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Investigation of the presence of antibodies to SARS-CoV-2 in cats owned by people with COVID-19

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ABSTRACT

Objective: Coronaviruses are viral agents with zoonotic potential that can cause disease in many animal species. Recently a novel coronavirus agent excited pandemia in humans. It was named SARS-COV-2. In addition to causing pandemia, it was reported that the agent has been detected in pets. Although it has reduced, its effectiveness since the pandemic, it continues to exist without losing its importance due to its potential. In this study, we aimed to research the presence of SARS CoV 2 Ig G specific antibodies in domestic cats whose owners were diagnosed with COVID 19.

Materials and Methods: SARS-COV-2 specific Ig G antibodies were searched by receptor-binding domain-ELISA (RBD ELISA) method in 88 domestic cats whose owners were diagnosed at most 3 months ago and approved by The Ministry of Health of the Republic of Türkiye and have positive COVID-19 results.

Results: As a result of the study, the seroprevalence of COVID-19 in cats was 2.27%. In the literature reviews, it was seen that there were a limited number of studies in our country.

Conclusion: This study is the first article conducted in Central Anatolia. The results show that cats can carry the potential for anthropozoonotic potential.

Keywords: *COVID-19, Cat, ELISA, Seroprevalence, Antibody*

INTRODUCTION

In 2019, a novel coronavirus agent, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was discovered in human in China (Wu et al., 2020). This originally zoonotic virus caused a pandemic all over the world and it was named SARS-CoV 2 and sparked off COVID 19 pandemic (Zhou et al., 2020). The novel coronavirus disease (SARS-CoV-2, COVID-19) induced over 4.5 million death and over 220 million cases in humans until September 2021 and SARS-CoV-2 has cost social and economic impacts global extensity (Murphy and Ly, 2021). SARS-CoV-2 is a member of genus Betocoronavirus and has single stranded RNA, as an enveloped virus (Yılmaz et al., 2021). SARS-CoV-2 is closely in sight with severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle Eastern respiratory syndrome virus (MERS-CoV) (Murphy and Ly, 2021). Between 2002-2013 years the zoonotic severe acute respiratory syndrome-CoV (SARS-CoV) and the Middle East respiratory syndrome-CoV (MERS-CoV) caused significant outbreaks in humans and they are broadly determined (Bennett et al., 2019). SARS CoV 2, SARS CoV and MERS CoV are members of genus Betocoronavirus and also SARS CoV 2 has amino acid sequences at the rate of %80 like SARS CoV that caused the 2003 SARS

outbreak with almost %10 fatality rate. Although SARS-Cov 2 has the same nucleotide level in proportion to %96.2 with the coronavirus RaTG13-which was identified in Yunnan province, China, in 2013 at horseshoe bats (Zhou et al., 2020). SARS-CoV and MERS-CoV are thought to derive from bats and transmit through intermediate hosts, such as camels and civet cats to humans (Wan et al., 2020). For coronaviruses are thought to spread to humans through animal hosts but for SARS CoV 2 the potential intermediate host(s) are still unknown (Ye et al., 2020).

Coronaviruses (CoVs) are grouped in four different genera according to their genetic characters and evolutionary structures (Masters and Pearlman, 2013). Genera diversity is grouped under the headings of alpha, beta, gamma and delta (Stout et al., 2020). Coronaviruses have enveloped RNA with single stranded genome that with 4 structural proteins (spike S, envelope E, membrane M, nucleocapsid N), several accessory proteins and 16 non-structural proteins (nsp1-nsp16) are encoded (V'kovski et al., 2020). Coronaviruses can cause disease both animals and humans. In humans, coronavirus can cause variable disease manifestations, ranging from common cold lesions to acute respiratory disease. For animals, SARS-CoV 2 has been detected, especially in ferrets, dromedario camels, cats and dogs, in line with the studies conducted in the last five years. In the clinical findings caused by SARS CoV 2 in the aforementioned animals, are various; as follows animals can show upper and lower respiratory system infection findings and digestive system disorders (Shi and Hu, 2008; Schulz et al., 2021). At the same time SARS can be asymptomatic in animals (Barrs et al., 2020). Both SARS CoV and CoV 2 agents originate from bats. They can use palm civets/racoon dogs and dromedary camels as an intermediate host (Song et al., 2019).

