Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Veterinary Microbiology 260 (2021) 109179

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

First detection and molecular analysis of SARS-CoV-2 from a naturally infected cat from Argentina

Nadia Analía Fuentealba [a,c], Gastón Moré [b,c], María Emilia Bravi [a,c], Juan Manuel Unzaga [b], Lorena De Felice [b], Marcos Salina [a], Mariana Viegas [c,e], Mercedes Soledad Nabaes Jodar [c,e], Laura Elena Valinotto [c,f], Fernando David Rivero [d], David Di Lullo [d], Marcelo Pecoraro [a], Carlos Javier Panei [a,c,*]

[a] Laboratorio de Virología, Facultad de Ciencias Veterinarias (FCV), Universidad Nacional de La Plata (UNLP), 60 & 118, La Plata, Buenos Aires, Argentina
[b] Laboratorio de Inmunoparasitología, FCV-UNLP, 60 & 118, La Plata, Buenos Aires, Argentina
[c] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina
[d] Instituto Multidisciplinario de Salud, Tecnología y Desarrollo (IMSaTeD), CONICET-UNSE, Santiago del Estero, Argentina
[e] Laboratorio de Virología, Hospital de Niños Ricardo Gutiérrez, Buenos Aires, Argentina
[f] Centro de investigaciones en Genodermatosis y Epidermólisis Ampollar (CIDEGEA), Hospital de Niños Dr. Ricardo Gutiérrez / Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina

ARTICLE INFO

Keywords:
SARS-CoV-2
COVID-19
Coronavirus
Pets
Cats
Transmission

ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the etiological agent of coronavirus disease 2019 (COVID-19), has rapidly spread worldwide. Studies of transmission of the virus carried out in animals have suggested that certain animals may be susceptible to infection with SARS-CoV-2. The aim of the present study was to investigate the infection of SARS-CoV-2 in pets (18 cats and 20 dogs) from owners previously confirmed as COVID-19-positive. Oropharyngeal and rectal swabs were taken and analyzed by real-time RT-PCR assays, while blood samples were taken for antibody detection. Of the total pets analyzed, one cat was found reactive to SARS-CoV-2 by real-time RT-PCR of an oropharyngeal and a rectal swab. This cat presented only sneezing as a clinical sign. Serological analysis confirmed the presence of antibodies in the serum sample from this cat, as well as in the serum from another cat non-reactive to real-time RT-PCR. Complete sequence and phylogenetic analysis allowed determining that the SARS-CoV-2 genome belonged to the B.1.499 lineage. This lineage has been reported in different provinces of Argentina, mainly in the Metropolitan Area of Buenos Aires. This study notifies the first detection of the natural infection and molecular analysis of SARS-CoV-2 in a cat from Argentina whose owner where COVID-19-positive. Although there is currently no evidence that cats can spread COVID-19, results suggest that health authorities should test pets with COVID-19-positive owners.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the etiological agent of coronavirus disease 2019 (COVID-19), has rapidly spread worldwide. Although the available data suggest that SARS-CoV-2 emerged from an animal source, there is currently not enough evidence to corroborate either the source or route of transmission from the original animal reservoir to a putative intermediate host and then to humans. Thus, several studies are being carried out to gain insights into the susceptibility of different animal species, including domestic animals, to SARS-CoV-2. Coronaviruses (CoVs) belong to the order *Nidovirales* and the family *Coronaviridae*, which is further divided into the subfamilies *Letovirinae* and *Orthocoronavirinae*. The members of the subfamily *Orthocoronavirinae* are classified into four genera: *Alpha*, *Beta*, *Gamma* and *Delta coronavirus* according to their antigenic properties and phylogenetic relationship (https://talk.ictvonline.org/). The CoVs, have a single-stranded positive-sense RNA genome of 27−31-Kb. About two thirds of its genome code for the replicase gene, which consists of two overlapping open reading frames (*ORFs*), denominated *ORF 1a* and *1b*, while other four *ORFs* code for the structural proteins of the virus. These

* Corresponding author at: Laboratorio de Virología, Facultad de Ciencias Veterinarias (FCV), Universidad Nacional de La Plata (UNLP), 60 & 118, La Plata, Buenos Aires, Argentina.
E-mail address: javierpanei@fcv.unlp.edu.ar (C.J. Panei).

