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Molecular screening of SARS-CoV-2 in dogs and cats from households with infected owners diagnosed with COVID-19 during Delta and Omicron variant waves in Iran

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Abstract

Objective: The emergence of SARS-CoV-2 infection in dogs and cats in different countries worldwide raises concerns that pets are at a higher risk for spreading or transmitting of SARS-CoV-2 to humans and other pets and increased the research works about the zoonotic aspects and natural routes of infection in companion animals. The current study aimed to detect the SARS-CoV-2 in household dogs and cats living with COVID-19 positive owners.

Methods: Deep oropharyngeal and rectal swabs were collected from 30 household pets (20 cats and 10 dogs) living with COVID-19 positive owners from April 2021 to 2022 in Kerman, Iran. All dogs' and cats' samples were tested by real-time reverse transcription polymerase chain reaction for detection of SARS-CoV-2.

Results: Two household cats out of 20 examined (10%) were positive for SARS-CoV-2, whereas none of the examined dogs were positive for SARS-CoV-2. The two cats positive for SARS-CoV-2 were symptomatic and suffered from severe anorexia with maximum contact with their infected owners.

Conclusion: This study reported the presence of SARS-CoV-2 in household cats in close contact with COVID-19 positive owners during the circulation of new SARS-CoV-2 variants (Delta and Omicron) in Iran and suggested that the transmission may have occurred from owners to their cats. Therefore, infected owners should eagerly limit close contact with their pets during COVID-19 illness.

KEYWORDS
One Health, pet owners, pets, SARS-CoV-2 delta and Omicron variant

1 | INTRODUCTION

SARS-CoV-2 is one of the most challenging health issues in the 21st century that became a priority worldwide. This coronavirus was first detected in December 2019, in Wuhan City, Hubei province, China (Hosie et al., 2021) and World Health Organization (WHO) declared it a pandemic on March 11, 2020 (Elaswad et al., 2020). Hence, other coronaviruses (Covs) are considered zoonotic, and one of the most important aspects of this situation based on the One Health approach is to survey the zoonotic potential of SARS-CoV-2 and the transmission of this virus between humans and animals (Temmam et al., 2020). SARS-CoV-2 is a member of the genus *Betacoronavirus*, family *Coronaviridae*, order *Nidovirales* (Hosie et al., 2021). Covs are enveloped, single-stranded Ribonucleic acid (RNA) viruses that are one of the major pathogens in medical and veterinary science, and this name (CoVs) comes from the spiky proteins that can be seen in the microscopic field (Alluwaimi et al., 2020). The *Coronaviridae* family has a large genome size compared with other RNA viruses (Mousavizadeh & Ghasemi, 2021). Covs can be categorized in different ways, however; a reclassification was published recently. According to this classification, Covs are categorized into four genera including *Alpha*, *Beta*, *Gamma*, and *Delta* Covs. The first two genera involve mammalian and human CoVs and the others related to the avian CoVs (King et al., 2011).

Feline CoVs are members of *Alpha* coronaviruses and can occur as low-virulent pathotype feline enteric coronavirus (FECV) or high-virulent pathotype feline infectious peritonitis coronavirus (FIPV) causing severe multi-systemic diseases. FECV-involved cats show mild diarrhea or upper respiratory signs even though they can experience subclinical illness. The occurrence of mutation in the genome of FECV can change that virus to the highly virulent form (FIPV) that may result in a lethal multi-systemic disease (Brown et al., 2009). FIPV can be categorized into two general forms, wet and dry, and both of them can severely affect wild and domestic felids (Kipar & Meli, 2014; Pedersen et al., 2009).

Dogs also infected with canine coronaviruses (CCoVs) (*Alphacoronavirus*) and canine respiratory coronaviruses (CRCoVs) (*Betacoronavirus*). CCoVs are also known as enteric coronaviruses and are categorized into two genotypes I–II, which result in a gastrointestinal upset in dogs. These genotypes cause mild and self-limiting gastroenteritis (Licitra et al., 2014). However, the highly virulent form (CCoV-IIa) has pantropic behavior that can cause a lethal disease, especially in young dogs, the infected dogs show lethargy, severe lymphopenia, hemorrhagic diarrhea, and neurologic signs (Decaro et al., 2007). CRCoV infection causes respiratory illness in dogs (Erles et al., 2003).

