

Risk Factors for SARS-CoV-2 Infection and Illness in Cats and Dogs¹

Dorothee Bienzle, Joyce Rousseau, David Marom, Jennifer MacNicol, Linda Jacobson, Stephanie Sparling, Natalie Prystajeky, Erin Fraser, J. Scott Weese

We tested swab specimens from pets in households in Ontario, Canada, with human COVID-19 cases by quantitative PCR for SARS-CoV-2 and surveyed pet owners for risk factors associated with infection and seropositivity. We tested serum samples for spike protein IgG and IgM in household pets and also in animals from shelters and low-cost neuter clinics. Among household pets, 2% (1/49) of swab specimens from dogs and 7.7% (5/65) from cats were PCR positive, but 41% of dog serum samples and 52% of cat serum samples were positive for SARS-CoV-2 IgG or IgM. The likelihood of SARS-CoV-2 seropositivity in pet samples was higher for cats but not dogs that slept on owners' beds and for dogs and cats that contracted a new illness. Seropositivity in neuter-clinic samples was 16% (35/221); in shelter samples, 9.3% (7/75). Our findings indicate a high likelihood for pets in households of humans with COVID-19 to seroconvert and become ill.

SARS-CoV-2 originated in horseshoe bats and probably reached humans through an unidentified intermediary host (1). The virus is aerosolized and highly transmissible among humans; new variants have arisen and spread in successive waves across the world since late 2019. Since a report of SARS-CoV-2 infection in a dog in March 2020 (2), an ever-increasing range of species has been shown to be susceptible to infection, including household cats, dogs, ferrets, and hamsters (3–10).

Author affiliations: Ontario Veterinary College, Guelph, Ontario, Canada (D. Bienzle, J. Rousseau, D. Marom, J. MacNicol, J.S. Weese); Toronto Humane Society, Toronto, Ontario, Canada (L. Jacobson); Toronto Animal Services, Toronto (S. Sparling); BC Centre for Disease Control Public Health Laboratory, Vancouver, British Columbia, Canada (N. Prystajeky); University of British Columbia, Vancouver (N. Prystajeky); Communicable Disease and Immunization Service, BC Centre for Disease Control, Vancouver (E. Fraser); University of British Columbia School of Population and Public Health, Vancouver (E. Fraser)

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Companion animals have closest contact with humans, creating ample opportunity for exposure. Experimental infections have suggested that most companion animals are infected only transiently, as indicated by PCR positivity or virus isolation (11,12). Conversely, detection of antibodies by ELISA or neutralizing antibody assay suggests infection rates of 0.2%–43.9% related to factors such as the likelihood and frequency of interaction with infected humans (13–16). Infections in animals are typically subclinical or associated with transient respiratory or gastrointestinal disease (17,18). In rare cases, death has been attributed to SARS-CoV-2 infection; however, defining the contribution of SARS-CoV-2 to death in animals with underlying conditions such as cancer, bacterial pneumonia, or obesity is challenging. On the other hand, minks are highly susceptible to infection and pneumonia, and mortality rates of 35%–55% caused by SARS-CoV-2 infection were reported from outbreaks among farmed mink in Utah (19). Captive minks also contracted viruses with a unique amino acid substitution in the spike (S) protein that were subsequently retransmitted to humans and to community cats and dogs, around mink farms in the Netherlands (5,20). Similarly, infected pet Syrian hamsters may also retransmit SARS-CoV-2 to humans (21). More than 30% of free-ranging white-tailed deer tested in Ohio were SARS-CoV-2 positive by PCR, and a similarly high proportion of white-tailed deer in Texas and other North America locations had neutralizing antibodies (22,23). Experimentally, white-tailed deer transmitted SARS-CoV-2 to other deer vertically and horizontally by direct contact (24). It has not yet been determined if infected deer experience illness or have increased illness and death rates or if transmission is sustained among wild deer populations. However, such high

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prevalence suggests SARS-CoV-2 may become endemic in some deer populations in North America.

SARS-CoV-2 is transmitted predominantly via aerosols, aided by proximity of infected and susceptible hosts, the degree of host susceptibility, and the concentration of infectious virions in air. Although most infections in animals originate from humans, neither risk factors for zoonotic transmission from humans to pets nor the frequency and nature of clinical illness in pets are well defined. We report the frequency of SARS-CoV-2 seropositivity in cohorts of pets from households, low-cost neuter clinics, and animal shelters in Ontario, Canada, and analyze household risk factors associated with seropositivity. The University of Guelph (Ontario, Canada) approved the studies by Animal Utilization Protocol 4411 and Research Ethics Board Protocol 20-04-002.

Methods

Swab Samples

Pet owners who had a diagnosis of SARS-CoV-2 infection or symptoms compatible with COVID-19 in the previous 3 weeks were invited to have their pet swabbed by study veterinarians during April 24, 2020–August 31, 2021. Dogs, cats, and ferrets of any age and clinical status were eligible for testing; the only exclusion criterion was medical or behavioral issues that precluded safe sampling. We obtained swab samples from the distal nares, oropharynx, and rectum, whenever possible. We placed swabs in inactivating media (PrimeStore; Longhorn Vaccines and Diagnostics, <https://lhnvd.com>) for a minimum of 12 hours, extracted RNA using Galvens Viral RNA Extraction (Montreal Biotech, <https://www.montreal-biotech.com>), and eluted into water.

We performed quantitative reverse transcription PCR to amplify SARS-CoV-2 cDNA with primers and probe in the viral N1 gene (Appendix 1, <https://wwwnc.cdc.gov/EID/article/28/6/22-0423-App1.pdf>). We submitted samples with positive results for amplification of segments of the envelope (E) and RNA-dependent RNA polymerase (RdRp) genes and whole-genome sequencing in additional laboratories.

Serum Samples

During June 8, 2020–November 30, 2021, we invited owners of pets who received a diagnosis of SARS-CoV-2 infection 2 weeks–3 months previously to have a blood sample of their pet analyzed for SARS-CoV-2 antibodies.

Veterinarians or veterinary technicians at Toronto Humane Society (THS) collected blood samples

from cats and dogs admitted to the shelter during June 18–November 28, 2020. Any animal that did not have health and behavioral reasons for exclusion was eligible for the study, regardless of origin (surrender, seizure, stray) or known history of SARS-CoV-2 exposure. Similarly, we collected samples through Toronto Animal Services (TAS) from unowned and owned cats admitted to a low-cost neuter clinic during January 21–July 6, 2021. We centrifuged all blood samples on site and shipped serum samples to Ontario Veterinary College (Guelph, Ontario, Canada). Serum samples were frozen in aliquots until batch analysis.

We constructed ELISA assays for the detection of cat and dog IgG and IgM to SARS-CoV-2 S protein (Appendix 1). Positive controls were from a SARS-CoV-2–experimentally-infected cat and 2 dogs with high titers; negative controls were cat and dog serum samples collected before 2019.

We tested the initial 42 serum samples and a subsequent 70 samples with IgG optical density (OD) >1.4 with the surrogate virus neutralization test (sVNT; GenScript, <https://www.genscript.com>) to determine blocking of the interaction of the receptor-binding domain (RBD) of SARS-CoV-2 with the ACE2 receptor. Following manufacturer instructions, we interpreted inhibition >20% relative to the kit positive control as indicating the presence of neutralizing antibodies.

Survey

We asked owners of household pets to complete an online 20-question survey concerning household demographics, the nature of the interaction with their pets, and the development of new illness in pets (Appendix 2, <https://wwwnc.cdc.gov/EID/article/28/6/22-0423-App2.pdf>). We also administered a questionnaire to owners of cats brought to the low-cost neuter clinic (Appendix 3, <https://wwwnc.cdc.gov/EID/article/28/6/22-0423-App3.pdf>). Questionnaires were not administered for unowned cats.

