SARS-CoV-2 infection in dogs and cats is associated with contact to COVID-19-positive household members

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Abstract

Several domestic and wild animal species are susceptible to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Reported (sero)prevalence in dogs and cats vary largely depending on the target population, test characteristics, geographical location and time period. This research assessed the prevalence of SARS-CoV-2-positive cats and dogs (PCR- and/or antibody positive) in two different populations. Dogs and cats living in a household with at least one confirmed COVID-19-positive person (household (HH) study; 156 dogs and 152 cats) and dogs and cats visiting a veterinary clinic (VC) (VC study; 183 dogs and 140 cats) were sampled and tested for presence of virus (PCR) and antibodies. Potential risk factors were evaluated and follow-up of PCR-positive animals was performed to determine the duration of virus shedding and to detect potential transmission between pets in the same HH. In the HH study, 18.8% (27 dogs, 31 cats) tested SARS-CoV-2 positive (PCR- and/or antibody positive), whereas in the VC study, SARS-CoV-2 prevalence was much lower (4.6%; six dogs, nine cats). SARS-CoV-2 prevalence amongst dogs and cats was significantly higher in the multi-person HHs with two or more COVID-19-positive persons compared with multi-person HHs with only one COVID-19-positive person. In both study populations, no associations could be identified between SARS-CoV-2 status of the animal and health status, age or sex. During follow-up of PCR-positive animals, no transmission to other pets in the HH was observed despite long-lasting virus shedding in cats (up to 35 days). SARS-CoV-2 infection in dogs and cats appeared to be clearly associated with reported COVID-19-positive status of the HH. Our study supports previous findings and suggests a very low risk of pet-to-human transmission within HHs, no severe clinical signs in pets and a negligible pet-to-pet transmission between HHs.

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cats. dogs. risk factors. households. prevalence. SARS-CoV-2

1 | INTRODUCTION

In December 2019, a novel coronavirus, severe acute respiratory syndrome coronavirus 2, SARS-CoV-2, was found in several pneumonia cases in humans in China (Wang et al., 2020). This originally zoonotic virus has spread among humans worldwide causing a pandemic. Sporadic animal infections after close contact with infected people have been reported, and subsequent virus circulation and transmission back to humans have been described in mink and pet hamsters (Yen et al., 2022; Oreshkova et al., 2020; Oude Munnink et al., 2021).

Cats and dogs are in close contact with their owners; therefore, it is important to determine if these animals might act as a potential reservoir for SARS-CoV-2 for humans. In experimental studies, cats appeared to be highly susceptible to the virus and shed oral, nasal and fecal viral-RNA for several days after inoculation (Bosco-Lauth et al., 2020; Shi et al., 2020). Dogs appeared less susceptible to SARS-CoV-2 in experimental studies (Shi et al., 2020). Besides experimental studies, several cases of natural SARS-CoV-2 infections in dogs and cats were reported. Few dogs and cats showed respiratory and gastrointestinal signs after SARS-CoV-2 infection, whereas most dogs and cats showed no symptoms at all (Patterson, Elia et al., 2020; Sit et al., 2020; Decaro et al., 2021).

The seroprevalence of SARS-CoV-2 infections in cats and dogs has been investigated in different populations since the beginning of the pandemic. Several studies - using different diagnostic tests - suggest that dogs and cats can become infected with SARS-CoV-2 when in close contact with their COVID-19-positive owners (Patterson, Smith et al., 2020; Sailleau et al., 2020; Fritz et al., 2021; Michael et al., 2021). Reported seroprevalence vary from 0.2% in Dutch dogs without known contact with COVID-19-positive persons (S. Zhao et al., 2021) to 53% in dogs living in COVID-19-positive households (HHs) in France (Fritz et al., 2021). In Northern Italy, 3.3% of dogs and 5.8% of cats in HHs had measurable SARS-CoV-2 antibody responses (Patterson, Elia et al., 2020). The wide variation in reported prevalences may be related to differences in the targeted animal population, different test characteristics, and the timing of sample collection. Besides these seroprevalence studies, few studies have reported virus detection by PCR. Virus shedding was observed in cats after natural infection and seems to be shorter (5-17 days) than in humans in the same HH (Neira et al., 2021). Very few studies have included viral detection as well as serological screening in dogs and cats.