https://doi.org/10.1016/j.vetmic.2021.109179
Received 13 May 2021; Accepted 1 July 2021
Available online 8 July 2021
0378-1135/

proteins are the spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins, necessary to complete the replication cycle of the virus. The S protein mediates viral binding to specific cell receptors and fusion between the envelope and the plasma membrane, and is the main inducer of neutralizing antibodies (Brian and Baric, 2005). The ability of CoVs to produce mutations in their genome facilitates the transmission from animals to humans and vice versa (Woo et al., 2009). These events could occur due to the accumulation of point mutations in different regions of the genome, and due to homologous recombination events between closely related genes of different circulating CoVs lineages in multiple host species, which have given rise to different viral strains (Graham and Baric, 2010; Ghosh and Malik, 2020; Terada et al., 2014; Vijgen et al., 2005, 2006). According to previous studies, SARS-CoV-2 is believed to have originated in bats (Zhou et al., 2020), similarly to that occurred with SARS-CoV and MERS-CoV, two other zoonotic coronaviruses reported in 2003 and 2012, respectively (Gautam et al., 2020). Since the species barrier jump from bats to humans is considered unlikely, the most probable hypothesis includes the existence of an intermediate host (Andersen et al., 2020). This highlights the importance of animals in the emergence of COVID-19 in China, which further emphasizes the need of a One-Health approach to tackle emerging diseases. Although there is currently no evidence that pets play a substantial role in spreading SARS-CoV-2, World Organization of Health (WHO) advises persons with suspected or confirmed COVID-19 to restrict contact with them during their illness (https://www.cdc.gov/coronavirus/2019-ncov/animals/pets-other-animals.html). The existence of hundreds of millions of companion animals living close to humans raises the question of their susceptibility to infection. A small number of animals worldwide, including dogs, cats, zoo tigers, zoo lions, ferrets and mink, have been reported as naturally infected with SARS-CoV-2 (OIE report: https://www.oie.int/en/scientific-expertise/specific-information-and-recommendations/questions-and-answers-on-2019novel-coronavirus/events-in-animals/; McAloose et al., 2020; Newman et al., 2020; Oreshkova et al., 2020; Sailleau et al., 2020; Segalés et al., 2020; Sit et al., 2020). Animals can be exposed to the virus by contact with SARS-CoV-2 infected humans, which can in turn result in reverse zoonosis. In addition, under experimental conditions, ferrets, minks and cats have been shown to be able to transmit the infection to animals of the same species (Shi et al., 2020; Ulrich et al., 2020; Hossain et al., 2021; Schlottau et al., 2020). Also, a high prevalence of SARS-CoV-2 antibodies has been reported in pets from COVID-19-positive owners (Fritz et al., 2020; Zhang et al., 2020). However, the extent of natural infections of animals with SARS-CoV-2 is still largely unknown. Thus, the aim of this study was to detect SARS-CoV-2 infection in cats and dogs living with SARS-CoV-2-positive owners.

2. Materials and methods

2.1. Samples

A total of 18 cats and 20 dogs were sampled for SARS-CoV-2 detection by real-time RT-PCR, from May to September 2020. Oropharyngeal (OP) and rectal (R) swabs were taken from each animal and resuspended in 1 mL of phosphate buffer solution (PBS). The age of the animals ranged from 18 months to 12 years old. The samples were taken at their homes, all of which were located in the Metropolitan Area of Buenos Aires (AMBA), Argentina. All the animals were in close contact with their COVID-19-positive owners. Most of the animals selected in this study had no clinical signs; however, some of them presented signs such as anorexia, lethargy, diarrhea, cough and sneezing.

To monitor the animals with doubtful or reactive SARS-CoV-2 by real-time RT-PCR in the first set of samples, a second set of OP and R swab samples was taken. These samples were processed as described below.

To analyze the presence of antibodies against SARS-CoV-2, a 3−5-ml blood sample was then collected from four cats and three dogs (cases in which it was possible to access their homes again), without anticoagulant for serum extraction at least 15 days after the first swab sampling.

All samples were obtained and conditioned according to the guide for the detection of SARS-CoV-2 in animals (https://www.argentina.gob.ar/sites/default/files/covid-19-guia-para-deteccion-sars-cov-2-en-animales.pdf). OP and R swab samples were stored at −80 °C, whereas serum samples were stored at -20 °C, until further analysis.

Each owner gave their written consent to allow the collection of samples from their pets, and all the protocols were approved by the Animal Care and Use Institutional Committee (CICUAL) from the Faculty of Veterinary Sciences of the Universidad Nacional de La Plata, Buenos Aires, Argentina, under the protocol code 105-4−20 P.

2.2. SARS-CoV-2 real-time RT-PCR detection

For molecular SARS-CoV-2 detection by real-time RT-PCR, the OP and R samples were thawed and vortexed for 10 s. Total RNA from each swab was extracted from 140 µL of supernatant, using the PURO Virus RNA kit (PB-L Productos Bio-Lógicos, Argentina), according to the manufacturer's instructions, and resuspended with a final elution volume of 50 µL. Along with each extraction routine, a process control sample was added (including only the kit solutions and ultrapure water as a sample) and used as control in each PCR. All the process was performed under a biosafety cabinet II. Finally, 5 µL of eluted RNA was used for each of two different real-time RT-PCR kits for SARS-CoV-2 detection. The kits used were DisCoVery SARS-CoV-2 RT-PCR detection kit Cy5 (AP-Biotech, Argentina; Safecare Biotech Hangzhou Co. Ltd., China) and GeneFinder™ COVID-19 Plus RealAmp kit (OSANG Healthcare, South Korea), according to the manufacturer's instructions. Both kits provide the reaction mix (containing all reagents for reverse transcription and amplification), the primer and probe mix, and the positive and negative controls. Briefly, the real-time RT-PCR reaction allows the amplification and detection of an internal control and two fragments of the viral target (*ORF1ab* and *N* gene) with the DisCoVery kit and three gene fragments or viral targets (*RdRp*, *E* and *N* genes) with the GeneFinder™ kit in a final volume of 20 and 25 µl, respectively. The reactions were carried out using the MIC qCycler equipment with the respective analysis software (Bio Molecular Systems, Australia). Each real-time RT-PCR routine was carried out with an extraction control (which should react in an equivalent way to a no template control). The remaining RNA samples were stored in 1.5-ml microtubes at -70 °C.