Statistical Analysis

For household cats, factors associated with PCR positivity were not evaluated because of the small sample size and low prevalence. We evaluated factors associated with seropositivity by univariable analysis using χ^2 , Fisher exact, or Wilcoxon tests as appropriate for the data. We categorized neuter-clinic cats by age: cats <6 months of age were kittens and cats >6 months adults. We calculated odds ratios and 95% CI. We did not perform multivariable analysis because of limitations in sample size.

We compared differences in seropositivity among different pet cohorts with Mann-Whitney tests. We

Table 1. Serology results from dogs and cats whose owners had received a diagnosis of SARS-CoV-2 infection or had symptoms compatible with COVID-19 in the previous 3 weeks, Ontario, Canada*

Test result	IgG	IgM	IgG and IgM	IgG or IgM
Dogs, n = 59				
>3 SD	26 (44)	26 (44)	21 (36)	31 (53)
>6 SD	22 (37)	16 (27)	16 (27)	24 (41)
Cats, n = 48				
>3 SD	29 (60)	29 (60)	22 (46)	35 (73)
>6 SD	23 (48)	13 (27)	11 (23)	25 (52)

*Values are no. (%). Results were >3 or >6 SD above the mean result for negative controls.

calculated correlation of ELISA OD with sVNT results using Prism version 9.3.1 (GraphPad, <https://www.graphpad.com>); $p < 0.05$ was considered significant.

Results

PCR

We collected a total of 283 swab specimens from 65 cats, 49 dogs, and 6 ferrets: 70 nasal, 90 oral, 107 rectal and 16 fur (dorsum) samples. Samples from 5 (7.7%) cats and 1 (2.0%) dog had positive PCR results. Each N1 PCR positive result (Ct <35.99) was confirmed by amplification with E, R, or RdRp primers. For all 6 animals testing positive, the nasal swab samples were positive; oral swab samples were positive from 2 of 3 tested, and rectal swab samples were positive from 1 of 3 tested. Swab samples from an additional 10 (15%) cats, 3 (6.1%) dogs, and 3 (50%) ferrets had nonnegative results. N1 PCR Ct values for those 16 samples were 36.00–39.00. Testing of other targeted regions at additional laboratories yielded similar nonnegative results.

One cat with an initial Ct of 21.56 was retested weekly 5 times after the first positive result and had positive results during the first 3 weeks. Another cat with an initial Ct of 24.11 tested positive again 1 week later (Ct 36.19) and negative thereafter.

We derived whole-genome sequences (>99.3% coverage) from 2 positive cats. Phylogenetic analysis assigned the sequences to Pangolin lineage A.23.1 and B.1.2, which had the highest similarity to human SARS-CoV-2 sequences derived in that time period from the corresponding geographic region.

Serology

Household Pets

We collected serum samples from 59 dogs and 48 cats from 77 households and 1 animal shelter (from recently surrendered cats). Median number of samples per household was 1 (range 1–4). We collected 7 samples from the humane society; those 7 samples were excluded from risk factor analysis because of the potential clustering effect and the lack of metadata about these animals. Dogs were a median of 5 years of age (range 5 months–14 years of age), and cats were a median of 6 years of age (range 1–19 years of age).

Seropositivity for IgG and IgM was 42%–62% using >3 SD above the mean of the negative control samples as a cutoff and 25%–48% at >6 SD (Table 1). At >6 SD, all IgM positive dogs were also IgG positive, whereas 12/48 (25%) cats were IgG positive but IgM negative.

For statistical analysis, we defined seropositivity as >3 SD for IgG, IgM, or both. We observed a significant association between seropositivity and owner-reported onset of new respiratory disease in dogs at the time of the owner's infection ($p = 0.04$), but not in cats (Table 2). Association of seropositivity and owner-reported new onset of clinical signs (respiratory, gastrointestinal, or systemic signs such as lethargy) approached significance in dogs ($p = 0.06$).

Not all risk factor data were available for all animals. Univariable risk factor analysis did not identify risk factors for dogs, but sleeping in the owner's bed was a risk factor for seropositivity in cats (OR 5.8, 95% CI 1.1–29.4). We determined no effect from the presence of multiple pets in the household (dogs $p = 0.33$, cats $p = 0.70$) or the number of persons with confirmed (dogs $p = 0.77$, cats $p = 0.64$) or confirmed and suspected (dogs $p = 0.92$, cats $p = 0.47$) COVID-19. We did not see an association between time the animal typically spent per day with the infected owner for either dogs ($p = 0.71$) or cats ($p = 0.53$).

When we defined seropositivity as >6 SD above the mean of negative controls, we saw no significant association between seropositivity and owner-reported

Table 2. Association of seropositivity for SARS-CoV-2 in pets with household risk factors and development of new illness, Ontario, Canada*

Variable	Dogs, n = 59			Cats, n = 48		
	Seropositive*	Seronegative	p value	Seropositive	Seronegative	p value
Kissed by owner	16/25 (64)	16/27 (59)	0.73	16/27 (59)	3/13 (30)	0.15
Licked hands/face of owner	19/25 (64)	22/25 (81)	0.63	13/27 (48)	3/13 (30)	0.46
Slept in/on bed	17/24 (68)	15/27 (56)	0.36	23/27 (85)	5/10 (50)	0.04
New respiratory signs	9/29 (31)	2/27 (7.4)	0.04	8/29 (28)	2/10 (20)	1.00
New clinical signs	12/29 (41)	5/27 (19)	0.06	12/29 (41)	2/10 (20)	0.28

*Seropositivity is defined by IgG, IgM or both against viral S protein. Results were positive if optical density is >3 SD above the mean of negative controls.

Table 3. Association of seropositivity for SARS-CoV-2 in pets with household risk factors and development of new illness, Ontario, Canada*

Variable	Dogs, n = 59			Cats, n = 48		
	Seropositive	Seronegative	p value	Seropositive	Seronegative	p value
Multiple pets	9/24 (38)	15/19 (44)	0.79	15/27 (56)	12/19 (63)	0.61
Kissed by owner	13/20 (65)	19/32 (59)	0.69	11/19 (58)	8/18 (44)	0.52
Licked hands/face of owner	16/20 (80)	25/32 (78)	1.00	10/19 (53)	6/18 (33)	0.32
Slept in/on bed	13/20 (65)	19/32 (59)	0.69	17/19 (76)	11/18 (61)	0.06
New respiratory signs	7/23 (30)	4/33 (12)	0.17	8/21 (38)	2/18 (11)	0.07
New clinical signs	11/23 (48)	6/33 (18)	0.018	12/21 (57)	2/18 (11)	0.006

*Seropositivity is defined by IgG, IgM or both against viral S protein. Results were positive if optical density is >6 SD above the mean of negative controls.

onset of new respiratory disease in the pet at the time of the owner’s infection for dogs (Table 3). However, we observed a significant association of seropositivity and owner-reported new onset of clinical respiratory, gastrointestinal, or systemic signs such as lethargy in the pet. We found the same association in cats.

