

BRIEF REPORT

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Serosurveillance of SARS-CoV-2 in companion animals in Sarawak, Malaysia

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Abstract

SARS-CoV-2 is a zoonotic betacoronavirus that was first reported at the dawn of 2019 in Wuhan, China and has since spread globally, causing an ongoing pandemic. Anthroponotic transmission was reported early, with confirmed infections reported in 26 species to date, including dogs and cats. However, there is a paucity of reports on the transmission of SARS-CoV-2 to companion animals, and thus, we aimed to estimate the seroprevalence of SARS-CoV-2 in dogs and cats in Sarawak, Malaysia. From August 2022 to 2023, we screened plasma samples of 172 companion animals in Sarawak, Malaysia, using a species-independent surrogate virus neutralization test. Our findings revealed the presence of neutralizing antibodies of SARS-CoV-2 in 24.5% (27/110) of dogs and 24.2% (15/62) of cats. To the best of our knowledge, this is the first report of the seroprevalence of SARS-CoV-2 in companion animals in Malaysia. Our findings emphasize the need for pet owners to distance themselves from their pets when unwell, and a strategy must be in place to monitor SARS-CoV-2 in companion animals to assess the potential impact of the virus on companion animals.

Keywords SARS-CoV-2, Seroprevalence, Dogs, Cats, Neutralizing antibodies, cPASS

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a member of the family *Coronaviridae* under the genus betacoronavirus, subgenus Sarbecovirus. The proximal origin of SARS-CoV-2 is *Rhinolopus* bats, with pangolin as the most probable intermediary host [1]. The epidemic was first reported in a cluster of patients with severe pneumonia in Wuhan, China, and quickly escalated into a full-blown pandemic due to the sustainable human-to-human transmission, absence of protective

immunity, efficient connectivity to the world, and the high rates of asymptomatic infections [2].

SARS-CoV-2 infection in animals is well-documented, with reported cases in 26 species, across 36 countries by the end of 2022 [3]. Not surprisingly, dogs and cats are susceptible to SARS-CoV-2 infections since they were readily infected by SARS-CoV in the 2003 epidemic [4]. Moreover, dogs and cats are at higher risk of infection due to their close physical relationship with humans [5]. As the pandemic progresses, SARS-CoV-2 contact tracing has been discontinued, and home quarantine measures are becoming more common, increasing the potential for anthroponotic transmission from humans to companion animals. At the end of 2022, SARS-CoV-2 has infected >5 million people in Malaysia, but no studies have been reported to assess the risk of SARS-CoV-2 exposure in animals, especially in dogs and cats. Therefore, this study aims to determine the exposure of

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SARS-CoV-2 in dogs and cats in Sarawak, Malaysia by screening for SARS-CoV-2 neutralizing antibodies.

Blood was collected from pet dogs and cats at the Animal Central Veterinary Clinic in Sarawak, Malaysia between August 2022 to January 2023. A total of 172 samples were collected, 64% (n=110 samples) and 36% (n=62 samples) were from dogs and cats, respectively. On average, dogs have a mean age of 3 years, while cats tend to be slightly older with a mean age of 4 years. Among the dogs, the majority of dogs are mongrels, accounting for 88.2% of the total. The remaining percentages are divided among various purebred dogs, including Pomeranians (3.6%), Golden Retrievers (1.8%), Shih Tzus (1.8%), Corgis (1.8%), Beagles (0.9%), and Huskies (0.9%). On the other hand, among the cats, mongrels make up a significant majority, representing 96.8% of the total.

The remaining percentages are occupied by Scottish Folds (1.6%) and British Shorthairs (1.6%). The collected blood was processed to obtain plasma, which was then screened for SARS-CoV-2 neutralizing antibodies using the species-independent surrogate virus neutralization test (sVNT) cPASS™ (Genscript, Nanjing, China) according to the manufacturer’s recommendations. The optical density (OD) was read at 450 nm using the SpectraMax ID3 device (Molecular Devices, California, US). The percentage inhibition (%) was calculated using the formula $(1 - OD_{\text{sample value}}/OD_{\text{negative control}}) \times 100\%$. The manufacturer’s positive cutoff value of $\geq 30\%$ inhibition was followed. The respective results are shown in Fig. 1.

