SHORT COMMUNICATION

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Long-term persistence of neutralizing SARS-CoV-2 antibodies in pets

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Abstract

We monitored the severe acute respiratory syndrome coronavirus 2 antibody response in seven dogs and two cats by using two multispecies ELISA tests, plaque reduction neutralisation test and virus neutralization. SARS-CoV-2 neutralizing antibodies in pets persisted up to 10 months since the first positive testing, thus replicating observations in COVID-19 human patients.

KEYWORDS
cat, dog, ELISA, long-term persistence, plaque reduction neutralization test, SARS-CoV-2 antibodies, virus neutralization

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been found to infect a plethora of mammals, including dogs and cats (Decaro, Balboni, et al., 2021). There are some reports of SARS-CoV-2 active infection and/or detection of specific antibodies in domestic carnivores (Colitti et al., 2021; Decaro, Vaccari, et al., 2021; Hamer et al., 2021; Patterson et al., 2020). While several studies have found that SARS-CoV-2 neutralizing antibodies can persist from 6–8 months to more than 12 months in humans (Chia et al., 2021; Dispinseri et al., 2021; Knies et al., 2021; Sonnleitner et al., 2021), no data are available about the persistence of the antibody response in dogs and cats. Here,

TABLE 1 Serological follow-up for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies in positive dogs and cats

Species	Animal ID	Age (years)	COVID-19 household	Sampling number	Date of collection	ELISA ID.vet[a]	ELISA In 3 Diagnostic[b]	$PRNT_{80}$[c]	VN[d]	Antibody persistence (months)
Dog	1	9	Yes	1	01 Apr 2020	Neg	Neg	1:40	1:20	≥10
				2	29 Jul 2020	Neg	Neg	<1:20	<1:10	
				3	08 Feb 2021	Neg	Neg	<1:20	1:10	
Dog	2	8	Yes	1	24 Apr 2020	Neg	Neg	<1:20	<1:10	≥9
				2	25 Jul 2020	Neg	Neg	1:40	<1:10	
				3	22 Jan 2021	Neg	Neg	<1:20	1:10	
Dog	3	11	Yes	1	24 Apr 2020	Neg	Neg	1:40	1:10	<3
				2	27 Jul 2020	Neg	Neg	<1:20	<1:10	
				3	22 Jan 2021	Neg	Neg	<1:20	<1:10	
Dog	4	11	Yes	1	24 Apr 2020	Neg	Neg	1:20	<1:10	<3
				2	25 Jul 2020	Neg	Neg	<1:20	<1:10	
				3	22 Jan 2021	Neg	Neg	<1:20	<1:10	
Dog	5	5	Yes	1	05 May 2020	Pos (209%)	Pos (57%)	1:160	1:160	≥9
				2	07 Aug 2020	Pos (263%)	Pos (76%)	1:320	1:10	
				3	06 Feb 2021	Pos (196%)	Pos (47%)	1:80	1:40	
Dog	6	1.5	Yes	1	25 May 2020	ND	ND	1:80	1:80	≥8
				2	30 Jul 2020	Neg	Neg	1:80	1:20	
				3	27 Jan 2021	Neg	Neg	1:40	1:40	
Dog	7	1.5	Yes	1	25 Nov 2020	Neg	Pos (23%)	1:80	1:10	≥3
				2	12 Dec 2020	Neg	Neg	1:80	1:20	
				3	28 Feb 2021	Neg	Pos (59%)	1:80	1:40	
Cat	1	17	No	1	12 May 2020	Pos (152%)	Pos (30%)	1:80	1:20	≥8
				2	28 Jul 2020	Neg	Neg	1:20	<1:10	
				3	26 Jan 2021	Neg	Neg	1:20	1:40	
Cat	2	7	Yes	1	03 Apr 2020	Pos (237%)	Neg	1:160	1:40	≥10
				2	30 Jul 2020	Pos (>QR)	Pos (123%)	1:640	1:80	
				3	08 Feb 2021	Pos (>QR)	Pos (138%)	1:160	1:80	

Abbreviations: ELISA, enzyme-linked immunosorbent assay; ND, not done; Neg, negative; Pos, positive; $PRNT_{80}$, plaque reduction neutralization test; >QR, above the quantification range; VN, virus neutralization.

[a]Values in brackets represent the ratio between the optical densities of the tested serum and the positive control (cut-off value = 50%).

[b]Values in brackets represent the ratio between the optical densities of the tested serum and the positive control (cut-off value = 20%).

[c]Antibody titre is expressed as the highest serum dilution with 80% reduction in plaques in inoculated VERO-E6 cells compared to the control, with 1:20 being the lowest serum dilution tested.

