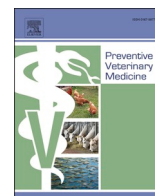




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Frequency of SARS-CoV-2 infection in dogs and cats: Results of a retrospective serological survey in Šumadija District, Serbia

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ABSTRACT

It has long been known that coronaviruses cause various infectious diseases in animals. Although SARS-CoV-2 is genetically related to viruses isolated from *Rhinolophus* bats, the exact origin, mode of transmission, and how the human species has become the epidemiological reservoir of the virus have not yet been established with certainty. Although the main route of transmission is human-to-human, there are considerable numbers of reported cases of infection in animal species, predominantly among pet animals. The aim of this retrospective study was to assess SARS-CoV-2 seropositivity in dogs and cats during the COVID-19 pandemic in Šumadija District, Serbia. We used serology to identify household contacts of pet animals with infected pet owners and the degree of association. The study presented in this paper is also the first study of this type in Serbia. The results of a retrospective serosurvey, which was conducted in dogs and cats with different exposure risk factors, were analyzed to find the possible modes of transmission between humans and animals. The relative frequency of SARS-CoV-2 infection in dogs was 1.45% bounded with a 95% confidence interval (CI) of 0.0007–7.73%, while in cats, it was 5.56% (95% CI: 0.77–4.13%). The relative frequency of SARS-CoV-2 infection in pet owners was 11% (95% CI: 6.25–18.63%). In pets that were in close contact with COVID-19 positive owners, the seropositivity was found to be 9%. Out of a total of five stray dogs and cats tested, seropositivity was observed in two animals. Detected SARS-CoV-2 infection in pets shows that these animals are susceptible to infection and that the most common means of virus transmission to pets is through contact with diseased owners. However, the presence of infection in stray dogs and cats is not clear and needs further research.

1. Introduction

Severe Acute Respiratory Syndrome Virus 2 (SARS-CoV-2) was first detected in Wuhan, China at the end of 2019 and spread around the world very quickly (Zhou et al., 2020). The current view is that SARS-CoV-2 originated from an animal source and then spilled over into the human population (Zhou et al., 2020). However, the exact origin and mode of pathogen spillover to the human population have not yet been established with certainty (Andersen et al. (2020); World Organization for Animal Health (Anon, 2021). Although the main route of transmission of the virus is human-to-human, there are considerable numbers

of reported cases of SARS-CoV-2 infection in animals, predominantly in dogs and cats (Anon, 2021; Ruiz-Arrondo et al., 2021; Decaro et al., 2021; Prince et al., 2021; Bosco-Lauth et al., 2020). In almost all confirmed cases of pet infection, companion animals were in close contact with infected people (Oreshkova et al., 2020) or were exposed to a contaminated environment (Barrs et al., 2020; Sit et al., 2020; Dias et al., 2021). However, there are reported cases of SARS-CoV-2 transmission from farmed mink to in-contact humans and companion animals (Oude Munnink et al., 2021; Van Aart et al., 2021) as well as detected cases of infections in stray dogs and cats where transmission most likely occurred through contact between animals (Villanueva-Saz et al., 2021;

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Dias et al., 2021; Barrs et al., 2020). Among stray animals, three main routes of infection are described, i.e., contact with infected animals, contact with infected humans, and exposure to a contaminated environment (Dias et al., 2021; Abrahão et al., 2021; Prado et al., 2020; Sit et al., 2020). In Brazil, significant contamination of public surfaces and the sewage system with viral RNA originating from SARS-CoV-2 has been demonstrated, pointing out that these environments can be a potential source of infection (Abrahão et al., 2021; Prado et al., 2020).

At this time, there are no available data that would unequivocally indicate the transmission of the virus from pets to humans. The risk of transmission from these animals to humans is low (Bosco-Lauth et al. (2020); European Centre for Disease Prevention and Control (ECDC, 2021; Anon, 2019, 2022a, 2021). The aims of this paper are to assess SARS-CoV-2 seropositivity in dogs and cats during the COVID-19 pandemic in the district of Šumadija, Serbia, and to compare the results of a serological survey with the findings of epidemiological surveillance of pet owners. The results of a retrospective serosurvey that was conducted in dogs and cats with different exposure risk factors were analyzed to find the possible modes of transmission between humans and animals.