We aimed to assess the prevalence of SARS-CoV-2 amongst cats and dogs (PCR and/or antibody positive) in two different populations (with and without known COVID-19-positive status of the HH) and potential risk factors for SARS-CoV-2 infection in these pets. Additionally, a follow-up of PCR-positive animals was performed to determine

the duration of virus shedding and to detect potential transmission between pets in the same HH.

2 MATERIALS AND METHODS

2.1 Study population and data collection

We performed two studies from July 2020 to April 2021 targeting two different populations of dogs and cats in the Netherlands: (I) Dogs and cats living in HHs with one or more COVID-19-positive persons (HH study) and (II) Dogs and cats visiting veterinary clinics (VCs) (VC study).

2.1.1 | HH study

COVID-19-positive pet owners were recruited via regional Municipal Health Service centers. If at least one person within the HH tested positive for SARS-CoV-2 by PCR, a leaflet with a participation request was sent. Interested pet owners could apply through e-mail after which they were contacted by telephone to provide more information on the study and to schedule a house visit. A mobile VC from the Dutch Stray Cat Foundation was used to visit the HHs for sampling. The mobile clinic was available twice a week and house visits were planned on a convenience basis, that is, routes were planned on willingness and availability of the pet owners and the geographical location.

2.1.2 | VC study

VCs were selected from the client database of the Veterinary Microbiological Diagnostic Center of Utrecht University and invited to participate by e-mail. All clinics willing to participate were provided with information on the study, sampling instructions, questionnaires and sampling material for 20 animals (10 dogs, 10 cats). Veterinarians were asked to select cats and dogs with symptoms potentially associated with SARS-CoV-2 (defined as fever, respiratory and/or gastrointestinal complaints) and from dogs and cats without symptoms (e.g., pets for elective surgery or vaccination).

2.2 Data collection

Pet owners signed an informed consent and filled out a short questionnaire on HH composition (including number of COVID-19-positive HH members), pets' characteristics (e.g., age, sex) and health status (Supporting Information File 1).

2.3 | Sampling procedures

Oropharyngeal and rectal swabs were taken and immediately immersed in separate RNA/DNA shield containers for transport. Blood samples were taken and collected in gel-and-clot activator tubes (Greiner Bio-One, Kremsmunster, Austria) to obtain serum.

To determine duration of virus shedding and potential transmission between pets, owners of PCR-positive pets were contacted for resampling 1–3 weeks after initial sampling. Not only the PCR-positive pet was resampled but also all other dogs and cats living in the same HH (defined as contact animals).

2.4 | Laboratory procedures

All samples were processed within 48 h after sampling. Oropharyngeal and rectal swabs were analysed for presence of SARS-CoV-2 RNA using real time reverse-transcription PCR targeting the envelope protein gene (E gene) as previously described (Corman et al., 2020). In-house validation was performed prior to the study using a panel consisting of HCoV-229E, HCoV-NL63, HCoV-OC43, MERS-CoV, SARS-CoV-1, SARS-CoV-2, FCoV (TN406HP), FIPV, CCV378 and BCV. Good performance and no cross reactivity with other coronaviruses was shown.

Serology was performed as previously described. In short, an indirect enzyme-linked immunosorbent assay (ELISA) detecting antibodies against two SARS-CoV-2 proteins (S1 and RBD) was performed. Positive ELISA results were confirmed by virus neutralization test (VNT) using luciferase-encoding VSV particles pseudo typed with S-protein of SARS-CoV-2 (S. Zhao et al., 2021). 'Seropositive' samples were defined as samples that were either ELISA positive for both SARS-CoV-2 proteins, or samples that were ELISA positive for at least one SARS-CoV-2 protein, combined with a positive VNT. All other samples were considered 'seronegative'.

