

Detection of SARS-CoV-2 antibodies in a pet dogs and cats living in COVID-19-positive households in Iraq

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Abstract

The goal of this study was to assess SARS-CoV-2 seroprevalence in pet dogs and cats during the coronavirus disease (COVID-19) pandemic in Iraq. Serum samples were collected from 180 animals, including 96 dogs and 84 cats. All animals included in this study were owned animals from a household with at least one COVID-19 patient. Serum samples from dogs and cats were screened for antibodies against SARS-CoV-2 using the commercial ELISA assay (ID Screen® SARS-CoV-2 Double Antigen Multi-species) according to the manufacturer's instructions. The seroprevalences for SARS-CoV-2 was 7.3% for dogs and 8.3% for cats but this difference was not significant. Also, no significant different in seropositivity between age groups, sex and breeds of pet animals. All positive animals did not show any clinical signs when contact with human SARS-CoV2 positive cases, suggesting that dogs and cats are likely asymptomatic carriers of SARS-CoV-2.

Keywords: SARS-CoV-2, dogs, cats, ELISA.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged in Wuhan, China, in December 2019 and caused a severe pandemic of coronavirus disease (COVID-19) (1,2), rapidly became a serious threat to human health worldwide (1,3,4). SARS-CoV-2 belongs to the *Betacoronavirus* genera in the subfamily *Orthocoronavirinae* of the family

Coronaviridae, in which SARS-CoV-1 and MERS-CoV are also in this group. SARS-CoV-2 is a zoonotic virus and natural infections by SARS-CoV-2 have been reported in pet dogs, cats, zoo tigers, and lions that show only mild respiratory signs (5-7). Almost all positive pets or captured animals shared households or have been in close contact with infected individuals (8,9). As companion animals are the potential sources and sentinels of a wide range of

infectious diseases, the determination of their susceptibility to and prevalence for natural SARS-CoV-2 infections has significant impacts on both animal and human health (10-12). World Organization for Animal Health defined COVID-19 as an emerging disease in animals and began promoting surveys on the prevalence of SARS-CoV-2 infections among animals (13). Few studies have been conducted to clarify the effects domestic animals have in sustaining the SARS-CoV-2 transmission cycle (5,14-16). As infections in animals can easily go unnoticed because they do not complain of fever, sore throat, etc., infections in the acute stage may be missed. Therefore, antibody detection is an excellent way of confirming the disease even after the animal has recovered from the illness. The optimal serological test would be one that can detect the SARS-CoV-2 antibody from different kinds of animal species. Serologic screening of SARS-CoV-2-specific antibodies in cats and dogs are needed for insights into the prevalence of this infection and possible modes of transmission. The goal of this study was to assess SARS-CoV-2 seroprevalence in pet dogs and cats during the coronavirus disease (COVID-19) pandemic in Iraq.

Materials and methods

Animal Serum Samples: Sampling from January to June 2022, a total of 180 animals, including 96 dogs and 84 cats. Convenience sampling was performed at private

veterinary clinics located in Baghdad and Basrah. The blood samples were collected via forelimb venipuncture, and sera were separated and stored at -20°C until further detection. Informed consent for the publication of their clinical details was obtained from the owners. All animals included in this study were owned animals from a household with at least one COVID-19 patient.

Antibody (IgM and IgG) detection by Enzyme-linked immunosorbent assay (ELISA): Serum samples from dogs and cats were screened for antibodies against SARS-CoV-2 using the commercial ELISA assay (ID Screen® SARS-CoV-2 Double Antigen Multi-species, IDVet, rue Louis Pasteur, Grabels, France). The ELISA was performed according to the manufacturer's instructions with an S/P % (sample to positive ratio) over 60% regarded as positive. Ratios from 50% to 60% were considered as doubtful, and those below 50% as negative.

Results

SARS-CoV-2 Seroprevalence in Domestic Dogs and Cats: A total of 14 (7.8%) of 180 serum samples from 96 dogs and 84 cats were positive by ELISA for SARS-CoV-2. Neutralizing antibodies were confirmed in 7/96 (7.3%) dogs and 7/84 (8.3%) cats (Figure 3.1). No significant difference in the prevalence of neutralizing antibodies between dogs and cats was recorded in this study (Table 1). All positive animals did not show any clinical signs when contact with a human SARS-CoV2 positive case.

Table 1 Serologic results for animal samples tested in ELISA kit to detect antibodies against nucleoprotein of SARS-CoV-2 virus.

Animal	Number of samples tested	Positive	Seroprevalence (%)	P value
Dog	96	7	7.3	0.7
Cat	84	7	8.3	
Total	180	14	7.8	

P > 0.05

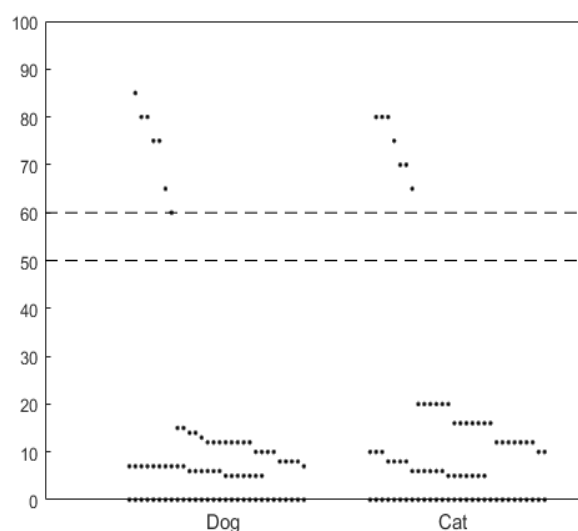


Figure 3.1. Distribution of sample to SARS-CoV-2 serology results among dogs and cats (Positive = ≥ 60 , Doubtful = $>50 - < 60$, negative = ≤ 50)