Univariable risk factor analysis did not identify an association of seropositivity with risk factors (Table 3). We saw no association between time the animal typically spent per day with the infected owner for either dogs (p = 0.73) or cats (p = 0.35). However, cats that spent <2 hours per day with their owner were significantly less likely to be seropositive (1/7 [16%] vs. 18/30 [67%]; p = 0.04). We did not see the same result for dogs (p = 0.51). We saw no effect from the presence of multiple pets in the household (dogs p = 0.61, cats p = 0.69) or the number of persons per household with confirmed (dogs p = 0.83, cats p = 0.74) or confirmed or suspected (dogs p = 0.84, cats p = 0.82) COVID-19. Overall, >1 animal was seropositive in 3 (16%) of the 19 households where >1 animal was sampled: 2 households in which 2 dogs were seropositive and 1 in which a dog and cat were seropositive.

We performed sVNT on 53 samples from household pets. Of those, 30/41 (76%) that were positive for IgG and/or IgM (6 SD) were also positive on sVNT compared with 0/12 IgG/IgM negative samples (p<0.0001). Despite the smaller sample size, we repeated risk factor analysis using the samples tested by sVNT. For dogs, licking hands or face of owners was associated with seropositivity (OR 10.5 95% CI 1.5–73; p = 0.017). In addition, we noticed an association between positivity and dogs spending 19–24 hours with owners (OR 13.3, 95% CI 1.3–135; p = 0.033). For cats, the association between positivity and being kissed by owners was significant (OR 18.7, 95% CI 1.6–223; p = 0.020).

Neuter-Clinic Cats

We collected serum samples from 221 cats during January–June 2021. Full animal information and history were not available for all cats. The median age of the 184 (83%) cats for which age was reported or estimated was 1.5 years (interquartile range 3.25 years). We

classified 32/184 (17%) cats as kittens and 152 (83%) as adults (Table 4). COVID contact status was known for 103 cats. We detected S IgG (>6 SD) in 35/221 (16%) cats. Monthly seropositivity rate was 0%–40%; we identified a significant association between month and seropositivity (p<0.0001) (Figure 1).

Univariable analyses were performed excluding animals whose exposure to persons with COVID-19 was unknown (Table 4). We identified animal source as a risk factor for seropositivity. Compared with cats originating from households, cats that were in a shelter, rescue or foster facility cats were 3.6 times as likely to be seropositive (95% CI 1.5–8.8; p = 0.005). We found no significant difference between feral and household cats or feral and shelter/rescue/foster cats.

Humane Society Animals

Of 67 cat and 8 dog samples from THS, 7/75 (9.3%) overall and 7/67 (10%) of cat samples were seropositive (>6 SD). We did not perform risk factor analysis because limited metadata were available.

Correlation of ELISA with sVNT

We identified a significant difference in the mean OD between household samples and those from both THS

Table 4. Characteristics of 221 cats at a neuter clinic tested for the presence of SARS-CoV-2 serum antibodies and univariable analysis results, Ontario, Canada

Characteristic	Seropositive, no. (%)	p value
Categorical age		0.12
Kitten	2/32 (6.3)	
Adult	27/152 (18)	
Sex		1.0
M	16/106 (15)	
F	12/78 (15)	
Not reported	7/37 (19)	
Animal source		0.01
Household pet	7/93 (8)	
Shelter/rescue/foster	23/102 (23)	
Feral	5/26 (19)	
Exposure to person with COVID		0.59
Yes	2/13 (15)	
No	6/90 (6.7)	
Unknown or declined to answer	27/118 (23)	

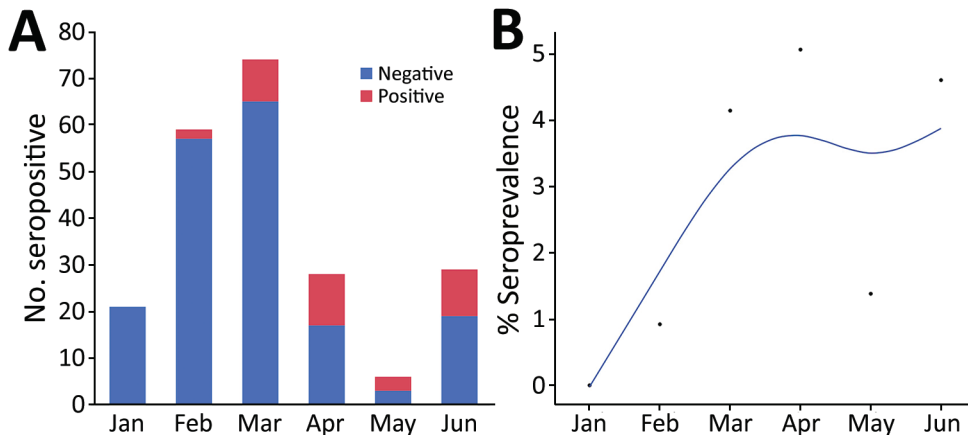


Figure 1. Seropositivity for SARS-CoV-2 in cats brought for care to a low-cost spay/neuter clinic during January – June 2021, Ontario, Canada. A) Test results for 221 cats shown by month. B) Positivity rate per month. The points indicate the proportion of positive test results among all test results over time. Blue line indicates the smoothed rate of seropositivity. The association between month and the change in seropositivity was significant ($p < 0.0001$).

and TAS. Differences between THS and TAS were not significant (Figure 2).

In addition to ELISA testing, we also assessed a subset of 112 serum samples (53 household and 59 from shelter and spay/neuter clinic) with the sVNT. We found a significant correlation between the ELISA OD and neutralization of virus binding ($\rho = 0.4188$, 95% CI 0.2529–0.5608; $p < 0.0001$) (Figure 3, panel A). The correlation between ELISA and sVNT results was higher for cats than dogs (Figure 3, panel B).

Discussion

Our findings suggest that transmission of SARS-CoV-2 from infected humans to their pets as indicated by seroconversion is common. PCR-based detection of SARS-CoV-2 in pets was uncommon within 3 weeks from owners being symptomatic or having a

diagnosis of COVID-19, which may reflect genuine brevity of infection in pets, as noted experimentally in cats (12). Other factors are variations in time intervals between owner infection and pet sampling and the challenge of obtaining representative samples from the nose in cats (12). Other studies of infection of cats from households of persons with COVID-19 had similarly low PCR-based prevalence (16,25–28). The timeframe required for owners to be diagnosed, contact the study team, and arrange a household visit likely resulted in false negative PCR results from samples being collected too late relative to onset of infection. The definition of COVID-19 symptoms and access to PCR testing for sick persons was limited early in the pandemic, and it is possible that pets in this study were infected concurrently or immediately after their owners but swabbed only after they had eliminated infection. Kittens 4–5 months old experimentally infected with 1×10^6 TCID₅₀ of SARS-CoV-2 intranasally and orally had detectable viral RNA for 10 days in nasopharyngeal swabs, 7 days in oropharyngeal swabs, and 14 days in rectal swabs, but such high viral challenge may not simulate typical human-cat household interactions (12). Subtle pulmonary lesions and viral RNA detectable until 6 days postinfection in experimentally infected cats suggest that, even with high viral inoculates, cats rarely get sick and can clear infection relatively quickly (29).

