Scientific Works. Series C. Veterinary Medicine. Vol. LXVIII (2), 2022
ISSN 2065-1295; ISSN 2343-9394 (CD-ROM); ISSN 2067-3663 (Online); ISSN-L 2065-1295

SARS-CoV-2 DETECTION IN THREE CATS (*Felis catus*) BY REAL-TIME REVERSE-TRANSCRIPTASE POLYMERASE-CHAIN-REACTION IN BUCHAREST, ROMANIA

Dana Mihaela CREȚU[1], Maria Rodica GURĂU[1], Mihai TURCITU[2], Stelian BĂRĂITĂREANU[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]Omnivet Impex SRL, 32 Louis Blanc St., District 1, Bucharest, Romania

Corresponding author email: otelea_maria@yahoo.com

Abstract

Since the COVID-19 pandemic began, there have been more and more studies and case-reports regarding the transmission of SARS-CoV-2 to several animal species. In cats, SARS-CoV-2 susceptibility was proved through experimental infection and natural settings (direct contact with infected humans). In this paper, the real-time reverse-transcriptase polymerase-chain-reaction (rRT-PCR) results obtained from four cats living in SARS-CoV-2-infected households in Bucharest (Romania) were described. All cases were indoor cats living in close contact with infected owners (in one household, owners were asymptomatic and, in the other households, owners had mild clinical signs). All the cats were lethargic and had a moderate loss of appetite. One cat was slightly dyspnoeic. The genomic material was extracted from deep oropharyngeal swabs using the QIAamp cador Pathogen Mini Kit. The rRT-PCR analysis used the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel and primers designed to detect the SARS-CoV-2 nucleocapsid (N) protein gene, supplied by Integrated DNA Technologies (IDT, USA). The sample from the cat living with an asymptomatic family was negative, while cats of owners with clinical signs provided positive results, as follows: $Ct_{(Cat\#1)}$=27.04, $Ct_{(Cat\#2)}$=24.13, and $Ct_{(Cat\#4)}$=35.0. Results revealed the risk of indoor cats' infection with SARS-CoV-2 in households where owners have COVID-19, especially if they show clinical signs.

Key words*: SARS-CoV-2, COVID-19, Coronavirus, feline diseases.*

INTRODUCTION

In December 2019, a new pandemical situation was declared, starting from Wuhan, China. The first official communications incriminated bats as an infectious reservoir, and the new virus was suspected of having the ability to cross the species barrier (Wang et al., 2020; Hosie et al., 2021a). SARS-CoV-2 is a novel Coronavirus responsible for the COVID-19 global spread. It is related with SARS-CoV-1 [the etiological agent of Severe Acute Respiratory Syndrome (SARS), first identified at the end of February 2003) and MERS-CoV [the etiological agent of Middle East Respiratory Syndrome (MERS), first reported in June 2012], but unlike these, SARS-CoV-2 has caused a pandemic still evolving, with still emerging strains and still rising questions (Likhacheva, 2006; de Groot et al., 2013; Turcu et al., 2021).

Coronaviruses are enveloped single-stranded, positive-sense RNA viruses. At least 39 distinct coronavirus species have been described and classified in 27 subgenera. They belong to genera *Alphaletovirus*, *Alphacoronavirus*, *Betacoronavirus*, *Deltacoronavirus*, and *Gammacoronavirus*, in subfamilies *Letovirinae* and *Orthocoronavirinae* of the family *Coronaviridae*, suborder *Cornidovirineae,* and order *Nidovirales*. SARS-CoV-1, SARS-CoV-2, and MERS-CoV belongs to the genus *Betacoronavirus* (Baraitareanu, 2020; Gaudreaulta, 2020).

