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Detection of neutralizing antibodies against SARS-CoV-2 in companion animals in Istanbul

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ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in late 2019 and rapidly spread throughout the world. After the susceptibility of various animals to SARS-CoV-2 infection was reported, the prevalence of COVID-19 infection in pet animals and their role in virus transmission became a source of concern. Seroprevalence of SARS-CoV-2 in pet animals has been reported from various parts of the world to date. In this study, 206 serum samples of household dogs and cats were obtained from a diagnostic laboratory in Istanbul. Ninety-nine of the samples were collected during the second wave (November and December 2020) of the pandemic, and 107 were collected between August and September 2021. Neutralizing antibodies against SARS-CoV-2 in companion animals was investigated by virus neutralization test. Among the 99 serum samples which were collected during the second wave (November and December 2020) of the pandemic, while no neutralizing antibodies were found in 61 sera of cats, one of 38 dogs was positive with a titer of 1/256. Similarly, 0/54 cats and 1/53 dogs were tested positive for the neutralizing antibodies among the samples collected between August and September 2021. This study is demonstrating the evidence of SARS-CoV-2 infection in dogs.

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Introduction

SARS-CoV-2 emerged in Wuhan, China in December 2019 (Zhu et al., 2020) and was defined as a pandemic by the World Health Organisation (WHO) in March 2020. During the rapid spread of SARS-CoV-2 to the entire world via person-to-person transmission, the susceptibility of animals and their role in transmission dynamics have arisen as a concern. The first COVID-19 report in animals was from Hong Kong, viral RNA was detected from oral and nasal swabs of a pomeranian dog with a history of a COVID-19 positive owner in February 2020 (Sit et al., 2020). According to the WOAH Situation Report (31.08.2022), 692 SARS-CoV-2 outbreaks in animals have been reported, affecting 25 species, including animals from zoos (Puma, Lion, Tiger, Gorilla, Ottor, Binturong, Fishingcat, and Coatimundi), pets (dogs, cats, ferrets), wild animals (white-tailed deer), and farm animals (Minks) (WOAH, 2022). Generally, infected companion animals had a

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history of a COVID-19 affected owner (Hamer et al., 2020; Segalés et al., 2020; Sit et al., 2020; Neira et al., 2021; Ruiz-Arrondo et al., 2021). The transmission of the virus from human-to-animal occurs as a result of the close contact between infected people and household animals. Recently, evidence of cat-to-human transmission reported that a pet cat infected a veterinary surgeon in Thailand (Sila et al., 2022) and mink-to-human transmission reported from the Netherlands (Munnink et al., 2021) after mink farms were infected with SARS-CoV-2. Transmission of animal-to-human cases indicate the importance of monitoring the SARS-CoV-2 infections in animals.

With the increasing number of reports stating that the SARS-CoV-2 RNA is detected by molecular methods in pet animals from various countries, further studies were conducted in which serological tests were performed to determine the prevalence of the disease in pet animals. Studies from China (Zhang et al., 2020), Croatia (Stevanovic et al., 2021), Italy (Patterson et al., 2020), Netherlands (Zhao et al., 2021), Germany (Michelitsch et al., 2020, 2021), United Kingdom (Smith et al., 2021), France (Laidoudi et al., 2021) Brazil (Calvet et al., 2021) and several countries (Fritz et al., 2020; Barua et al., 2021; Schulz et al., 2021; Yilmaz et al., 2021) have shown that pet animals develop antibodies against SARS-CoV-2.

In this study, the presence of neutralizing antibodies was investigated in serum samples of cats and dogs which were collected in the second wave of the pandemic and during the dominance of variants.

Materials and Methods

Samples: In this study, 206 sera (91 dogs and 115 cats) were tested, which were provided from a veterinary diagnostic laboratory, where the samples were sent from veterinary clinics for hematological tests for other reasons under the cold chain. Samples were provided in two apart periods. The first group consisted of samples from 61 cats and 38 dogs, which were collected in November and December 2020. As the second group, 54 cat sera and 53 dog sera were collected in August and September 2021. Bio-information of animals was not available in both groups.

Test Virus: hCoV-19/Turkey/Pen07/2020 strain (GISAID Access. ID: EPI_ISL_491476) which was isolated in Pendik Veterinary Control Institute from an oropharyngeal swab of a SARS-CoV-2 patient before (not published), were used in neutralization test.