SARS-CoV-2 (COVID-19) that emerged in 2019 is considered the third coronaviral disease after severe acute respiratory syndrome coronavirus (SARS-CoV) in 2003 and Middle-East respiratory syndrome coronavirus (MERS-CoV) in 2012. Although SARS, MERS, and SARS-CoV-2 can cause fatal respiratory diseases in humans, other human CoVs may result in asymptomatic or mild illnesses. Both SARS-CoV and SARS-CoV-2 could infect humans and different animal species including pets and farm animals through binding to angiotensin converting enzyme 2 (ACE2) receptors on host cells resulting in clinical or subclinical illnesses (Sreenivasan, 2021). Epidemiological studies indicated that dogs and cats are susceptible to SARS-CoV-2, even though the global number of naturally infected animals is far less than the number of people with coronavirus disease 2019 (COVID-19) (Bosco-Lauth et al., 2020). These studies suggested that dogs and cats acquired the infection from COVID-19 infected owners through direct contact. However, there are no studies that reported that dogs and cats can transmit SARS-CoV-2 to other animals and humans, under natural conditions (Leroy et al., 2020; Newman et al., 2020).

The emergence of SARS-CoV-2 infection in companion animals (dogs and cats) in different countries worldwide raises concerns that pets are at a higher risk for spreading and transmitting SARS-CoV-2 to humans and other animals, and this could threaten the public health (Leroy et al., 2020). The ongoing COVID-19 pandemic drastically challenged the healthcare system and caused over 4.5 million deaths worldwide. Iran was one of the nations that were hit by the hardest pandemic and involved in the Sixth COVID-19 wave. This study aimed to detect the SARS-CoV-2 infection in household dogs and cats living with COVID-19 positive owners by real-time reverse transcription polymerase chain reaction (RT-qPCR) during Delta and Omicron variant waves in Iran.

2 | MATERIALS AND METHODS

2.1 | The study subjects and sampling

Sixty samples (oropharyngeal and rectal swabs) were collected from 30 household pets (20 cats and 10 dogs) belonging to different breeds during the period from April 2021 to 2022, coinciding with the geographic SARS-CoV-2 Delta and Omicron variant waves in humans in Kerman, Iran. The inclusion criteria were restricted to only dogs and cats living in the same households with COVID-19 positive owners whose infection was confirmed by presenting a COVID-19 positive PCR test from a referral laboratory. The samples were collected only from animals whose owners gave informed consent to participate in the study. There was no limitation for age, sex, and health status of the enrolled dogs and cats. However, pregnant animals were not included in the study.

All swab samples were collected from dogs and cats 1–3 weeks after the owners' COVID-19 infection was confirmed by PCR. Oropharyngeal swabs were inserted deeply into the posterior pharynx and tonsillar areas. Moreover, the rectal swabs were inserted 1–2 cm past the anal verge and the swab was rotated gently 360°. Each swab was placed in Eppendorf tubes (Tamadkala, Iran) containing 300 μL of sterile viral transport medium that consisted of 200 mL of double distilled sterile water mixed with protein stabilizer, antibiotic, and buffer solution (VTM; Payvand Clinical & Specialty Laboratory, Iran). The collected swab samples were transferred to the reference COVID-19 biosafety level 3 containment laboratory for further processing and RNA extraction.

For each client, data were collected via a detailed questionnaire. Pet's signalment, current health status, past medical history, and the relationship between pets and their owners during COVID-19 involvement (level of contact) were recorded. The level of contact was determined in terms of low, moderate, and high. High contact with pets was defined as kissing, hugging, sharing sleeping place, food processing, and hand feeding without mask. In moderate contact, shared room with pets, food processing and hand feeding without mask were recorded. In low contact, the owners followed the quarantine rules and have no close contact with their pets. Food processing and feeding without close contact, litter box cleaning for cats (with mask) or taking dogs for walk and defecation (with mask) were done. The owner's gender, clinical signs during the infection period, and the number of infected family members were documented.