Since the COVID-19 pandemic years began there are more and more studies and case reports regarding the transmission of SARS-CoV-2 to several species. First, the bat was recorded as a natural reservoir, but along with new researches, new animals proved to be receptive to this virus. In the early days of the pandemic, studies like the one conducted by Xiao et al. (2020) showed the presence in pangolins of a virus strongly related (until 99%) with SARS-CoV-2. The most studied species are cats, dogs, ferrets, and minks. In addition, experiments have shown that birds

such as ducks and chickens, as well as pigs, are not susceptible to the virus (Hosie et al., 2021a).
The most frequently studied and proven natural interspecies SARS-CoV-2 infections are human-cat and cat-to-cats. There is more evidence of natural infections of animals resulting from close coexistence with infected people, but also infections between animals of the same species demonstrated by experimental infections (Braun et al., 2021).
Hong Kong reported the first SARS-CoV-2 infections in a cat: a domestic short-haired cat that lived with the owner confirmed with COVID-19. Subsequently, numerous scientific reports have reported this in Belgium, the USA, France, Spain, Germany, the UK, Italy, Switzerland, Russia, Denmark, Sweden, Chile, Japan, Brazil, and Argentina (Barrs et al., 2020; Garigliany et al., 2020; Michelitsch et al., 2020; Musso et al., 2020; Newman et al., 2020; Sailleau et al., 2020; Segalés et al., 2020; Hosie et al., 2021a, 2021b; Klaus et al., 2021; Ruiz-Arrondo et al., 2021).
Recent analytical evaluation of real-time reverse-transcriptase polymerase-chain-reaction (rRT-PCR) for SARS-CoV-2 supports the gold standard value of this test method in COVD-19 diagnostic (Rahbari et al., 2021). Moreover, the studies of Rahbari et al. (2021) and Lee et al. (2021) provided information about sources of error (e.g., sampling, storage, processing, RNA extraction, cDNA synthesis, amplification, interpretation, and analysis and test reporting) and findings of the CDC investigation conducted to identify the causes of the N1 and N3 false-positive reactivity (Lee et al., 2021; Rahbari et al., 2021). This paper describes the results obtained by rRT-PCR for SARS-CoV-2 in indoor cats (*Felis catus*) that were in close contact with infected owners.

MATERIALS AND METHODS

Samples, animals, and housing
Deep oropharyngeal swabs from four cats (#1, #2, #3, and #4) that were living in the same apartment with SARS-CoV-2 infected owners were collected by their owners during quarantine. The swabs were safely collected by the owners who had medical training (veterinarians for cats #1, #2, #3, and a human anaesthetist for cat #4).
All samples were refrigerated (2-8°C) until the owners left the isolation period and brought the samples to the laboratory themselves (swabs were not older than 3 days after sampling).
Cat #1 is Siamese two years old female, Cat #2 is British Shorthair two years and 8 months old female, Cat #3 is British Shorthair 1 year and 2 months old female, and Cat #4 is European shorthair two years old female.
All cats were lethargic, with moderate loss of appetite. Cat #1 was slightly dyspnoeic, but with no radiologic findings (Figure 1). For cat #1 basic biochemical and haematological assays were performed, but there no changes were found.
The cats lived indoors, in apartments, with no outside access. The owners of cat #4 were two doses-vaccinated, asymptomatic, and PCR-positive. The owners of the cats #1, #2, and #3 were two doses-vaccinated, with a mild flu-like syndrome, and PCR-positive. No owner needed hospitalization.

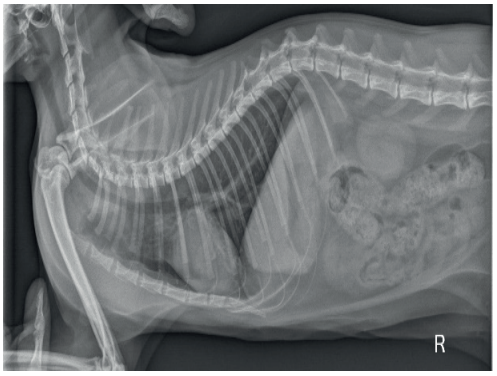

Figure 1. No pulmonary X-ray abnormalities (left, latero-lateral incidence) in a SARS-CoV-2 positive cat with mild respiratory syndrome. Cat #1 (Siamese breed, 2 years old, female).

rRT-PCR
For this analysis, it was used the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic-Panel (https://www.fda.gov/media/134922/download) Briefly, this test method use oligo-nucleotide primers (2019-nCoV_N1-F; 2019-nCoV_N1-R; 2019-nCoV_N2-F; and 2019-nCoV_N2-R;) and probes (2019-nCoV_N1-P label FAM, BHQ-1; and2019-nCoV_N2-P label FAM, BHQ-1) which detect the SARS-CoV-2 nucleocapsid (N) protein gene

(www.fda.gov/media/134922/download). The primers and probes were supplied by Integrated DNA Technologies (IDT, USA). The extraction of the ARN was made manually using QIAamp cador Pathogen Mini Kit (Qiagen, USA), following the manufacturer's extraction protocol. The amplification of the DNA was accomplished using qScript XLT One-Step RT-qPCR Tough Mix (Quantabio, USA) and a Light Cycler 2.0 analyser.