VNT: Serum samples were heat-inactivated at 56°C for 30 min. The tests were performed in Vero cell culture (3×10^5 cell/ml) grown in 96-well microplates. Serial two-fold dilutions of serum were mixed with the same volume of virus suspensions containing 100 TCID50 virus and incubated at 37°C for 1h. The serum and virus suspension inoculated to four wells of Vero cells and incubated at 37°C for 3 days. Neutralizing antibody titers were recorded as the reciprocal of the highest serum dilution that inhibited 50% of the cytopathic effect per well. With a titer of ≥ 16 samples were considered positive (Zhao et al., 2021). The tests were performed in BSL-3 laboratories of Pendik Veterinary Control Institute.

Statistical Analysis: The number of seropositive animals among samples collected between two separate periods were tested for significant using chi-square test and values of $P \leq 0.05$ were considered significant.

Results

The first cohort was including 99 sera samples collected during the second wave (November and December 2020) of the pandemic. Among these, one serum sample from a dog out of 99 pet animals tested positive for neutralizing antibodies with a titer of 1/256. Neutralizing antibodies were not detected from 61 cat sera, while 1/38 dog sera were positive.

The second cohort consisted of 107 sera (54 cats, 53 dogs), collected in August and September 2021, in which one dog sera was positive with a titer of 1/32. None of the 54 cat sera gave positive results in VNT.

No significant difference detected between the two periods of pandemic in number of seropositive animals ($P>0.05$).

Discussion

SARS-CoV-2 emerged from China and spread to the whole world rapidly. During the spread of the disease via person-to-person transmission, studies have been carried out on the susceptibility of animals to SARS-CoV-2 due to the possibility of inter-species transmission. Experimental infection studies showed that cats, ferrets, dogs, white-tailed deer, golden hamsters, and fruit bats are susceptible to SARS-CoV-2 (Schlottau et al., 2020; Shi et al., 2020; Sia et al., 2020; Palmer et al., 2021). The experimental study on cattle demonstrated that cattle showed low susceptibility and the transmission didn't occur from the infected cattle to naive ones (Ulrich et al., 2020). Also, natural infections revealed that minks, tigers, otters, lions, and gorillas are susceptible as well (WOAH, 2022).

As a result of the quarantine process applied during the pandemic, people have spent more time with pet animals than pre-pandemic period. Close contact of COVID-19 positive owners with pets have let the transmission of the virus to the animals. To

date, SARS-CoV-2 positive companion animals have been reported from various countries. Although it was a question of curiosity whether pet animals play a role in the transmission of the virus to humans, it is now the general opinion that reverse zoonotic cases from humans to companion animals are common. Since investigating SARS-CoV-2 infections in animals by molecular methods is challenging because of the low chance of sampling in the viral shedding period, serological surveillance studies were conducted to put forward the prevalence of SARS-CoV-2 infections in pet animals.

In this study, we investigated the presence of neutralizing antibodies against SARS-CoV-2 in the sera of 91 dogs and 115 cats. The samples were obtained from a diagnostic laboratory, which were sent from the veterinary clinics for hematological tests during routine veterinary visits of pets in Istanbul. Our results are consistent with a study conducted in the United Kingdom, in which none of the 96 serum samples collected from cats during the first wave were positive for VNT (Smith et al., 2021). Although seroprevalence of cats from COVID-19 positive households were remarkably high, such as 23.5% in France (Fritz et al., 2020), 43.8% in the USA (Hamer et al., 2020) and 20% in Brazil (Calvet et al., 2021), studies investigating the seroprevalence of cats without the information of their owners' COVID-19 status, as in this study, resulted in low seropositivity (0.36-1.36%) in Germany (Michelitsch et al., 2020, 2021), the USA (Barua et al., 2021), Thailand (Udom et al., 2021), and Croatia (Stevanovic et al., 2021). Focusing on Istanbul, the sampling area of this study, Yilmaz et al. (2021) investigated the seroprevalence of domestic cats and found three positive with surrogate virus neutralizing test, out of 34 ELISA-positive sera among 155 samples. The discordance between the results of this study and ours may be due to the different testing methods used between the studies.

In the present study, 91 dog sera were tested with VNT, and 2 (2.2%) samples were positive, with titers of 1/256 and 1/32, respectively. Our results are in line with the study from Italy (Patterson et al., 2020), in which after excluding the known COVID-19 status households, the samples collected from the unknown status of COVID-19 environments showed 2.3% positivity by neutralizing tests in dogs during the early pandemic. Results of studies that conducted in the United Kingdom (Smith et al., 2021), the Netherlands (Zhao et al., 2021), France (Fritz et al., 2020), the USA (Barua et al., 2021), and Thailand (Udom et al., 2021) remained below 2% seropositivity.