Longitudinal samples were rarely available; however, serial sampling for 1 cat revealed prolonged PCR positivity. That cat had chronic upper respiratory disease; whether the condition played a role in the prolonged PCR positivity is unclear. Despite the duration of PCR positivity, it is unlikely that the cat was infectious because the relatively high PCR Ct values would be consistent with low-level shedding of viral nucleic acids. Similar prolonged PCR positivity

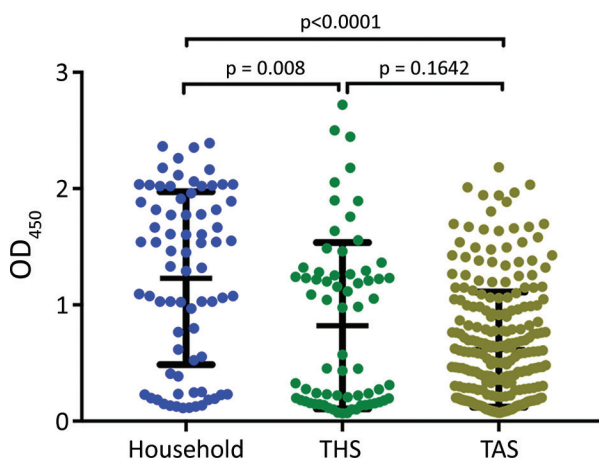
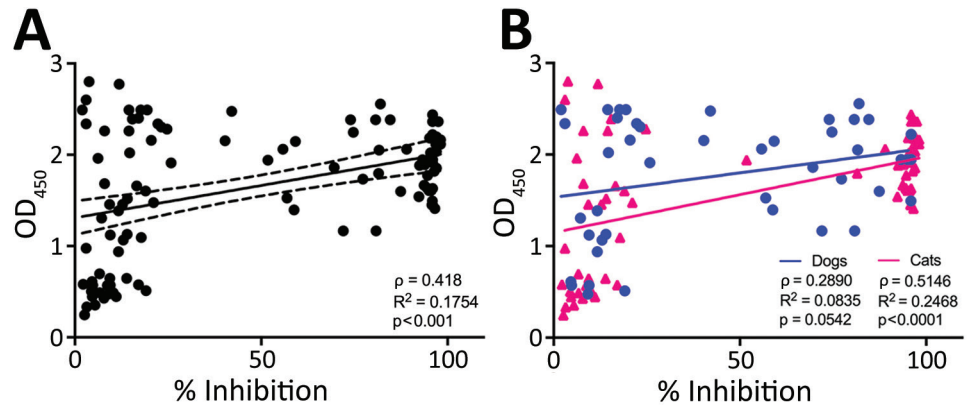


Figure 2. Mean serum SARS-CoV-2 spike protein IgG as measured by ELISA for samples from household cats, from cats in a shelter (THS), and from cats brought to a spay/neuter clinic for care (TAS), Ontario, Canada. The mean and SD are indicated. Differences were significant for household vs. shelter cats and household vs. clinic cats, but not for shelter vs. clinic cats. OD₄₅₀, optical density at 450 nm; THS, Toronto Humane Society; TAS, Toronto Animal Services.

Figure 3. Results of IgG ELISA in relation to percentage inhibition of binding of the SARS-CoV-2 receptor binding domain (RBD) to the ACE2 receptor in cat and dog serum samples measured with a surrogate virus neutralization assay, Ontario, Canada. A) Surrogate virus neutralization test results correlated with IgG ELISA results. B) Percentage of inhibition for dog (blue circles) and cat (pink triangles) samples.



The solid line shows correlation and dashed lines 95% CI. Correlation is higher for cat than dog samples. OD₄₅₀, optical density at 450 nm.

has been reported for a cat exposed in a retirement home (30) and for tigers and lions in zoos (31). More data regarding the duration of positivity in naturally infected dogs and cats, and whether infectious virus is shed, are needed.

Seroprevalence was much higher than PCR positivity. We expected this finding because serologic data represent historical exposure and there is not a need to sample animals within a narrow infection window. Seroprevalence detected in other studies was 0.4%–30% or higher; in most instances such variability could be attributed to the extent of pets' exposure to infected humans (6,9,32–34).

Without broadly accepted definitions, the parameters and interpretation of serologic assays for SARS-CoV-2 vary widely (13,28,35–37). We designed traditional ELISAs detecting IgG and IgM for S protein. We used a range of negative serum samples from before 2019, as well as serum from cats with feline infectious peritonitis caused by enteric α coronavirus. The negative controls yielded consistently low ODs for S protein IgG and IgM; we interpreted results from exposed animals at 3 SD and 6 SD above the mean of the least diluted negative controls to enable comparison with other studies (12,13). A relatively high proportion of dogs and cats had antibodies to S protein, which could indicate infection or exposure. Results of the sVNT, most likely to reflect infection, correlated with S protein ELISA results in this and other studies (38). Some serum samples had high S antibodies despite lack of neutralization; this pattern could indicate exposure rather than infection or postinfection persistence of antibodies broadly reactive with S protein but not neutralizing RBD binding. The cause of the discrepant results cannot be determined from samples collected at a single time point that was potentially days or

weeks postexposure. Furthermore, development of antibodies to SARS-CoV-2 is affected by host age, immunocompetence, and comorbidities, which could not be controlled in this surveillance study (36); even experimentally infected young cats had inconsistent antibody responses (12).

Risk factor analyses identified plausible associations presumably linked to the duration and closeness of human–animal contact. Limited risk factor information for dogs and cats has been reported (16, 28,37,39); however, association of seropositivity and proximity or sleeping with infected owners has been reported for dogs (16) and in a study where canine and feline data were combined (40). In our study, the same risk factors were not identified when using different serologic cutoffs or tests, which was likely a result of small sample sizes.

The substantially higher seroprevalence in cats exposed to infected persons gives more credence to the seropositivity data. Yet, the prevalence of seropositivity was still moderately high in cats with no known exposure to infected people. The lack of metadata makes this challenging to interpret, because it is possible that cats from the humane society or neuter clinic had previously been exposed to infected humans (28).

PCR positivity rate was too low for robust comparison of sample sites. However, all positive animals had positive nasal swab specimens, despite the challenges that can be encountered collecting good nasal swabs, especially from cats. Adding oral, rectal, or fur swab specimens did not increase diagnostic yield. Further study of sampling sites under field conditions would identify sampling approaches that maximize diagnostic yield while minimizing the number of sites that must be sampled. These data are preliminary but support the

importance of collecting nasal swab specimens as part of or all of the sample set.

Our study's first limitation was sample size; enrollment was hampered by low human COVID-19 infection rates in the study region throughout the main sampling times and by difficulties identifying exposed households in an appropriate timeframe. Lack of a coordinated One Health approach concurrently investigating human and animal exposures was a problem; local or provincial public health agencies had little interest in leading research or performing a joint study. The timing of sampling also affected PCR results. More complete validation of the specificity of serologic assays with a samples from animals with diverse other infectious and inflammatory conditions remains to be done. Ideally, the timeframe for sampling would have been more condensed to focus testing on animals whose owners were more recently infected (e.g., 1–2 weeks after the onset of the owner's infection).

These data indicate relatively common transmission of SARS-CoV-2 from humans to animals and that certain human–animal contacts (e.g., kissing the pet, pet sleeping on the bed) appear to increase the risk. We inferred that infections in dogs and cats reflect direct transmission from humans to animals, given the pandemic nature of this virus in humans and limited contact of most household pets with other animals (41). Intra-household transmission cannot be ruled out as a cause of some infections; however, multiple seropositive animals were only identified in 3/19 (16%) households where multiple animals were tested. We did not specifically investigate whether this relates to differences in individual animal susceptibility or animal–owner contact.

The relevance of human–pet transmission of SARS-CoV-2 needs further study. We observed an association between infection and clinical disease in both dogs and cats; in most cases, disease was very mild and self-limiting. Clinical data from this study are consistent with other studies indicating limited overall health risk to otherwise healthy dogs and cats (17,18,42). The zoonotic risk posed by dogs is probably low based on the lower infection rate and lack of evidence of transmission experimentally (43). Risk is probably higher for cats; cat–cat transmission has been identified, but the actual risk for cat–human transmission is unknown (44). Our findings support the occurrence of human–dog and human–cat transmission and highlight the need for further study of the animal and human health consequences of spillback of this zoonotic pathogen into animals.