[d]Antibody titre is expressed as the highest serum dilution giving 100% reduction of cytopathic effect in inoculated VERO-E6 cells, with 1:10 being the lowest serum dilution tested.

we report the results of a longitudinal study in SARS-CoV-2 seropositive pets demonstrating the persistence of neutralizing antibodies for up to 10 months in some animals.

The pets included seven dogs and two cats, which had SARS-CoV-2 neutralizing antibodies according to previous studies (Decaro, Vaccari, et al., 2021; Patterson et al., 2020) or at a first screening (Table 1). The age of the tested animals ranged from 1.5 to 11 years and from 7 to 17 years for dogs and cats, respectively. All seven dogs and one of two cats were from COVID-19 positive households, but none of the sampled animals had developed COVID-19 clinical signs. Only one dog

(Dog 7) had been found to shed SARS-CoV-2 RNA by real-time PCR (Decaro, Vaccari, et al., 2021). For pets living in COVID-19 households, sera collection was initially carried out between 7 and 60 days after SARS-CoV-2 molecular detection in their owners. Sera were collected at different time points according to the owners' convenience and were tested with two commercial multispecies ELISA kits, ID Screen SARS-CoV-2 Double Antigen Multi-species ELISA (ID.vet, Grabels, France) and Eradikit[T] COVID19 Multispecies (In3Diagnostic, Turin, Italy), with a plaque reduction neutralization test (PRNT) (Patterson et al., 2020) and with virus neutralization (VN) (Zhang et al., 2020). The results

showed that four of seven dogs and two of two cats had SARS-CoV-2 neutralizing antibodies at 8 months or more after the first positive testing (Table 1). For one dog that had tested positive for SARS-CoV-2 by real-time RT-PCR (Decaro, Vaccari, et al., 2021), sera were available only for the first 3 months after infection and displayed antibodies through PRNT and VN at all time points. The remaining two dogs tested positive by PRNT and VN only at the first sampling, which may account for a shorter duration of the humoral immunity rather than for an older infection, since these animals were infected during the first wave of the COVID-19 pandemic (Patterson et al., 2020). A great discrepancy was observed between serological tests based on ELISA and neutralization tests. Five dogs that were seropositive by PRNT and/or VN at one or more time points were completely negative by both commercial ELISA tests. The dog (Dog 7) that had been found positive for SARS-CoV-2 RNA (Decaro, Vaccari, et al., 2021) was constantly seropositive by both PRNT and VN, but invariably negative by the ID.vet ELISA and positive at two out of three time points by the In3Diagnostic ELISA. In contrast, the discrepancy between PRNT and VN was less evident, being generally restricted to few sera with low neutralizing antibody titres. The discrepancy between ELISA and neutralization tests may be related to a lower sensitivity of ELISA or, alternatively, to a lack of specificity of neutralization assays. However, the latter hypothesis could be ruled out since previous experiments have demonstrated that pre-pandemic sera that were antibody positive for endemic coronaviruses of dogs and cats test negative by SARS-CoV-2 neutralization assays (Decaro, Vaccari, et al., 2021; Patterson et al., 2020; Zhang et al., 2020). The lower sensitivity of ELISA compared to VN or PRNT may be due to a different kinetic between the antibody response raised against the viral nucleoprotein (the antigen used in both ELISA tests) and that directed against the spike protein (the main target of neutralizing antibodies).

Interestingly, for Cat 1 there was no evidence for exposure to COVID-19 positive human patients. Therefore, it is likely that this cat was infected by an asymptomatic owner with undiagnosed SARS-CoV-2 infection or, alternatively, it acquired the virus from other infected people or animals.

Despite the increasing number of reports of SARS-CoV-2 infection in dogs and cats, no long-term monitoring has been carried out so far to evaluate the persistence of specific antibodies in pets. To our knowledge, the antibody response in pets has been monitored for a maximum of 2–3 months after infection, displaying relatively stable or increasing titres and no evidence of seroreversion (Hamer et al., 2021). Zhang et al. (2020) followed two cats for about 4 months, finding that neutralizing antibodies peaked after 10 days from the first sampling and then decreased to detection limit in 110 days. Our study, which was conducted using four different serological assays, demonstrates that similar to humans, dogs and cats may develop a long-term neutralizing antibody response against SARS-CoV-2. At which extent the presence of neutralizing antibodies is able to protect these animals from SARS-CoV-2 reinfection is currently unknown, thus requiring further studies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. The study was authorized by the Ethics Committee of the Department of Veterinary Medicine, University of Bari (approval number 15/2020).

DATA AVAILBALE STATEMENT

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

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