



Diagnosis of COVID -19 by PCR method from the nasopharyngeal samples of symptomatic domestic cats and dogs

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ABSTRACT

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The coronavirus disease 2019 (COVID-19) is caused by the acute respiratory syndrome of the coronavirus-2 (SARS-CoV-2). Due to its severity and spread, the World Health Organization considered it an epidemic. Coronavirus can infect several species of animals and can spread to people and then spread between them. This study aimed to isolate COVID-19 from the nasopharynx of cats and dogs. This cross-sectional study was conducted over 3 months, Spring 2022. saliva and nasopharynx swab samples were taken from 36 domestic dogs (N=30) and cats (N=6) that were symptomatic (with respiratory and/or digestive symptoms) in the clinic of the Faculty of Veterinary Medicine of Tehran University. Also, a questionnaire specific to the owners of these animals was considered, including questions such as age, sex, history of most recently COVID-19, and clinical symptoms. COVID-19 was detected by the real-time polymerase chain reaction (Real-Time PCR) method. In this study 7 dog parents (23.3%) were recently infected with COVID-19, of which 4 had contact with their pets. Also, one cat parent had been recently infected and had contact with other pets during his illness. No COVID-19 was isolated from the symptomatic pets, but some clinical symptoms were reported in them; the most common symptoms seen in dogs were digestive and then respiratory ones. In cats, the most involved systems were respiratory and then Gastrointestinal. All saliva and nasopharynx samples were negative. It will require more samples of pets of more individuals with confirmed SARS-CoV-2 infection in future studies. Nevertheless, avoiding close contact should be considered as long as they remain ill, and using face masks and basic health measures should be observed.

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1. Introduction

Coronaviruses are a large family of viruses, and SARS-CoV-2 which is initiated from a type of bat, can infect several species of animals moreover can spread to people and then spread between them [1-3]. COVID-19 virus transmission is mainly through airborne particles, which is facilitated by factors such as the proximity of infected hosts, the level of host susceptibility, and the concentration of infectious virions in the air [1]. Most COVID-19 animals became infected after contact with COVID-19 people, including their owners or other close contacts [2]. There is no evidence that animals play a significant role in the spread of SARS-CoV-2 to humans [2]. With the possibility of animal-to-human transmission of SARS-CoV-2 in the most vulnerable populations, pet ownership may pose little risk to the elderly [3]. Among the animals reported to be infected in several countries, are companion animals, including cats and dogs [2, 4]. Since companion animals have the closest contact with humans, this provides sufficient opportunity for exposure [1]. It should be noted that people are substantially more likely to catch COVID-19 from each other than animals; therefore there is no need to harm COVID-19 animals [2]. To protect public and animal health is strongly recommended to quarantine domesticated mammals, including dogs and cats, in appropriate facilities, from confirmed COVID-19 households or as close contact with COVID-19 persons [5]. It is essential for risk communication in communities to monitor SARS-CoV-2 in domestic animals in high-risk areas, especially those in close contact with the owners [4]. Experimental infections have shown that most of these animals are only transiently infected, as indicated by positive PCR or virus isolation [6, 7]. There is a probability that the virus could mutate by infecting animals and spread a new strain to persons and then among people (called spillback) [2]. To understand how this virus spreads between humans and animals, more studies are needed. Despite different research about the spread of COVID-19 from animal to human, we decided to isolate COVID-19 from the nasopharynx of cats and dogs in the clinic of the Faculty of Veterinary Medicine of Tehran University in the spring of 2022.

2. Method

Our study was conducted cross-sectionally during 3 months from March 20 to June 20, 2022. Domestic dogs and cats suspected of having coronavirus that were brought to the veterinary clinic, and samples were taken from the saliva and nasopharynx of the pets and their owners under sterile conditions. In the care environment, it is transferred to the Pediatric Infectious Research Center (PIRC). The swab samples were taken from 36 domestic dogs (N=30) and cats (N=6) that were symptomatic (with respiratory and/or digestive symptoms) in the clinic of the Faculty of Veterinary Medicine of Tehran Uni-

versity; This was done with the consent of their owners. According to the pre-determined questionnaire for pets, variables such as animal type, breed, sex, age, weight, clinical symptoms, number of days of symptoms, living indoors/outdoors, contact with other animals, presence of other pets in the house, etc. were asked. Also, a questionnaire specific to the owners of these animals was considered, including questions such as age, sex, history of most recently COVID-19 and the clinical symptoms, recent PCR test and result, and contact with the pet during infection.