2.5 | Data analysis

Excel and SAS (version 9.4) were used to manage and organize the collected data. R software (version 3.5) was used to perform the statistical analyses. Seropositive pets and/or pets with a positive PCR were considered SARS-CoV-2 positive. Chi-square tests were performed to test for differences in percentage of SARS-CoV-2 positives between dogs and cats and to test for differences in percentage of SARS-CoV-2-positive pets (dogs and cats separately) and different HH composition (HH study). Logistic regression analyses (uni- and multivariable) were performed to identify associations between potential determinants and SARS-CoV-2 positivity in pets. Logistic mixed regression analyses (random intercept per HH) were performed (stratified for dogs and cats) to assess associations between SARS-CoV-2 status and age, sex, health status, underlying chronic disease status, SARS-CoV-2-relevant symptoms (defined as fever, respiratory and/or gastrointestinal complaints) (Supporting Information File 2).

3 | RESULTS

3.1 | HH study

In total 311 pets (156 dogs, 155 cats) from 196 HHs were enrolled in the HH study. Three cats were excluded due to missing blood samples. Descriptives and data analyses were therefore based on a total of 308 pets (156 dogs, 152 cats) from 195 HHs (Table 1).

Thirteen pets (seven dogs; six cats) tested PCR positive for SARS-CoV-2. All PCR-positive animals tested positive in the oropharyngeal swab, two (one dog and one cat) in the rectal swab as well. In total 50 animals (21 dogs; 29 cats) tested seropositive for SARS-CoV-2. Five of the 13 PCR-positive pets were seropositive as well. So, in total 58 animals (18.8%; 27 dogs; 31 cats) from 46 different HHs (23.6%) were SARS-CoV-2 positive based on PCR results and/or serology (Table 2; Supporting information file 4). SARS-CoV-2 prevalence in cats (20.4%) was not statistically different from that in dogs (17.3%) ($\chi = 0.30$, p = .58).

As a result of convenience sampling, the interval between the positive test result of owner(s) and initial sampling of the animal(s) was variable and ranged from 2 to 210 days (median 18 days). The interval between the positive test result of the owner and all PCR-positive animals was less than 21 days.

The composition of the HH (single cat/dog, multi cat/dog or mixed cat/dog) and HH size were not associated with SARS-CoV-2 prevalence in cats and dogs. However, in multi-person HHs (i.e., HHs with more than one person), SARS-CoV-2 prevalence in pets was significantly higher in HHs with two or more COVID-19-positive persons compared with HHs with only one positive person (29.8% in cats and 35.4% in dogs versus 8.9% in cats and 4.3% in dogs). This was significant for both dogs (N = 156, $\chi = 25.49$, p < .001) and cats (N = 152, $\chi = 10.65$, p = .01; Table 1).

SARS-CoV-2-relevant symptoms were reported in 36 cats and 53 dogs (including both SARS-CoV-2-positive and -negative animals), no association was identified with SARS-CoV-2 status. For gender, age and underlying chronic diseases no associations with SARS-CoV-2 status were identified either (Supporting Information File 2).

3.2 VC study

In total, samples from 344 animals (196 dogs, 148 cats owned by 327 different owners) were submitted by 34 VCs. Three dogs and one cat were excluded due to missing blood samples. Additionally only one animal per owner was included to avoid bias of multiple pets per owner. Therefore, descriptives and data analyses were based on a total of 323 animals (183 dogs, 140 cats) from 323 owners.

Four pets (three cats; one dog) tested PCR positive for SARS-CoV-2. All tested positive in the oropharynx, one cat tested positive in the rectal swab as well. The three PCR-positive cats were also seropositive, the PCR-positive dog was seronegative. Nine cats and five dogs tested seropositive. So, in total 15 animals (4.6%; six dogs; nine cats) from

TABLE 1 Characteristics derived from the questionnaires of the household and veterinary clinic study with the number of cats and dogs and percentage SARS-CoV-2 positives per category