2.2 Molecular detection of SARS-COV-2 in household dogs and cats

The collected swab samples from dogs and cats were transferred to a reference COVID-19 laboratory for RNA extraction using QIAamp Viral RNA Mini kit (Qiagen, Hilden, Germany cod; 52904) according to the manufacturert's instructions and the extracted RNA was preserved at −70°C. The extracted RNA from dogs' and cats' swab samples was then transferred to a reference microbiology laboratory and RT-qPCR was done by SENMURV Mulristar-2 SARS-COV-2 RT-PCR kit (Stem Cell Technology, Iran; Cat-No. BONCOV-MS2200PB) following the manufacturert's instructions. RT-qPCR was performed in a multiplex method to detect specific SARS-COV-2 nucleoprotein (N) and RNA-dependent RNA polymerase (*RdRp*) genes using Texas Red and FAM fluorophores (Thermo Fisher Scientific, Germany), respectively. In this method, the *RNaseP* gene was considered as the internal control for sampling and extraction steps and was evaluated with HEX fluorophore. Each RT-qPCR reaction consisted of 20× RTase (1 μl), 4× CAPITAL buffer (5 μl), primer/probe Mix (3 μl), nuclease-free water (6 μl), and extracted viral RNA (5 μl). The cycling steps of RT-qPCR included reverse transcription (50°C, 20 min, 1 cycle), pre-denaturation (95°C, 10 min, 1 cycle) denaturation (95°C, 10 s), annealing and acquisition on Texas Red, FAM and HEX channels (95°C, 40 s). Denaturation and annealing were performed for 45 cycles. Reverse transcription and amplification was done in LightCycler® 96 System (Roche Diagnostics, Germany). Finally, descriptive analysis of the data was conducted using the computer program IBM SPSS statistics for Windows Version 28 (IBM Corp. 2021, Armonk, NY, USA).

3 | RESULTS

In this study, the results of RT-qPCR revealed that the N and *RdRp* genes of SARS-COV-2 were detected in 2 out of 20 (10%) examined oropharyngeal swabs from household cats living with COVID-19 positive family member (Table 1 & Figure 1). However, N and *RdRp* genes of SARS-COV-2 couldn't be detected in the examined rectal swabs from the investigated cats by RT-qPCR.

Table 1 showed that none of the oropharyngeal or rectal swabs collected from household dogs were positive for the N and *RdRp* genes of SARS-COV-2 using RT-qPCR.

CT values of RT-qPCR products related to *N* and *RdRp* genes are shown in Figure 1. The weight of these products was too low, so it was not possible for Sanger sequencing.

The two infected cats (4 years old) were anorectic and fed by their owners. One of them, female domestic short hairs cat, had a high contact rate with her owner (kissing, hugging, sharing sleeping place, food processing, and hand feeding) and the other male Angora cat had a moderate contact rate with her owner (shared room, food processing, and hand feeding). The median number of days between a COVID-19 positive PCR test result in infected owners and pet samples' collection was illustrated in Table 1. The SARS-COV-2 positive domestic short hairs cat in this study had a median of 7 days and Angora cat had a median of 10 days. All dog and cat owners enrolled in this study had a COVID-19 positive PCR test and the infection occurred during Delta wave.

In the current study, all the investigated dogs and cats for SARS-CoV-2 were symptomatic and had clinical signs. The clinical signs in the participated dogs ranged from decreased appetite (5), tracheobronchitis and dry cough (3) diarrhea (3) conjunctivitis (1), and sneezing (1). Moreover, the clinical signs in the investigated cats include loss or decreased appetite (20), conjunctivitis, nasal and eye discharge (9), laryngitis and vocal change (5), diarrhea (2), and fever (3) (Table 1).