2. Materials and methods

2.1. Samples

The sera used in this study were from dogs ($n = 69$) and cats ($n = 36$) that required treatment in a veterinary clinic. All sera from dogs and cats were sampled by veterinarians during health care visits for various reasons in the Kragujevac Veterinary Clinic between the 10th of June 2020 and the 18th of December 2021 (Fig. 1), and the sera were tested in the microbiological laboratory at the Institute of Public Health in Kragujevac. The samples were accompanied by a set of anamnestic data, i. e., species, breed, gender, age, date of sampling, veterinary practice, and date received. All pet owners filled out a questionnaire regarding their demographic and epidemiological information, including data on SARS-CoV-2 infection, pet species, pet's living environment (indoors or outdoors), possible exposure to COVID-19, clinical symptoms, and the reason for visiting the clinic. The survey was conducted after obtaining the consent of the pet owners.

According to the possible risk of virus transmission, pets were first classified into two groups. Group A ($n = 59$) consisted of companion animals kept exclusively indoors, while group B ($n = 41$) consisted of animals kept outdoors. Within these strata, animals were further divided according to the type of animals examined. A total of 22 indoor cats and 10 outdoor cats were examined. Also, 37 blood sera of indoor dogs and 31 sera of outside dogs were examined. Within these groups, pets were then subdivided according to the risk of exposure to the SARS-CoV-2

virus. Those considered to be at particularly increased risk of infection were labeled A_{exposed} , as these were pets whose owners had confirmed SARS-CoV-2 infection in the last six months and were in constant contact with their pets (animals kept exclusively indoors). Pets that were kept outdoors (and were in contact with owners who had COVID-19 in the last six months before blood sampling) were labeled B_{exposed} . A total of 11 pet sera that met these criteria were examined, six in the A_{exposed} subgroup and five in the B_{exposed} subgroup (see Supplementary Material Table 6).

2.2. SARS-CoV-2 double antigen enzyme-linked immunosorbent assay (ELISA)

Dog and cat serum samples were screened for SARS-CoV-2 specific antibodies by commercial indirect ELISA (ID Screen® SARS-CoV-2 Double Antigen Multi-species IDVet, Grabels, France), intended to detect nucleocapsid-binding antibodies of SARS-CoV-2 in the sera of various species. The assay was performed according to the manufacturer's instructions.

2.3. Statistical analysis

The analyses were performed using the statistical software packages XLSTAT Version 2014.5.03., IBM SPSS Version 26.0.0.0. and OpenEpi Version 3, (https://www.openepi.com/Menu/OE_Menu.htm). Confidence intervals were calculated with the Wilson score method. Fisher's exact test and Mid- P exact were used to determine the significance of nominal data. The strength of the association between the variables was assessed using odds ratios (OR) with a 95% confidence interval (CI). P -values < 0.05 were considered significant. In multiple comparisons, to avoid spurious P -values, the Bonferroni post hoc procedure was applied. Binary logistic regression (BLR) analysis was used to model the relationship between predictors and dependent variables, i.e., species exposed to the virus (humans, dogs, and cats) and SARS-CoV-2 infection (for more information see Supplementary material).

3. Results

Out of 105 samples, three sera were positive, one was doubtful, and 101 were negative. Out of the total of three positive sera, two were of feline and one was of canine origin. In the group of tested stray dogs and cats ($n = 5$), two positive findings were obtained.

The relative frequency of SARS-CoV-2 infection in dogs was 1.45% (95% CI: 0.0007–7.73%), while in cats, it was 5.56% (95% CI: 0.77–4.13%). The frequency of SARS-CoV-2 infection in pet owners was 11% bounded with a 95% CI of 6.25–18.63% (Table 1). There was significant variation between relative frequencies of SARS-CoV-2 infection