Determinants		Household study				Veterinary clinics study			
		Cats (n = 152)		Dogs (n = 156)		Cats (n = 140)		Dogs (n = 183)	
		n	% SARS-CoV-2 positive	n	% SARS-CoV-2 positive	n	% SARS-CoV-2 positive	n	% SARS-CoV-2 positive
Sex	Male	79	21.5	79	16.5	70	10.0	97	2.0
	Female	73	19.2	77	18.2	70	2.9	85	4.7
Age [†]	Junior	20	30.0	26	7.7	35	2.9	43	2.3
	Adult	104	17.3	99	22.2	62	8.1	102	3.9
	Senior	28	25.0	31	9.7	42	7.1	35	2.9
SARS-CoV-2-relevant symptoms [‡]	Yes	36	25.0	54	14.8	60	8.3	83	3.6
	No	116	19.0	102	18.6	80	5.0	100	3.0
Underlying disease [§]	Yes	15	33.3	2	0.0	NA NA	NA	NA	NA
	No	137	19.0	154	17.5				
Multipet household	Yes	114	20.2	76	22.4	NA	NA	NA	NA
	No	38	21.1	80	12.5				
COVID -19 in household	Yes	152	100.0	156	100.0	23	21.7	28	17.9
	No	0	0.0	0	0.0	117	3.4	155	0.6
Number of COVID-19 positive persons in HH	Single person HH [¶]	23	8.7	22	4.5	NA	NA	NA	NA
	Multiperson HH, 1 positive	45	8.9	69	4.3	NA	NA	NA	NA
	Multiperson HH, >1 positive	84	29.8	65	35.4				
Reason for visiting veterinary clinic	Clinical	NA	NA	NA	NA	61	8.2	86	3.5
	Preventive [#]					79	5.1	97	3.0

[†]Age cats: young <1 years old, adult 1–10 years old, senior >10 years old; age dogs: young <2 years old, adult 2–10 years old, senior >10 years old. [‡]SARS-CoV-2-relevant symptoms were defined as fever, respiratory and/or gastro-intestinal symptoms.

[§]Underlying diseases were defined as any chronic underlying diseases, for example diabetes, obesity, immune deficiency.

[¶]Including households with missing data on number of persons per household.

[#]Preventive, for example for vaccination, check-up or elective surgery.

TABLE 2 Number and percentage of SARS-CoV-2-positive cats and dogs based on PCR and serology in the household and veterinary clinic study

			PCR positive		Seropositive		SARS-CoV-2 positive	
		Total number	n	% (CI)	n	% (CI)	n	% (CI)
Household study	Cats	152	6	4.0 (1.5-8.4)	29	19.1 (13.2–26.2)	31	20.4 (14.3-27.7)
	Dogs	156	7	4.5 (1.8-9.0)	21	13.5 (8.5–19.8)	27	17.3 (11.7–24.2)
Veterinary Clinic study †	Cats	140	3	2.1 (0.4-6.1)	9	6.4 (3.0-11.9)	9	6.4 (3.0-11.9)
	Dogs	183	1	0.6 (0.0-3.0)	5	2.7 (0.9–60.3)	6	3.3 (0.7–5.9)

[†]10 out of 15 SARS-CoV-2-positive animals in the veterinary clinic study were exposed to COVID-19-positive persons.

15 different owners were considered SARS-CoV-2 positive (Table 2; Supporting Information File 4). Prevalence in cats (6.4%) was not statistically different from prevalence in dogs (3.3%) (χ = 1.11, p = .29).

All PCR-positive animals (three cats, one dog) and six of the 11 seropositive animals (two cats, four dogs) were exposed to COVID-19-

positive persons in their HH. SARS-CoV-2 positivity was much higher in exposed animals (19.6%; 10 out of 51) than in animals with an unknown history of exposure to COVID-19-positive persons (1.8%; five out of 272). Consequently, a strong association between SARS-CoV-2 positivity among pets and the reported COVID-19 status of the HH (positive

versus unknown) was observed (OR 31.3 [4.8–614], p = .002 for dogs; OR 7.5 [1.83–32.9], p = .005 for cats; Table 1).

The reason for visiting the VC were clinical complaints in 147 animals (45.5%; 86 dogs, 61 cats) and preventive, for example, an elective procedure or vaccination, in 176 animals (54.5%; 97 dogs, 79 cats). Potential SARS-CoV-2-relevant symptoms were reported in 60 cats and 83 dogs, no association was identified with SARS-CoV-2 status. For gender, age and the reason for visiting the VC, also no associations with SARS-CoV-2 status were identified (Supporting Information File 3).