Discussion

Because SARS-CoV-2 can infect cats and dogs, virus might spread in this population and animals might act as a reservoir with the possibility of animal-to-human transmission. Although so far, the pandemic has been driven by human-to-human transmission, it

is useful to know whether domestic animals can play a role in the maintenance and spread of SARS-CoV-2 infections. For these studies, verified serologic assays that detect virus-specific antibody responses in cats and dogs are needed. In our study of samples from domestic animals with known SARS-

CoV-2 exposure, we determined seroprevalences for SARS-CoV-2 of 7.3% for dogs and 8.3% for cats. It has been demonstrated that multiple animal species, including dogs and cats, are susceptible to SARS-CoV-2 infection under experimental conditions or natural settings (12). However, little is known about the prevalence of SARS-CoV-2 infection in companion animals in households. An early study in Wuhan, China, after the COVID-19 outbreak showed a 15% seroprevalence of SARS-CoV-2 IgG in cats (17). A large-scale study conducted on 919 companion animals in Italy found 3.3% of dogs and 5.8% of cats with measurable SARS-CoV-2 Abs but no animals tested PCR positive¹⁸. The direct human-to-cat transmission was identified in 6 out of 50 cats from COVID-19 households or close contacts in Hong Kong (19). In experimental conditions, cats are more susceptible to SARS-CoV-2 infection (14) and develop much higher neutralizing antibody titer than dogs (20). Despite these data, no significant difference in the prevalence of neutralizing antibodies between cat and dog population was recorded in this study. Also, there is no significant difference in seropositivity between age groups, sex and breeds of pet animals. All positive animals did not show

any clinical signs when contact with human SARS-CoV2 positive case, suggesting that dogs and cats are likely asymptomatic carriers of SARS-CoV-2, as has previously been reported (21,22). Similarly, experimental studies have shown no clinical signs in any of the inoculated cats (19,23). Moreover, other authors have described cases of natural infection that never showed clinical signs compatible with SARS-CoV-2 infection (20,24). Thus, infected cats with no symptoms could act as a silent intermediate host of SARS-CoV-2. However, in a case of natural infection reported in Belgium, the cat presented clinical signs, such as lethargy, anorexia, loss of appetite, vomiting, and diarrhea, and later showed sneezing, productive cough, difficulty breathing, and emaciation (25). Concerning the infection of dogs with SARS-CoV-2, two cases have been reported in Hong Kong in February and March 2020 (5). In both cases, dogs were placed under quarantine after their owner was hospitalized due to COVID-19. Samples taken from the dogs were tested positive for SARS-CoV-2 by RT-PCR and serological analysis. Both animals did not exhibit any specific clinical signs. An experimental infection that dogs have low susceptibility to SARS-CoV-2 (14). In summary, our study

provides the first results of serological tests of pet cats and dogs in Iraq during the COVID-19 pandemic. These results justify the need to adopt control measures in SARS-CoV-2–infected pet owners to reduce viral transmission to their companion animals. COVID-19 rapidly crossed the international borders to involve almost all world countries Iraq was not exceptional (26, 27, 28,29,30).

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الكشف عن الأجسام المضادة لـ SARS-CoV-2 في الكلاب والقطط الأليفة التي تعيش في منازل فيها إصابات بـ COVID-19 في العراق

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الخلاصة

كان الهدف من هذه الدراسة هو تقييم الانتشار المصلي لـ SARS-CoV-2 في الكلاب والقطط الأليفة أثناء جائحة فيروس كورونا (COVID-19) في العراق. تم جمع 180 عينة مصل الدم منهم 96 كلباً و 84 قطّة. كانت جميع الحيوانات المشمولة في هذه الدراسة حيوانات مملوكة لأسرة بها مريض واحد على الأقل من COVID-19. تم فحص عينات المصل من الكلاب والقطط بحثاً عن الأجسام المضادة ضد SARS-CoV-2 باستخدام اختبار ELISA التجارية (SARS-CoV-2 ID Screen® 2 Double Antigen Multi-types) وفقاً لتعليمات الشركة المصنعة. كان معدل الانتشار المصلي لـ SARS-CoV-2 7.3% للكلاب و 8.3% للقطط ولكن هذا الاختلاف لم يكن معنوياً. أيضاً، لا يوجد اختلاف معنوي في الإيجابية المصلية بين الفئات العمرية والجنس وسلالات الحيوانات الأليفة. لم تظهر جميع الحيوانات الموجبة أي علامات سريرية عند التماس بحالة إصابة بشرية بفيروس SARS-CoV-2، مما يشير أن الكلاب والقطط من المحتمل أن تكون ناقلة لفيروس SARS-CoV-2.