2.3. Next-generation sequencing of SARS-CoV-2 and phylogenetic analysis

For the sequencing of samples found positive by real-time RT-PCR, the following two-step strategy was used:

1- Specific in-house designed amplicons developed by the Artic Network group (University of Birmingham, UK) (Josh Quick. nCoV-2019 sequencing protocol. 2020. protocols.io. dx.doi.org/10.17504/protocols.io.bbmuik6w).

Complementary DNA (cDNA) was obtained from previously extracted RNA as mentioned above. This strategy allowed the entire genome to be generated from only 11 µL of RNA. cDNA was amplified with the Q5™ Hot Start High-Fidelity DNA Polymerase (New England BioLabs). Each sample was separately subjected to two amplification reactions in multiplex format with 48 pairs of primers each. In this way, fragments of approximately 400 bp (+/- 40 bp) were generated, covering the entire viral genome.

The Illumina Nextera Flex DNA Library Prep Kit was then used for library preparation. Briefly, the protocol consists of an enzymatic fragmentation which also adds the Illumina sequencing adapters (tagging in beads) corresponding to the selected equipment. Then, the indexes were added through a low number of PCR cycles.

For the quality control, fragments were analyzed by the Fragment Analyzer kit (Agilent, Santa Clara, CA, USA) and fluorometry

quantifications were performed with the Qubit kit (Thermo Fisher Scientific, Waltham, MA, USA). For the sequencing process, a NextSeq500 platform with a NextSeq 500/550 Mid Output Kit v2.5 cartridges (300 cycles) was used.

2- Mapping-Alignment and complete genome sequencing obtaining.

The resulting reads were aligned with the BWA-MEM software to the SARS-CoV-2 reference genome with the ID EPI_ISL_402124 (GISAID), hCoV-19/Wuhan/WIV04/2019. Finally, the consensus sequence for each sample analyzed was generated in FASTA format with the help of the pileup command of the samtools software (Li et al., 2009) and the consensus command of bcftools (Li, 2011). To validate this consensus sequence, a second method, the *de novo* assembly with the MEGAHIT software (Li et al., 2015), was used to ensure that there was coincidence with the one previously obtained. Finally, a visual verification was performed using the IGV software.

2.4. Phylogenetic analysis

Regarding the viral data processing and analysis, the resulting reads of the sequencing process were aligned with the BWA-MEM software to the SARS-CoV-2 reference genome with the ID EPI_ISL_402124, and the consensus sequence for the sample was generated in FASTA format with a mean read depth of 4000X, covering around 94.01 % of the full-length genome (sequence data have been deposited in GISAID with accession number: EPI_ISL_1914577)

For the analysis of the cat sequence, a rapid lineage assignment was carried out using the Pangolin COVID-19 Lineage Assigner program (https://pangolin.cog-uk.io/). This tool allows determining the lineage through identification of complex patterns by using machine learning.

The lineage assigned was confirmed by phylogenetic analysis. The cat sequence was analyzed together with reference sequences from different lineages (https://github.com/hCoV-2019/pangolin) and sequences with the best alignment score by BLAST (10 hits per unknown sequence, against the GISAID database as of April 20th 2021, Supplementary data). The alignments were built with the MAFFT program (https://mafft.cbrc.jp/alignment/server/) with default parameters. The appropriate evolutionary model was selected with ModelFinder (Kalyaanamoorthy et al., 2017) according to Bayesian Information criteria, and the phylogenetic analysis was performed by Maximum Likelihood with the IQ program -TREE v. 2.1.2 COVID-edition (Minh et al., 2020). The reliability of the groups obtained was evaluated by the SH-like approximate likelihood ratio test (1000 replicates) (Guindon et al., 2010) and Ultrafast bootstrap Approximation (10,000 replicates) (Hoang et al., 2018). The SH-like / UFB values for the relevant groups are indicated in the tree branches.

2.5. Detail of non-synonymous substitutions (amino acid changes) in the proteins encoded in the cat SARS-CoV-2 genome

To determine the non-synonymous substitutions in comparison with the reference sequence hCoV-19/Wuhan/WIV04/2019, the CoV surver tool was used and corroborated by visual inspection. The changes were compared with those reported in SARS-CoV-2 sequences worldwide using the GISAID COV-GLUE tool (Singer et al., 2020) and the outbreak.info tool.