The samples underwent processing by gently vortex the nasopharyngeal swab in Viral Transport Medium (VTM) and assigning them unique codes. RNA was extracted using a specialized total RNA extraction kit, employing a silicon column under the prescribed protocol (SIMBIO-LAB. Lot. No010502-2326-202N100).

The Spike and Envelope genes, known as the S and E genes, were identified as viral genes using a multiplex real-time PCR technique (COVITEC, Iran) as a follow; reverse transcription: 55°C for 10 minutes, initial denaturation 95°C for 3 minutes and 50 cycles denaturation: 95°C for 15 seconds and annealing, elongation and data collection 60°C for 1 minutes.

The statistical analyses included: Descriptive statistics that presented as Means, Standard deviations, Minimum and maximum values or percentages. Statistical analysis was conducted with SPSS (version 24).

3. Results

From 36 domestic dogs (N=30) and cats (N=6), nasopharyngeal swab samples were taken.

From 30 dogs (18 males, 12 females), the mean age was 10.57 (Min-Max: 2-72) months, with a mean weight of 9.600 (Min-Max: 1-45Kg). The breeds of dogs according to their number are listed in Table 1.

Table 1: Number of dogs by breed

Breeds	Num
Spitz	7
Free-ranging dog	5
German	5
Terrier	3
Pamer Spitz	2
Others (Alabai, Bulldog, Pomeranian, Pejdar, Dogo, Dobermann, Sarabi, Shih Tzu terrier)	8

Nineteen dogs (63.3%) had Gastrointestinal (GI) symptoms, 17 (56.7%) respiratory and 1 (3.3%) had neurological signs. Eleven dogs (36.7%) showed up with malaise, 11 (36.7%) with anorexia, 8 (26.7%) with nausea, 6 (20%) with rhinorrhea, 5 (16.7%) with sneeze, 4 (13.3%) with fever and 3 (10%) with cough. The mean days of symptoms duration were 4.97 (Min-Max: 1-30); and 20 (66.7%) of all had contact with other pets on those days. 13 (43.3%)

of dogs were kept outdoor and 17(56.7%) indoor. All of the dogs had negative COVID-19 PCR tests.

Of the dog parents (21 males, 9 female), 7(23.3%) were infected by SARS-CoV-2 recently (5 with positive PCR (in the last month), 1(3.3%) with involved CT scan, and 1 without any PCR test or CT scan). Whereas 7(23.3%) parents had negative PCR. While the parents were infected *and/or* their test was positive, 4(57.14%) of them had contact with their pets. Six (20%) of the pet parents had cough, 4(13.3%) of them had fever, 4(13.3%) had headache, 3(10%) had sneeze, and 3(10%) people had myalgia. 5(16.7%) of these parents had respiratory involvements and 2(6.7%) had GI signs.

All of the 6 cats (6 female), were of the DSH breed. 3(50%) of them were 2 months, 2(33.3%) were 1 month and one of them was 48 months old. The mean weight was 1.630 kg (Min-Max:800gr-5Kg). Of all these cats, 3(50%) had respiratory signs, 2(33.3%) GI signs and 1 had a neurological sign. All the cats showed anorexia, 5(83.3%) malaise, 2(33.3%) rhinorrhea, 2(33.3%) had sneeze and 1 cough. The symptoms showed up in 3-60 days with a mean of 14.3. Four (66.7%) of them had contact with other pets. Two (33.3%) cats were kept outdoors and the rest were indoors. Our 6 cats, entirely had negative COVID-19 PCR tests. Cat's parents (5 males, 1 female) were between 25-65 years old with a mean of 43.5. One parent had been recently infected, who had positive COVID-19 PCR, and showed cough, sneeze, and myalgia. And while his disease had contact with other pets.

4. Discussion

Some studies have been conducted to investigate COVID-19 in domestic animals in Iran. In this study, in addition to information about domestic cats and dogs, we also evaluated the symptoms and variables related to their owners. Several observations including the study of Kumar et al have confirmed that pets can be infected with SARS-CoV-2 [8]. According to studies, cats are more susceptible to contracting COVID-19 than dogs [9]. The feline ACE2 is more similar to humans than to canines, therefore this is the reason that cats are more affected by SARS-CoV-2 infection [10]. Even though in Hong Kong, 8 cats and 17 dogs from COVID-19 households were tested, the positivity of 2 dogs was confirmed by molecular and serological methods, and the virus was isolated in one case [11, 12]. Our study reported just isolation of COVID-19 from dogs and cats without comparing the susceptibility between these pets; detection of the virus was done by Real-Time PCR.