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About the Author

Dr. Bienzle is a professor of veterinary pathology at Ontario Veterinary College. Her research interests include infectious diseases of companion animals.

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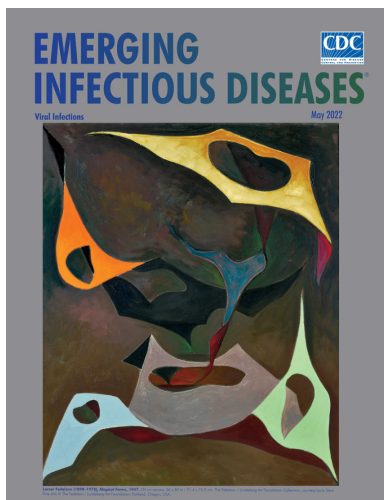
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Address for correspondence: Dorothee Bienzle, Department of Pathobiology, University of Guelph, Rm 3822, Bldg 89 419 Gordon St, Guelph, ON, N1G2W1, Canada; email: dbienzle@uoguelph.ca

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Viral Infections

- Invasive Group A *Streptococcus* Outbreaks Associated with Home Healthcare, England, 2018–2019
- Genomic Epidemiology of Global Carbapenemase-Producing *Escherichia coli*, 2015–2017
- Risk for Asymptomatic Household Transmission of *Clostridioides difficile* Infection Associated with Recently Hospitalized Family Members
- Estimating Relative Abundance of 2 SARS-CoV-2 Variants through Wastewater Surveillance at 2 Large Metropolitan Sites, United States
- Effectiveness of BNT162b2 Vaccine Booster against SARS-CoV-2 Infection and Breakthrough Complications, Israel
- Effects of Tick-Control Interventions on Tick Abundance, Human Encounters with Ticks, and Incidence of Tickborne Diseases in Residential Neighborhoods, New York, USA
- Pertactin-Deficient *Bordetella pertussis* with Unusual Mechanism of Pertactin Disruption, Spain, 1986–2018
- Determining Existing Human Population Immunity as Part of Assessing Influenza Pandemic Risk



- Pathogens that Cause Illness Clinically Indistinguishable from Lassa Fever, Nigeria, 2018
- Duration of Infectious Virus Shedding by SARS-CoV-2 Omicron Variant–Infected Vaccinees
- Intercontinental Movement of Highly Pathogenic Avian Influenza A(H5N1) Clade 2.3.4.4 Virus to the United States, 2021
- Rapid Replacement of SARS-CoV-2 Variants by Delta and Subsequent Arrival of Omicron, Uganda, 2021
- SARS-CoV-2 Antibody Prevalence and Population-Based Death Rates, Greater Omdurman, Sudan
- Evidence of Prolonged Crimean-Congo Hemorrhagic Fever Virus Endemicity by Retrospective Serosurvey, Eastern Spain
- Lack of Evidence for Crimean–Congo Hemorrhagic Fever Virus in Ticks Collected from Animals, Corsica, France
- Highly Pathogenic Avian Influenza A(H5N8) Clade 2.3.4.4b Viruses in Satellite-Tracked Wild Ducks, Ningxia, China, 2020
- Novel Hendra Virus Variant Circulating in Black Flying Foxes and Grey-Headed Flying Foxes, Australia
- Multisystem Inflammatory Syndrome in Children after SARS-CoV-2 Vaccination
- Disparities in First Dose COVID-19 Vaccination Coverage among Children 5–11 Years of Age, United States
- Severe Multisystem Inflammatory Symptoms in 2 Adults after Short Interval between COVID-19 and Subsequent Vaccination
- Imported Monkeypox from International Traveler, Maryland, USA, 2021

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Risk Factors for SARS-CoV-2 Infection and Illness in Cats and Dogs

Appendix 1

Materials and Methods

PCR and Whole-Genome Sequencing

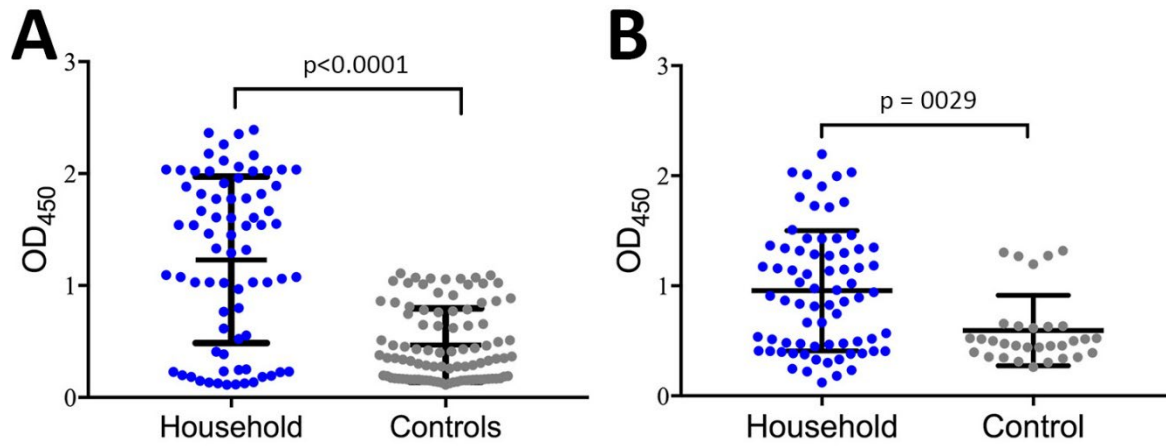
RNA was reverse transcribed and SARS-CoV-2 cDNA was amplified by qPCR (AgPath-ID, Applied Biosystems) with primers to the viral N1 gene 3' GAC CCC AAA ATC AGC GAA AT 5' and 3' CAG ATT CAA CTG GCA G 5', and detected with the probe 3' ACC CCG CAT TAC GTT TGG 5' labeled with fluorescein amidite (FAM). Viral RNA was also amplified with primers to the N2 gene 3' TTA CAA ACA TTG GCC GCA AA 5' and 3' TTC TTC GTA ATG TCG CGC 5' and detected with the FAM-labelled probe 3' ACA ATT TGC CCC CAG CGC TTC 5'. Primers and probe were based on the SARS-CoV-2 sequence GenBank MT226610.1. Host cellular RNA extracted from swabs was amplified with primers 3' CAA TTT CCA ATG CCC TCA AYT T 5' and 3' CAC ATC GTA TGG GCC TCT TAT T 5' directed against the ribonuclease P/MRP subunit p30 (RPP30) and detected with an Affinity Plus probe TC+T+A+GTG+C+T+GC. All oligonucleotides were obtained from Integrated DNA Technologies (Coralville, IA). Nucleic acids were amplified and quantified in a Light Cycler 480 (Roche, Mississauga, ON). The positive control consisted of inactivated SARS-CoV-2/Canada/VIDO-01/2020, kindly provided by the National Microbiology Laboratory (Winnipeg, MB). Only samples with Ct values <35.99 for RP30 were considered to yield interpretable viral PCR results. Samples with viral Ct values <35.99 were considered positive, samples with Ct values of 36–39.00 were considered non-negative.