2.6. Serological analysis

The serum samples collected from the cats and dogs chosen for the present study were used to determine the presence of antibodies against SARS-CoV-2. Antibody detection were carried out at the Facultad de Farmacia y Bioquimica, Universidad Nacional de Buenos Aires, under the service modality using an ELISA 2.0 kit, developed by researchers from NANOBIOTEC and the LIE group (Chair of Immunology-IDEHU). The kit detects the specific antibodies against the S protein of SARS-CoV-2 in serum samples from different species.

3. Results

3.1. SARS-CoV-2 detection

The RNA extraction kit used includes an RNA carrier which allows the monitoring of the process by amplification of the internal control in the real-time RT-PCR in all samples. One out of the 18 cats analyzed in this study was detected as reactive by real-time RT-PCR from the OP and R swab samples. The *ORF1ab* and *N* genes were detected from the OP swab sample with a cycle threshold (Ct) of 35 and 30 respectively, while the *N* gene was detected from the R swab with a Ct 37.5, using the Cy5 DisCoVery SARS-CoV-2 RT-PCR detection kit. The results were then analyzed with a second kit of real-time RT-PCR, the GeneFinder™ COVID-19 Plus RealAmp kit. The results obtained showed the amplification for the three viral targets, *Rdrp*, *N* and *E* genes, from OP samples with a Ct of 34.5, 31.5 and 36.5 respectively. Therefore, both kits amplified the OP sample, while the R sample was not reactive with the second kit.

The SARS-CoV-2-reactive cat was a 1.5-year-old female of mixed breed, living in a household in La Plata city, Buenos Aires, Argentina. The animal presented only sneezing for about 3 days after close contact with its COVID-19-positive owners. One of the owners developed symptoms on August 23rd 2020 and was then diagnosed COVID-19-positive by real-time-RT-PCR two days later, on August 25th, whereas the other owner was confirmed as COVID-19-positive on August 28th 2020. Both owners were in close contact with the animal during the course of the disease. The samples were taken from the cat on September 5th, 2020.

To determine how long, the cat remained reactive to SARS-CoV-2 by the molecular assay, additional OP and R swab samples were taken 6 days after the first samples. In this case, the presence of the genome of the virus was not detected by either real-time RT-PCR kit.

None of the dog samples analyzed in this study were found reactive to SARS-CoV-2 by real-time PCR with either kit. In addition, none of the dogs showed clinical signs, but some owners mentioned that their dogs were lethargic.

3.2. Next-generation sequencing of SARS-CoV-2 and phylogenetic analysis

The phylogenetic analysis determined that the cat SARS-CoV-2 genome identified belonged to the B.1.499 lineage. To study the genetic relationship of the cat sequence with sequences of the same lineage, a phylogenetic tree of the lineage B.1.499 was constructed (Fig. 1). In this phylogenetic tree, the cat sequence (blue) is included in a moderately supported cluster with sequences from Buenos Aires City (orange) and sequences from the Province of Buenos Aires (red) detected from June 2020 to December 2020. As compared with the Wuhan reference sequence, and based on 94.01 % of the genome covered, the SARS-CoV-2 cat sequence here found showed seventeen nucleotide mutations (Table 1).

3.3. Serological analysis

Serum samples from all the animals studied in the present study were taken at least 15 days after the first swab sampling, except the sample from the cat reactive to SARS-CoV-2 by molecular assay, which was taken 35 days after the first swab sample. The serological analysis by ELISA of the serum sample from this cat showed a strong positive signal against protein S of SARS-CoV-2. The serum sample from another cat also showed antibodies against SARS-CoV-2, indicating that the animal was also infected with the virus. The other two cats analyzed were negative by this technique.

The serum sample of the cat positive by real-time RT-PCR gave a positivity index (PI) value of 101.79. These values, clearly above the standard cut-off criteria (PI $\geq$ 10), revealed the presence of specific