RESULTS AND DISCUSSIONS

The nucleotide-chain sequence for SARS-CoV-2 was identified and amplified in oropharyngeal swabs from three cats (#1, #2, and #3) and the Cat #4 sample was negative.
The Ct values of Cat #1 and Cat #2 were 27.04 (Figure 2) and 24.13 (Figure 3), which represents a medium-low amount of viral genome in the analysed samples.

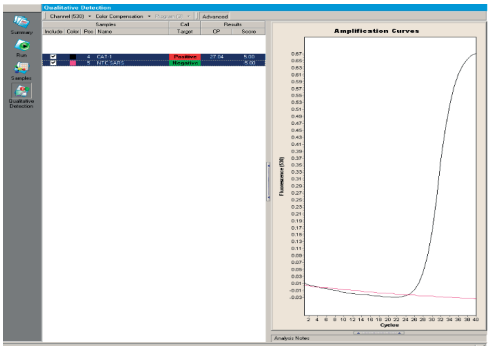

Figure 2. Amplification curve of rRT-PCR: oropharyngeal swab from Cat #1 (Siamese breed, 2 years old, female): Ct=27.07

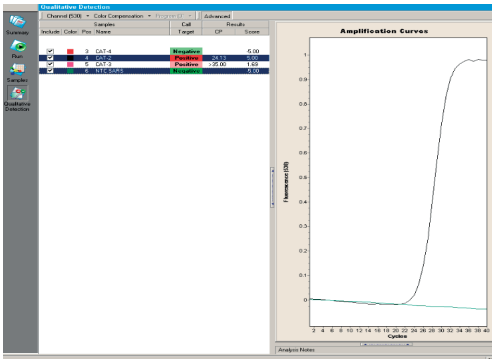

Figure 3. Amplification curve of rRT-PCR: oropharyngeal swab from cat #2 (British Shorthair breed, 2 years and 8 months old, female): Ct=24.13

A Ct=35.0 (Figure 4) value was found in the sample from cat #3 that can be related to a very small amount of virus in the extracted swab.
For the cat #4 no SARS-CoV-2 genome was detected in the received sample, meaning that the qPCR result was negative (Figure 5).

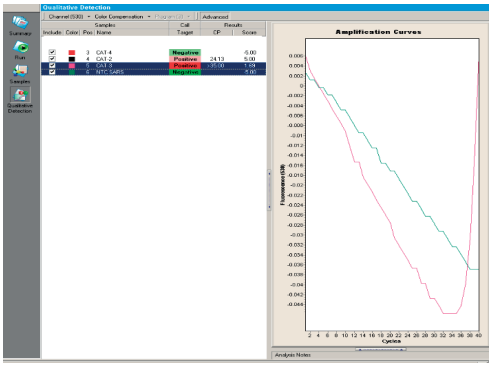

Figure 4. Amplification curve of rRT-PCR: oropharyngeal swab from cat #3 (British Shorthair breed, 1 year and 2 months old, female): Ct=35.0

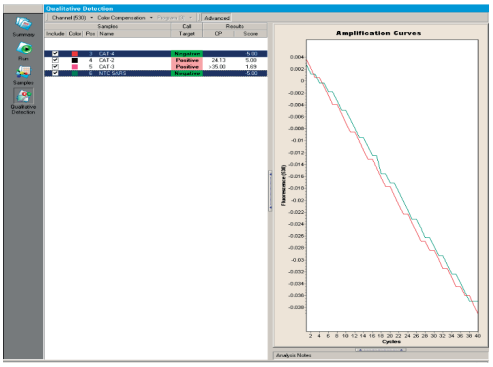

Figure 5. Amplification curve of rRT-PCR: oropharyngeal swab from cat #4 (European shorthair breed, 2 years old, female): Ct>40