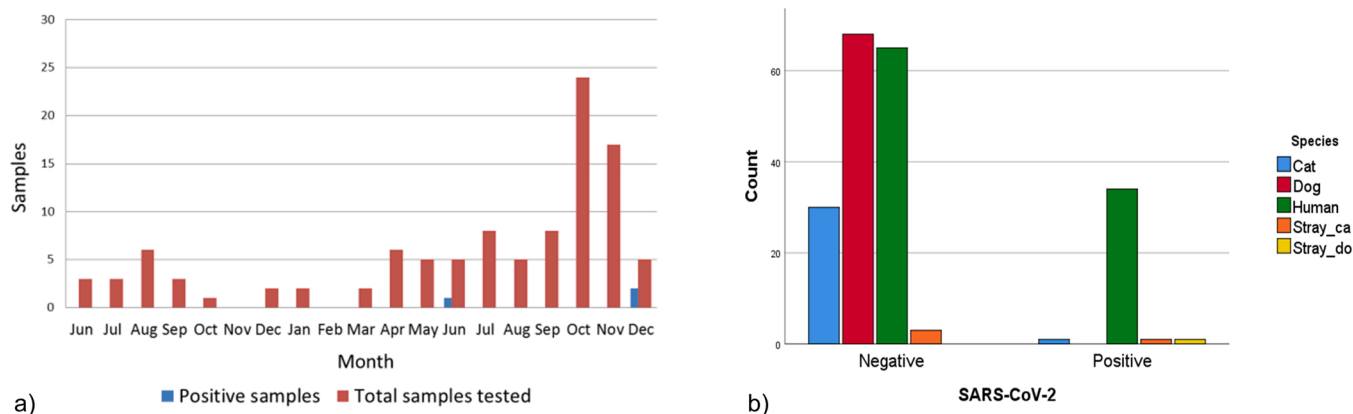


Fig. 1. Distribution of sera examined during the study. a) Monthly distribution of dog and cat sera tested in 2020 and 2021. b) Distribution by species of pets and pet owners that tested positive or negative to SARS-CoV-2 infection.

Table 1

Frequency of SARS-CoV-2 infection in pet owners and animals tested and statistical significance of between-species differences (Fisher's exact test).

Disease status/species	Pet owners	Cats	Dogs	Fisher's exact test (P)
D(+)	11	2	1	0.0398
D(-)	89	34	68	
Total samples	100	36	69	
Relative frequency	11.00% (95CI: 6.25–18.63%)	5.56% (95CI: 1.54–18.14%)	1.45% (95CI: 0.0007–7.73%)	

D(+) - disease or SARS-CoV-2 infection confirmed by PCR (humans) or ELISA test (animals); D(-) - infection not present or negative ELISA test (animals)

among cats and dogs and pet owners (Fisher's exact test: $P = 0.0398$). Bonferroni's post hoc test confirmed that seropositivity in cats did not differ significantly from that observed in pet owners (Mid- P exact = 0.3702) and dogs (Mid- P exact = 0.3081). However, this was not the case with dogs compared to pet owners. The seropositivity in dogs was significantly lower than the frequency of SARS-CoV-2 infection in pet owners (Mid- P exact = 0.0156). The Bonferroni adjusted critical P -value was set as 0.017 (see [Supplementary Material Table 1–3](#)).

BLR analysis proved a negative association between animal species and the registered number of SARS-CoV-2 positive animals compared to humans as a reference category. The regression coefficients for the model predictor variables were negative and significant, indicating that dogs and cats are less susceptible to SARS-CoV-2 infection than humans. The regression coefficient for the cats and dogs was $B_{\text{cat}} = -2.155$ (S.E. = 0.758; $P = 0.004$) and $B_{\text{dog}} = -3.571$ (S.E. = 1.029; $P = 0.001$), respectively. Regarding OR, an exponential value of B_{cat} of 0.116 indicates that cats are 8.62 times less likely to be infected with SARS-CoV-2 than humans, while an exponential value of B_{dog} of 0.028 shows that dogs are 35.7 less likely to be infected with SARS-CoV-2 than humans ([Table 2](#); [Supplementary Material Table 9](#)).

In the group of dogs and cats ($n = 68$), stratified according to pets that were mostly kept indoors (A) and those that were kept outdoors (B) (n), one seropositive case was registered in the stratum of pets kept indoors (see [Supplementary Material Table 5](#)). In the group of dogs and cats ($n = 11$) belonging to the stratum of animals that were kept indoors, and close to their owners whose infection had been confirmed in the last six months after blood sampling, the presence of specific antibodies was proven in a cat that was in direct contact with the infected owner and permanently kept indoors (see [Supplementary Material Tables 6 and 7](#)).

4. Discussion

Studies on SARS-CoV-2 infection in pets show that these animals are susceptible to infection and that the most common means of virus transmission is contact with owners with proven COVID-19 ([Prince et al., 2021](#)). Cases of infection of pets have been registered in many countries ([Ruiz-Arondo et al., 2021](#); [Barrs et al., 2020](#); [Michelitsch et al., 2020](#); [Smith et al., 2021](#); [Pomorska-Mól et al., 2021](#)). In our study, by comparing the SARS-CoV-2 seropositivity in different pet species and confirmed COVID-19 cases of their owners, we found that these differences are statistically significant. Based on BLR analysis, it was found that pets are significantly less susceptible to SARS-CoV-2 infection than their owners. We found that 28.9% of differences in the number of registered human and animal cases can be explained by the type of species exposed to the virus (Nagelkerke R square, $R = 0.289$). The study

Table 2

The results of the regression analysis (predicted variable: SARS-CoV-2 infection; explanatory variable: species).

