

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

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26 **Abstract**

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

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41 **Key words:** COVID-19, SARS-CoV-2, cats, serology, neutralizing antibodies, One Health

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54 **Introduction**

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

64 Serological testing is a valuable tool for screening antibody levels associated with
65 pathogen exposure. As with other viral infections, host neutralizing serum antibodies may
66 block the binding of viral proteins to cell surface receptors. In humans, SARS-CoV-2
67 neutralizing antibodies have been determined to inversely correlate with disease severity
68 and can predict the probability of re-infections [9]. In animals, reported prevalence of
69 neutralizing serum antibodies against SARS-CoV-2 in cats varies, with as low as 0.002% in
70 Germany, 0.2% in Brazil, 5.8% in Italy, and 10.8% in Wuhan, China [10-13]. In Peru, one of
71 the most affected countries by the COVID-19 pandemic, no previous studies have been
72 conducted investigating the seroprevalence or prevalence of SARS-CoV-2 among domestic
73 cats. In this report we demonstrate the presence of serum neutralizing antibodies against the
74 receptor binding-domain (RBD) of the SARS-CoV-2 viral spike protein in cats whose owners
75 confirmed previous infection with SARS-CoV-2.

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77 **Materials and Methods**

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

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93 **Results and Discussion**

94 A total of 41 samples from a serum bank of 700 were selected for screening of serum
95 neutralizing antibodies. The median age of the animals was 12 months (IQR: 8 months – 46
96 months), 53.7% were female (22/41), and 87.8 % (36/41) were classified as domestic
97 shorthair. 53.7% (22/41) came from the district of Comas while the remainder 46.3% came
98 from Miraflores (5/41), Surco (5/41), San Juan de Miraflores (3/41), Independencia (2/41),
99 San Juan de Lurigancho (1/41), San Luis (1/41), and San Martín de Porres (1/41). Out of the
100 41 cat samples, 22 cats (53.7%) lived in a single household (household C) in which cats
101 were sampled on two different dates, and 2 cats lived in household D. Age, sex, breed, and
102 district of all animals are shown in **Supplementary Table 1**. Out of the 41 samples, 17.1%
103 (7/41) and 31.7% (13/41) were positive for the presence of serum neutralizing antibodies,
104 using the cut-off value of 30% and 20%, respectively (**Figure 1**). Out of 13 positive samples,

105 38.4% (5/13) showed clinical signs including sneezing and dyspnea, cough, vomit, or
106 depression. Interestingly, one of the animals with the highest percent neutralization (73.06%)
107 showed all the symptoms described. Only 8 out 22 cats in household C had evidence of
108 serum neutralizing antibodies. This suggests that infection may not be homogenous among
109 cohabiting animals, and this could be associated with other factors such as health state,
110 immunity, proximity to the infected owner(s), among others.

111 Our results show compelling evidence of SARS-CoV-2 exposure in domestic cats
112 and it is the first report of such an event in Peru. Percent seropositivity in this population of
113 cats is high compared to other studies published, such as that of Italy and Wuhan, China, in
114 which 5.8% of 191 cats 10.8% of 102 cats had neutralizing antibodies, respectively [12, 13].
115 However, these studies were not exclusively done on a pet population living with COVID-19
116 infected owners. In a longitudinal cohort study of pets living with COVID-19 owners, 43.8% of
117 16 cats developed neutralizing antibodies against SARS-CoV-2 [14]. Limited sample size
118 and a convenience sample do not permit prevalence estimation.

119 Serum neutralization activity is commonly tested using plaque reduction
120 neutralization tests. However, the commercial assay utilized in this preliminary study has
121 shown a high correlation with serum neutralization activity using plaque reduction and has
122 shown robust internal validity parameters for both humans, cats, dogs and hamster sera [15,
123 16]. Additionally, this commercial assay offers logistical and biosafety advantages for
124 researchers working in resource-limited settings that do not have access to a BSL-3
125 containment required for SARS-CoV-2 manipulation.

126 These animals sought routine veterinary care that was not associated with
127 symptomatic respiratory disease in most of the cases, demonstrating potential asymptomatic
128 infection in cats, and consequently, potential viral reservoirs. In one study, over 25% of
129 households sampled had pets with neutralizing antibodies. Few case studies of natural
130 infection in cats document severe clinical outcomes, and those that have revealed that co-
131 morbidities likely played a contributing factor in illness or death [14].

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133 **Conclusions**

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

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140 **Ethics Statement**

141 The authors confirm that the ethical policies of the journal, as noted on the journal's
142 author guidelines page, have been adhered to and the appropriate ethical review committee
143 approval has been received.

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151 **Conflict of interests**

152 None noted.

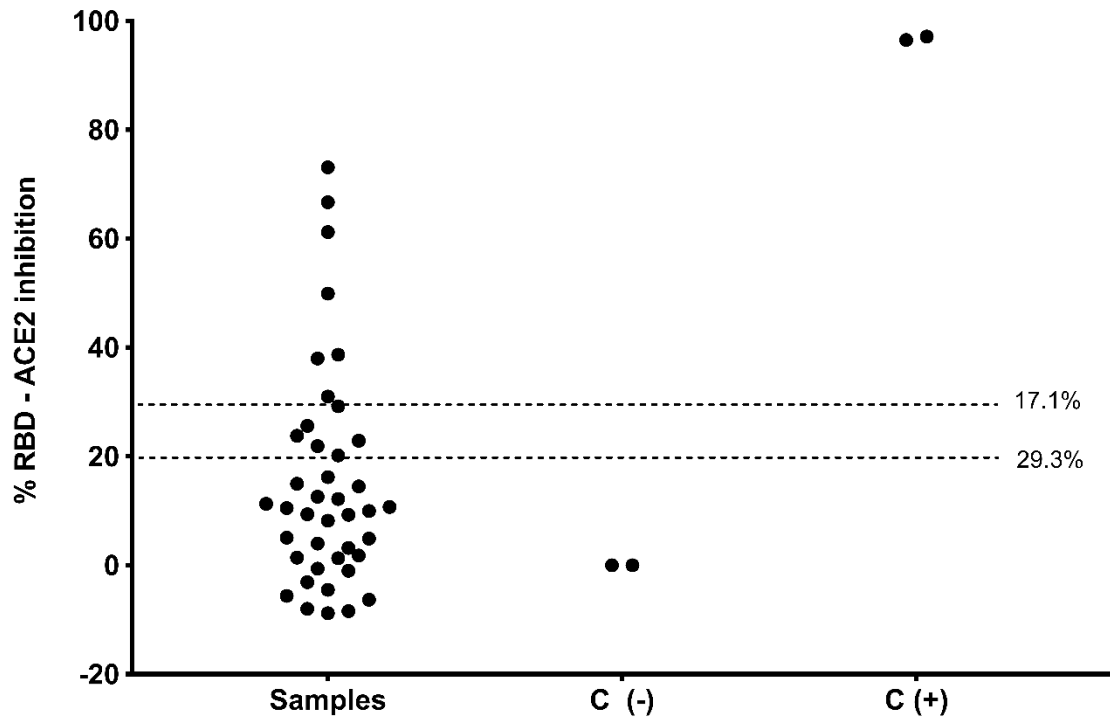
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- 207

208 **Figure 1. Percent inhibition against SARS-COV-2 receptor binding-domain (RBD) in**
209 **serum of domestic cats whose owners had a history of COVID-19 (n = 41).**



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211 C: controls, (-): negative, (+): positive. The 17.1% and 29.3% shows the frequency of cats
212 with neutralizing antibodies with 30% and 20% of cut-off values of inhibition, respectively.

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