The rRT-PCR used in our study proved to be a very useful, quick, and accurate diagnostic tool for an infection with the new Coronavirus, which, at least for the animals included in our study, has no characteristic symptoms. Also, the amount of sample required by this assay is very small and a properly sampled swab is sufficient to extract the required RNA.
Our results support the previous reports of cats and dogs' natural infections with SARS-CoV-2 in households with confirmed COVID-19 human subjects (Musso et al., 2020; Segalés et al., 2020; Hamer et al., 2021; Hosie et al.,

2021a; Klaus et al., 2021; Tewari et al., 2021) and demonstrate that cohabitation of a SARS-CoV-2-positive humans with a cat can lead to transmission of the virus to the cat. However, the role that cats play in COVID-19 epidemiology should be determined in further research studies because the relationship between humans, animals, and the environment is complex and dependent (Hernández et al., 2020).

The possible susceptibility of the cat to SARS-CoV-2 was considered because Martina et al. (2003) demonstrated the ability of SARS-CoV to infect ferrets and cats (Martina et al., 2003) and by first reports of Chen et al. (2020), Halfmann et al. (2020), and Shi et al. (2020) made early in the COVID-19 pandemic.

Moreover, Zhang et al. (2020) study provided serological evidence for SARS-CoV-2 infection in pets with the main recommendation to investigate the route of transmission of SARS-CoV-2 from humans to cats (Zhang et al., 2020).

In our study, only the cats living with the symptomatic owners provided positive results, possibly due to a higher amount of virus spread by owners with clinical signs, which increased the risk of contamination of these cats (statement also supported by the fact that the negative cat belongs to the infected owners without clinical signs).

Our data support the recommendation of Zhang et al. (2020) to implement preventive measures to maintain a suitable distance between owners with COVID-19 and companion cats (Zhang et al., 2020).

Spada et al. (2021) surveyed stray colony and shelter cats from the Lombardy region in pre- and during pandemic SARS-CoV-2 infection and obtained negative molecular results and very low seroprevalence. These results indicate that the likelihood of cats that do not live in close contact with humans (free-ranging stray or shelter cats) becoming infected is very low, as well as their role of them in the transmission of SARS-CoV-2 during the pandemic (Spada et al., 2021). However, cats living in households where people are infected with SARS-CoV-2 may act as vectors and close contact with these cats should be avoided, especially if owners showed signs of COVID-19 (Hosie et al., 2021b). Therefore, veterinarians should pay special attention to contact with cats and other pets that require medical care during the quarantine period of owners with COVID-19.

CONCLUSIONS

The risk of indoor cats' infection with SARS-CoV-2 in households where owners have COVID-19 is high, especially if they show clinical signs. In infection with SARS-CoV-2, cats can become lethargic, lose their appetite and be slightly dyspnoeic. In our study the cats recovered without treatment. Further research on the epidemiology of COVID-19 to establish the role that companion animals play in SARS-CoV-2 spreading should be carried out.

REFERENCES

Baraitareanu, S. (2020). Coronavirus infections: a brief review. *Revista Romana de Medicina Veterinara*, *30*(1).71–76.

Barrs, V.R., Peiris, M., Tam, K., Law, P., Brackman, C. J., To, E., Yu, V., Chu, D., Perera, R., & Sit, T. (2020). SARS-CoV-2 in quarantined domestic cats from COVID-19 households or close contacts, Hong Kong, China. *Emerging infectious diseases, 26*(12), 3071–3074.

Braun, K.M., Moreno, G.K., Halfmann, P.J., Hodcroft, E.B., Baker, D.A., Boehm, E.C., Weiler, A.M., Haj, A.K., Hatta, M., Chiba, S., Maemura, T., Kawaoka, Y., Koelle, K., O'Connor, D.H., & Friedrich, T.H. (2021). Transmission of SARS-CoV-2 in domestic cats imposes a narrow bottleneck. *PLOS Pathogens, 17*(2). e1009373.

