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Communication

[*Comunicação*]

First reported cases of SARS-CoV-2 infection in owned dogs in Belo Horizonte, Brazil

[*Primeiros casos de infecção por SARS-CoV-2 em cães com tutores em Belo Horizonte, Brasil*]

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was described as an RNA virus of the coronavirus disease 2019 (COVID-19) in human beings (Zhu and others, 2020), and detected in different animal species worldwide, including dogs, cats, wild felids, minks, and non-human primates .

The first SARS-CoV-2 report in dog occurred in Hong Kong, following by two other dogs from COVID-19 positive owners that were tested and identified as asymptomatic (SARS-CoV-2…, 2022). In this same city, a clinically healthy cat tested positive for SARS-CoV-2 on RT-PCR (COVID-19…, 2022). Among the several animal species experimentally infected with SARS-CoV-2, cats and ferrets were found as highly susceptible, while dogs presented low susceptibility (Shi and others, 2020). Companion animals that have been infected by SARS-CoV-2 at time were considered to be most likely in contact with infected humans with a high viral load (Zou and others, 2020).

Despite SARS-CoV-2 infection in companion animals having been described worldwide, dog susceptibility under natural settings has not been fully established. Thus, the aim of this study was to assess dogs and cats in domiciliary contact with owners diagnosed with SARS-CoV-2 infection in Belo Horizonte city, capital of Minas Gerais State, the sixth most populated city in Brazil.

This was a prospective study in dogs and cats housed by owners in isolation due to a positive laboratory diagnosis for SARS-CoV-2, located in the Belo Horizonte city, Minas Gerais state, southeastern Brazil. Home visits took place between January and July 2021, after prior contact to volunteers and their signed authorization, following safety rules regarding the use of personal protective equipment during visits. Oropharyngeal and rectal swabs were collected and placed in a transport medium for the detection of SARS-CoV-2 through the RT-qPCR.

This study was conducted in Belo Horizonte, capital of the Minas Gerais State, southeastern Brazil, ranking 6th in population with around 2,530,701 inhabitants, 4th in Gross Domestic Product (GDP) and 20th in Human Development Index (HDI) out of the 5,570 Brazilian cities. Belo Horizonte climate has been classified as tropical with a dry season (Aw) according to the Köppen climate classification. The average annual temperature has been around 22°C, characterized by a moderately hot summer with high precipitation and pleasant winter with practically no precipitation (Cidades@, 2022).

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The swab samples were tested for SARS-CoV-2-specific RNA,and confirming molecular. Molecular diagnosis for SARS-CoV-2 has been performed as previously described (Epifanio and others 2021). RNA was extracted from all swab samples using a commercially available kit (Maxwell® RSC simply RNA Tissue Kit, Promega Co., Madison, WI, USA) in automated platform (Maxwell® RSC 48, Promega Co., Madison, WI, USA), following the manufacturer recommendation. A quantitative reverse transcription polymerase chain reaction (RT qPCR) was performed to identify the presence of SARS-CoV-2, targeting two viral genome regions: 2019 nCoV_N1 (F: GACCCCAAAATCAGCGAAAT, R: TCTGGTTACTGCCAGTTGAATCTG and P: ACCCCGCATTACGTTTGGTGGACC) and 2019 nCoV_N2 (F: TTACAAACATTGGCCGCAAA, R: GCGCGACATTCCGAAGAA and P: ACAATTTGCCCCCAGCGCTTCAG) as previously described (Epifanio *et al.,* 2021). RT qPCR with cycle threshold value (CT-value) results less than 40 were considered as positive. Samples were tested in duplicate and considered positive for SARS-CoV-2 when the two genes were amplified. All samples with only one target amplified were considered inconclusive.

The present study was approved by the Ethics Committee for Animal Use (protocol number 4879280420) of Federal Rural University of Pernambuco, Brazil. The Brazil National Research Council's guidelines for the Care and Use of Laboratory Animals were followed.

Overall, 3/16 (18.7%) dog samples were positive, and all 10 cat samples were negative for SARS-CoV-2 by RT-qPCR. The oropharyngeal swab from dog 1 had the highest viral load (Target nCoV_N1: CT 33.2, 66.76 copies of RNA/uL; Target nCoV_N2: CT 31.14, 77.3 RNA copy number/ uL), from the dog 2 had the lowest viral load (Target nCoV_N1: CT 35.47, 2.41 RNA copy number/uL; Target: CT 34.28, 10.71 RNA copy number/uL) and dog 3 was intermediate (Target nCoV_ N1: CT 32.72, 26.58 RNA copy number/uL; Target nCoV_N2: CT 33.57, 52.06 RNA copies/uL). Among the three positive dog samples, median Ct values were 33.79 for target gene nCoV_N1 and 32.99 for target gene nCoV_N2, with no viral genome successfully sequenced.