In Kumar et al study, Symptoms in most recorded cases of SARS-CoV-2 infection in dogs and cats were variable [8]. Most animals were asymptomatic, however, some animals had mild respiratory distress [8]. On April 23 in New York state, two domestic cats were reported as the first confirmed cases of positive COVID-19 in companion animals in the USA [8]; they both showed symptoms of

upper respiratory disease including cough and nasal discharge [13]. Also from Hong Kong, there was a positive cat without any symptoms [8]. In North Carolina, USA, there was a report of a dog with a COVID-19 positive test and mild respiratory symptoms, but another cat and dog from the same COVID-19 household tested negative [8]. In a COVID-19 Belgian family, a cat showed respiratory and gastrointestinal symptoms and shed the virus in its feces and gastric fluid [8]. A study at a veterinary clinic in France reported no infections in 9 cats and 12 dogs in close contact with COVID-19 patients [14]. In a Spanish study, from the 23 pets, there was only one asymptomatic cat with RT-PCR positive and the remaining pets were negative [15]. Also, in our study, we couldn't isolate any COVID-19 from the symptomatic pets.

According to a French study in June 2020, in the screening of 11 dogs and 22 cats from owners suspected or with a history of SARS-CoV-2 infection [16], one cat infected by SARS-CoV-2 was identified, which was positive by RT qPCR on a rectal swab, however, a nasopharyngeal swab test was negative; and it showed mild respiratory and digestive symptoms. In the present study, just nasopharyngeal samples were examined; Against the previous French study, we did not use rectal swabs for the detection of Coronavirus. Although in our study, the PCR tests of all animals were negative, some disease symptoms were reported in them separately. Some factors have an important role in the detection of the COVID-19 virus such as the best time for collection of the samples, and the kind of samples (nasopharynx, nose, rectal swab...). According to some studies, one of the possible consequences of SARS-CoV-2 infection in dogs and cats can be myocarditis [17]. The most clinical symptoms seen in dogs were digestive symptoms and then respiratory ones. In cats, the most involved systems were respiratory and then GI [18]. Therefore, paying attention to the relevant symptoms in future examinations and investigations can be helpful.

In some studies, it was specifically established that cats exposed to SARS-CoV-2 in laboratory conditions can become infected and capable of transmitting the disease to other felines [3, 9]. In our study 7 dog parents (23.3%) were recently infected with covid-19, of which 4 had contact with their pets. Also, one cat parent had been recently infected and had contact with other pets during his illness. As mentioned in the Kumar et al study, to minimize the potential risk of transmission from pets, animal owners and veterinarians should be vigilant in handling animals with respiratory disease [8].

Pharyngeal sampling in the case of animals faces difficulties, veterinarian colleagues had an important role in this study. Their opportunity for rectal sampling was limited, also because of ethics, we couldn't take samples from all of the pets in the clinic. In that period, there was no proper situation to do animal serological tests. It would be better to have conditions detection of disease by serology method, using the swab rectal, too. It is suggest-

ed the detection of the virus from pets can be followed up and evaluated in the continuation of the study.

This research was one of the first studies of COVID-19 on symptomatic pets during the pandemic using Real-Time PCR in Iran. All of the saliva and nasopharyngeal samples were negative. It will require more samples of pets of more individuals with confirmed SARS-CoV-2 infection in future studies. According to the studies conducted and the nature of the COVID-19 virus, it is unknown that the disease is transmitted through the contact of animals with their owners. Therefore, as long as they are sick, close contact should be avoided and the use of face masks and basic hygiene measures should be observed. The role of pets in these outbreaks is unknown. Further research is needed to clarify these aspects.

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Authors' contributions

AM, SMH, LA, and SHA conceptualized and designed the study, conducted focus groups, designed the data collection tool, collected all the data, and carried out analyses. SMH, NM, and SHA drafted the manuscript, revised the final manuscript, and agreed to be accountable for all aspects of the work. AMA, BS, AB, and SHA helped interpret the data, critically reviewed the manuscript for important intellectual content, reviewed the final version, and agreed to be accountable for all aspects of the work. All authors read and approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Ethical declarations

This study was reviewed and approved by the Research Institute for Children's Health of Shahid Beheshti University of Medical Sciences Ethics Committee (ethical code IR.SBMU.RICH.REC.1400.052). Verbal consent was obtained from all the pet owners.

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