11[a]**	***p* value[b]**	**Ab negative *n* = 24[a]**	**Ab positive *n* = 2[a]**	***p* value[b]**	**Ab negative *n* = 78[a]**	**Ab positive *n* = 13[a]**	***p* value[b]**
Surveillance stream			**< 0.001**			0.4			**< 0.001**
Case-based recruitment	18 (33%)	10 (91%)		5 (21%)	1 (50%)		23 (29%)	11 (85%)	
Event-based recruitment	36 (67%)	1 (9.1%)		19 (79%)	1 (50%)		55 (71%)	2 (15%)	
Animal housing situation			>0.9						0.7
Multipet house	40 (74%)	8 (73%)		24 (100%)	2 (100%)		64 (82%)	10 (77%)	
Single-pet house	14 (26%)	3 (27%)					14 (18%)	3 (23%)	
Age (years)			**0.011**			0.4			**0.003**
< 1	13 (24%)	0 (0%)		8 (33%)	0 (0%)		21 (27%)	0 (0%)	
1–3	19 (35%)	2 (18%)		12 (50%)	1 (50%)		31 (40%)	3 (23%)	
4–7	17 (31%)	4 (36%)		3 (13%)	1 (50%)		20 (26%)	5 (38%)	
8+	5 (9.3%)	5 (45%)		1 (4.2%)	0 (0%)		6 (7.7%)	5 (38%)	
Animal sex			**0.002**			0.08			**< 0.001**
Female—intact	26 (48%)	1 (9.1%)		13 (54%)	0 (0%)		39 (50%)	1 (7.7%)	
Female—spayed	4 (7.4%)	5 (45%)		1 (4.2%)	1 (50%)		5 (6.4%)	6 (46%)	
Male—neutered	17 (31%)	2 (18%)		7 (29%)	1 (50%)		24 (31%)	3 (23%)	
Male—intact	7 (13%)	3 (27%)		3 (13%)	0 (0%)		10 (13%)	3 (23%)	
Symptoms			0.7			> 0.9			0.5
Symptomatic	12 (27%)	4 (36%)		2 (9.5%)	0 (0%)		14 (22%)	4 (33%)	
Asymptomatic	32 (73%)	7 (64%)		19 (90%)	1 (100%)		51 (78%)	8 (67%)	
Unknown	10	0		3	1		13	1	
Species									0.3
Canine							54 (69%)	11 (85%)	
Feline							24 (31%)	2 (15%)	

[a] *n* (%).

[b] Fisher's exact test.

TABLE 3 | The demographic details of each pet in a seven-pet household associated with multiple SARS-CoV-2 positive animals, Harris County, Texas, 2021. Ab = antibody (anti-SARS-CoV-2 neutralizing antibodies as measured by the $PRNT_{90}$ tests.

ID	Species	Antibody status	Age (years)	Weight (lbs)
A	Canine	Positive	4–7	50
B	Canine	Positive	8+	15
C	Canine	Positive	4–7	45
D	Canine	Positive	8+	20
E	Canine	Negative	1–3	20
F	Feline	Negative	< 1	5
G	Feline	Negative	1–3	10

TABLE 4 | Play interactions among seven pets in a single household were characterized. Pets A, B, C and D tested seropositive, while pets E, F and G tested seronegative. 'True' indicates that the animals were noted to play/interact together, while 'false' indicates that they were not noted to play/interact together.

Animal	A	B	C	D	E	F	G
A	—	—	—	—	—	—	—
B	False	—	—	—	—	—	—
C	True	False	—	—	—	—	—
D	True	False	True	—	—	—	—
E	True	False	True	False	—	—	—
F	False	False	True	False	True	—	—
G	False	False	True	False	True	True	—

contributed to selection bias, as many owners declined enrolment and questioned the benefit of subjecting their pet to the test, given they did not perceive any negative impact on pet health. Indeed, in an international study to identify the beliefs and attitudes about COVID-19 that were predictive of taking health precautions, the belief that taking health precautions would be effective for avoiding COVID-19 was significant (Clark et al. 2020). Given the uncertain links between having one's pet tested for infection and avoiding future disease in pets or humans, some individuals may not have perceived enough benefit to participate. Additionally, there were logistical challenges as participating owners were required to bring their pet to the HCPRC for testing. While centrally located within Harris County (Figure 1), HCPH only serves unincorporated regions of the county and as a result may not have been convenient to access for all potential participants. Another potential source of bias was the available appointment times. Because the animal control officers use the sally port to bring in animals every afternoon, appointments were only offered from 8 a.m. to noon during Monday–Friday and select Saturdays. For the event-based stream, there was a bias in the socioeconomic status of the participating households as participation in the free/low cost spay neuter events occurred predominantly by those with financial hardships and households with a lower socioeconomic status. Indeed, cost was cited as the most common reason individuals chose to use non-profit spay and neuter clinics over private practice clinics (White, Scarlett, and Levy 2018).

This pilot project features a rapid-response veterinary surveillance system with novel aspects including leadership by a local public health authority, use of existing events for access to samples and involved academic collaborations for laboratory support. We found that the amount of staffing resources needed to successfully contact/recruit the pet owner, coordinate an appointment, collect the specimens, send out the specimens and inform the pet owner of the results far exceeds the amount that is usually available in a local health department. Local health departments cannot be the sole implementing agency in a surveillance system like this, underscoring the need for a One Health approach involving collaboration. Partnerships with academic/research institutions, local veterinary partners and local medical systems can facilitate such surveillance programs (Dacso et al. 2022). A successful One Health approach considers the changes in the human–companion animal relationship (Overgaauw et al. 2020) and looks at domestic pets as a potential part of the transmission cycle of zoonotic disease within a household instead of focusing on only animal meat products and wild animals. This has been successfully implemented in response to increased cases of *Brucella canis* in China (Zhou et al. 2020b) and Europe (Djokic et al. 2023). Given that 75% of the world's emerging infectious diseases are zoonotic (Jones et al. 2008), companion animal disease surveillance is a compelling approach for learning about emerging threats to public health. Moving forward, a One Health approach that incorporates local health departments along with other stakeholders may allow for a better understanding of transmission patterns in households and the community.

Author Contributions

Sarah J. Smith: conceptualization, data curation, formal analysis, funding acquisition, investigation, project administration, resources, writing–original draft, writing–review and editing. **Brendan Sullivan**: conceptualization, funding acquisition, resources, writing–review and editing. **Amanda Hall**: conceptualization, resources, writing–review and editing. **Lisa Auckland**: data curation, project administration, writing–review and editing. **Wendy Tang**: formal analysis, methodology, writing–review and editing. **Gabriel Hamer**: funding acquisition, methodology, resources, supervision, writing–review and editing. **Sarah Hamer**: funding acquisition, resources, supervision, writing–review and editing.

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NIAID, NIH: SARS-Related Coronavirus 2, Isolate USAIL1/2020, NR 52381.

Ethics Statement

This project was approved by the Centers for Disease Control and Prevention's IACUC (protocol # 3163BARMULX-A1). All pet owners signed a consent form approving testing of their pet and for the data to be used.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data used are available through a Freedom of Information Act request to Harris County Public Health.

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