4 | DISCUSSION

A One Health approach is informative for the prevention and control of SARS-CoV-2 transmission. The animal origin of the novel coronavirus SARS-CoV-2 led to a discussion about the possible transmission of the disease to humans through animals. The investigation of the reservoir and intermediate hosts for SARS-CoV-2 can help in understanding the epidemiology and dynamics of COVID-19 pandemics that raises the alarm that the disease could be a reverse zoonosis and the infected animals may act as potential transmitters to humans (Ye et al., 2020). Hence, dogs and cats are the most common species taken as home pets, several studies were performed to detect the transmission of SARS-CoV-2 between pets and their owners (Barrs et al., 2020; Hamer et al., 2021). Over 10 countries to date have documented natural infections of dogs and cats, often associated with exposure to a person with COVID-19 (OIE, 2020). Focused studies on pet animals with known exposure to COVID-19 infected people are critical for understanding the potential for companion animal's infection and their risk to serve as reservoirs for the virus.

The prevalence rate (10%) of SARS-COV-2 in household cats living with COVID-19 positive owners in this study coincided with the reports from different countries: Hong Kong, China (Barrs et al., 2020), Texas, USA (Hamer et al., 2021), Italy (Patterson et al., 2020), France (Sailleau et al., 2020), and Spain (Ruiz-Arrondo et al., 2021). The former studies suggested that cats were infected from their owners. On the contrary, higher prevalence rates were recorded in Wuhan, China

TABLE 1 Demographic and clinical characteristics of pets screened for SARS-COV-2 living with COVID-19 infected owners in Iran

					RT-qPCR results (Ct value)					
Sample ID	Pet species	Breed	Age	Gender	*RdRp* (Texas red)	*N* (FAM)	*RNaseP*[a] (Hex)	Infected family members /Contact rate with COVID-19 infected owners	Medical history of the examined pets	Days from owners' COVID-19 diagnosis to pet sample collection
1	Cat	Persian	5 years	Male	-	-	28.51	2/low	Mild anorexia, conjunctivitis, nasal and eye discharge	16
2	Cat	DSH	6 months	Female	-	-	31.56	4/low	Sever anorexia, laryngitis and vocal change	10
3	Cat	Persian	4 years	Male	-	-	31.11	2/Moderate	Sever anorexia	20
4	Cat	DSH	4 years	Female	20.36	17.50	27.90	1/ high	Sever anorexia	7
5	Cat	Persian	3 years	Female	-	-	28.99	1/low	Mild anorexia	8
6	Cat	DSH	10 months	Female	-	-	28.68	3/ low	Mild anorexia, laryngitis and vocal change	21
7	Cat	DSH	2.5 years	Male	-	-	24.74	4/Moderate	Mild anorexia and diarrhea	25
8	cat	Scottish fold	9 months	Female	-	-	31.95	4/low	Mild anorexia, laryngitis and vocal change and fever	10
9	Cat	Angora	4 years	Male	21.56	19.89	25.60	1/Moderate	Sever anorexia	10
10	Cat	DSH	4 years	Male	-	-	24.80	2/low	Mild anorexia, conjunctivitis, nasal and eye discharge	12
11	Cat	DSH	3 years	Female	-	-	27.05	4/low	Mild anorexia, conjunctivitis, nasal and eye discharge	8
12	Cat	Scottish fold	3.5 years	Female	-	-	28.58	2/low	sever anorexia conjunctivitis, nasal and eye discharge	10
13	Cat	Persian	4 years	Male	-	-	30.15	2/low	Mild anorexia, conjunctivitis, nasal and eye discharge	11
14	Cat	DSH	11 months	Female	-	-	30.88	2/moderate	Mild anorexia, conjunctivitis, nasal and eye discharge	25
15	Cat	DSH	18 months	Male	-	-	26.10	2/low	Mild anorexia and diarrhea	15
16	Cat	DSH	3 years	Male	-	-	27.42	2/low	Mild anorexia, conjunctivitis, nasal and eye discharge	18
17	Cat	DSH	7 years	Male	-	-	31.36	2/moderate	Mild anorexia, laryngitis, vocal change and fever	20
18	Cat	DSH	2 years	Male	-	-	28.33	4/low	Mild anorexia, conjunctivitis, nasal and eye discharge	10
19	Cat	DSH	5 years	Male	-	-	30.74	3/low	Sever anorexia, laryngitis, vocal change and fever	7

(Continues)

TABLE 1 (Continued)