It has been reported that infected people with Coronavirus transmit the agent to their pets (Fritz et al., 2021; Zhao et al., 2021). It is still unclear for animals how they play role as hosts for the SARS CoV 2 virus. The SARS CoV 2 agent can infect animals both naturally and experimentally (Rowe et al., 2004). Detection of the prevalence of the agent in terms of the potential for human-to-animal and animal-to-human transmission has an important place in disease control policies. For this reason, in this study, the presence of SARS CoV 2 IgG specific antibodies among domestic cats, whose owners were diagnosed with COVID 19 and contact with them, was investigated via quantitative ELISA method.

MATERIALS and METHODS

Study Population

The study involved cats admitted to Animal Hospital, Veterinary Faculty, Kırıkkale University for clinical examinations. In line with anamnesis, cats of both gender and any breed owned by people had COVID-19 in the last 3 months diagnosed using PCR by the Turkish Ministry of Health were included in the study. All cats were older than 6 months age and were household cats with no access to the outdoors. In total 88 cats met the criteria.

Sampling

Blood sampled were taken from vena cephalica antebrachiums into 2 ml serum tubes. Blood samples were centrifuged at 5000 rpm/10 min to obtain serum. Serum samples were stored at -20°C until analyses.

Detection of antibodies with ELISA

We used quantitative ELISA kits to detect Ig G antibodies against S1 antigen (inc. RBD) of SARS-CoV-2 (Anti-SARS-CoV-2 Quantivac ELISA Ig G, Germany) in the Laboratory of Infectious Diseases and Clinical Microbiology of Medical Faculty, Kırıkkale University. The assay was run in accordance with the recommendations of the manufacturer. The RBD-ELISA prove high sensitivity (100%) and specificity (98.23%) with the OD cut off value of 0.493. The test includes a 6-point calibration curve. According to the manufacturer's instructions, high results of relative units per milliliter were considered positive with up to a reader value of 0.337 optic density (OD). OD values above the cutoff value were taken as positive. Also, for the RU unit, results above 10 RU/ml were assessed as positive, and the binding antibody units per milliliter (BAU/ml) level was >3.2 BAU/ml.

Statistical analyses

Descriptive statistical analyses were made to determine the prevalence of COVID-19 in cats using SPSS Statistics 23.

Ethical statement

The study was approved by the Animal Experiments Local Ethics Committee of Kırıkkale University (Decision Number: 2021/04/20)

RESULTS

In total, 88 cats were evaluated with the RBD-ELISA. Two cat had positive results including all criteria (OD, RU/ml, BAU/ml) (Table 1). Positive cats OD levels were over 0.337 (0.389, 0.851 OD value). For RU levels, positive two cats had higher results than 10 RU/ml (11.79, 28.78 RU/ml). Also, these cats' BAU results were above positive criteria levels (>3.2) (37.74, 92.11 BAU/ml). SARS-CoV-2 seroprevalence was 3.41 % in cats in this study (Table 2).

Table 1. SARS-CoV-2 Ig G antibodies results with ELISA.

Sample	O.D. Value[1]	RU/ml Value[2]	BAU/ml Value[3]	Result
Cat 10	0.389*	11.79	37.74	Positive
Cat 68	0.851*	28.78	92.11	Positive

*Cause of positivity above cut off value OD (0.337)
[1]Optic Density, [2]Relative Unit, [3]Binding Antibody Unit

Table 2. Prevalance of COVID 19 results in cats.

Sample	Number	Ratio (%)
Negative	85	96.59
Positive	2	3.41
Total	88	100

DISCUSSION

It has been reported that COVID 19 can infect many animal species. SARS-CoV were experimentally isolated from masked palm civets, monkeys, mice, pigs and chickens, guinea pigs, golden syrian hamsters and ferrets (Hosie et al., 2021; Hossain et al., 2021). SARS-CoV prevalance was high in masked palm civets. Although these animals are consumed by humans, it is determined that animals develop neutralized antibodies (Wang et al., 2005; Sun et al., 2020). Palm civets were identified accidental hosts at temporary period (Kuiken et al., 2003; Wang et al., 2005) and it was thought that they can be occasional direct sources of human infections (Shi and Hu, 2008). Ferrets had quite high prevalance to SARS- CoV-2 after experimental inoculation and they could transmit the virus other ferrets (Shi et al., 2020). Dogs can be infected experimentally but they cannot spread the agents (Schlottau et al., 2020). There are several studies that were reported COVID 19, were detected cats whose owners had positive result with COVID 19 (Hossain et al., 2021). First SARS spread it was stated that natural infection was seen in cats (Rowe et al., 2004; He et al., 2013). In a study conducted in domestic cat in an apartment block where 100 people with SARS-CoV positivity, it was reported the agent was found in eight cats. However, it is stated that there are deficiencies in the direction of this transmission from humans to animals (Martina et al., 2003). In studies that in experimentally and natural infected cats, it was notified that human can be reservoir for animal infections (Hossain et al., 2021). Cat-human transmission can possible theorically but there is not enough evidence to demonstrate this situation (Barrs et al., 2020). In this study 3 cats were positive and the both of them lived same house. In line with this information, we thought that the positive cases in our study contributed to literature in terms of contamination.