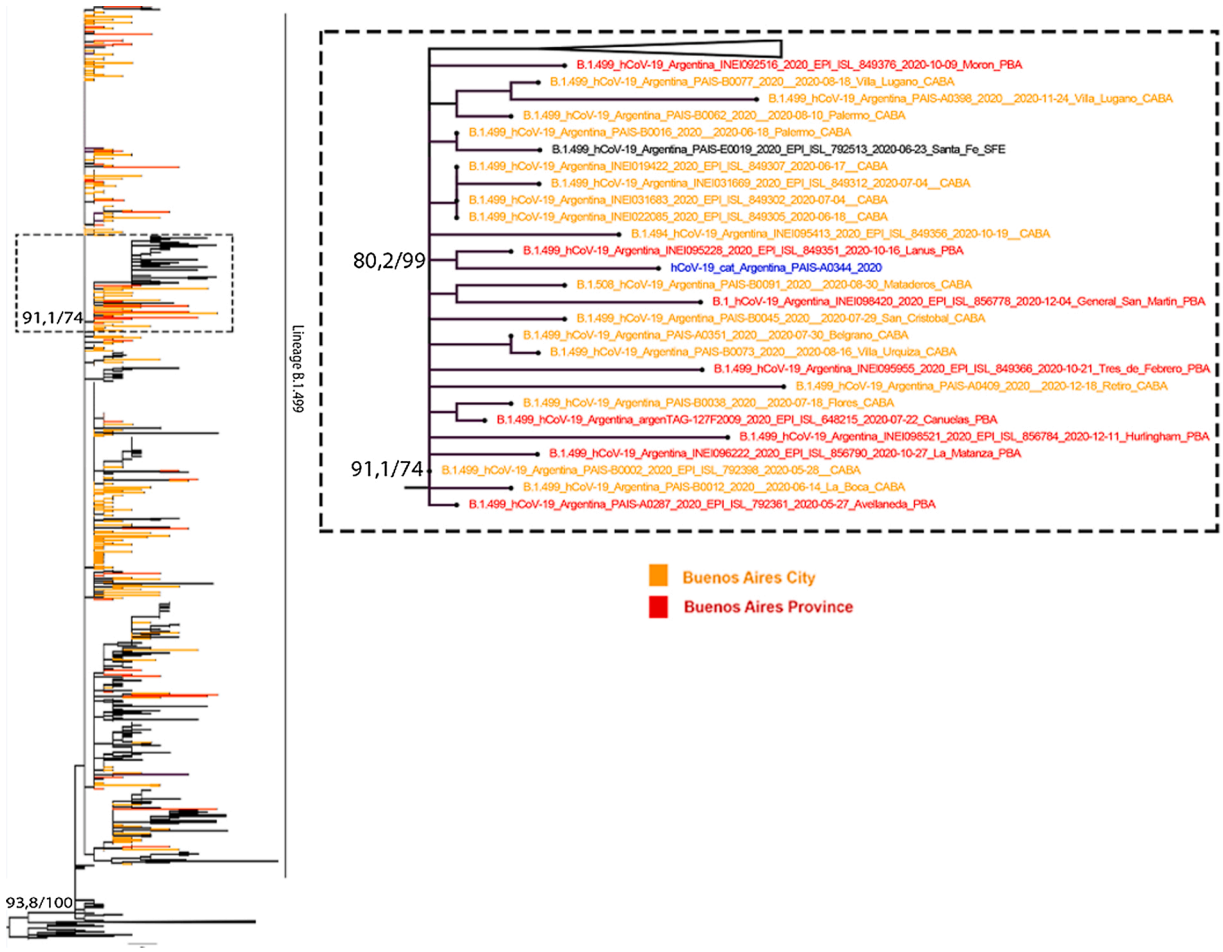

Fig. 1. Phylogenetic tree of the SARS-CoV-2 lineage B.1.499. Cat sequence in blue, sequences from Buenos Aires City in orange and sequences from the Province of Buenos Aires in red (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 1
Mutationes in the genome of SARS-CoV-2 detected from the reactive cat.

Protein	SNP	aa Replacement
nsp2	C1059T	T85I
nsp2	A2304G	K500R
nsp2	T2308C	SYN
nsp3	C3037T	SYN
nsp3	C6525T	T1269I
nsp4	T8570A	W6R
nsp7	C11916T	S25L
nsp12	C14408T	P323L
nsp12	C15960T	SYN
nsp14	C18929T	P297L
nsp14	C18998T	A320V
S	A23403G	D614G
S	G23522C	E654Q
ORF 3a	G25563T	Q57H
M	C26586T	SYN
N	C28863T	S197L
ORF 10	G29540A	SYN

Non-structural proteins (nsp); Open reading frame (ORF); Amino acid (aa); Single Nucleotide Polymorphism (SNP).

antibodies against SARS-CoV-2. This result, combined with the positive real-time RT-PCR data obtained 35 days before serology, strongly suggests that SARS-CoV-2 actively replicated in this animal, inducing a robust anti-spike-protein antibody response. The other cat detected as positive by ELISA showed PI values of 21.07.

In all dog sera analyzed, no presence of specific antibodies against SARS-CoV-2 was detected by ELISA.