Chen, J., Huang, C., Zhang, Y., Zhang, S., & Jin, M. (2020). Severe acute respiratory syndrome coronavirus 2-specific antibodies in pets in Wuhan, China. *The Journal of infection, 81*(3). e68–e69.

de Groot, R.J., Baker, S.C., Baric, R.S., Brown, C.S., Drosten, C., Enjuanes, L., Fouchier, R.A., Galiano, M., Gorbalenya, A.E., Memish, Z.A., Perlman, S., Poon, L.L., Snijder, E.J., Stephens, G.M., Woo, P.C., Zaki, A.M., Zambon, M., & Ziebuhr, J. (2013). Middle East respiratory syndrome coronavirus (MERS-CoV): announcement of the Coronavirus Study Group. *Journal of virology*, *87*(14). 7790–7792.

Garigliany, M., Van Laere, A. S., Clercx, C., Giet, D., Escriou, N., Huon, C., van der Werf, S., Eloit, M., & Desmecht, D. (2020). SARS-CoV-2 Natural transmission from human to cat, Belgium, March 2020. *Emerging infectious diseases*, *26*(12). 3069–3071.

Gaudreaulta, N.N., Trujilloa, J.D., Carossinob, M., Meekinsa, D.A., Morozova, I., Maddena, D.W., Indrana, S.V., Bolda, D., Balaramana, V., Kwona, T., Artiagaa, B.L., Coola, K., García-Sastrec, A., Maa, W., Wilsong, W.C., Henningsona, J., Balasuriyab,

U.B.R., & Richt, J.A. (2020). SARS-CoV-2 infection, disease and transmission in domestic cat. *Emerging Microbes & Infections, 9*(1). 2322-2332.

Halfmann, P.J., Hatta, M., Chiba, S., Maemura, T., Fan, S., Takeda, M., Kinoshita, N., Hattori, S. I., Sakai-Tagawa, Y., Iwatsuki-Horimoto, K., Imai, M., & Kawaoka, Y. (2020). Transmission of SARS-CoV-2 in domestic cats. *The New England journal of medicine, 383*(6). 592–594.

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. (2021). 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. *Viruses*, *13*(5). 938.

Hernández, M., Abad, D., Eiros, J.M., & Rodríguez-Lázaro, D. (2020). Are animals a neglected transmission route of SARS-CoV-2? *Pathogens, 9*(6). 480.

Hosie, M.J., Epifano, I., Herder, V., Richard, J. Orton, R.J., Stevenson, A., Johnson, N., MacDonald, E., Dunbar, D., McDonald, M., Howie, F., Tennant, B., Herrity, D., Da Silva Filipe, A., Streicker, D.G., the COVID-19 Genomics UK (COG-UK) consortium#, Willett, B.J., Murcia, P.R., Jarrett, R.F., Robertson, D.L., & Weir, W. (2021a). Detection of SARS-CoV-2 in respiratory samples from cats in the UK associated with human-to-cat transmission. *Veterinary Record 188*(8). e247.

Hosie, M.J., Hofmann-Lehmann, R., Hartmann, K., Egberink, H., Truyen, U., Addie, D. D., Belák, S., Boucraut-Baralon, C., Frymus, T., Lloret, A., Lutz, H., Marsilio, F., Pennisi, M. G., Tasker, S., Thiry, E., & Möstl, K. (2021b). Anthropogenic infection of cats during the 2020 COVID-19 pandemic. *Viruses*, *13*(2). 185.

Klaus, J., Meli, M.L., Willi, B., Nadeau, S., Beisel, C., Stadler, T., ETH SARS-CoV-2 Sequencing Team, Egberink, H., Zhao, S., Lutz, H., Riond, B., Rösinger, N., Stalder, H., Renzullo, S., & Hofmann-Lehmann, R. (2021). Detection and genome sequencing of SARS-CoV-2 in a domestic cat with respiratory signs in Switzerland. *Viruses, 13*(3). 496.

Lee, J.S., Goldstein, J.M., Moon, J.L., Herzegh, O., Bagarozzi, D.A., Jr, Oberste, M.S., Hughes, H., Bedi, K., Gerard, D., Cameron, B., Benton, C., Chida, A., Ahmad, A., Petway, D.J., Jr, Tang, X., Sulaiman, N., Teklu, D., Batra, D., Howard, D., Sheth, M., Kuhnert W., Bialek S.R., Hutson C.L., Pohl J., & Carroll, D.S. (2021). Analysis of the initial lot of the CDC 2019-Novel Coronavirus (2019-nCoV) real-time RT-PCR diagnostic panel. *PloS one*, *16*(12). e0260487.

