



Evidence of neutralizing antibodies against SARS-CoV-2 in domestic cats living with owners with a history of COVID-19 in Lima – Peru

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ABSTRACT

SARS-CoV-2 can infect a variety of wild and domestic animals worldwide. Of these, domestic cats are highly susceptible species and potential viral reservoirs. As such, it is important to investigate disease exposure in domestic cats in areas with active community transmission and high disease prevalence. In this report we demonstrate the presence of serum neutralizing antibodies against the receptor binding-domain (RBD) of the SARS-CoV-2 in cats whose owners had been infected with SARS-CoV-2 in Lima, Peru, using a commercial competitive ELISA SARS-CoV-2 Surrogate Virus Neutralization Test. Out of 41 samples, 17.1% (7/41) and 31.7% (13/41) were positive, using the cut-off inhibition value of 30% and 20%, respectively. Not all cats living in a single house had detectable neutralizing antibodies showing heterogenous exposure and immunity among cohabiting animals. This is the first report of SARS-COV-2 exposure of domestic cats in Lima, Peru. Further studies are required to ascertain the prevalence of SARS-COV-2 exposure among domestic cats.

1. Introduction

The new human coronavirus, SARS-CoV-2, has been shown to mainly infect humans. However, SARS-CoV-2 infection has also been detected in a variety of animals, including wild cats, minks, ferrets, domestic dogs and cats [1–6]. Cats and minks may be considered the most susceptible species because of the higher similarity of the angiotensin-converting enzyme 2 (ACE2) between these species and humans [7]. Although the majority of infected cats are asymptomatic, some animals may develop clinical disease, and the virus can be experimentally transmitted between individuals [8]. Therefore, SARS-CoV-2 could have a direct impact on animal health, while the possibility of cats becoming zoonotic reservoirs has not been totally discarded.

Serological testing is a valuable tool for screening antibody levels associated with pathogen exposure. As with other viral infections, host

neutralizing serum antibodies may block the binding of viral proteins to cell surface receptors. In humans, SARS-CoV-2 neutralizing antibodies have been determined to inversely correlate with disease severity and can predict the probability of re-infections [9]. In animals, reported prevalence of neutralizing serum antibodies against SARS-CoV-2 in cats varies, with as low as 0.002% in Germany, 0.2% in Brazil, 5.8% in Italy, and 10.8% in Wuhan, China [10–13]. In Peru, one of the most affected countries by the COVID-19 pandemic, no previous studies have been conducted investigating the seroprevalence or prevalence of SARS-CoV-2 among domestic cats. In this report we demonstrate the presence of serum neutralizing antibodies against the receptor binding-domain (RBD) of the SARS-CoV-2 viral spike protein in cats whose owners confirmed previous infection with SARS-CoV-2.

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2. Materials and methods

Blood samples of cats seeking routine veterinary care were collected between August 2020 and April 2021 from three veterinary clinics in Lima, Peru. All cat owners signed an informed consent authorizing the use of the samples for research purposes. Samples were centrifuged at 3500 rpm for 5 min and the serum supernatant was transferred to microcentrifuge tubes and was stored at -20°C . Samples from cats whose owners confirmed previous COVID-19 disease (clinical signs with positive IgG/IgM rapid test or qRT-PCR) during veterinary anamnesis were conveniently selected. To test the serum samples for the presence of neutralizing antibodies against the RBD of the viral spike protein, a commercial competitive ELISA cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit also known as SARS-CoV-2 Surrogate Virus Neutralization Test (sVNT) Kit was used according to the manufacturer's instructions (Genscript, New Jersey, USA). Percent serum neutralization was calculated as follows: $= (1 - \text{OD value of sample} / \text{OD value of negative control}) \times 100\%$. A cut-off value of 20% and an updated 30% of inhibition were used to establish positivity. This study was approved by the Universidad Peruana Cayetano Heredia Animal Care and Use Ethical Committee (N° 027-08-20).