One of the aims of this study was also to make a comparative assessment between the original SARS-CoV-2 virus circulating period in the second wave and during the dominance of SARS-CoV-2 variants in the late pandemic, which was expected to exhibit higher positivity. According to studies in Germany and the United Kingdom, the seropositivity was higher in the second wave than in the first wave (Michelitsch et al., 2020, 2021; Smith et al., 2021), which is probably due to the cumulative increase of people with a history of COVID-19, reflecting on animals. Additionally, since it is known that the variants are more transmissible (Davies et al., 2021; Public Health England, 2021), it could have resulted in higher rates of infection in companion animals. But these hypotheses are in contrast with our results, which showed that there was not a significant difference between the two periods in this study ($P>0.05$). This might be due to the limited number of our samples.

In this study, both cats and dogs were subjected to investigation. Experimental infection studies have shown that cats are highly susceptible to SARS-CoV-2 and can shed the virus for a prolonged period; however, dogs have low susceptibility, but develop antibodies (Bosco-Lauth et al., 2020; Shi et al., 2020). Based on this information, some seroprevalence studies in companion animals have focused solely on cats. The studies that investigated both cats and dogs generally showed no significant difference on seropositivity between the two species. Companion cats spend most of their lifetime inside the house. In contrast to cats, dogs take their daily walks outside, may interact with other dogs and people closely in common areas, such as parks, etc. As they are more social, in case of the virus shed by dogs, they may be considered potentially more spreader than cats. Since there is still a lack of knowledge about SARS-CoV-2 and it is a highly mutagenic virus, following the transmission of the virus among pet animals is important, but dogs should not be excluded from the seroprevalence studies.

VNT was the method of our study, which requires at least biosafety level 3 conditions. Despite the infectious virus used in some studies (Michelitsch et al., 2020; Patterson et al., 2020; Zhang et al., 2020; Smith et al., 2021) to determine the seroprevalence, some others used pseudotyped viruses with SARS-CoV-2 spike protein (Fritz et al., 2021; Zhao et al., 2021) and surrogate virus neutralization tests (Barua et al., 2021; Yilmaz et al., 2021), which enabled to investigate the neutralizing activity of sera without the need of high-containment laboratories. ELISA is also one of the widely used method that showed more positivity than neutralization tests for the same samples in several studies (Michelitsch et al., 2020; Zhang et al., 2020; Zhao et al., 2021) which might be because of

non-neutralizing but binding antibodies can be detected with this method (Tan et al., 2020), but sometimes it is because of the low specifity of in-house ELISA methods. For this reason, serum samples that are detected positive by in-house ELISA methods should be confirmed by neutralization tests. The variety of antibody detection methods limits to draw a precise comparison among the studies on seroprevalence. In this study the samples were not tested with other coronaviruses since it has been reported previously that there was no cross-reactivity between SARS-CoV-2 and canine respiratory coronavirus (CRCoV),feline coronavirus (FCoV) and canine enteric coronavirus (CeCoV) (Michelitsch et al., 2020; Patterson et al., 2020; Zhang et al., 2020; Smith et al., 2021; Stevanovic et al., 2021).

Conclusion

This study conducted in Istanbul, the biggest city of Turkey which was affected severely by COVID-19. The results of this study shows the evidence of SARS-CoV-2 infection in dogs. Companion animals are susceptible to SARS-CoV-2 and can develop neutralizing antibodies. COVID-19 positive people should avoid close contact with the animals they live with at home, and they should include the companion animals in-home quarantine procedures. The results of this study reinforce the importance of the One Health approach.

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Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical statement: Pendik Veterinary Control Institute Animal Experiments Local Ethics Committee decided that no ethical approval was required for this study.

References

Barua, S., Hoque, M., Adekanmbi, F., Kelly, C., Jenkins-Moore, M., Torchetti, M.K., Chenoweth, K., Wood, T., & Wang, C. (2021) Antibodies to SARS-CoV-2 in dogs and cats, USA. *Emerging Microbes & Infections* 10, 1669-1674.

Bosco-Lauth, A.M., Hartwig, A.E., Porter, S.M., Gordy, P.W., Nehring, M., Byas, A.D., VandeWoude, S., Ragan, I.K., Maison, R.M., & Bowen R.A. (2020). Experimental infection of domestic dogs and cats with SARS-CoV-2: Pathogenesis, transmission, and response to reexposure in cats. *Proceedings of the National Academy of Sciences of the United States of America.* 117, 26382-26388.