Likhacheva, A. (2006). SARS revisited. *The virtual mentor: VM*, *8*(4). 219–222.

Martina, B.E., Haagmans, B.L., Kuiken, T., Fouchier, R.A., Rimmelzwaan, G.F., Van Amerongen, G., Peiris, J.S., Lim, W., & Osterhaus, A.D. (2003). Virology: SARS virus infection of cats and ferrets. *Nature*, *425*(6961). 915.

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*(4), 772.

Musso, N., Costantino, A., La Spina, S., Finocchiaro, A., Andronico, F., Stracquadanio, S., Liotta, L., Visalli, R., & Emmanuele, G. (2020). New SARS-CoV-2 infection detected in an Italian pet cat by RT-qPCR from deep pharyngeal swab. *Pathogens (Basel, Switzerland), 9*(9). 746.

Newman, A., Smith, D., Ghai, R. R., Wallace, R.M., Torchetti, M.K., Loiacono, C., Murrell, L.S., Carpenter, A., Moroff, S., Rooney, J.A., & Barton Behravesh, C. (2020). First reported cases of SARS-CoV-2 infection in companion animals - New York, March-April 2020. *MMWR. Morbidity and mortality weekly report*, *69*(23), 710–713.

Rahbari, R., Moradi, N., & Abdi, M. (2021). rRT-PCR for SARS-CoV-2: Analytical considerations. *Clinica Chimica Acta; International Journal of Clinical Chemistry*, *516*. 1–7.

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*(2), 973–976.

Sailleau, C., Dumarest, M., Vanhomwegen, J., Delaplace, M., Caro, V., Kwasiborski, A., Hourdel, V., Chevaillier, P., Barbarino, A., Comtet, L., Pourquier, P., Klonjkowski, B., Manuguerra, J. C., Zientara, S., & Le Poder, S. (2020). First detection and genome sequencing of SARS-CoV-2 in an infected cat in France. *Transboundary and emerging diseases, 67*(6), 2324–2328.

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*(40). 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., & Bu, Z. (2020). Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. *Science (New York, N.Y.)*, *368*(6494). 1016–1020.

Spada, E., Vitale, F., Bruno, F., Castelli, G., Reale, S., Perego, R., Baggiani, L., & Proverbio, D. (2021). A pre- and during pandemic survey of SARS-CoV-2 infection in stray colony and shelter cats from a high endemic area of northern Italy. *Viruses, 13*(4). 618.

Tewari D., Boger L., Brady S., Livengood J., Killian M.L., Surendran Nair M., Thirumalapura N., Kuchipudi S.V., Zellers C., Schroder B., Torchetti M., Carpenter A., Kunkel A., & Brightbill K. (2021). Transmission of SARS-CoV-2 from humans to a 16-

year-old domestic cat with comorbidities in Pennsylvania, USA. *Veterinary Medicine Science* 10.1002/vms3.695. Advance online publication. 1–8.

Turcu, C., Pastiu, A.I., Cocostirc, V., Lucaci, F., & Pusta, D.L. (2021). COVID-19 - a brief review on diagnosis, treatment and vaccination. *Revista Romana De Medicina Veterinara*, *31*(1). 81–86.

Wang, M.Y., Zhao, R., Gao, L.J., Gao, X.F., Wang, D.P., & Cao, J.M. (2020). SARS-CoV-2: structure, biology, and structure-based therapeutics development. *Frontiers in cellular and infection microbiology, 10.* 587269.

Xiao, K., Zhai, J., Feng, Y., Zhou, N., Zhang, X., Zou, J.J., Li, N., Guo, Y., Li, X., Shen, X., Zhang, Z., Shu, F., Huang, W., Li, Y., Zhang, Z., Chen, R.A., Wu, Y.J., Peng, S.M., Huang, S.M., Xie, W.J., Cai, Q.H., Hou, F.H., Chen, W., Xiao, L., & Shen, Y. (2020). Isolation of SARS-CoV-2-related coronavirus from Malayan pangolins. *Nature, 583*. 286–289.

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*(1). 2013–2019.