3. Results and discussion

A total of 41 samples from a serum bank of 700 were selected for screening of serum neutralizing antibodies. The median age of the animals was 12 months (IQR: 8 months – 46 months), 53.7% were female (22/41), and 87.8% (36/41) were classified as domestic shorthair. 53.7% (22/41) came from the district of Comas while the remainder 46.3% came from Miraflores (5/41), Surco (5/41), San Juan de Miraflores (3/41), Independencia (2/41), San Juan de Lurigancho (1/41), San Luis (1/41), and San Martín de Porres (1/41). Out of the 41 cat samples, 22 cats (53.7%) lived in a single household (household C), and 2 cats lived in household D. Age, sex, breed, neuter status and district of all animals are shown in Table 1. Animals living in households C and D attended the veterinary clinic for neutering. The rest of the animals attended the veterinary clinics seeking for care based on unspecific clinical signs. Out of the 41 samples, 17.1% (7/41) and 31.7% (13/41) were positive for the presence of serum neutralizing antibodies, using the cut-off value of 30% and 20%, respectively (Fig. 1).

Out of 13 positive samples, 38.4% (5/13) showed clinical signs including sneezing and dyspnea, cough, vomit, or depression. Interestingly, one of the animals with the highest percent neutralization (73.06%) showed all the symptoms described. A subclinical infection, with a prolonged period of oral and nasal viral shedding that is generally not accompanied by symptomatology has been reported in cats [14]. Only 8 out of 22 cats in household C had evidence of serum neutralizing antibodies. This suggests that infection may not be homogenous among cohabiting animals, and this could be associated with other factors such as health state, immunity, proximity to the infected owner(s), among others.

Our results show compelling evidence of SARS-CoV-2 exposure in domestic cats and it is the first report of such an event in Peru. Percent seropositivity in this population of cats is high compared to other studies published, such as that of Italy and Wuhan, China, in which 5.8% of 191 cats 10.8% of 102 cats had neutralizing antibodies, respectively [12,13]. However, these studies were not exclusively done on a pet population living with COVID-19 infected owners. In a longitudinal cohort study of pets living with COVID-19 owners conducted in Texas, USA, 43.8% of 16 cats developed neutralizing antibodies against SARS-CoV-2 [15], percentage that is closer to the results obtained in this study.

Limited sample size and a convenience sample do not permit prevalence estimation and seroconversion dynamics. Another limitation is the prolonged time between the onset of COVID-19 in owners and sampling of animals, which could have affected the sensitivity of the test. The immunity duration in pets is currently unknown.

Experimentally infected cats develop neutralizing activity by seven days post-viral infection, but seroconversion is evident by 14 days with a plateaued stage at day 42 [16].

Serum neutralization activity is commonly tested using plaque reduction neutralization tests (PRNT) as gold standard. However, the commercial assay utilized in this preliminary study has shown a high correlation with serum neutralization activity using PRNT and has shown robust internal validity parameters for both humans, cats, dogs and hamster sera [17,18]. Additionally, this test has already been used in a large-scale serological survey in cats in Thailand using 20% of inhibition as the cut-off value [19]. Our study reports results based on both a 20% and a 30% inhibition cut-off value, based on the manufacturer's recommendation. Moreover, moderate to high sensitivity and specificity of the sVNT has been reported using this cut-off value correction (30%) in samples from multiple animal species (ferrets, cats, minks, hamster). Specifically, the specificity was 99% and 100% using 20% and 30% cut-off values, respectively [20]. The sensitivity of the sVNT using a cut-off value of 20% was 98.9% and 97% when compared to PRNT₉₀ and PRNT₅₀, respectively, yet sensitivity decreased to 93.9% when using a 30% of cut-off value (compared to PRNT₅₀) [17]. Additionally, this commercial assay offers logistical and biosafety advantages for researchers working in resource-limited settings that do not have access to a BSL-3 containment required for SARS-CoV-2 manipulation.