3.3 | Follow-up of PCR-positive animals

Two owners of PCR-positive animals at T1 in the HH study waived further cooperation, resulting in the resampling (T2) of 19 animals (11 PCR-positive animals and eight contact animals). The median time between initial sampling (T1) and resampling (T2) was 21 days (range: 7-35 days). All PCR-positive animals were already or became seropositive (at T1 and/or T2), confirming an active infection with SARS-CoV-2. All six PCR-positive dogs tested PCR negative at T2, whereas four cats still tested PCR positive at resampling (Supporting Information File 4). The three cats and four contact animals were resampled for a second time (T3). All cats turned PCR negative (range time interval T2 and T3: 14–30 days); the fourth cat was lost for follow-up. No contact animal turned PCR positive or showed seroconversion during the follow-up. In the VC study, two out of four PCR-positive animals (one cat, one dog) were resampled. Both animals were resampled 12 days after T1. both tested PCR negative and seropositive at T2 (Supporting Information File 4).

4 DISCUSSION

In 23.6% (46 out of 195) of the HHs with at least one confirmed COVID-19-positive person, SARS-CoV-2-infected pets were found; 31 out of 152 cats and 24 out of 156 dogs. A study in France showed a high seroprevalence in COVID-19-positive HHs as well (21-53%, depending on the cut-off criteria chosen) and suggested a transmission route from owner to animal (Fritz et al., 2021). SARS-CoV-2 prevalence was much lower (4.6%) in the studied population of dogs and cats visiting VCs. Excluding animals from the VC study exposed to COVID-19-positive owners resulted in an even lower SARS-CoV-2 prevalence (1.8%) in pets without known exposure to COVID-19 persons. A large serosurvey during the first wave in the Netherlands among cats and dogs with an unknow history of COVID-19 exposure showed a low seroprevalence as well (0.4% in cats and 0.2% in dogs; (S. Zhao et al., 2021)). The apparent difference in prevalence between pets with and without known exposure to COVID-19-positive persons supports that transmission takes place from humans to animals.

The cross-sectional design of the study does not allow to determine the actual direction of transmission within the HHs. Transmission between cats has been shown in experimental studies (Bosco-Lauth et al., 2020; Shi et al., 2020), but in our follow-up no transmission from PCR-positive animals to contact animals within the same HH was observed. This suggests that within-HH transmission between pets is not very efficient; however, only eight contact animals were resampled, so no firm conclusions can be drawn based on this limited number. Transmission from animals to humans has been shown for minks and hamsters, and recently cat-to-human transmission was suspected in Thailand (Yen et al., 2022; Oude Munnink et al., 2021). Despite this incident, the role of dogs and cats in the transmission of SARS-CoV-2 to humans seems insignificant, which is also supported by a risk assessment that assessed the risk of cat-human transmission within HHs negligible to very low, depending on the intensity of cat-human interactions (Allendorf et al., 2022).

Several studies in humans have proven that the risk of SARS-CoV-2 transmission within HHs can be high and that a larger HH size is associated with seropositivity (Reukers et al., 2022; Warszawski et al., 2022). Large variations in transmissibility and superspreading by a minority of individuals have been observed in humans (Toth et al., 2021). In our study, we did not find a difference in SARS-CoV-2 prevalence in pets for different HH sizes or composition (i.e., number of humans, cats and/or dogs). However, the percentage of SARS-CoV-2-positive pets was significantly higher in multi-person HHs with two or more positive persons compared with multi-person HHs with just one positive person. Whether this is caused by a human super spreader infecting multiple HH members (human and animal), a higher risk of exposure to a SARS-CoV-2-positive person or other factors remains unknown.