All samples with Ct <39 were submitted for additional amplifications with primers to the viral E (envelope) and RdRp (RNA-dependent RNA polymerase) genes at the Animal Health Laboratory (Guelph, ON) and the Canadian Food Inspection Agency (CFIA) at the National Microbiology Laboratory (NML), Winnipeg, MB. Whole-genome sequencing and analysis was

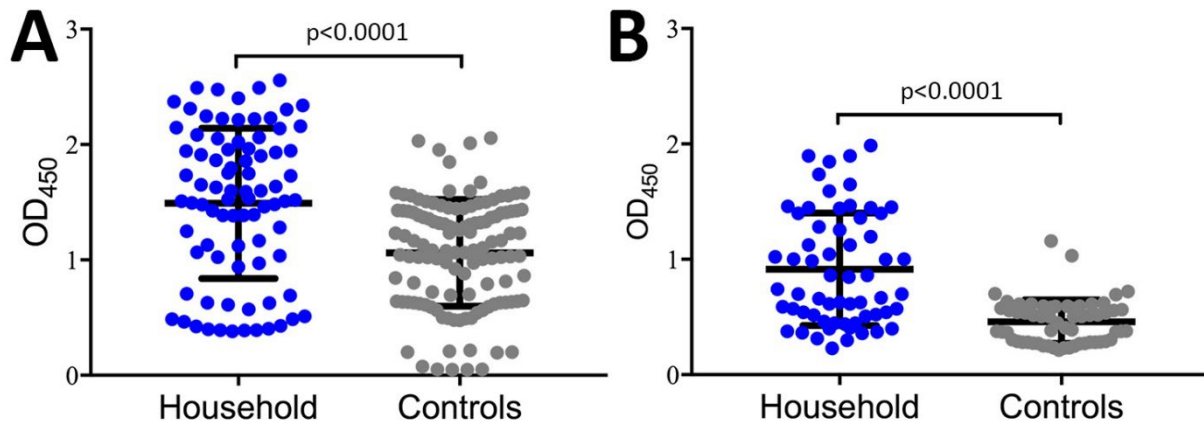
performed at the NML or the British Columbia Centre for Disease Control Public Health Laboratory using an amplicon method (Artic V3 or Freed 1200bp) on an Illumina instrument. Sequences were analyzed alongside human SARS-CoV-2 sequences from the household or the region to assess virus relatedness.

ELISA

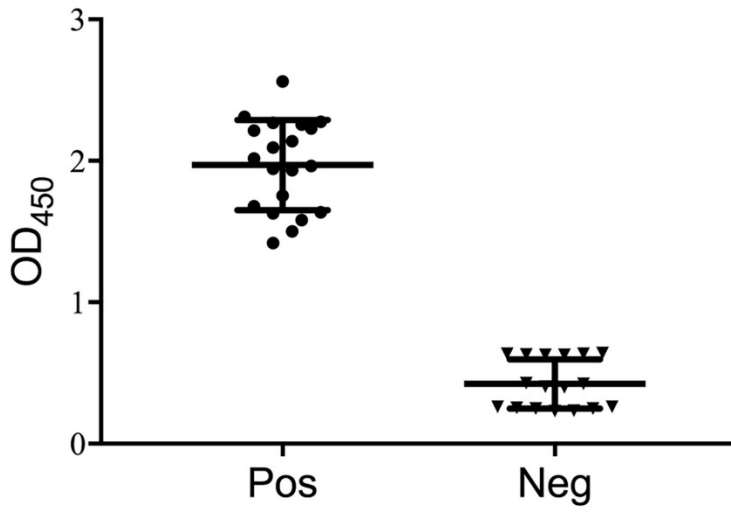
Enzyme-linked immunosorbent assays (ELISA) for the detection of cat and dog IgG and IgM antibodies to SARS-CoV-2 S protein were constructed. Briefly, adsorption immunoassay plates (96-well, ThermoFisher, Mississauga, ON) were coated overnight at 4° C with 2 µg/mL of His-tagged SARS-CoV-2 S1 (GenScript, Piscataway, NJ). Next day, wells were washed 3×, blocked with 3% skim milk in Tris buffer for 1 hour, and washed 3×; then 60 µL of five 3-fold dilutions (1:100, 1:300, 1:900, 1:2,700, and 1:8,100) of each serum sample was added. Plates were incubated for 2 hours, washed 3×, and secondary antibodies conjugated to horseradish peroxidase (HRP) and diluted 1:5,000 were added for 1 hour. Wells were washed 3×, and HRP activity was visualized by adding trimethyl benzidine (TMB) substrate. Reactions were stopped with sulfuric acid, and optical density (OD) at 450 nm was read. Secondary antibodies consisted of goat anti-dog IgG, goat anti-dog IgM, goat anti-cat IgG and goat anti-cat IgM (all from Abcam, Waltham, MA). Control samples consisted of serum from a SARS-CoV-2 experimentally-infected cat (kindly provided by Y. Kawaoka, Madison, WI; positive feline control, used at 1:5,000 in ELISA), 3 different batches of pooled cat serum from 2016 or 2017, 2 serum samples from cats with feline infectious peritonitis, 1 serum sample from a cat with osteomyelitis and hyperglobulinemia (Appendix. Figure 1). Control dog samples were from 2017, 2018, and 2019 (negative controls, Appendix Figure 2). One dog sample with high OD was used across all ELISA plates as a positive control (Appendix Figure 3). Each ELISA plate included 16 wells that were not coated with recombinant protein (blank), 5 replicate 1:100 dilutions of species-specific negative control samples, and five 3-fold dilutions of the positive control and test samples, starting at a 1:100 dilution.



Appendix Figure 1. Optical density for IgG and IgM to S protein in household cat samples relative to control samples.



Appendix Figure 2. Optical density for IgG and IgM to S protein in household dog samples relative to control samples.



Appendix Figure 3. Repeatability of optical density for a positive dog sample relative to negative samples, IgG to S protein.

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Risk Factors for SARS-CoV-2 Infection and Illness in Cats and Dogs

Appendix 2

Online survey

A survey given to owners of household pets is shown on the following pages.

COVID serology

Start of Block: Default Question Block

Q1 Evaluation of antibodies against SARS-CoV-2, the cause of COVID-19, in pets

exposed to infected people This study will evaluate whether or how commonly SARS-CoV-2 can be found in animals. You are being invited to participate because you have indicated that one or more people in your household had COVID-19. The study aims to help us better understand transmission of this virus between people and their pets. Participants must be 18 years of age or older. This study is funded by the Ontario Animal Health Network.

If you agree to take part in this study, you will be asked to complete a short (5 minute) survey. You will be asked to provide your name, phone number or email address, information about COVID-19 in your household and pet contact information. You will not be asked to identify specific people that are known or suspected to have COVID-19. Information from this survey will help understand COVID-19 in animals and transmission of the virus between people and animals. This survey is voluntary and all identifying information will be treated as confidential. Only Drs. Weese and Bienzle will have access to the data. No identifying or individual results will be released and identifying information will be stored on an encrypted device. Although there is a risk that you may be concerned about how your responses will be evaluated or about providing information about COVID-19 diagnoses, there are no right or wrong answers. You will not be asked any details about anyone's medical status beyond whether people in contact with the pet are known or suspected to be infected, and this information will be confidential. You will not be contacted about your survey results or participation, but will be informed of the testing results of your pet.

By entering this survey, you indicate that you have read the information provided and consent to participate. There are no direct benefits to you, but results will improve our understanding SARS-CoV-2. You may choose to skip any question(s) you do not want to answer, apart from your name and one form of contact. You can stop completing the survey at any time. If you wish to have your answers removed or if you have any questions, you may contact Dr. Weese at 519-824-4120 ext 54064 or jsweese@uoguelph.ca. After Mar 1, 2021, we will not be able to remove data.

A summary of results will be available at <http://www.wormsandgermsblog.com> upon completion of the study and be part of a scientific paper. Data will be retained on a secure drive until publication of results, then survey results will be destroyed. We encourage you to print a copy of this consent page.

