PLOS ONE

Neutralizing antibodies for SARS-CoV-2 in stray animals from Rio de Janeiro, Brazil

Helver Gonçalves Dias[1], **Maria Eduarda Barreto Resck**[2], **Gabriela Cardoso Caldas**[3], **Alessandro Fioretti Resck**[4], **Natália Valente da Silva**[5], **Aline Marcele Vieira dos Santos**[5], **Thiago das Chagas Sousa**[5], **Maria Halina Ogrzewalska**[5], **Marilda Mendonça Siqueira**[5], **Alex Pauvolid-Corrêa**[5,6], **Flavia Barreto dos Santos**[1]*

1 Laboratório de Imunologia Viral, Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, Brasil, 2 Laboratório de Mosquitos Transmissores de Hematozoários, Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, Brasil, 3 Laboratório de Morfologia e Morfogênese Viral, Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, Brasil, 4 Clínica Veterinária Animal Help, Rio de Janeiro, Brasil, 5 Laboratório de Vírus Respiratórios e Sarampo, Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, Brasil, 6 Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, Texas, United States of America

* flaviab@ioc.fiocruz.br

Abstract

The epidemic of coronavirus disease 2019 (COVID-19), caused by a novel *Betacoronavirus* named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) became a public health emergency worldwide. Few reports indicate that owned pets from households with at least one human resident that was diagnosed with COVID-19 can be infected by SARS-CoV-2. However, the exposure to SARS-CoV-2 of pets from households with no COVID-19 cases or stray animals remains less assessed. Using real-time reverse transcriptase polymerase chain reaction (RT-PCR) and plaque reduction neutralization test (PRNT$_{90}$), we investigated the infection and previous exposure of dogs and cats to SARS-CoV-2 during the ongoing COVID-19 epidemic in Rio de Janeiro, Brazil. From June to August 2020, 96 animals were sampled, including 49 cats (40 owned and 9 stray) and 47 dogs (42 owned and 5 stray). Regarding owned pets, 75.6% (62/82) belonged to households with no COVID-19 cases. Samples included serum, and rectal and oropharyngeal swabs. All swabs were negative for SARS-CoV-2 RNA, but serum samples of a stray cat and a stray dog presented neutralizing antibodies for SARS-CoV-2, with PRNT$_{90}$ titer of 80 and 40, respectively. Serological data presented here suggest that not only owned pets from households with COVID19 cases, but also stray animals are being exposed to SARS-CoV-2 during the COVID-19 pandemic.

Introduction

The novel *Severe acute respiratory syndrome coronavirus 2* (SARS-CoV-2) that belongs to the *Betacoronavirus* genus rapidly spread worldwide causing an unprecedented pandemic of coronavirus disease (COVID-19). Patients usually have clinical symptoms such as fever, cough, difficulty breathing, loss of taste or smell, headache and pneumonia, and also others clinical

nonrespiratory manifestations, which can progress to severe clinical presentations and death [1, 2].

Phylogenetic analyzes showed that SARS-CoV-2 has a possible zoonotic origin, with species of bat as reservoirs [1, 3, 4]. Regarding domestic animals, it has been experimentally shown that cats are not only susceptible to SARS-CoV-2 infection, but they also have the capability to transmit the virus to others co-housed cats [5–7]. Furthermore, cats that were experimentally infected by SARS-CoV-2 mounted an effective immune response [5]. On the other hand, dogs seem to be less susceptible to SARS-CoV-2 infection presenting lower seroprevalence and limited capacity to transmit the virus [5, 8].

Since the beginning of the pandemic, different countries have reported the detection of RNA and/or specific antibodies for SARS-CoV-2 in dogs and felines [9–16]. The large majority of these studies have tested pets from households with at least one human case of COVID-19. The exposure of animals from households with no confirmed COVID-19 cases, or the exposure of stray animals to SARS-CoV-2 is being less investigated.