Most SARS-CoV-2-positive dogs and cats in our study were seropositive and not PCR positive. This can be explained by the sometimes long interval between the first SARS-CoV-2-positive test of the human HH member and sampling of the pet. PCR-positive pets were only detected in HHs with recently infected owners (less than 3 weeks). In humans, long-lasting viral shedding (>4 weeks) has been reported and appears to be associated with severity of disease and age (Long et al., 2021). In our study, we only collected data on the animal health status, so human health data could not be assessed. After experimental infection, shedding of viral RNA has been reported up to 21 days in cats (Bosco-Lauth et al., 2020; Gaudreault et al., 2020). At the follow-up sampling of PCR-positive animals, four cats showed long-lasting viral shedding (up to 35 days). However, in this study, the exact start of shedding was not known and the number of resampled animals was too low to determine a reliable duration of shedding. In humans, antibodies have been reported for over ten months after a SARS-CoV-2 infection (Sonnleitner et al., 2021). Data on the duration of persistence of neutralizing antibodies in cats and dogs are scarce. A study in seven dogs and two cats showed a long-term antibody response against SARS-CoV-2 up to 8 months (Decaro et al., 2021). The longest period in our study between the SARS-CoV-2-positive test of the owner and detecting antibodies in a pet was 112 days. In pets that were sampled long after exposure to their positive owner, antibody titres might have decreased under the detection limit.

Dogs and cats appeared equally at risk for infection in our study as prevalence among dogs and cats were not statistically different. This contrasts experimental studies where dogs appeared less susceptible than cats (Shi et al., 2020). An Italian study suggested that a greater interaction between owners and their dogs compared with cats might explain the higher transmission risk from humans to dogs (Patterson, Smith et al., 2020). A serological survey of 920 dogs in Wuhan also suggests that in COVID-19-positive HHs dogs have a higher risk of infection (Y.Zhao et al., 2021).

Mild clinical signs have been reported in cats after experimental infection, whereas dogs did not show any clinical signs. Some case reports describe clinical symptoms in dogs and cats with SARS-CoV-2, mostly from the respiratory and gastro-intestinal tract (Miró et al., 2021; Colitti et al., 2022). In our HH study, about one-third of owners reported clinical signs in their pets, but this was not associated with the presence of viral RNA and/or antibodies against SARS-CoV-2. Other studies confirm that generally SARS-CoV-2 does not cause any or only mild clinical signs in dogs and cats. (Bosco-Lauth et al., 2020; Gaudreault et al., 2020; Sit et al., 2020; Decaro et al., 2021). In our study, no association was found between SARS-CoV-2 status of pets and age or reported underlying diseases.

Our study was conducted in 2020/2021 when the alpha- and delta variant were most abundant in the Netherlands. Other variants of SARS-CoV-2 might be more transmissible or pathogenic among animal species, therefore future (passive) surveillance in dogs and cats is recommended to monitor trends or increases in (sero)prevalence in pets.

In conclusion, dogs and cats in close contact with COVID-19positive owners have a high risk of getting infected with SARS-CoV-2. No or only mild clinical signs were observed, pet-to-pet transmission within HHs was not detected and pet-to-human transmission seemed negligible. Despite these reassuring findings, our government applied the precautionary principle and advises COVID-19-positive persons to avoid close contact with their pets and to keep dogs and cats indoors as much as possible during the isolation or quarantine period.

AUTHOR CONTRIBUTIONS

M. K. J., H. E., J. W., A. S., M. L. and E. M. B. designed the study. E. M. B. supervised the study. H. K., T. P., M. d. J., C. R., E. B. and Y. d. G. visited the households and collected samples from dogs and cats. E. M. B., M. K. J., E. B. and Y. d. G. processed data from test results and questionnaires; L. S. and M. d. G. perfomed statistical analyses. N. S., S. Z., M. B. S., H. E., E. M. B. and B. D. developed, validated and performed the laboratory tests (PCR, ELISA and VNT). M. K. J. and E. M. B. interpreted the data and wrote the manuscript draft. All authors reviewed the draft manuscript and approved the final version.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

Sampling of the animals was approved by the Animal Care and Ethics Committee of Utrecht University, in accordance with the Dutch law on experimental animals (approval number AVD1080020209666). All pet owners signed an informed consent before sampling.

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