					RT-qPCR results (Ct value)					
Sample ID	Pet species	Breed	Age	Gender	*RdRp* (Texas red)	*N* (FAM)	*RNaseP*[a] (Hex)	Infected family members /Contact rate with COVID-19 infected owners	Medical history of the examined pets	Days from owners' COVID-19 diagnosis to pet sample collection
20	Cat	Persian	2 years	female	-	-	24.54	3/low	Mild anorexia, conjunctivitis, nasal and eye discharge	12
21	Dog	Chihuahua	1 years	Male	-	-	29.43	4/low	Conjunctivitis	10
22	Dog	Shi-Tzu	5 years	Female	-	-	30.13	2/moderate	Sever anorexia	15
23	Dog	Terrier	1 years	Female	-	-	28.08	3/low	Mild anorexia and diarrhea	7
24	Dog	Pekingese	6 years	Female	-	-	25.03	1/High	Diarrhea	25
25	Dog	Maltese	5 years	Male	-	-	26.43	3/low	Tracheobronchitis and dry cough	7
26	Dog	King Charles spaniel	6 months	Female	-	-	30.88	1/High	Mild Anorexia	20
27	Dog	Pekingese	2 years	Male	-	-	27.42	3/low	Mild anorexia	5
28	Dog	Pomeranian	4.5 years	Male	-	-	31.38	2/low	Tracheobronchitis, dry cough and severe anorexia	4
29	Dog	Shi-Tzu	4 years	Female	-	-	30.05	3/low	Diarrhea and sneezing	5
30	Dog	Pomeranian	4 years	Male	-	-	24.16	2/low	Tracheobronchitis and dry cough	4

Abbreviation: DSH, domestic short hairs.
[a]Internal control for sampling and extraction steps.