Companion animals, especially dogs and cats, are in close contact with humans and inhabit the same environment. Because of that, they may be highly exposed to human pathogens. It is estimated there are around 78.1 million dogs and cats as pets in Brazil. Of those, about 5% are animals in vulnerable conditions [17]. The large number of stray animals represents an important concern in the context of public health and animal welfare [9, 16].

The Brazilian Ministry of Health notified the first human confirmed case of COVID-19 in the country on February 26, 2020. By late October, about 5.5 million cases and more than 159 thousand deaths had been reported in the country [18]. Brazil has the most cases and deaths in Latin America and those numbers are probably underestimated. The Southeast Region is the most populated area of the country and concentrates the largest number of COVID-19 cases and where São Paulo and Rio de Janeiro are the most affected cities [19]. Even with the largest number of COVID-19 cases in South America, the exposure of companion animals to SARS-CoV-2 in Brazil has been poorly explored [20].

In the present study, we investigated the presence of SARS-CoV-2 RNA in oropharyngeal and rectal swabs by real-time RT-PCR, and the presence of neutralizing antibodies specific to SARS-CoV-2 in sera by plaque reduction neutralization test ($PRNT_{90}$) in cats and dogs from Rio de Janeiro, during the ongoing COVID-19 epidemic.

Materials and methods

Ethical statement

This study was approved by the Oswaldo Cruz Institute's Animal Care and Use Committee (protocol number 013/2020) in compliance with the requirements of Brazilian Law 11794/2008, and additional approvals from veterinary clinics were specific to each partner.

Sampling

From June to August 2020, a total of 96 animals, including 49 cats (*Felis catus*) and 47 dogs (*Canis lupus familiaris*), were sampled in the city of Rio de Janeiro, Brazil. From those, 85.4% (82/96) were owned pets and 14.6% (14/96) were stray animals. Among owned animals, 75.6% (62/82) were from a household with no history of COVID-19 case and 24.4% (20/82) from a household with history of a human COVID-19 case. Convenience sampling was performed at two veterinary clinics located in the North and West zones of the city. Blood samples as well as oropharyngeal and rectal swabs were collected from each animal. After initial medical evaluation, animals were eligible for research and the owners were invited to participate. It was not an inclusion criterium that the owner had been diagnosed with COVID-19. Animals older

than 14 years, less than 4 months, or pregnant were not included in the study. After acceptance, the owners underwent a quick interview with questions related to the animal's general health status and the socio-environmental factors of the dwelling. The stray animals included in the research were captured by non-governmental organizations and assisted at both veterinary clinics.

The oropharyngeal and rectal swabs were collected and placed in tubes containing viral transport medium (VTM). VTM was prepared with 2% fetal bovine serum (FBS) (Gibco— 10270106), antibiotics (100 μg/mL for gentamicin and 0.5 μg/mL for amphotericin B) and Hanks Balanced Salt Solution [21]. Blood collection was performed through femoral venipuncture in tubes with no anticoagulants. Serum was separated from blood samples by centrifugation, and then stored at -70˚C freezers.

Laboratory testing

Real-time RT-PCR for SARS-CoV-2. RNA extraction of VTM samples from oropharyngeal and rectal swabs was performed using the ZR-Viral RNA kit (Zymo Research, Irvine, USA) according to the manufacturer's instructions. RNA samples were then tested for SARS-CoV-2 by two commercial kits, the Allplex™ 2019-nCoV Assay (Seegene Inc., Taewon, Republic of Korea) that targets the envelope (E), polymerase (RdRP) and nucleocapsid (N) genes, and the Biomanguinhos RT-qPCR kit (FIOCRUZ, Brazil) that targets the E gene, according to manufacturer's instructions.

Plaque reduction neutralization test ($PRNT_{90}$). The PRNT is a highly specific serologic test and can be carried out on samples to confirm the presence of neutralizing antibodies to SARS-CoV-2 [22, 23]. All serum samples were heat-inactivated and tested by for their ability to neutralize plaque formation by SARS-CoV-2. The assay was performed in a Multi-user Research Facility of Biosafety Level 3 Platform of Instituto Oswaldo Cruz/FIOCRUZ. The reference SARS-CoV-2 used for $PRNT_{90}$ was isolated from a patient from Rio de Janeiro and belongs to lineage B.1. Full genome sequence is deposited at GISAID (EPI_ISL_414045).