4. Discussion

At the beginning of September 2020, the Faculty of Veterinary Sciences of the National University of La Plata, Buenos Aires, Argentina, reported to the local health authorities the first case of a domestic cat infected with SARS-CoV-2, detected by real-time RT-PCR. This result was reported to the World Organization for Animal Health (OIE), being the first case of SARS-CoV-2 detected in an animal from Argentina. Different species, including domestic and wild animals, naturally infected with this novel coronavirus have been reported around the world (https://www.oie.int/en/scientific-expertise/specific-information-and-recommendations/questions-and-answers-on-2019novel-coronavirus/events-in-animals/). Moreover, experimental studies have demonstrated that cats are susceptible to SARS-CoV-2 infection and able to transmit the virus among them (Halfmann et al., 2020; Shi et al., 2020). The confirmed positive cat detected in this study was a 1.5-year--old female of mixed breed. The first samples (OP and R swabs) were taken on September 5th 2020, 11 days after the first positive diagnosis of its owners. Although the exact moment of infection cannot be determined, the animal was detected as reactive to SARS-CoV-2 in these samples, with Ct values over 30, which could indicate a moderate viral load. Nevertheless, it also suggests that a potential replicative virus was present. Similar results have been previously found in two cats in Spain (Segalés et al., 2020). In the present study, a second set of OP and R samples was taken from the cat on September 11th, and, in this case, the real-time RT-PCR results were not reactive in either swab. These results

suggest a rapid clearance of the virus from the respiratory and intestinal tracts, which is consistent with experimental infection of cats, where the presence of viral RNA was detected until 5–8 days post-infection (Shi et al., 2020). The viral RNA expressed in the nasal turbinate, trachea, lung, and small intestine indicated that the virus replicated well in the upper respiratory tract of those cats. Viral RNA has also been detected in tonsils, lymph nodes, spleen, bone marrow, liver, kidney, heart, and olfactory bulb (Gaudreault et al., 2020; Shi et al., 2020). In natural infection of cats, it has been reported that the viral RNA may persist for about 10–11 days in nasal swabs (Barrs et al., 2020; Garigliany et al., 2020; Sailleau et al., 2020).

Although the SARS-CoV-2-positive cat studied in the present study did not show complications of its health during the course of the infection, it was clinically monitored until the real-time RT-PCR was not-reactive. Similarly, experimental studies have shown no clinical signs in any of the inoculated cats (Gaudreault et al., 2020; Shi et al., 2020). Moreover, other authors have described cases of natural infection that never showed clinical signs compatible with SARS-CoV-2 infection (Barrs et al., 2020; Segalés et al., 2020). Thus, infected cats with no symptoms could act as a silent intermediate host of SARS-CoV-2. However, in a case of natural infection reported in Belgium, the cat presented clinical signs, such as lethargy, anorexia, loss of appetite, vomiting, and diarrhea, and later showed sneezing, productive cough, difficult breathing, and emaciation (Garigliany et al., 2020).

The phylogenetic analysis here performed allowed determining that the cat SARS-CoV-2 genome belonged to the B.1.499 lineage. This lineage, fully Argentine and initially detected in March 2020, has been reported in different provinces of the country, mainly in the region of the Metropolitan Area of Buenos Aires. In the analysis of genetic relationship with sequences of the same lineage, the cat sequence was included in the cluster with sequences from Buenos Aires City and sequences from the Province of Buenos Aires. This cluster included different cities of Buenos Aires province, all of which are located close to the city of La Plata, where the cat sample was obtained. Furthermore, within this cluster, the cat sequence formed a highly supported subcluster with a sequence that was detected in October 2020 in Lanús, a city within the Metropolitan Area of Buenos Aires. These two cities are connected by a large flow of people, a fact that supports this finding. When compared to the Wuhan reference sequence (Wu et al., 2020) and based on 94.01 % of the genome covered, the SARS-CoV-2 cat sequence showed seventeen nucleotide mutations. Twelve out of these seventeen mutations have resulted in amino acid mutations, which have already been described in other sequences worldwide, except for the NSP2_K500R and NSP4_W6R combination, which was here observed for the first time and has not been previously reported. In particular, for the Spike region, the two changes observed (S_D614 G and S_ E654Q) have already been reported in other sequences of the B.1.499 lineage from Argentina.

In this study, serological analysis by ELISA showed a strong positive signal against SARS-CoV-2 for the serum sample taken from the cat found reactive by real-time RT-PCR, 35 days (October 16th) after detection. The OD values, clearly above the standard cut-off criteria (PI $\geq$ 10), revealed the presence of specific antibodies against protein S of SARS-CoV-2. These serological results strongly suggests that SARS-CoV-2 actively replicated in this animal. The same has been observed by other authors (Sailleau et al., 2020), who confirmed the natural infection of a cat in France and detected the presence of specific antibodies against SARS-CoV-2 eight days after the beginning of signs. In addition, in the present study, a cat that was not reactive by real-time RT-PCR showed specific antibody responses against SARS-CoV-2 by ELISA, indicating that the animal was infected by the virus. The cat began with clinical signs of lethargy and cough 4 days after the positive diagnosis of one of its owners, and the samples were taken 17 days after the SARS-CoV-2 reactive real-time RT-PCR of its owners. The negative results for the detection of viral RNA may have been due to the rapid clearance of the virus from the respiratory and intestinal tracts, as reported by other authors (Barrs et al., 2020; Garigliany et al., 2020; Sailleau et al., 2020; Shi et al., 2020).