We discovered neutralizing antibodies in 24.4% of animals with equal seroprevalence in dogs (24.5%) and cats (24.2%) [Table 1]. Most of the neutralizing antibodies can

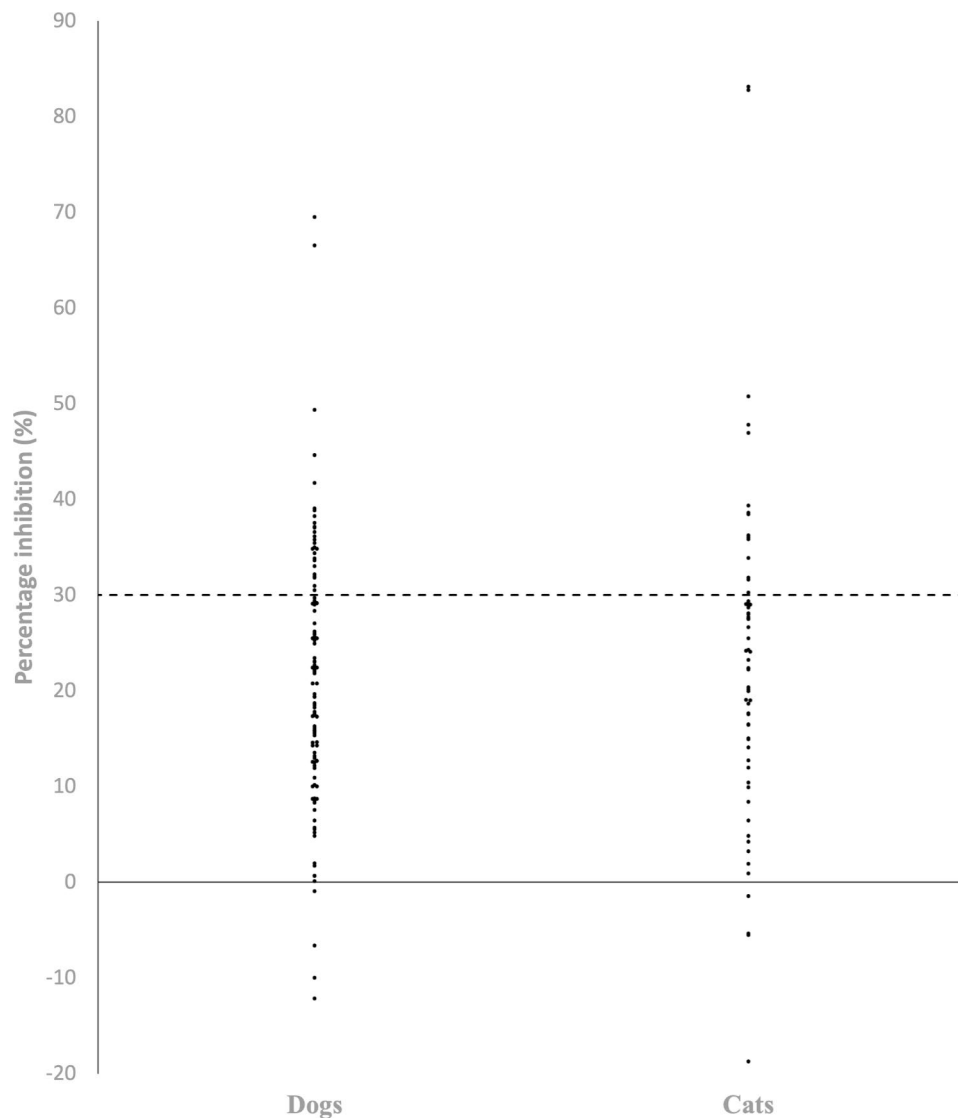


Fig. 1 The measurement of neutralizing antibodies in dogs and cats’ plasma represented as percentage inhibition. Horizontal dashed line represents the positive cutoff of $\geq 30\%$ inhibition.