Vero cells (ATCC, CCL 81) were maintained in 5% CO_2 atmosphere at 37˚C in 199 Medium supplemented with 10% FBS (Gibco—10270106), buffered with sodium bicarbonate (0,075g/mL), 100 U/mL penicillin, 0,1 mg/mL streptomycin, 40 ug/mL gentamicin and 0.025 ug/mL amphotericin B. Briefly, inactivated aliquots were screened at a dilution of 1:10 in two-day old Vero CCL-81 cells seeded in 6-well plates. Those that neutralized SARS-CoV-2 by at least 90% were further tested in duplicate at serial two-fold dilutions to determine 90% end-point titers. Serum samples were considered seropositive to SARS-CoV-2 when a serum dilution of at least 1:20 reduced no less than 90% of the formation of SARS-CoV-2 viral plaques [15, 24, 25]. Because the potential circulation of other coronaviruses in the region could generate cross-reacting neutralizing antibodies, we used a conservative threshold for detection of neutralizing antibodies of 90%, and we considered seropositive only samples that presented $PRNT_{90}$ titers of 20 or greater.

Results

All oropharyngeal (n = 96) and rectal (n = 96) swabs were submitted to both real-time RT-PCR protocols and tested negative for SARS-CoV-2 RNA. To investigate previous SARS-CoV-2 exposure, serum samples of all 96 animals were tested by $PRNT_{90}$ for the detection of SARS-CoV-2-neutralizing antibodies. From those, one cat (70S) and one dog (64S), presented $PRNT_{90}$ titer of 80 and 40, respectively. Both individuals were stray animals. They were taken to the veterinary clinics by volunteers who capture abandoned animals for neutering and general health care, and then put them up for adoption. None of those animals had respiratory

symptoms or fever at the time of sampling. The cat 70S is a female with 3,3kg, no breed defined (NBD), and one year old. The dog 64S is a female with 13,5 kg, NBD and eight years old. Both animals were sampled in the Barra da Tijuca neighborhood, located in the west zone of Rio de Janeiro.

Discussion

Based on the serological data presented here, we suggest exposure of a stray cat and a stray dog to SARS-CoV-2, during the ongoing COVID-19 epidemic in Rio de Janeiro, Brazil. Our results corroborate the evidence observed in other countries that not only owned pets, but also stray animals are being exposed worldwide [10–16, 25–27].

Infection is likely to occur only when the animal is in close contact with people who are actively shedding virus or in contaminated environments [11, 13, 15]. Both cat 70S and dog 64S may have been exposed to SARS-CoV-2 by three main routes: 1) contact with other infected animals, 2) contact with infected humans, and finally 3) exposure to a contaminated environment. SARS-CoV-2 RNA has already been detected on public surfaces and in the sewage system in large Brazilian cities, pointing out that these environments can be a potential source of infection [28, 29].

In Wuhan, China, a high seroprevalence for SARS-CoV-2 was observed in cats sampled between January and March 2020, mainly due to the high viral transmission in that period [16]. The same high seroprevalence may be observed in other cities, but more studies are needed to draw a global eco-epidemiological picture [11, 30].

Human-animal transmission events are not restricted to domestic animals. Several large felines in the Bronx Zoo in New York, USA developed symptoms of respiratory illness and tested positive for SARS-CoV-2. The source of infection was identified as a zoo employee, who was actively shedding virus [31]. Despite these results, reverse transmission, that is, from pets to human, has never been demonstrated. At least so far, only one episode of probable direct transmission of animals to humans has been reported and involved an outbreak of respiratory disease in farmed minks in the Netherlands [32]. As observed for other respiratory viruses, as influenza, mink replicates SARS-CoV-2 efficiently and are able to transmit the virus, in addition to developing lung lesions similar to those observed in humans. For those reasons, this species has been used as an excellent model for drug and vaccine testing [33].