Calvet, G.A., Pereira, S.A., Ogrzewalska, M., Pauvolid-Corrêa, A., Resende, P.C., Tassinari, W de S., de Pina Costa, A., Keidel, L.O., da Rocha, A.S.B., da Silva, M.F.B., dos Santos, S.A., Lima, A.B.M., de Moraes, I.C.V., Mendes, A.A.V., das C Souza, T., Martins, E.B., Ornellas, R.O., Corrêa, M.L., da S Antonio, I.M., Guaraldo, L., do C Motta, F., Brasil, P., Siqueira, M.M., Gremião, I.D.F., & Menezes, R.C. (2021). Investigation of SARS-CoV-2 infection in dogs and cats of humans diagnosed with COVID-19 in Rio de Janeiro, Brazil. *PLoS One.* 16.

Davies, N.G., Abbott, S., Barnard, R.C., Jarvis, C.I., Kucharski, A.J., Munday, J.D., Pearson, C.A.B., Russell, T.W., Tully, D.C., Washburne, A.D., Wenseleers, T., Gimma, A., Waites, W., Wong, K.L.M., van Zandvoort, K., Silverman, J.D., Diaz-Ordaz, K., Keogh, R., Eggo, R.M., Funk, S., Jit, M., Atkins, K.E., & Edmunds, W.J. (2021). Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England. *Science.* 372.

Fritz, M., Rosolen, B., Krafft, E., Becquart, P., Elguero, E., Vratskikh, O., Denolly, S., Boson, B., Vanhomwegen, J., Gouilh, M.A., Kodjo, A., Chirouze, C., Rosolen, S.G., Legros, V., & Leroy, E.M. (2020). High prevalence of SARS-CoV-2 antibodies in pets from COVID-19+ households. *One Health.* 11, 100192.

Hamer, S.A., Pauvolid-Corrêa, A., Zecca, I.B., Davila, E., Auckland, L.D., Roundy, C.M., Tang, W., Torchetti, M., Killian, M.L., Jenkins-Moore, M., Mozingo, K., Akpalu, Y., Ghai, R.R., Spengler, J.R., Behravesh, C.B., Fischer, R.S.B., & Hamer, G.L. (2020). SARS-CoV-2 Infections and Viral Isolations among Serially Tested Cats and Dogs in Households with Infected Owners in Texas, USA. *Viruses.* 13, 938.

Laidoudi, Y., Sereme, Y., Medkour, H., Watier-Grillot, S., Scandola, P., Ginesta, J., Andréo, V., Labarde, C., Comtet, L., Pourquier, P., Raoult, D., Lou Marié, J., & Davoust, B. (2021). SARS-CoV-2 antibodies seroprevalence in dogs from France using ELISA and an automated western blotting assay. *One Health.* 13.

Michelitsch, A., Hoffmann, D., Wernike, K., & Beer, M. (2020). Occurrence of antibodies against SARS-CoV-2 in the domestic cat population of Germany. *Vaccines.* 8, 1–10.

Michelitsch, A., Schön, J., Hoffmann, D., Beer, M., & Wernike, K. (2021). The second wave of sars-cov-2 circulation—antibody detection in the domestic cat population in Germany. *Viruses.* 13, 1009.

Munnink, B.B.O., Sikkema, R.S., Nieuwenhuijse, D.F., Molenaar, R.J., Munger, E., Molenkamp, R., Van Der Spek, A., Tolsma, P., Rietveld, A., Brouwer, M.,

Bouwmeester-Vincken, N., Harders, F., Van Der Honing, R.H., Wegdam-Blans, M.C.A., Bouwstra, R.J., GeurtsvanKessel, C., Van Der Eijk, A.A., Velkers, F.C., Smit, L.A.M., Stegeman, A., Van Der Poel, W.H.M., & Koopmans, M.P.G. (2021). Transmission of SARS-CoV -2 on mink farms between humans and mink and back to humans. *Science. 371*, 172-177.

Neira, V., Brito, B., Agüero, B., Berrios, F., Valdés, V., Gutierrez, A., Ariyama, N., Espinoza, P., Retamal, P., Holmes, E.C., Gonzalez-Reiche, A.S., Khan, Z., van de Guchte, A., Dutta, J., Miorin, L., Kehrer, T., Galarce, N., Almonacid, L.I., Levican, J., van Bakel, H., García-Sastre, A., & Medina, R.A.(2021). A household case evidences shorter shedding of SARS-CoV-2 in naturally infected cats compared to their human owners. *Emerging Microbes & Infections. 10*, 376-383.

