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A one-year extensive molecular survey on SARS-CoV-2 in companion animals of Turkey shows a lack of evidence for viral circulation in pet dogs and cats

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ABSTRACT

Current evidence have now demonstrated that SARS-CoV-2 infects a wide array of mammalian animals; however, the full range of hosts and the viral circulation in companion animals remains to be clarified. In this context, as no such evidenced cases have been reported from Turkey, we aimed to screen for SARS-CoV-2 nucleic acid in housed dogs and cats clinically evaluated for respiratory symptoms and reared in different locations of Samsun province in the black sea region of Turkey from July 2020 to July 2021. Nasal swabs were collected from a total of 415 pets (65 cats and 350 dogs) aged between 1 and 9 years old. All the specimens were tested for SARS-CoV-2 RNA presence by real-time RT-PCR targeting two genomic regions of SARS-CoV-2, but none showed positive results. Our findings suggest that SARS-CoV-2 does not circulate in local pets and is not responsible for respiratory symptoms. However, further comprehensive molecular and serological surveys are required to have a better picture of the zoonotic, reverse zoonotic and pathogenic consequences of the ongoing COVID-19 pandemic in Turkey.

1. Introduction

SARS-CoV-2, a *Coronaviridae* family and *Orthocoronavirinae* subfamily virus, is the causative agent of the ongoing COVID-19, being responsible for the first ever known coronavirus pandemic (Liu et al., 2020). This virus can cause fatal infections in humans and was speculated to be emerged from an animal origin, similarly to its close predecessors SARS-CoV and MERS-CoV that previously caused respiratory outbreaks in different regions of the globe. Moreover, SARS-CoV-2 has a close identity (96.2%) to a horseshoe bats (*Rhinolophus* spp.) coronavirus (RaTG13) that was early discovered in 2013 in the Chinese Yunnan province, but never previously observed in animals (Abdel-Moneim and Abdelwhab, 2020).

The involvement of animals in the COVID-19 pandemic and the possibility of new animal reservoirs that may contribute to virus evolution is still under consideration, especially in light of recent reports that evidenced the circulation of SARS-CoV-2 in several companion, zoo and wild animals (Chandler et al., 2021; Decaro et al., 2021). Most of those reports emphasized the role of domestic animals as they co-habit with people in close contact, qualifying them to be able to amplify this emerging virus. As such, domestic animals may disperse, drive and sustain a novel and continuous evolution of the virus. (Chandler et al., 2021; Decaro et al., 2021; El Zowalaty and Jarhult, 2020).

SARS-CoV-2 infection in pets, particularly in dogs and often in cats, is

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associated with clinical signs such as: anorexia, respiratory symptoms, lacrimation, and nasal discharges without a fatal prognosis (Leroy et al., 2020, Klaus et al., 2021). To raise knowledge on the possibility of SARS-CoV-2 circulation in pets of Turkey, we have investigated the infection status of dogs and cats that developed respiratory symptoms in the period between 2020 and 2021 and lived in close proximity to local inhabitants of northern Turkey.

2. Materials and methods

2.1. Samples

Nasal swabs samples were obtained from a total of 415 pet animals (65 cats and 350 dogs) aging one to nine years old, after consulting in veterinary clinics in Samsun province of Turkey (Fig. 1). Only animals presenting a case history of respiratory symptoms between July 2020 and July 2021 were included. Collected samples were kept in sterile viral transport media and transferred under a cold chain to the virology laboratories of Samsun Veterinary Control Institute and Faculty of Veterinary Medicine, Ondokuz Mayis University, for immediate RNA extraction and PCR testing.

Limited data on these pet owners' health status were collected since they were unwilling to provide information about the status of previous or ongoing SARS-CoV-2 infection. In this context, 11 of the 65 cats' owners confirmed their recent COVID-19 positivity, whereas, among the 350 dogs' owners, only 47 indicated their current SARS-CoV-2 condition. Substantially, in this study, the analyzed companion animals were household and had no chance of outdoor contact.

2.2. Nucleic acid extraction and real-time RT-PCR

A total of 415 swab samples were tested for SARS-CoV 2 presence. Firstly the swab samples were vortexed and centrifuged for 10 min at 2500g. Nucleic acid extraction of all samples was performed using a commercial extraction kit according to the manufacturer's instructions. (Invitrogen, Van Allen Way Carlsbad, CA, USA). All nucleic acid samples were screened by RT-qPCR using BIO-RAD iTaq Universal Probes One Step Kit (BIO-RAD, USA) targeting the N1 and N2 genomic regions of SARS-CoV-2, according to CDC (2020), as seen in Table 1. Real-Time RT-PCR mixture was performed in 25 µl total reaction volume containing 10µl 2X buffer, 0.5µl RT enzyme, 2µl RNAse-free water, 5µl RNA, 500 nM from each primer and 125 nM FAM-BHQ1 labeled probe (Table 1). The thermal profile consisted of; 50°C for 10 min, 95°C for 3 min, 40 cycles at 95°C for 5 seconds and 60°C for 10 seconds. RNAse-free water was used as a negative control, while the RNA of a previously identified anonimized COVID-19 human case obtained from our university teaching hospital was utilized as a positive control for this test. This assay was carried out using CFX96 Touch Real-Time PCR Detection System (BIO-RAD, USA).