One of the strengths of this study was the convenience sampling in two veterinary clinics that did not specifically include only pets owned by COVID-19 patients. The inclusion of stray animals has revealed an important aspect for the surveillance of SARS-CoV-2 in urban centers and may be considered as indicator of environment contamination. Animals were sampled between June and August, when the epidemic reached about 100,000 human confirmed cases of COVID-19 in the city of Rio de Janeiro [34]. Another strength of this study is the use of both highly sensitive and highly specific molecular and serological methods for detection of SARS-CoV-2 infection. Molecular methods included two different well-established real-time RT-PCR protocols for SARS-CoV-2 diagnosis. Antibody detection in sera was accomplished by $PRNT_{90}$ that utilized a highly conservative threshold of 90% neutralization, which is considered one of the most specific serological tests for the differentiation of viral infections in convalescent serum samples [35].

Limitations of our study include the lack of sampling in other regions of the city, and the absence of other coronaviruses in the $PRNT_{90}$ as differential diagnosis. The *Coronaviridae* family has different species of coronaviruses, including the feline enteric coronavirus (FECV) and the feline infectious peritonitis coronavirus (FIPV) that infect cats, and the canine coronavirus (CCoV) and the canine respiratory coronavirus (CRCoV) that infect dogs [34]. The

potential serological cross-reactivity between SARS-CoV-2 and these other coronaviruses remains poorly investigated. A recent study suggests little or absent cross-reactivity between SARS-CoV-2 and feline infectious peritonitis virus type I or II [16]. However, unless tested by other coronaviruses, cross-reactivity cannot be fully discarded. The circulation of canine and feline coronaviruses in Rio de Janeiro has already been reported [16, 36–38]. Because of that, instead of using a 50% neutralization criterion as it has been reported elsewhere [22, 39], we decided to adopt the most conservative criteria of 90% neutralization for detection of SARS-CoV-2 neutralizing antibodies, even at cost of some false negatives. If a 50% neutralization was used as positivity criteria, cat 70S and dog 64S would present $PRNT_{50}$ titers $\geq$320, but no other animal would be seropositive.

In conclusion, the results presented here suggest that stray animals were not currently infected, but may have been exposed to SARS-CoV-2 during the ongoing COVID-19 epidemic in Rio de Janeiro, Brazil. Present findings are in agreement with previous investigations that suggest human-animal transmission of SARS-CoV-2 [9–16]. For that reason, the investigation of SARS-CoV-2 in animal populations by a One Health approach is necessary and should be encouraged [40]. It is important to emphasize that, based on current data, COVID-19 patients must maintain preventive measures of social isolation and pets, such as cats and dogs, should be included in this care. Further studies are needed to establish the role of pets and other animals in the ongoing COVID-19 pandemic. We reinforce that any attempt to abandon or mistreat animals is condemnable and is not justified.

Supporting information

S1 Database.
(XLSX)

Acknowledgments

Author Contributions

Conceptualization: Helver Gonçalves Dias, Alex Pauvolid-Corrêa, Flavia Barreto dos Santos.

Data curation: Helver Gonçalves Dias, Alex Pauvolid-Corrêa.

Formal analysis: Helver Gonçalves Dias, Maria Eduarda Barreto Resck, Alex Pauvolid-Corrêa.

Funding acquisition: Marilda Mendonça Siqueira, Flavia Barreto dos Santos.

Investigation: Helver Gonçalves Dias, Maria Eduarda Barreto Resck, Gabriela Cardoso Caldas, Alessandro Fioretti Resck, Natália Valente da Silva, Aline Marcele Vieira dos Santos, Thiago das Chagas Sousa, Maria Halina Ogrzewalska, Alex Pauvolid-Corrêa.

Methodology: Helver Gonçalves Dias, Gabriela Cardoso Caldas, Natália Valente da Silva, Aline Marcele Vieira dos Santos, Thiago das Chagas Sousa, Maria Halina Ogrzewalska, Alex Pauvolid-Corrêa.

Project administration: Flavia Barreto dos Santos.