5. Conclusion

Studies suggest that the SARS-CoV-2 pandemic outbreak involves a diversified host range. Although there is no evidence that domestic animals play a role in the onward transmission of the virus, the testing of pets with COVID-19-positive owners is suggested. The evidence suggests that human-to-animal transmission of SARS-CoV-2 can occur, and it is thus recommended that infected owners should avoid close contact with their pets. However, more studies will be needed to see whether cats and other domestic animals with co-morbidities may show a more severe clinical course of SARS-CoV-2 infection.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgments

We thank all the owners of the animals here studied, who, despite having COVID-19, allowed us the possibility of taking samples from their pets to carry out this study. We also thank Dr. Dolores Oliva Delosa and her team from the Municipal Zoonosis Department of the city of La Plata, and Dr. Gustavo Martinez and his team from the Urban Zoonoses Department of the Ministry of Health of the Province of Buenos Aires.

This study was funded by the Agencia Nacional de Promoción a la Investigación, Desarrollo Tecnológico e Innovación Productiva (I+D+i), Argentina, Project 444-468.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetmic.2021.109179.

References

Andersen, K.G., Rambaut, A., Lipkin, W.I., Holmes, E.C., Garry, R.F., 2020. The proximal origin of SARS-CoV-2. Nat. Med. 26, 450–452. https://doi.org/10.1038/s41591-020-0820-9.

Barrs, V.R., Peiris, M., Tam, K.W.S., Law, P.Y.T., Brackman, C.J., To, E.M.W., Yu, V.Y.T., Chu, D.K.W., Perera, R.A.P.M., Sit, T.H.C., 2020. SARS-CoV-2 in quarantined domestic cats from COVID-19 households or close contacts, Hong Kong, China. Emerg. Infect. Dis. 26, 3071–3074. https://doi.org/10.3201/eid2612.202786.

Brian, D.A., Baric, R.S., 2005. Coronavirus genome structure and replication. In: Enjuanes, L. (Ed.), Coronavirus Replication and Reverse Genetics. Current Topics in Microbiology and Immunology. Springer, Berlin, Heidelberg, pp. 1–30. https://doi.org/10.1007/3-540-26765-4_1.

Fritz, M., Rosolen, B., Krafft, E., Becquart, P., Elguero, E., Vratskikh, O., Denolly, S., Boson, B., Vanhomwegen, J., Gouilh, M., Kodjo, A., Chirouze, C., Rosolen, S., Legros, V., Leroy, E.M., 2020. High prevalence of SARS-CoV-2 antibodies in pets from COVID-19+ households. One Health 11. https://doi.org/10.1016/j.onehlt.2020.100192.

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. Emerging Infect. Dis. 26, 3069–3071. https://doi.org/10.3201/eid2612.202223.

Gaudreault, N.N., Trujillo, J.D., Carossino, M., Meekins, D.A., Morozov, I., Madden, D. W., Indran, S.V., Bold, D., Balaraman, V., Kwon, T., Artiaga, B.L., Cool, K., García-Sastre, A., Ma, W., Wilson, W.C., Henningson, J., Balasuriya, U., Richt, J.A., 2020. SARS-CoV-2 infection, disease and transmission in domestic cats. Emerg. Microbes Infect. 9, 2322–2332. https://doi.org/10.1080/22221751.2020.18336.

Gautam, A., Kaphle, K., Shrestha, B., Phuyal, S., 2020. Susceptibility to SARS, MERS, and COVID-19 from animal health perspective. Open Vet. J. 10, 164–177. https://doi.org/10.4314/ovj.v10i2.6.

Ghosh, S., Malik, Y.S., 2020. Drawing comparisons between SARS-CoV-2 and the animal coronaviruses. Microorganisms 8, 1840. https://doi.org/10.3390/microorganisms8111840.

Graham, R.L., Baric, R.S., 2010. Recombination, reservoirs, and the modular spike: mechanisms of coronavirus cross-species transmission. J. Virol. 84, 3134–3146. https://doi.org/10.1128/JVI.01394-09.

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. 59, 307–321. https://doi.org/10.1093/sysbio/syq010.

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. N. Engl. J. Med. 383, 592–594. https://doi.org/10.1056/NEJMc2013400.

Hoang, D.T., Chernomor, O., von Haeseler, A., Minh, B.Q., Vinh, L.S., 2018. UFBoot2: improving the ultrafast bootstrap approximation. Mol. Biol. Evol. 35, 518–522. https://doi.org/10.1093/molbev/msx281.

Hossain, M.G., Javed, A., Akter, S., Saha, S., 2021. SARS-CoV-2 host diversity: an update of natural infections and experimental evidence. J. Microbiol. Immunol. Infect. 54 (2), 175–181. https://doi.org/10.1016/j.jmii.2020.06.0066.

Kalyaanamoorthy, S., Minh, B., Wong, T., Von Haeseler, A., Jermiin, L.S., 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat. Methods 14, 587–589. https://doi.org/10.1038/nmeth.4285.