The animals evaluated in our study sought routine veterinary care that was not associated with symptomatic respiratory disease in most of the cases, demonstrating potential asymptomatic infection in cats, and consequently, potential viral reservoirs. In some studies, over 21% and 25% of infected households sampled had pets with neutralizing antibodies [15,21]. Few case studies of natural infection in cats document severe clinical outcomes, and those that have revealed that comorbidities likely played a contributing factor in illness or death [15]. Seroprevalence studies should be conducted in affected regions to monitor SARS-CoV-2 asymptomatic infections in pets such as cats, ferrets, or dogs, especially in companion animals living with COVID-19 patients [22].

4. Conclusions

It is crucial to monitor SARS-CoV-2 exposure and infection in domestic animals using rapid and affordable point-of-care serological and molecular assays that can be used by veterinarians serving low-income communities. Cats have the potential to serve as sentinels for undetected community transmission, and in this scenario, veterinarians play a key role as first-line responders.

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 and the appropriate ethical review committee approval has been received.

Conflict of interests

None noted.

CRediT authorship contribution statement

Luis M. Jara: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Methodology. **Cusi Ferradas:** Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Methodology. **Francesca Schiaffino:** Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Methodology. **Camila Sánchez-Carrión:** Investigation, Writing – review & editing. **Martínez-Vela:** Investigation, Writing – review & editing. **Alexandra Ulloa:** Investigation, Writing – review & editing. **Gisela Isasi-Rivas:**

Table 1
Data of cats with serum neutralizing antibodies against SARS-COV-2 in Lima (2020–2021).

Household	Sample Date (MM/DD/YY)	Age (months)	Sex	Breed	Neuter status	District	Time since onset of clinical symptoms of COVID-19 in owners (weeks)	Percent Inhibition (%)
A	1/20/2021	7	Male	Domestic Shorthair	Intact	San Juan de Miraflores	Not available	73.06
A	1/21/2021	28	Female	Domestic Shorthair	Intact	San Juan de Miraflores	Not available	66.69
B	4/01/2021	8	Female	Domestic Shorthair	Spayed	San Juan de Miraflores	Not available	3.2
C	10/9/2020	8	Female	Domestic Shorthair	Intact	Comas	12	29.15
C	10/9/2020	12	Female	Domestic Shorthair	Intact	Comas	12	25.59
C	10/9/2020	11	Female	Domestic Shorthair	Intact	Comas	12	23.81
C	10/9/2020	36	Male	Domestic Shorthair	Intact	Comas	12	22.88
C	10/9/2020	10	Female	Domestic Shorthair	Intact	Comas	12	20.24
C	10/9/2020	5	Male	Domestic Shorthair	Intact	Comas	12	10.69
C	10/9/2020	5	Male	Domestic Shorthair	Intact	Comas	12	4.92
C	10/9/2020	24	Male	Domestic Shorthair	Intact	Comas	12	9.27
C	10/9/2020	12	Female	Domestic Shorthair	Intact	Comas	12	-3.14
C	10/9/2020	12	Female	Domestic Shorthair	Intact	Comas	12	-0.64
C	10/9/2020	24	Female	Domestic Shorthair	Intact	Comas	12	14.47
C	10/9/2020	10	Female	Domestic Shorthair	Intact	Comas	12	16.18
C	10/9/2020	60	Female	Domestic Shorthair	Intact	Comas	12	3.99
C	10/9/2020	5	Female	Domestic Shorthair	Intact	Comas	12	1.8
C	10/24/2020	9	Male	Domestic Shorthair	Intact	Comas	12	61.22
C	10/24/2020	6	Male	Domestic Shorthair	Intact	Comas	12	49.87
C	10/24/2020	11	Male	Domestic Shorthair	Intact	Comas	12	38.03
C	10/24/2020	8	Female	Domestic Shorthair	Intact	Comas	12	-8.38
C	10/24/2020	8	Female	Domestic Shorthair	Intact	Comas	12	-8.75
C	10/24/2020	24	Female	Domestic Shorthair	Intact	Comas	12	12.58
C	10/24/2020	7	Female	Domestic Shorthair	Intact	Comas	12	8.18
C	10/24/2020	6	Female	Domestic Shorthair	Intact	Comas	12	-5.59
D	1/27/2021	24	Female	Domestic Shorthair	Intact	Independencia	3	38.73
D	1/27/2021	60	Male	Domestic Shorthair	Intact	Independencia	3	30.96
F	8/17/2020	84	Male	Russian Blue	Neutered	Miraflores	8	21.88
F	8/31/2020	72	Female	Russian Blue	Spayed	Miraflores	10	9.41
F	8/31/2020	12	Male	Bengal	Neutered	Miraflores	10	11.26
F	8/31/2020	12	Male	Bengal	Neutered	Miraflores	10	15.04
G	2/14/2021	46	Female	Maine Coon	Spayed	Miraflores	10	10.48
H	12/29/2020	96	Female	Domestic Shorthair	Intact	Surco	24	5.06
I	1/13/2021	6	Male	Domestic Shorthair	Neutered	Surco	Not available	-7.98
J	4/03/2021	58	Male	Domestic Shorthair	Neutered	Surco	8	1.31
K	1/02/2021	64	Male	Domestic Shorthair	Neutered	Surco	Not available	-4.48
L	2/06/2021	42	Female	Domestic Shorthair	Intact	Surquillo	8	-6.33
M	2/06/2021	77	Male	Domestic Shorthair	Neutered	San Luis	28	1.39
N	1/03/2021	124	Male	Domestic Shorthair	Neutered	San Juan de Lurigancho	Not available	12.19