Resources: Marilda Mendonça Siqueira, Flavia Barreto dos Santos.

Supervision: Alessandro Fioretti Resck, Alex Pauvolid-Corrêa.

Validation: Maria Halina Ogrzewalska, Marilda Mendonça Siqueira.

Writing – original draft: Helver Gonçalves Dias.

Writing – review & editing: Marilda Mendonça Siqueira, Alex Pauvolid-Corrêa, Flavia Barreto dos Santos.

References

1. Ren L-L, Wang Y-M, Wu Z-Q, Xiang Z-C, Guo L, Xu T, et al. Identification of a novel coronavirus causing severe pneumonia in human: a descriptive study. Chin Med J (Engl). 2020 May 5; 133(9):1015–24. https://doi.org/10.1097/CM9.0000000000000722 PMID: 32004165

2. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020 15; 395(10223):497–506. https://doi.org/10.1016/S0140-6736(20)30183-5 PMID: 31986264

3. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet. 2020 22; 395 (10224):565–74. https://doi.org/10.1016/S0140-6736(20)30251-8 PMID: 32007145

4. Zhou P, Yang X-L, Wang X-G, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020; 579(7798):270–3. https://doi.org/10.1038/s41586-020-2012-7 PMID: 32015507

5. Bosco-Lauth AM, Hartwig AE, Porter SM, Gordy PW, Nehring M, Byas AD, et al. Experimental infection of domestic dogs and cats with SARS-CoV-2: Pathogenesis, transmission, and response to reexposure in cats. Proc Natl Acad Sci U S A. 2020 20; 117(42):26382–8. https://doi.org/10.1073/pnas.2013102117 PMID: 32994343

6. Gaudreault NN, Trujillo JD, Carossino M, Meekins DA, Morozov I, Madden DW, et al. SARS-CoV-2 infection, disease and transmission in domestic cats. Emerg Microbes Infect. 2020 Dec; 9(1):2322–32. https://doi.org/10.1080/22221751.2020.1833687 PMID: 33028154

7. Halfmann PJ, Hatta M, Chiba S, Maemura T, Fan S, Takeda M, et al. Transmission of SARS-CoV-2 in Domestic Cats. N Engl J Med. 2020 6; 383(6):592–4. https://doi.org/10.1056/NEJMc2013400 PMID: 32402157

8. Shi J, Wen Z, Zhong G, Yang H, Wang C, Huang B, et al. Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. Science. 2020 29; 368(6494):1016–20. https://doi.org/10.1126/science.abb7015 PMID: 32269068

9. Farnia P, Aghajani J, Farnia P, Ayoubi S, Ghanavi J, Nadji SA, et al. Evidence for SARS-CoV-2 circulating among stray dogs and cats: Should we worry about our pets during the COVID-19 Pandemic? Biomed Biotechnol Res J. 2020; 4(5):49–55.

10. United States Department of Agriculture. Confirmed Cases of SARS-CoV-2 in Animals in the United States [Internet]. 2020. https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/sa_one_health/sars-cov-2-animals-us

11. Barrs VR, Peiris M, Tam KWS, Law PYT, Brackman CJ, To EMW, et al. SARS-CoV-2 in Quarantined Domestic Cats from COVID-19 Households or Close Contacts, Hong Kong, China. Emerg Infect Dis. 2020 Sep 16; 26(12). https://doi.org/10.3201/eid2612.202786 PMID: 32938527

12. Ruiz-Arrondo I, Portillo A, Palomar AM, Santibáñez S, Santibáñez P, Cervera C, et al. 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. Transbound Emerg Dis. 2020 Aug 18; https://doi.org/10.1111/tbed.13803 PMID: 32810370

13. Sailleau C, Dumarest M, Vanhomwegen J, Delaplace M, Caro V, Kwasiborski A, et al. First detection and genome sequencing of SARS-CoV-2 in an infected cat in France. Transbound Emerg Dis. 2020 Jun 5;