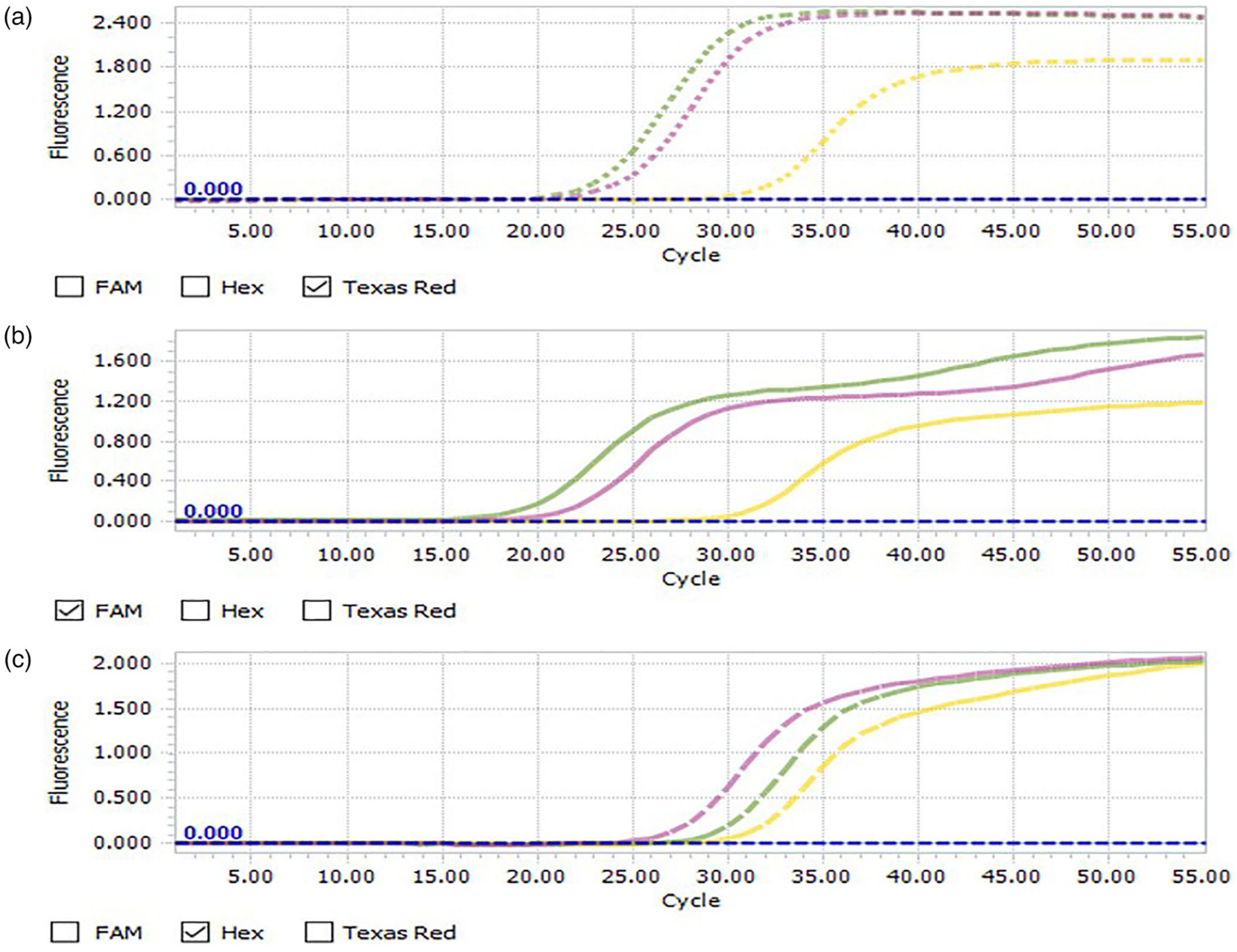

FIGURE 1 RT-qPCR amplification curves for SARS-CoV-2 in cat positive samples. Green: sample 4, purple: sample 9, and yellow: positive control. (a) Amplification curves with Texas Red fluorophore for *RdRp* gene [Ct: 20.36 (sample 4), 21.56 (sample 9), and 30.14 (positive control)]. (b) Amplification curves with FAM fluorophore for *N* gene [Ct: 17.50 (sample 4), 19.89 (sample 9), and 29.93 (positive control)]. (c) Amplification curves with Hex fluorophore for *RNaseP* gene [Ct: 25.60 (sample 9), 27.90 (sample 4), and 29.99 (positive control)]

(Chen et al., 2020; Zhang et al., 2020), France (Fritz et al., 2021), and Brazil (Calvet et al., 2021). However, none of the 99 examined household cats owned by COVID-19 infected owners showed molecular and serological evidence of SARS-CoV-2 in Italy (Stranieri et al., 2021).

Eventually, the current study revealed that SARS-COV-2 couldn't be detected in the oropharyngeal or rectal swabs collected from household dogs living with COVID-19 infected owners (Table 1). These findings are in agreement with previous studies in France (Sailleau et al., 2020) and Spain (Ruiz-Arrondo et al., 2021). Although a high prevalence of SARS-COV-2 in oropharyngeal, nasal and rectal swab of dogs living in the same household with COVID-19 infected owners was previously documented in Hong Kong, China (Sit et al., 2020), Italy (Patterson et al., 2020), Wuhan, China (Chen et al., 2020), France (Fritz et al., 2021), and Brazil (Calvet et al., 2021).

The receptor binding domain (RBD), which lies in the spike (S) protein of SARS-CoV-2, has the affinity to bind to ACE2 receptors and allows SARS-CoV-2 to enter the respiratory mucosa in humans as well as different animal species (Lai et al., 2020; Shi et al., 2020). This probably indicates the possibility of SARS-CoV-2 transmission from humans to animals. Thus, the findings of the current study suggest that SARS-CoV-2 in pets was acquired from COVID-19 positive owners and this could be related to the identity and similarity between the total genome, nucleotides, and amino acids of S protein in animal's and human's SARS-CoV-2 (Salajegheh Tazerji et al., 2020).

The higher susceptibility of cats to SARS-CoV-2 than dogs and other mammalian species could be related to the high similarity between human and feline ACE2 receptors that may result in a higher binding affinity of SARS-CoV-2 RBD to feline ACE2 than canine ACE2 (Lan et al., 2020; Shang et al., 2020). Previous studies reported that cats are highly susceptible to SARS-CoV-2 than dogs (Bosco-Lauth et al., 2020; Patterson et al., 2020; Shi et al., 2020).