Palmer, M.V., Martins, M., Falkenberg, S., Buckley, A., Caserta, L.C., Mitchell, P.K., Cassmann, E.D., Rollins, A., Zylich, N.C., Renshaw, R.W., Guarino, C., Wagner, B., Lager, K., & Diel, D.G. (2021). Susceptibility of White-Tailed Deer (Odocoileus virginianus) to SARS-CoV-2. *Journal of Virology, 10;95*(11):e00083-21.

Patterson, E.I., Elia, G., Grassi, A., Giordano, A., Desario, C., Medardo, M., Smith, S.L., Anderson, E.R., Prince, T., Patterson, G.T., Lorusso, E., Lucente, M.S., Lanave, G., Lauzi, S., Bonfanti, U., Stranieri, A., Martella, V., Solari Basano, F., Barrs, V.R., Radford, A.D., Agrimi, U., Hughes, G.L., Paltrinieri, S., & Decaro, N. (2020). Evidence of exposure to SARS-CoV-2 in cats and dogs from households in Italy. *Nature Communications.* 11, 6231.

Public Health England, (2021). SARS-CoV-2 variants of concern and variants under investigation in England: technical briefing 10. Retrieved from : https:// assets.publishing.service.gov.uk/government/ uploads/system/uploads/attachment_data file/984274Variants_of_Concern_VOC_Technical_Briefing_10_England.pdf

Ruiz-Arrondo, I., Portillo, A., Palomar, A.M., Santibáñez, S., Santibáñez, P., Cervera, C., & Oteo, J.A. (2021). Detection of SARS-CoV-2 in pets living with COVID-19 owners diagnosed during the COVID-19 lockdown in Spain: A case of an asymptomatic cat with SARS-CoV-2 in Europe. *Transboundary and Emerging Diseases.* 68, 973-976.

Schlottau, K., Rissmann, M., Graaf, A., Schön, J., Sehl, J., Wylezich, C., Höper, D., Mettenleiter, T.C., Balkema-Buschmann, A., Harder, T., Grund, C., Hoffmann, D., Breithaupt, A., & Beer, M. (2020). SARS-CoV-2 in fruit bats, ferrets, pigs, and chickens: an experimental transmission study. The *Lancet Microbe. 1*, e218-e225.

Schulz, C., Martina, B., Mirolo, M., Müller, E., Klein, R., Volk, H., Egberink, H., Gonzalez-Hernandez, M., Kaiser, F., von Köckritz-Blickwede, M., & Osterhaus, A. (2021). SARS-CoV-2-specific antibodies in domestic cats during first COVID-19 Wave, Europe. Emerging Microbes & Infections. 27, 3115-3118.

Segalés, J., Puig, M., Rodon, J., Avila-Nieto, C., Carrillo, J., Cantero, G., Terrón, M.T., Cruz, S,, Parera, M., Noguera-Julián, M., Izquierdo-Useros, N., Guallar, V., Vidal, E., Valencia, A., Blanco, I., Blanco, J., Clotet, B., & Vergara-Alert, J. (2020). Detection of SARS-CoV-2 in a cat owned by a COVID-19-affected patient in Spain. *Proceedings of the National Academy of Sciences of the United States of America. 117*, 24790 -24793.

Shi, J., Wen, Z., Zhong, G., Yang, H., Wang, C., Huang, B., Liu, R., He, X., Shuai, L., Sun, Z., Zhao, Y., Liu, P., Liang, L., Cui, P., Wang, J., Zhang, X., Guan, Y., Tan, W., Wu, G., & Chen, H., and Bu, Z.(2020). Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. *Science. 368*, 1016-1020.

Sia, S.F., Yan, L.M., Chin, A.W.H., Fung, K., Choy, K.T., Wong, A.Y.L., Kaewpreedee, P., Perera, R.A.P.M., Poon, L.L.M., Nicholls, J.M., Peiris, M., & Yen, H.L. (2020) Pathogenesis and transmission of SARS-CoV-2 in golden hamsters. *Nature. 583*, 834-838.