You do not waive any legal rights by agreeing to take part in this study. This study has been reviewed by the University of Guelph Research Ethics Board for compliance with federal guidelines for research involving human participants. If you have any questions about your rights as a research participant in this study (REB#20-04-002) please contact: Manager,

Research Ethics, University of Guelph, reb@uoguelph.ca; 519-824-4120 ext 56606. By continuing on to the survey, you are indicating your consent to participate in this survey.

Q2 What is your name?

Q3 What is the best phone number or email address to reach you at to provide you with test results?

Q4 At which veterinary clinic was your pet tested?

Q5 How many people currently live in your household?

Q6 How many people in your household were diagnosed with COVID-19?

Q7 How many were not tested but were told by a healthcare provider or public health personnel that they likely had COVID-19?

Q8 Approximately what date was COVID-19 first diagnosed or suspected in the household?

End of Block: Default Question Block

Start of Block: Block 2

Q74 Do you have one or more dogs that were tested?

Yes (1)

No (2)

Skip To: Q28 If Do you have one or more dogs that were tested? = No

Q11 Dog's name (if you have more than one dog, pick one and we'll ask about the others later)

Q12 During the time that someone in the household had COVID-19, to the best of your recollection, which of the following occurred with this dog? (Check all that apply)

- Went on walks on a leash (1)
 - Went to a dog park on a leash (2)
 - Went to an off-leash park (3)
 - Was off your property without supervision (4)
 - Spent time unsupervised in a fenced yard or tied up (5)
 - Visited a veterinary clinic (6)
 - Visited a human healthcare facility (7)
 - Went to a groomer (8)
 - Went to a kennel (9)
-

Q13 When people in your household had COVID-19, which of the following likely occurred?
(please check all that apply)

- Slept in or on the bed of an infected person (1)
 - Licked the face of an infected person (2)
 - Licked the hands of an infected person (3)
 - Was kissed by an infected person (4)
 - Sat on the lap of, or beside, an infected person (5)
-

Q14 When people in your household had COVID-19, approximately how much time did this dog spend in the same room as an infected person on an average day?

- Less than 2 hours (1)
 - 2-6 hours (2)
 - 7-12 hours (3)
 - 13-18 hours (4)
 - 19-24 hours (5)
-

Q15 Around the time that people in the household had COVID-19, did this dog have any new occurrences of the following? (please check all that apply)

- Cough (1)
 - Difficulty breathing (2)
 - Vomiting (3)
 - Diarrhea (4)
 - Decreased appetite (5)
 - Decreased energy (6)
-

Q16 Do you have another dog?

- Yes (1)
- No (2)

Skip To: Q28 If Do you have another dog? = No

Q17 Dog #2's name

Q18 During the time that someone in the household had COVID-19, to the best of your recollection, which of the following occurred with this dog? (Check all that apply)

- Went on walks on a leash (1)
 - Went to a dog park on a leash (2)
 - Went to an off-leash park (3)
 - Was off your property without supervision (4)
 - Spent time unsupervised in a fenced yard or tied up (5)
 - Visited a veterinary clinic (6)
 - Visited a human healthcare facility (7)
 - Went to a groomer (8)
 - Went to a kennel (9)
-

Q19 When people in your household had COVID-19, which of the following likely occurred?
(please check all that apply)

- Slept in or on the bed of an infected person (1)
 - Licked the face of an infected perso (2)
 - Licked the hands of an infected person (3)
 - Was kissed by an infected person (4)
 - Sat or the lap of, or beside, an infected person (5)
-

Q20 When people in your household had COVID-19, approximately how much time did this dog spend in the same room as an infected person on an average day?

- Less than 2 hours (1)
 - 2-6 hours (2)
 - 7-12 hours (3)
 - 13-18 hours (4)
 - 19-24 hours (5)
-

Q21 Around the time that people in the household had COVID-19, did this dog have any new occurrences of the following? (please check all that apply)

- Cough (1)
 - Difficulty breathing (2)
 - Vomiting (3)
 - Diarrhea (4)
 - Decreased appetite (5)
 - Decreased energy (6)
-

Q22 Do you have another dog?

- Yes (1)
- No (2)

Skip To: Q28 If Do you have another dog? = No

Q23 Dog #3's name

Q24 During the time that someone in the household had COVID-19, to the best of your recollection, which of the following occurred with this dog? (Check all that apply)

- Went on walks on a leash (1)
 - Went to a dog park on a leash (2)
 - Went to an off-leash park (3)
 - Was off your property without supervision (4)
 - Spent time unsupervised in a fenced yard or tied up (5)
 - Visited a veterinary clinic (6)
 - Visited a human healthcare facility (7)
 - Went to a groomer (8)
 - Went to a kennel (9)
-

Q25 When people in your household had COVID-19, which of the following likely occurred?
(please check all that apply)

- Slept in or on the bed of an infected person (1)
 - Licked the face of an infected perso (2)
 - Licked the hands of an infected person (3)
 - Was kissed by an infected person (4)
 - Sat or the lap of, or beside, an infected person (5)
-

Q26 When people in your household had COVID-19, approximately how much time did this dog spend in the same room as an infected person on an average day?

- Less than 2 hours (1)
 - 2-6 hours (2)
 - 7-12 hours (3)
 - 13-18 hours (4)
 - 19-24 hours (5)
-

Q27 Around the time that people in the household had COVID-19, did this dog have any new occurrences of the following? (please check all that apply)

- Cough (1)
 - Difficulty breathing (2)
 - Vomiting (3)
 - Diarrhea (4)
 - Decreased appetite (5)
 - Decreased energy (6)
-

Q28 Do you also have a cat that was tested?

- Yes (1)
- No (2)

Skip To: End of Survey If Do you also have a cat that was tested? = No

End of Block: Block 2

Start of Block: Block 4

Q29 Cat's name (if you have more than one, pick one and we'll ask about the others next)

Q30 What best describes your cat

- Indoor exclusively (1)
 - Mainly indoor with some outdoor access (2)
 - Spends large amounts of time both indoors and outdoors (3)
 - Mainly outdoor (4)
 - Outdoor exclusively (5)
-

Q31 When people in your household had COVID-19, which of the following likely occurred?
(please check all that apply)

- Slept on/in the bed of an infected person (1)
 - Licked the face or hands of an infected person (2)
 - Was kissed by an infected person (3)
 - Sat on the lap of, or beside, an infected person (4)
-

Q34 When people in the household had COVID-19, approximately how much time did this cat spend in the room of an infected person on an average day?

- Less than 2 hours (1)
- 2-6 hours (2)
- 7-12 hours (3)
- 13-18 hours (4)
- 19-24 hours (5)

Q33 Around the time that people in the household had COVID-19, did this cat have any new occurrences of the following? (please check all that apply)

- Cough (1)
 - Difficulty breathing (2)
 - Vomiting (3)
 - Diarrhea (4)
 - Decreased appetite (5)
 - Decreased energy (6)
-

Q75 Do you have another cat that was tested?

- Yes (1)
- No (2)

Skip To: End of Survey If Do you have another cat that was tested? = No

Q43 Cat #2's name

Q44 What best describes your cat

- Indoor exclusively (1)
 - Mainly indoor with some outdoor access (2)
 - Spends large amounts of time both indoors and outdoors (3)
 - Mainly outdoor (4)
 - Outdoor exclusively (5)
-

Q45 When people in your household had COVID-19, which of the following likely occurred?
(please check all that apply)

- Slept on/in the bed of an infected person (1)
 - Licked the face or hands of an infected person (2)
 - Was kissed by an infected person (3)
 - Sat on the lap of, or beside, an infected person (4)
-

Q46 When people in the household had COVID-19, approximately how much time did this cat spend in the room of an infected person on an average day?