Table 1
Primers specific to two regions of the Nucleocapsid (N) gene of SARS-CoV-2 (CDC 2020).

Primers and Prop	Sequences
2019-nCov_N1- F	5-GAC CCC AAA ATC AGC GAA AT-3
2019-nCov_N1-R	5-TCT GGT TAC TGC CAG TTG AAT CTG-3
nCov-N1- P	5-FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1-3
2019-nCov_N2- F	5-TTA CAA ACA TTG GCC GCA AA-3
2019-nCov_N2- R	5-GCG CGA CAT TCC GAA GAA-3
2019-nCov_N2 P	5-FAM-ACA ATT TGC CCC CAG CGC TTC AG-BHQ1-3

3. Results

In our study, a total of 415 swab samples taken from cats and dogs showing respiratory system symptoms were tested by real-time RT-PCR for the presence of SARS-CoV 2 RNA. All samples tested negative for both regions of the SARS-CoV-2 genome.

4. Discussion

Many studies strengthen domestic animals' conjectural effect and investigate the possibility of their zoonotic and reverse-zoonotic role in the COVID-19 pandemic (Kiros et al., 2020). On top of these reports came the companion animals, in particular cats and dogs, as they are closely linked with their human owners, which may qualify them as possible sources of infection (Meekins et al., 2021). Despite the relatively large sample size and previous or recent circulation of COVID-19 in some owners, we have not detected any SARS-CoV-2 positive pet animal in northern Turkey. However, a few reports have shown a few domestic animals infected by SARS-CoV-2, particularly ferrets, dogs and cats, likely favored by similar angiotensin-converting enzyme 2 (ACE2) receptors (Shi et al., 2020; Zhai et al., 2020). Like human ACE2, the viral receptors in the animal hosts favor COVID-19 infection, which may indicate a low species barrier as this receptor tolerates many amino acid changes with no decrement in its receptor function (Shi et al., 2020; Zhai et al., 2020).

Moreover, to demonstrate the contagious feature of the virus, many scoring studies indicated that ACE2 is expressed in dogs' respiratory

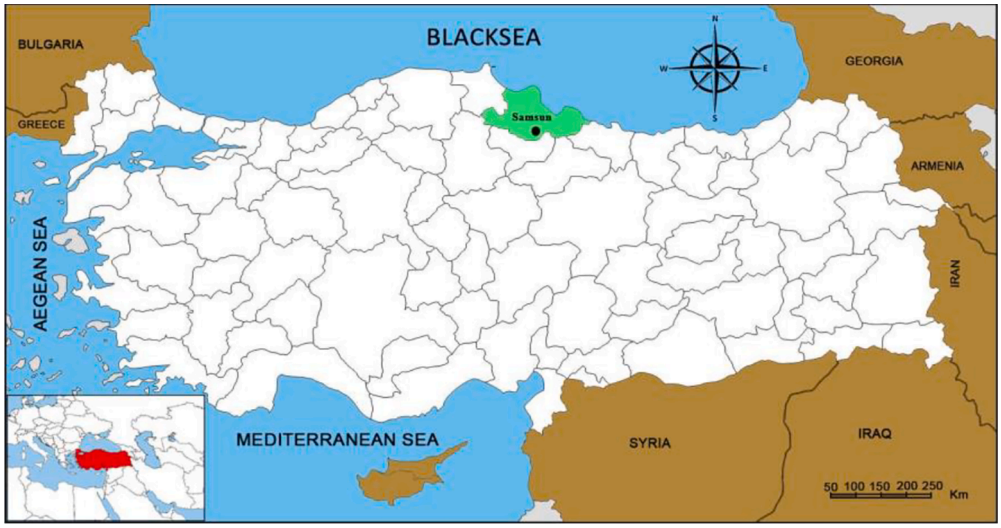

Fig. 1. Location of Samsun province in northern Turkey, where samples were collected for this study.

