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

Short communication

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

Nicola Decaro,[1] Andrea Grassi,[2] Eleonora Lorusso,[1] Edward I. Patterson,[3] Alessio Lorusso,[4] Costantina Desario,[1] Enyia R. Anderson,[5] Violetta Vasinioti,[1] Christida E. Wastika,[5] Grant L. Hughes,[5] Fabrizia Valleriani,[4] Barbara Colitti,[6] Dominga Ricci,[7] Domenico Buonavoglia,[1] Sergio Rosati,[6] Nicola Cavaliere,[8] Saverio Paltrinieri,[9] Stefania Lauzi,[9] Gabriella Elia,[1] Canio Buonavoglia[1]

[1] Department of Veterinary Medicine, University of Bari, Valenzano, Italy

[2] I-VET srl, Laboratorio di Analisi Veterinarie, Flero, Italy

[3] Brock University, St. Catharines, ON, Canada

[4] Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy

[5] Liverpool School of Tropical Medicine, Liverpool, UK

[6] Ambulatorio Veterinario Ricci Dott.ssa Dominga, Andria, Italy

[7] Department of Veterinary Sciences, University of Turin, Grugliasco, Torino, Italy

[8] Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italy

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[9] Department of Veterinary Medicine, University of Milan, Lodi, Italy

Corresponding author: Nicola Decaro, Department of Veterinary Medicine, University of Bari, Strada provinciale per Casamassima Km 3, 70010 Valenzano (Bari), Italy, tel. +390804679832, e-mail nicola.decaro@uniba.it.

Abstract

We monitored the SARS-CoV-2 antibody response in 7 dogs and 2 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: Dog; cat; SARS-CoV-2 antibodies; long-term persistence; ELISA; plaque reduction neutralization test; virus neutralization.

Text

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been found to infect a plethora of mammals, including dogs and cats (Decaro et al., 2021a). There are some reports of SARS-CoV-2 active infection and/or detection of specific antibodies in domestic carnivores (Patterson et al., 2020; Colitti et al., 2021; Decaro et al., 2021b; Hamer et al.,

2021). 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, 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 7 dogs and 2 cats, which had SARS-CoV-2 neutralizing antibodies according to previous studies (Patterson et al., 2020; Decaro et al., 2021b) 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 7 dogs and 1 of 2 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 et al., 2021b). 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™ 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 4 of 7 dogs and 2 of 2 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 et al., 2021b), sera were available only for the first three 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 at least one time point were completely negative by both commercial ELISA tests. The dog (Dog 7) that had been found positive for SARS-CoV-2 RNA (Decaro et al., 2021b) was constantly seropositive by both PRNT and VN, but invariably negative by the ID.vet ELISA and positive at 2 out of 3 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 titers. 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 our 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 (Patterson et al., 2020; Zhang et al., 2020; Decaro et al., 2021b). 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 titers 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 4 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.

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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Conflict of interest

The authors of this manuscript declare that there are no conflicts of interest.

Ethical approval

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).

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Table 1. Serological follow-up for 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 In3Diagnostic[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	

[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 titer 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 titer 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.

ELISA, enzyme-linked immunosorbent assay; $PRNT_{80}$, plaque reduction neutralization test; VN, virus neutralization;

ND, not done; Neg, negative; Pos, positive; >QR, above the quantification range.