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Table 1 (continued)

Household	Sample Date (MM/DD/YY)	Age (months)	Sex	Breed	Neuter status	District	Time since onset of clinical symptoms of COVID-19 in owners (weeks)	Percent Inhibition (%)
O	10/13/2020	63	Male	Domestic Shorthair	Neutered	San Martín de Porres	1	10.05
P	2/02/2021	9	Male	Domestic Shorthair	Neutered	Surco	Not available	-1.02

Different capital letters represent different households. Percent inhibition (%) in bold denotes positivity (>20%).

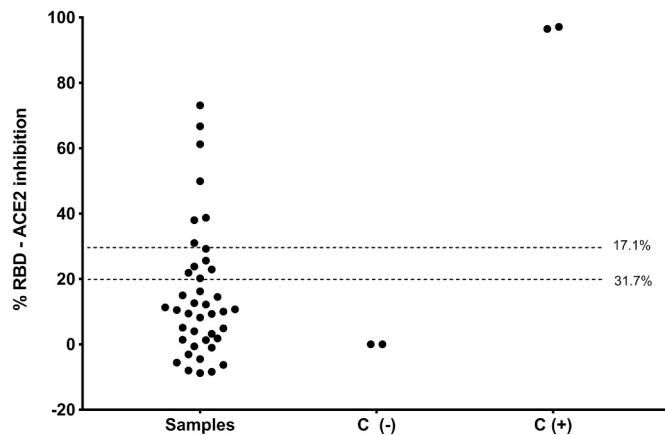


Fig. 1. Percent inhibition against SARS-CoV-2 receptor binding-domain (RBD) in serum of domestic cats whose owners had a history of COVID-19 ($n = 41$). C: controls, (-): negative, (+): positive. The 17.1% (7/41) and 31.7% (13/41) shows the frequency of cats with neutralizing antibodies with 30% and 20% of cut-off values of inhibition, respectively.

Investigation, Writing – review & editing, Methodology. **Angela Montalván**: Investigation, Writing – review & editing, Methodology. **Luis Guevara Sarmiento**: Conceptualization, Formal analysis, Writing – review & editing, Methodology. **Manolo Fernández**: Conceptualization, Data curation, Formal analysis, Writing – review & editing, Methodology. **Mirko Zimic**: Conceptualization, Formal analysis, Funding acquisition, Writing – review & editing, Methodology.

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