14. Segalés J, Puig M, Rodon J, Avila-Nieto C, Carrillo J, Cantero G, et al. Detection of SARS-CoV-2 in a cat owned by a COVID-19-affected patient in Spain. Proc Natl Acad Sci U S A. 2020 6; 117(40):24790–3. https://doi.org/10.1073/pnas.2010817117 PMID: 32948692

15. Sit THC, Brackman CJ, Ip SM, Tam KWS, Law PYT, To EMW, et al. Infection of dogs with SARS-CoV-2. Nature. 2020; 586(7831):776–8. https://doi.org/10.1038/s41586-020-2334-5 PMID: 32408337

16. Zhang Q, Zhang H, Gao J, Huang K, Yang Y, Hui X, et al. A serological survey of SARS-CoV-2 in cat in Wuhan. Emerg Microbes Infect. 2020 Dec; 9(1):2013–9. https://doi.org/10.1080/22221751.2020.1817796 PMID: 32867625

17. Instituto Pet Brasil. País tem 3,9 milhões de animais em condição de vulnerabilidade [Internet]. 2019. http://institutopetbrasil.com/imprensa/pais-tem-39-milhoes-de-animais-em-condicao-de-vulnerabilidade/

18. Ministério da Saúde do Brasil. Painel de casos de doença pelo coronavírus 2019 (COVID-19) no Brasil pelo Ministério da Saúde [Internet]. 2020 [cited 2020 Oct 10]. https://covid.saude.gov.br/. (2020).

19. The Lancet null. COVID-19 in Brazil: "So what?" Lancet. 2020 9; 395(10235):1461. https://doi.org/10.1016/S0140-6736(20)31095-3 PMID: 32386576

20. Bonilla-Aldana DK, Holguin-Rivera Y, Perez-Vargas S, Trejos-Mendoza AE, Balbin-Ramon GJ, Dhama K, et al. Importance of the One Health approach to study the SARS-CoV-2 in Latin America. One Health. 2020 Dec; 10:100147. https://doi.org/10.1016/j.onehlt.2020.100147 PMID: 32665970

21. Centers for Disease Control and Prevention (CDC). Preparation of viral transport medium SOP#: DSR-052-02. 2020; https://www.cdc.gov/csels/dls/locs/2020/new_sop_for_creating_vtm.html,

22. Okba NMA, Müller MA, Li W, Wang C, GeurtsvanKessel CH, Corman VM, et al. Severe Acute Respiratory Syndrome Coronavirus 2-Specific Antibody Responses in Coronavirus Disease Patients. Emerg Infect Dis. 2020 Jul; 26(7):1478–88. https://doi.org/10.3201/eid2607.200841 PMID: 32267220

23. World Health Organization. Population-based age-stratified seroepidemiological investigation protocol for COVID-19 virus infection [Internet]. 2020. https://apps.who.int/iris/handle/10665/331656

24. Russell PK, Nisalak A, Sukhavachana P, Vivona S. A plaque reduction test for dengue virus neutralizing antibodies. J Immunol. 1967 Aug; 99(2):285–90. PMID: 6031202

25. Algaissi A, Hashem AM. Evaluation of MERS-CoV Neutralizing Antibodies in Sera Using Live Virus Microneutralization Assay. Methods Mol Biol. 2020; 2099:107–16. https://doi.org/10.1007/978-1-0716-0211-9_9 PMID: 31883091

26. Newman A, Smith D, Ghai RR, Wallace RM, Torchetti MK, Loiacono C, et al. First Reported Cases of SARS-CoV-2 Infection in Companion Animals—New York, March-April 2020. MMWR Morb Mortal Wkly Rep. 2020 Jun 12; 69(23):710–3. https://doi.org/10.15585/mmwr.mm6923e3 PMID: 32525853

27. Hamer SA, Pauvolid-Corrêa A, Zecca IB, Davila E, Auckland LD, Roundy CM, et al. Natural SARS-CoV-2 infections, including virus isolation, among serially tested cats and dogs in households with confirmed human COVID-19 cases in Texas, USA [Internet]. Zoology; 2020 Dec [cited 2021 Feb 9]. http://biorxiv.org/lookup/doi/10.1101/2020.12.08.416339 PMID: 33330861