Table 1 Seropositivity of dogs and cats against SARS-CoV-2 in Sarawak, Malaysia, August 2022 – January 2023

Results	Animal		Total no.(%)
	Dog no.(%)	Cat no.(%)	
NEG	83 (75.5)	47 (75.8)	130 (75.6)
POS	27 (24.5)	15 (24.2)	42 (24.4)
TOTAL	110	62	172

block the binding of SARS-CoV-2 RBD to human ACE2 (hACE2) by 30.0–49.3% (dogs) and 30–50.7% (cats). Two dogs and two cats exhibited the highest inhibition levels at 66.5–69.5% and 82.8–83.1%, respectively.

The seropositivity of SARS-CoV-2 in dogs and cats in Malaysia is higher than reported in Italy [6, 7], Poland [8, 9], France [10], Croatia [11], United States [12–14], South Korea [15], China [16], and Thailand [17] with seroprevalence of 0–16% and 0–18.9%, respectively but their sampling were done earlier between March 2020 to February 2022. On the other hand, the seroprevalence rates in Canada and Argentina on samples taken in 2021 indicated high exposure rates of 35.9–41% in dogs and 42.9–52% in cats. It is not surprising that the seropositivity in China was negligible in 2021 due to their on-going zero-COVID policy since the beginning of the pandemic. However, human infections soared to 61% within a month after the cessation of the zero-COVID policy [18] and the rapid increase in human cases may positively correlate with the rise in anthroponotic transmission to pets.

Nearly all researchers have reported higher seropositivity in cats than dogs which can be explained by the higher affinity of SARS-CoV-2 receptor binding domain (RBD) to cat's angiotensin-converting enzyme 2 (cACE2) than in dog's ACE2 (dACE2). In our study, we observed a higher seropositivity in dogs compared to cats, which may be attributed to the gradual adaptation of recent SARS-CoV-2 variants to the dACE2 receptor. This finding is consistent with previous research demonstrating that SARS-CoV-2 variants carrying the D614G and E484K mutations exhibit an enhanced spike protein affinity for dACE2 [19, 20]. Furthermore, the presence of mouse-adapted mutations in the SARS-CoV-2 Omicron variant has raised a plausible speculation that SARS-CoV-2 replicated in rodents, and acquired functional mutations before spilling back to humans [21–23]. Notably, rodents were previously unsusceptible to infection by earlier SARS-CoV-2 variants [24–26].

A standardized reference assay is absent in seroprevalence studies of SARS-CoV-2 in animals, and the estimated seroprevalence by different researchers may be systematically biased due to the difference in the sensitivity and specificity of the type of serologic assays employed. Lessons from the SAR-CoV-2 vaccination in

humans revealed that approximately 40% of vaccinees who received the whole inactivated vaccine might not mount an immune response against the nucleocapsid [2]. Thus, researchers who employed the anti-N enzyme-linked immunosorbent assay (N-ELISA) may have underestimated the true seroprevalence in their animals, which agrees with the reduced sensitivity of the N-ELISA in comparison to sVNT in dogs and cats. In addition, N-ELISA was found to correlate poorly with the virus neutralization test (VNT), unlike the higher concordance Spike subunit-1 (S1)- and RBD-based ELISA [27]. The sVNT assay seems to be a plausible platform for standardization as it is species-independent, does not require biocontainment facilities, and is not known to cross-react with antibodies against other known coronaviruses except SARS-CoV [27, 28].

The current knowledge suggests that ACE2 is solely used by Sarbecoviruses such as SARS-CoV and SARS-CoV-2. However, SARS-CoV-2-like viruses have been discovered in bats and Malayan pangolin (*Manis javanica*) in South East Asia. Notably, the bat coronavirus (BANAL-52, -103, and -236) and Malayan pangolin coronavirus (Mp-CoV-GX) can use ACE2 to establish infections in both cell lines and transgenic hACE2-transgenic mice in the laboratory settings [29, 30]. Following this, the discovery of neutralizing antibodies in cats and white-tailed deer to the SARS-CoV-2 RBD before the pandemic in the United States suggests that epizootic viruses that use ACE2 may also be present in geolocations distant from the epicenter of SARS-CoV-2 [31].