Sila, T., Sunghan, J., Laochareonsuk, W., Surasombatpattana, S., Kongkamol, C., Ingviya, T., Siripaitoon, P., Kositpantawong, N., Kanchanasuwan, S., Hortiwakul, T., Charernmak, B., Nwabor, O.F., Silpapojakul, K., & Chusri, S. (2022). Suspected Cat-to -Human Transmission of SARS-CoV-2, Thailand, July-September 2021. *Emerging Infectious Diseases.* 28, 1485-1488.

Sit, T.H.C., Brackman, C.J., Ip, S.M., Tam, K.W.S., Law, P.Y.T., To, E.M.W., Yu, V.Y.T., Sims, L.D., Tsang, D.N.C., Chu, D.K.W., Perera, R.A.P.M., Poon, L.L.M., Peiris, M. (2020) Infection of dogs with SARS-CoV-2. *Nature 586*, 776-778.

Smith, S.L., Anderson, E.R., Cansado-Utrilla, C., Prince, T., Farrell, S., Brant, B., Smyth, S., Noble, P-J.M, Pinchbeck, G.L., Marshall, N., Roberts, L,, Hughes, G.L., Radford, A.D., Patterson, E.I. (2021). SARS-CoV-2 neutralising antibodies in dogs and cats in the United Kingdom. *Current Research in Virology Science. 2*, 100011.

Stevanovic, V., Vilibic-Cavlek, T., Tabain, I., Benvin, I., Kovac, S., Hruskar, Z., Mauric, M., Milasincic, L., Antolasic, L., Skrinjaric, A., Staresina, V., Barbic, L. (2021). Seroprevalence of SARS-CoV-2 infection

among pet animals in Croatia and potential public health impact. *Transboundary and Emerging Diseases. 68*, 1767-1773.

Tan, C.W., Chia, W.N., Qin, X., Liu, P., Chen, M.I.C., Tiu, C., Hu, Z., Chen, V.C.W., Young , B.E., Sia, W.R., Tan, Y.J., Foo, R., Yi, Y., Lye, D.C., Anderson, D.E., & Wang, L.F. (2020). A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2–spike protein–protein interaction. *Nature Biotechnology. 38*, 1073-1078.

Udom, K., Jairak, W., Chamsai, E., Charoenkul, K., Boonyapisitsopa, S., Bunpapong, N., Techakriengkrai, N., Amonsin, A. (2021). Serological survey of antibodies against SARS-CoV-2 in dogs and cats, Thailand. *Transboundary and Emerging Diseases.* 1-8.

Ulrich, L., Wernike, K., Hoffmann, D., Mettenleiter, T.C., & Beer, M. (2020). Experimental Infection of Cattle with SARS-CoV-2. Emerg Infect Dis. 26, 2979-2981.

WOAH, (2022). SARS-CoV-2 in Animals - Situation Report 11. Retrieved from: https://www.woah.org/app/uploads/2022/09/sars-cov-2-situation-report-16.pdf

Yilmaz, A., Kayar, A., Turan, N., Iskefli, O., Bayrakal, A., Roman-Sosa, G., Or ,E., Tali, H.E., Kocazeybek, B., Karaali, R., Bold, D., Sadeyen, J.R., Lukosaityte, D., Chang, P., Iqbal, M., Richt, J.A., Yilmaz, H. (2021). Presence of Antibodies to SARS-CoV-2 in Domestic Cats in Istanbul, Turkey, Before and After COVID-19 Pandemic. *Frontiers Veterinary Science. 8*, 1123.

Zhang, Q., Zhang, H., Gao, J., Huang, K., Yang, Y., Hui, X., He, X., Li, C., Gong, W., Zhang, Y., Zhao, Y., Peng, C., Gao, X., Chen, H., Zou, Z., Shi, Z.L., Jin, M. (2020). A serological survey of SARS-CoV-2 in cat in Wuhan. *Emerging Microbes & Infections.* 9, 2013-2019.

Zhao, S., Schuurman, N., Li, W., Wang, C., Smit, L.A.M., Broens, E.M., Wagenaar, J.A., van Kuppeveld, F.J.M., Bosch, B.J., Egberink, H. (2021). Serologic screening of severe acute respiratory syndrome coronavirus 2 infection in cats and dogs during first coronavirus disease wave, the Netherlands. *Emerging Infectious Diseases. 27*, 1362-1370.

Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., Zhao, X., Huang, B., Shi, W., Lu, R., Niu, P., Zhan, F., Ma, X., Wang, D., Xu, W., Wu, G., Gao, G.F., & Tan, W. (2020). A Novel Coronavirus from Patients with Pneumonia in China, 2019. *New England Journal of Medicine*. 382, 727-733.