- Less than 2 hours (1)
- 2-6 hours (2)
- 7-12 hours (3)
- 13-18 hours (4)
- 19-24 hours (5)

Q47 When people in the household had COVID-19, approximately how much time did this cat spend in the room of an infected person on an average day?

- Less than 2 hours (1)
- 2-6 hours (2)
- 7-12 hours (3)
- 13-18 hours (4)
- 19-24 hours (5)

Q48 Around the time that people in the household had COVID-19, did this dog have any new occurrences of the following? (please check all that apply)

- Cough (1)
 - Difficulty breathing (2)
 - Vomiting (3)
 - Diarrhea (4)
 - Decreased appetite (5)
 - Decreased energy (6)
-

Q78 Do you have another cat that was tested?

- Yes (1)
- No (2)

Skip To: End of Survey If Do you have another cat that was tested? = No

Q49 Cat #3's name

Q50 What best describes your cat

- Indoor exclusively (1)
 - Mainly indoor with some outdoor access (2)
 - Spends large amounts of time both indoors and outdoors (3)
 - Mainly outdoor (4)
 - Outdoor exclusively (5)
-

Q51 When people in your household had COVID-19, which of the following likely occurred?
(please check all that apply)

- Slept on/in the bed of an infected person (1)
- Licked the face or hands of an infected person (2)
- Was kissed by an infected person (3)
- Sat on the lap of, or beside, an infected person (4)

Q53 When people in the household had COVID-19, approximately how much time did this cat spend in the room of an infected person on an average day?

- Less than 2 hours (1)
 - 2-6 hours (2)
 - 7-12 hours (3)
 - 13-18 hours (4)
 - 19-24 hours (5)
-

Q54 Around the time that people in the household had COVID-19, did this dog have any new occurrences of the following? (please check all that apply)

- Cough (1)
- Difficulty breathing (2)
- Vomiting (3)
- Diarrhea (4)
- Decreased appetite (5)
- Decreased energy (6)

End of Block: Block 4

Start of Block: Block 4

Risk Factors for SARS-CoV-2 Infection and Illness in Cats and Dogs

Appendix 3

Questionnaire

The questionnaire given to owners of cats brought to a low-cost neuter clinic is reproduced on the following pages.

Toronto Animal Services COVID serology

Start of Block: Default Question Block

Q1 Evaluation of antibodies against SARS-CoV-2, the cause of COVID-19, in cats You are invited to participate in a study of SARS-CoV-2 infection in cats being coordinated by Drs. Scott Weese and Dorothee Bienzle of the Ontario Veterinary College, University of Guelph. The study aims to help us better understand transmission of this virus between people and their pets. Participants must be 18 years of age or older. This study is funded by the Public Health Agency of Canada.

If you agree to take part in this study, you will be asked to complete a short (5 minute) survey. If you wish to receive your cat's results, you will be asked to provide your name, phone number or email address. Otherwise, no identifying information will be collected. The questionnaire will ask about COVID-19 in your household and pet contact information. You will not be asked to identify specific people that are known or suspected to have COVID-19. This survey is voluntary and all identifying information will be treated as confidential. Only Drs. Weese and Bienzle will have access to the data. No identifying or individual results will be released and identifying information will be stored on an encrypted device. Although there is a risk that you may be concerned about how your responses will be evaluated or about providing information about COVID-19 diagnoses, there are no right or wrong answers. You will not be asked any details about anyone's medical status beyond whether people in contact with the pet are known or suspected to be infected, and this information will be confidential. You will not be contacted about your survey results or participation, but will be informed of the testing results of your pet. By entering this survey, you indicate that you have read the information provided and consent to participate. There are no direct benefits to you, but results will improve our understanding SARS-CoV-2. You may choose to skip any question(s) you do not want to answer. You can stop completing the survey at any time. If you wish to have your answers removed or if you have any questions, you may contact Dr. Weese at 519-824-4120 ext 54064 or jsweese@uoguelph.ca. After July 1, 2021, we will not be able to remove data.

A summary of results will be available at <http://www.wormsandgermsblog.com> upon completion of the study and be part of a scientific paper. Data will be retained on a secure drive until publication of results, then survey results will be destroyed. We encourage you to print a copy of this consent page.

You do not waive any legal rights by agreeing to take part in this study. This study has been reviewed by the University of Guelph Research Ethics Board for compliance with federal guidelines for research involving human participants. If you have any questions about your rights as a research participant in this study (REB#20-04-002) please contact: Manager, Research Ethics, University of Guelph, reb@uoguelph.ca; 519-824-4120 ext 56606. By continuing on to the survey, you are indicating your consent to participate in this survey.

Q79 Would you like to receive results of your cat's test? If so, we will ask for your contact information.

Yes (1)

No (2)

Skip To: Q29 If Would you like to receive results of your cat's test? If so, we will ask for your contact informa... = No

Q2 What is your name?

Q3 What is the best phone number or email address to reach you at to provide you with test results?

Q29 Cat's name

Q83 Cat's age (or approximate age)

Q85 Cat's sex

Male (1)

Female (2)

Q84 Which best describes this cat?

- This is your cat (lives in the same household as you) (1)
- You are fostering this cat (3)
- This cat is currently at a shelter or rescue (4)
- Other (please specify) (5) _____

Q30 What best describes this cat

- Indoor exclusively (1)
- Mainly indoor with some outdoor access (2)
- Spends large amounts of time both indoors and outdoors (3)
- Mainly outdoor (4)
- Outdoor exclusively (5)
- Unknown background (6)
- Lives in a shelter (7)

Q5 How many people currently live in your household?

Q6 Was anyone in your household diagnosed with COVID-19 during the time the cat was present?

- Yes (4)
- No (5)
- Maybe, COVID was suspected but testing was not performed (6)
- Don't know/prefer not to answer (7)

Skip To: End of Block If Was anyone in your household diagnosed with COVID-19 during the time the cat was present? = No

Skip To: End of Block If Was anyone in your household diagnosed with COVID-19 during the time the cat was present? = Don't know/prefer not to answer

Q8 Approximately what date was COVID-19 first diagnosed or suspected in the household?

End of Block: Default Question Block

Start of Block: Block 4

Display This Question:

If Was anyone in your household diagnosed with COVID-19 during the time the cat was present? = Yes

Q34 When people in the household had COVID-19, approximately how much time did this cat spend in the room of an infected person on an average day?

- Less than 2 hours (1)
 - 2-6 hours (2)
 - 7-12 hours (3)
 - 13-18 hours (4)
 - 19-24 hours (5)
-

Display This Question:

If Was anyone in your household diagnosed with COVID-19 during the time the cat was present? = Yes

Q33 Around the time that people in the household had COVID-19, did this cat have any new occurrences of the following? (please check all that apply)

- Cough (1)
- Difficulty breathing (2)
- Vomiting (3)
- Diarrhea (4)
- Decreased appetite (5)
- Decreased energy (6)

Display This Question:

If Was anyone in your household diagnosed with COVID-19 during the time the cat was present? = Yes

Q45 When people in your household had COVID-19, which of the following likely occurred? (please check all that apply)

- Slept on/in the bed of an infected person (1)
- Licked the face or hands of an infected person (2)
- Was kissed by an infected person (3)
- Sat on the lap of, or beside, an infected person (4)

Q81 Thank you for your participation. Test results will be provided to you within a few weeks if you provided contact information. If you have any questions, please contact Dr. Scott Weese at jsweese@uoguelph.ca

End of Block: Block 4

Start of Block: Block 4