28. Abrahão JS, Sacchetto L, Rezende IM, Rodrigues RAL, Crispim APC, Moura C, et al. Detection of SARS-CoV-2 RNA on public surfaces in a densely populated urban area of Brazil: A potential tool for monitoring the circulation of infected patients. Sci Total Environ. 2020 Oct 2;142645. https://doi.org/10.1016/j.scitotenv.2020.142645 PMID: 33069469

29. Prado T, Fumian TM, Mannarino CF, Maranhão AG, Siqueira MM, Miagostovich MP. Preliminary results of SARS-CoV-2 detection in sewerage system in Niterói municipality, Rio de Janeiro, Brazil. Mem Inst Oswaldo Cruz. 2020; 115:e200196. https://doi.org/10.1590/0074-02760200196 PMID: 32725059

30. Patterson EI, Elia G, Grassi A, Giordano A, Desario C, Medardo M, et al. Evidence of exposure to SARS-CoV-2 in cats and dogs from households in Italy. bioRxiv. 2020 Jul 23;

31. United States Department of Agriculture. USDA Statement on the Confirmation of COVID-19 in a Tiger in New York [Internet]. 2020 [cited 2020 Oct 10]. https://www.aphis.usda.gov/aphis/newsroom/news/sa_by_date/sa-2020/ny-zoo-covid-19

32. Oreshkova N, Molenaar RJ, Vreman S, Harders F, Oude Munnink BB, Hakze-van der Honing RW, et al. SARS-CoV-2 infection in farmed minks, the Netherlands, April and May 2020. Euro Surveill. 2020; 25(23). https://doi.org/10.2807/1560-7917.ES.2020.25.23.2001005 PMID: 32553059

33. Shuai L, Zhong G, Yuan Q, Wen Z, Wang C, He X, et al. Replication, pathogenicity, and transmission of SARS-CoV-2 in minks. National Science Review. 2020 Dec 8;nwaa291.

34. Secretaria Estadual de Saúde do Rio de Janeiro. Painel Coronavírus COVID-19 [Internet]. 2020 [cited 2020 Oct 10]. http://painel.saude.rj.gov.br/monitoramento/covid19.html#

35. Lee WT, Girardin RC, Dupuis AP Ii, Kulas KE, Payne AF, Wong SJ, et al. Neutralizing Antibody Responses in COVID-19 Convalescent Sera. J Infect Dis. 2020 Oct 26;

36. Castro TX, de CN Cubel Garcia R, Fumian TM, Costa EM, Mello R, White PA, et al. Detection and molecular characterization of caliciviruses (vesivirus and norovirus) in an outbreak of acute diarrhea in kittens from Brazil. The Veterinary Journal. 2015 Oct; 206(1):115–7. https://doi.org/10.1016/j.tvjl.2015.06.014 PMID: 26189893

37. Castro TX, de CN Cubel Garcia R, Gonçalves LPS, Costa EM, Marcello GCG, Labarthe NV, et al. Clinical, hematological, and biochemical findings in puppies with coronavirus and parvovirus enteritis. Can Vet J. 2013 Sep; 54(9):885–8. PMID: 24155496

38. Oliveira FR, Pereira J de A, Gonçalves RPM. Sepse em felino associada à peritonite infecciosa felina. Acta Veterinaria Brasilica. 2015; 9:296–300.

39. Kiyong'a AN, Cook EAJ, Okba NMA, Kivali V, Reusken C, Haagmans BL, et al. Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Seropositive Camel Handlers in Kenya. Viruses. 2020 3; 12(4). https://doi.org/10.3390/v12040396 PMID: 32260186

40. Leroy EM, Ar Gouilh M, Brugère-Picoux J. The risk of SARS-CoV-2 transmission to pets and other wild and domestic animals strongly mandates a one-health strategy to control the COVID-19 pandemic. One Health. 2020 Dec; 10:100133. https://doi.org/10.1016/j.onehlt.2020.100133 PMID: 32363229