systems at low levels (Zhai et al., 2020), but in other reports was proven that it possesses a 23 susceptibility score (Alexander et al., 2020), making them little vulnerable to be infected, particularly when compared to cats which have a 27 score of susceptibility to SARS-CoV-2 (Shi et al., 2020; Alexander et al., 2020). Cats, especially at their younger ages (less than 100 days), are more susceptible to the infection contracted naturally through direct contact with humans or other animals (OIE—WOAH, 2021). Interestingly, the subsequent transmission of the virus can be continued by nasal shedding, even in asymptomatic cats (Halfmann et al., 2020). Contrary to our survey, many reports have demonstrated SARS-CoV-2 positivity in stray and households cats living in active outbreak locations or with infected individuals (Klaus et al., 2021; Barrs et al., 2020; Ruiz-Arrondo et al., 2021; Hamer et al., 2021; Calvet et al., 2021; Hosie et al., 2021; Zhang et al., 2020; Stevanovic et al., 2021; Colitti et al., 2021; Dias et al., 2021; Dileepan et al., 2021; Fritz et al., 2021). Furthermore, wild and captive cats (leopards, tigers, cougars and lions) became positive for COVID-19 infection after exposure to infected human workers at the zoo (OIE—WOAH, 2021; McAloose et al., 2020).

Although reports showed SARS-CoV-2 naturally infected cats, these cases appear to be rare, indicating inadequate transmission of the virus from people to cats and from cats to cats (Shi et al., 2020; Alexander et al., 2020). Interestingly, 23% was the maximum cat seroprevalence ever recorded in published survey studies reported in animals living in proximity to their keepers who were infected as well by SARS-CoV-2 at the outbreak peak time (Fritz et al., 2021). Similar to our findings, many surveillance studies in cats exposed to the virus in intensive outbreaks areas could not detect SARS-CoV-2 nucleic acid (Stranieri et al., 2021; Sanchez-Montes et al., 2021; Temmam et al., 2020). On the other hand, the only report from our country confirmed the detection of anti-SARS-CoV-2 antibodies prior to and within the COVID-19 pandemic in domestic cats of Istanbul (Yilmaz et al., 2021), which may indicate the rising risk from those animals toward the community, especially when their owners contract COVID-19 without implementing any measures of prevention to break the cycle of transmission.

Research in experimentally infected dogs showed minimal replication of SARS-CoV-2 after intranasal administration, and isolation attempts during all study period from tissue and swab specimens were not successful (Shi et al., 2020; Bosco-Lauth et al., 2020). SARS-CoV-2 RNA was detected but in rectal swabs of two dogs out of five from one of the aforementioned experiments (Shi et al., 2020). Additionally, no record of respiratory symptoms and histopathological changes were ever noticed in the above-mentioned dogs, as well as the absence of infection transmission from these animals to other dogs that comingled in the same area. All of this supports the minute possibility of dogs to become infected with SARS-CoV-2 (Shi et al., 2020; Bosco-Lauth et al., 2020).

Also, remarkably, dogs were proven in many studies to contract COVID-19 infection naturally throughout the globe (OIE—WOAH, 2021), particularly in the case of Hong Kong two dogs that were able to shed SARS-CoV-2 via nasal and oral route; however, no infectivity was detected in other householded animals with those dogs (Sit et al., 2020). Moreover, many low seroprevalence studies for this virus in dogs were carried out in different countries at the peak time of the pandemic (Hamer et al., 2021; Calvet et al., 2021; Stevanovic et al., 2021; Colitti et al., 2021; Dileepan et al., 2021; Fritz et al., 2021). On the other hand, similar to our outcomes, some survey studies in dogs were not able to show seroconversion or viral transmission (Temmam et al., 2020; Sailleau et al., 2020).

For the above, the fact that SARS-CoV-2 was not found in the screened pet animals is not surprising. Owners that replied to our questionnaire reported having their last infection by SARS-CoV-2 infection one week to three months prior to the sampling process of this study. If pets would have been infected, it is likely that they had cleared the infection by the time of the study. Aditionally, the single sampling process we followed without repetitions can be considered another limitation of this survey, as some of the results may be underestimated.

5. Conclusion

Given the fact that 648 million and above individual cases of COVID-19 have been recorded globally to date, the occurrence of natural infection in pets might be a possibility that deserves further study. However, the endemic nature of this virus in pet animals is still under questioning, as the present research here shows. Nevertheless, preparedness for spillover events between dogs, cats and humans is of particular interest since they generally share the same locations, such as animal hospitals, catteries, shelters and houses.

Our findings indicated that local pets in northern Turkey were not excreting SARS-CoV-2. Further comprehensive molecular and serological surveys in different national and international contexts are required to have a better picture of the zoonotic, reverse zoonotic and pathogenic consequences of the ongoing COVID-19 pandemic and to accurately clarify any possible role of pets as an intermediate host in expanding cycle of the infection.

Ethics committee approval

All sampling operations and procedures were conducted in accordance with the Declaration of Helsinki and based on ethical legislative rules that consisted with the national regulations for animal experiments and prior to sampling a written informed consent was taken from animal owners following approval of the Samsun Veterinary Control Institute Ethics Committee as well as legal permission was also taken from General Directorate of Food and Control, Ministry of Agriculture and Forestry, Republic of Turkey (No: E.71037622-280.05-3452924, Date: 18/11/2021).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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