Within the scope of One Health, determining the natural and intermediate hosts of COVID 19 plays a key role in identifying the spread potential of the COVID 19 and establishing control policies. It is still unclear where cats are as a host and what role they play in case of transmission (Leroy et al., 2020). Bessière et al. (2021) examined five cats whose owners had COVID 19 positive results. They isolated the viral agent in 2 cats and it was seen that 1 cat had COVID 19 antibody (Bessière et al., 2021). Similarly, Hosie et al. (2021), researched 2 COVID 19 positive cats thought to be transmitted from humans to cats, reported that it is not clear what role cats play in the epidemiology of COVID 19, but they emphasized the need to clarify this point in the one health concept (Hosie et al., 2021). In this study, the presence of COVID 19 antibodies was determined in cats in contact with humans. It is recommended that more comprehensive studies are required, especially in terms of the anthropozoonotic feature of COVID 19, as well as its epidemiological importance.

There are several studies reported that serological surveys of COVID-19 pet populations rates ranging from 0% - 15.8% in cats and dogs (Chen et al., 2020; Deng et al., 2020). In Europe, canine coronavirus prevalence is lower than feline coronavirus (Stavisky et al., 2010). Feline coronavirus prevalence is 50% in healthy Swiss cats and 37% in Japan (Taharaguchi et al., 2012). Barrs et al. (2020) stated that the prevalence of SARS-CoV-2 was 12 % of 50 cats in Hong Kong at in their study (Barrs et al., 2020). Zhang et al. (2020) reported that, 15 of 102 (14.7%) cats were positive about SARS-CoV-2 for positive antibodies that recognized by ELISA (Zhang et al., 2020). During the first COVID-19

wave in Europe, a study was organized on identifying SARS-CoV-2 specific antibodies in domestic cats, and in this study SARS-CoV-2 seroprevalence was found 4,2 % in Germany. Also, the ratio was about COVID-19 announced 3.3% in the UK, 4.2% in Italy and 6.4% in Spain (Schulz et al., 2021). There are different epidemiological studies have been conducted that have taken samples on routine veterinary checks, where the dispersion of COVID-19 was not known at people. A study of 919 companion animals, 5.8% of cats was shown that had neutralizing antibodies (Patterson et al., 2020). Along these lines, in another study demonstrated that only 0.69% of samples contained antibodies at Germany (Michelitsch et al., 2020). Furthermore, in the study which was realized in 20 veterinary community students, 18 of them displayed COVID-19 symptoms and 2 students had positive COVID-19 results, was reported that none of these students' cats and dogs developed antibodies (Wu et al., 2020). In this study we found the prevalence of COVID-19 ratio as 3.41 % in 88 cats. We took to the study only the cats which had had COVID-19 positive results with their owner in the last three months. Our results are close to the data at Europe. This closeness in the results was considered significant in terms of the similarity of our living conditions, cats' similar environmental status, nutritional habits and routine veterinary practices, geographical proximity and the application of similar control policies during the COVID-19 pandemic.

CONCLUSION

As a result, the transmission from human to animals and animals to humans have not been clarified in the spread and control policies of COVID 19 which still continues to exist. Determining how the cats play a role in transmission remains an important point in controlling COVID 19. Certainly, we believe that studies on the presence of COVID 19 in cats, increasing the data reservoir on the spread and transmission potential of the disease will help identify what role cats play in COVID 19. In addition, we thought that, we contribute veterinarians by answering the question of what kind of path they should follow for controlling COVID 19 both human and animal care by informing patient owners.

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