In the current study, the two cats positive for SARS-CoV-2 were symptomatic and suffered from severe anorexia. These cats belonged to single lonely cat owners and had very close contact with them during infection period. Therefore, COVID-19 infected owners should limit close contact with pets during illness. Previous studies reported the occurrence of clinical signs in all dogs and cats infected with SARS-CoV-2 (Calvet et al., 2021; Carlos et al., 2021; OIE, 2020; Sailleau et al., 2020). On the other hand, most of the naturally infected dogs and cats with SARS-CoV-2 were asymptomatic as previously reported (Fritz et al., 2021; Hamer et al., 2021; Sit et al., 2020).

Natural transmission of SARS-COV-2 circulating between humans and domestic cats was also reported in the northwest of Iran (Mohebali et al., 2022). One out of 124 cats infected with SARS-COV-2 was symptomatic and showed clinical signs including respiratory symptoms (cough, wheezing, cough, dyspnea, and runny nose), gastrointestinal symptoms (diarrhea and vomiting), and severe anorexia and cachexia. Finally, the infected owned cat died due to the severity of infection.

Previous studies revealed that experimentally infected cats with SARS-COV-2 could shed the virus nasally, orally, or rectally and potentially transmit the infection to other non-infected ones cohoused with them via aerosols or direct contact (Bosco-Lauth et al., 2020; Halfmann et al., 2020). However, dogs don't appear to shed the virus (Bosco-Lauth et al., 2020).

Epidemiological studies reported that SARS-CoV-2 could be transmitted from owners to their pets by hand-mouth, eye conjunctiva, or by touching the nose with hands contaminated by saliva or respiratory droplets (Chen, 2020). Kissing, petting, or hugging pet animals may facilitate the transmission (Leroy et al., 2020).

The small number of sampled pets was one of the limitations of this study; however, a large number of infected owners for 25 dogs and 30 cats had expressed their desire over the phone call for participation but unfortunately asymptomatic animals (which mostly reported during Omicron wave) were not referred and this may be because their owners had less concerns about the health status of their pets. On the other hand, we missed some cases during the Delta wave due to the severity of the disease and its complication in infected owners, which eliminated the chance of referring the case at the right sampling time.

Based on these limitations, we lose the chance of screening the asymptomatic cases that had close contact with their owners during the Omicron wave. Moreover, based on the literature reviews, comparison between pathogenicity of different SARS-CoV-2 variant in dogs and cats was not done in experimental studies till now and could be a topic of interest for further studies.

Most of the serological surveys done in dogs and cats during two years period of COVID-19 pandemic have been performed on the sera collected blindly from a large-scale population referred for routine diagnostics of pet cats and dogs for illness, wellness, or chronic disease and based on the literature reviews, dogs and cats tested positive from 11 to 51 days after the human index COVID-19 case onset of symptoms (Calvet et al., 2021; Dileepan et al., 2021).

In this study, the referred cases with confirmed owner SARS-CoV-2 infection were admitted at different times (as soon as 1–3 weeks after the owners' COVID-19 infection was confirmed by PCR) and due to the low probability of antibody response in the first days of exposure, the serological examination was not performed in this minor population.

In conclusion, the occurrence of SARS-CoV-2 in cats in this study during the circulation of new SARS-CoV-2 variants in Iran (Delta and Omicron) suggested that the virus was transmitted from COVID-19 positive owners to cats and these findings have potential implications for future zoonotic transmission that could threaten human health. Although there is no evidence that dogs or cats can transmit SARS-CoV-2 to humans, the usual precautionary measures should be urgently considered as part of global control and "One Health" approach. Pet owners are at higher risk and should follow hygienic measures during contact with their pets, and COVID-19 infected owners should limit close contact with pets during illness. Furthermore, continued investigations should be carried out to elucidate the pathway of SARS-CoV-2 transmission from pets-humans-pets, which is an important implication in the prevention of COVID-19 in humans in close contact with these animals.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

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All authors read and approved the final manuscript.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the manuscript.

ETHICS STATEMENT

All applicable international, national and institutional guidelines for the care and use of animals were followed. This study was approved by Research Ethics Committees of the Veterinary Faculty of the Shahid Bahonar, Kerman University (IR.UK.VETMED.REC.1401.003).

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