Dog 1 was a 2-year-old neutered male boxer, asymptomatic at the time of sampling, with two positive owners for SARS-CoV-2. The positive dog lived with another dog and a cat, which were negative for SARS-CoV-2 in RT-qPCR in three swabs each. Owners referred that the dog presented no behavioral or physiological changes, comorbidities, or other diseases, confirmed by physical examination. Owners have also reported the habit of frequent playing, kissing, and keeping close contact with their dog, with no changes after SARS-CoV-2 diagnosis. As mentioned, a second sampling was performed after 14 days by oropharyngeal swab and resulted negative.

Dog 2 was a 10-year-old neutered male mongrel, with chronical clinical signs at the time of sampling, living with another dog and eight cats. The dog and only one cat were sampled and both tested negative for SARS-CoV-2 by RT-qPCR. Despite the positive dog presenting cachexia, dehydration, diarrhea, and general weakness, such condition initiated few weeks prior to diagnosis, worsening within past few days. The owner lived alone and kept the dog in close contact for basic care and cleaning, with no perceptible changes after COVID-19 diagnosis. The dog coinfection was diagnosed as clostridiosis through rectal swab, performed along with the first SARS-CoV-2 detection. The dog was euthanized by the owner's decision 10 days after the first sampling, due to poor animal condition and owner financial restraint.

Dog 3 was a single 15-year-old neutered female mongrel, asymptomatic at the time of home sampling. The owner referred close dog contact, caresses, kisses, same bed sleeping, with no behavior change after COVID-19 diagnosis. The dog had monitored and regularly treated chronic renal disease with polyuria and polydipsia. At physical examination, dog presented only a small heart murmur. A second sampling was also performed after 14 days by oropharyngeal swab and resulted negative.

This study has been the first report of companion animals naturally infected by SARS-CoV-2 in countryside Brazil, located in the sixth largest metropolitan area in Brazil. The positivity in dogs (3/16; 18.7%) and cats (0/10; 0.0%) herein

was lower than previously reported in owned pets of Rio de Janeiro, southeastern Brazil with 8/29 (28.0%) dogs and 4/10 (40.0%) cats positive for SARS-CoV-2 by RT-qPCR and/or sequencing (Calvet and others, 2021). The higher positivity showed in this study when compared herein could be associated to sequencing of secondary target from samples with only one positive-target, which has increased the positivity rates from 20.0% to 40.0% for cats, and 13.8% to 28.0% for dogs (Calvet and others, 2021). No viral genome or fragments were effectively sequenced herein, probably associated with low viral load of dog swab samples, with median CT values of 33.79 for target gene nCoV_N1 and 32.99 for target gene nCoV_N2, but still higher than recommended by most commercial sequencing kit protocols of Ct 30.0 or lower.

Despite dog positivity for SARS-CoV-2 (18.7%) herein was higher than cat positivity (0%), a longitudinal study conducted in Texas, USA has shown 1/59 (1.7%) dog and 3/17 (17.6%) cats positive by RT-PCR and sequencing, with RNA isolated from the oropharyngeal/ nasopharyngeal swabs of dog and cat similar to the variant diffused among owners (Hamer and others, 2020). In addition, a total of 15/451 (3.3%) household dogs and 11/191(5.8%) household cats have shown SARS-CoV-2 neutralizing antibodies in Italy (Patterson and others, 2020). Likewise, anti-SARS-CoV-2 antibodies were detected in 7/148 (4.73%) dogs and 15/69 (21.74%) cats in Portugal (Barroso *et al.,* 2022). Among seropositive, 11/22 (50.0%) pets were likely infected due to human-animal contact, and 5/15 (33.3%) infected cats due to cat-to-cat transmission (Barroso and others, 2022). In northeastern Brazil, 0/16 (0.0%) dogs and 2/15 (13.3%) cats were positive for SARS-CoV-2 by RT-qPCR, both after cat-human interaction without protection during owner self-isolation (Epifanio and others, 2021).

Despite dogs having been considered less susceptible to SARS-CoV-2 infection than cats under experimental conditions, close-contact habits such as kissing, hugging and bed sharing may increase the risk of infection to dogs, as indicated by 3/3 infected dogs herein with close human contact. Sampling was conducted during clinical manifestation of COVID-19 in dog owners, which may have improved SARS-CoV-2 detection, considering the relatively short period of virus detection with around 6 days post infection in dogs and 7-13 post-infection days in cats (Shi and others, 2020).

Positive owners and positive dogs for SARS-CoV-2 have already been associated to their close relationship (Hamer and others, 2020; Carpenter and others, 2021). As in the present study, the clinical signs in pet have been reportedly mild, non-existent or non-directly related to SARS-CoV-2 infection, with mostly an outcome of spontaneous animal healing (Hamer and others, 2020). Such variation in clinical symptomatology should be further investigated to fully establish the physiopathology of SARS-CoV-2 infection in companion animals.

In summary, dogs herein were more likely positive for SARS-CoV-2 than cats, which may be associated to closer human-animal contact reported by owners during COVID-19 infection, including care and cleaning, bed sharing, hugging, and kissing. Further studies should be conducted to fully establish dog susceptibility in different settings of human-animal interaction.

Keywords: SARS-CoV-2, pet, epidemiology, zoonoses, cat

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