Dogs and cats can acquire an infection directly from their owners and other means. For instance, they are not strictly indoor or outdoor but usually both, with some of them having free-ranging time outside their respective homes. They may become exposed to SARS-CoV-2 by encountering virus-contaminated food waste, face coverings, cigarette butts, and fecal materials. Their allogrooming, coprophagic and hunting behavior may further predispose them to infections, bearing in mind that some dogs and cats do hunt for rodents and cockroaches, which might serve as transmission vectors. Furthermore, dogs and cats can also serve as passive transmission vectors as viral nucleic acid can readily be detected on their paws, skin, and coat [4, 32, 33].

The manuscript has several limitations that should be acknowledged: [1] the sample size is relatively small which may lead to underestimation or overestimation of the true seropositivity rate; [2] the study does not provide direct evidence for the transmission route of SARS-CoV-2 between animals, humans and inanimate, or vice versa. While the study focused on seropositivity rates, it does not explore the specific mechanisms by which transmission may occur. Further research is needed to investigate the possible routes of transmission, such as

respiratory droplets, fomites, or close contact, to better understand the dynamics of interspecies transmission; [3] it should be noted that other potential confounding factors were not studied in detail in this manuscript. Factors such as the age, breed, and overall health status of the animals, as well as their living conditions and geographical location, could influence the seroprevalence rates. Future studies should consider incorporating a more comprehensive analysis of these confounding factors to obtain a more nuanced understanding of the relationship between SARS-CoV-2 seropositivity and various animal characteristics; [4] virological and molecular investigations were not conducted in this study. This limitation restricts the feasibility of establishing correlations between key spike protein mutations and their affinity towards the ACE2 receptors in respective animals. Performing such investigations in future research would provide valuable insights into the molecular interactions and potential implications.

In conclusion, our study in Sarawak, Malaysia demonstrates a significant level of anthroponotic transmission of SARS-CoV-2, as evidenced by nearly equal seropositivity rates in both dogs and cats. To mitigate the risk of infecting pets, owners who test positive for SARS-CoV-2 should consider maintaining distance from their animals. These findings underscore the importance of implementing a long-term serological and molecular surveillance program to monitor the prevalence and evolutionary trends of SARS-CoV-2 in companion animals. Such a program would provide valuable insights for public health measures and help ensure the well-being of both humans and their animal companions in the face of ongoing transmission dynamics.

Abbreviations

RBD	Receptor binding domain
ACE2	Angiotensin-converting enzyme 2
hACE2	Human angiotensin-converting enzyme 2
cACE2	Cat angiotensin-converting enzyme 2
dACE2	Dog angiotensin-converting enzyme 2
COVID	Coronavirus disease
sVNT	Surrogate virus neutralization test
VNT	Virus neutralisation test
ELISA	Enzyme-linked immunosorbent assay
N	Nucleocapsid
S	Spike
SARS	Severe acute respiratory syndrome
CoV	Coronavirus

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Authors' contributions

C.S.T and A.L.L.L conceived the idea, wrote the proposal, obtained funding and obtained animal ethics committee approval. D.B.B. and L.T.T. conducted sampling, documentation and archiving of samples. H.S.P. performed sample processing, laboratory analysis and tabulation of data for preliminary analysis. L.L.T. performed the data analysis. C.S.T. drafted the manuscript, A.L.L.L., D.B.B., L.T.T., H.S.P. improved the manuscript. All authors have read and approved the manuscript.

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Data availability

Data is available via the corresponding author upon reasonable requests.

Declarations

Ethics approval and consent to participate

The study was approved by the Universiti Malaysia Sarawak Animal Ethics Committee no. UNIMAS/AEC/R/F07/070 and carried out in compliance with relevant local guidelines and regulations.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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