	B	S.E.	Wald	Sig.	Exp (B)	95% C.I. for Exp (B)	
						Lower	Upper
Human			19.048	0.000			
Dogs	-2.155	0.758	8.078	0.004	0.116	0.026	0.512
Cats	-3.571	1.029	12.039	0.001	0.028	0.004	0.211
Constant	-0.648	0.212	9.374	0.002	0.523		

of seropositivity showed a small proportion of companion animals in Serbia tested positive for SARS-CoV-2 specific antibodies. None of the tested animals showed clinical symptoms of infection with the SARS-CoV-2 virus. Compared to the pet owners, a lower percentage of SARS-CoV-2 infection in pets, especially in dogs, was observed. In comparison in a similar study conducted in Germany, a lower percentage of seropositive cats has been reported (0.69%) ([Michelitsch et al., 2020](#)) than in our study. However, this German study was conducted at the beginning of the pandemic, which could be the reason for the low percentage of infected cats. On the other hand, in a study conducted in Wuhan, China, 14.7% of cats tested from January to March 2020 were positive ([Zhang et al., 2020](#)), while in France the seroprevalence was much higher, being 23.5% in cats and 15.4% in dogs ([Fritz et al., 2021](#)). In our study, the seropositivity in pets that were in close contact with owners with COVID-19 was a slightly lower frequency (9% of the examined pets were seropositive). In a similar study in Italy, a significantly higher seropositivity was found in dogs living with COVID-19-positive pet owners, compared to that of dogs living with owners without confirmed SARS-CoV-2 infection. They found a prevalence of 12.8% in exposed dogs and 1.53% in the unexposed group. Opposite to findings in dogs, the difference in prevalence between exposed and unexposed cats was not statistically significant (4.5% vs. 2.6%) ([Patterson et al., 2020](#)). Unlike the Italian study, in our study, cats that were in close contact with people suffering from COVID-19 most often became infected (more so than dogs). However, our observations coincide with the findings of authors from the United States, where the average seroprevalence in pet cats and dogs was 8% and 3%, respectively ([Dileepan et al., 2021](#)).

In our study, out of a total of five sera of stray dogs and cats tested, seropositivity was observed in two animals. In Serbia, there is a significant number of stray dogs and cats, especially in large cities. It is possible that these animals during their food search come into contact with discarded protective masks that were widely used during the epidemic and most often ended up together with communal waste in containers for waste disposal, but it is not possible to exclude any other transmission means, i.e., contact with infected people or animals. The results obtained support the assumption of significant infection in stray dogs and cats and are in line with findings in other countries ([Dias et al., 2021](#); [Farnia et al., 2020](#); [Villanueva-Saz et al., 2021](#); [Zhang et al., 2020](#); [Shi et al., 2020](#)). The observed differences that appear between some studies may be explained by the age of tested animals or by the characteristics of investigated populations, i.e., free-range animals and animals originating from animal shelters. These differences significantly determine the nature and frequency of contacts.

The epidemiological results of our study indicate that the human-to-companion animal transmission of SARS-CoV-2 probably occurs. This study gives no evidence that companion animals might serve as a significant source of infection for people. Although transmission among animals does not pose a major epidemiological risk to humans at this time, considering the mutation rate of SARS-CoV-2 and the ability to adapt to new hosts, it is important to remain vigilant and monitor the infection in animals, especially among companion animals, and to monitor and evaluate the potential and extent of the zoonotic capacity of the virus and potential reverse zoonosis of SARS-CoV-2 in the future. We note the current omicron strain of SARS-CoV-2 often gives a milder clinical picture without the need for medical care, while existing

serological tests in humans do not differentiate vaccine-induced antibodies from those resulting from natural infection. Under these conditions, monitoring of SARS-CoV-2 infection in pets could be useful as an additional instrument for monitoring SARS-CoV-2 circulation in the human population.

5. Conclusions

SARS-CoV-2 infection in pets shows that these animals are susceptible to infection and that the most common means of infecting them is contact with owners diagnosed with COVID-19. However, the presence of infection in stray animals is not clear and further research is needed to understand the mode of transmission to these animals.

Companion animals that are in close contact (indoor) with infected owners are at a higher risk of becoming infected than other, unexposed animals. There is no evidence of recurrent infection from companion animals to humans.

The results show that cats are more susceptible to SARS-CoV-2 infection than dogs. This study gives no evidence that companion animals might serve as a significant source of infection for humans. However, the monitoring of SARS-CoV-2 infection in pets could be useful as an additional instrument for monitoring SARS-CoV-2 circulation in the human population.

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Conflict of interests

The authors of this research paper have no financial or personal interests that could have influenced this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.prevetmed.2022.105755.

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