Li, H., 2011. A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. Bioinformatics (Oxford, England) 27, 2987–2993. https://doi.org/10.1093/bioinformatics/btr509.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., 2009. 1000 genome project data processing subgroup. The sequence alignment/map format and SAMtools. Bioinformatics (Oxford, England). 25, 2078–2079. https://doi.org/10.1093/bioinformatics/btp352.

Li, D., Liu, C.M., Luo, R., Sadakane, K., Lam, T.W., 2015. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics (Oxford, England). 31, 1674–1676. https://doi.org/10.1093/bioinformatics/btv033.

McAloose, D., Laverack, M., Wang, L., Killian, M.L., Caserta, L.C., Yuan, F., Mitchell, P.K., Queen, K., Mauldin, M.R., Cronk, B.D., Bartlett, S.L., Sykes, J.M., Zec, S., Stokol, T., Ingerman, K., Delaney, M.A., Fredrickson, R., Ivančić, M., Jenkins-Moore, M., Mozingo, K., Diel, D.G., 2020. From people to panthera: natural SARS-CoV-2 infection in tigers and lions at the Bronx Zoo. mBio 11. https://doi.org/10.1128/mBio.02220-20 e02220-20.

Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., von Haeseler, A., Lanfear, R., 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Mol. Biol. Evol. 37, 1530–1534. https://doi.org/10.1093/molbev/msaa015.

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 Morb. Mortal. Wkly. Rep. 69, 710–713. https://doi.org/10.15585/mmwr.mm6923e3.

Oreshkova, N., Molenaar, R.J., Vreman, S., Harders, F., Oude Munnink, B.B., Hakze-van der Honing, R.W., Gerhards, N., Tolsma, P., Bouwstra, R., Sikkema, R.S., Tacken, M. G., de Rooij, M.M., Weesendorp, E., Engelsma, M.Y., Bruschke, C.J., Smit, L.A., Koopmans, M., van der Poel, W.H., Stegeman, A., 2020. SARS-CoV-2 infection in farmed minks, the Netherlands, April and May 2020. Euro surveillance: bulletin Europeen sur les maladies transmissibles = Eur. Commun. Dis. Bull. 25, 2001–2005. https://doi.org/10.2807/1560-7917.ES.2020.25.23.2001005.

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. Transbound. Emerg. Dis. 67 (6), 2324–2328. https://doi.org/10.1111/tbed.13659.

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. Lancet Microbe 1, e218–e225. https://doi.org/10.1016/S2666-5247(20)30089-6.

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. Proc. Natl. Acad. Sci. U. S. A. 117, 24790–24793. https://doi.org/10.1073/pnas.2010817117.

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., et al., 2020. Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. Science (New York, N.Y.). 368, 1016–1020. https://doi.org/10.1126/science.abb7015.

Singer, J., Gifford, R., Cotten, M., Robertson, D., 2020. CoV-GLUE: a web application for tracking SARS-CoV-2 genomic variation. Preprints. https://doi.org/10.20944/preprints202006.0225.v1, 2020060225.

Sit, T., Brackman, C.J., Ip, S.M., Tam, K., Law, P., To, E., Yu, V., Sims, L.D., Tsang, D., Chu, D., Perera, R., Poon, L., Peiris, M., 2020. Infection of dogs with SARS-CoV-2. Nature 586, 776–778. https://doi.org/10.1038/s41586-020-2334-5.

Terada, Y., Matsui, N., Noguchi, K., Kuwata, R., Shimoda, H., Soma, T., Mochizuki, M., Maeda, K., 2014. Emergence of pathogenic coronaviruses in cats by homologous recombination between feline and canine coronaviruses. PLoS One 9. https://doi.org/10.1371/journal.pone.0106534 e106534.

Ulrich, L., Wernike, K., Hoffmann, D., Mettenleiter, T.C., Beer, M., 2020. Experimental infection of cattle with SARS-CoV-2. Emerging Infect. Dis. 26, 2979–2981. https://doi.org/10.3201/eid2612.203799.

Vijgen, L., Keyaerts, E., Moës, E., Thoelen, I., Wollants, E., Lemey, P., Vandamme, A.M., Van Ranst, M., 2005. Complete genomic sequence of human coronavirus OC43: molecular clock analysis suggests a relatively recent zoonotic coronavirus transmission event. J. Virol. 79, 1595–1604. https://doi.org/10.1128/JVI.79.3.1595-1604.2005.

Vijgen, L., Keyaerts, E., Lemey, P., Maes, P., Van Reeth, K., Nauwynck, H., Pensaert, M., Van Ranst, M., 2006. Evolutionary history of the closely related group 2 coronaviruses: porcine hemagglutinating encephalomyelitis virus, bovine coronavirus, and human coronavirus OC43. J. Virol. 80, 7270–7274. https://doi.org/10.1128/JVI.02675-05.

Woo, P.C., Lau, S.K., Huang, Y., Yuen, K.Y., 2009. Coronavirus diversity, phylogeny and interspecies jumping. Exp. Biol. Med. (Maywood, N.J.). 234, 1117–1127. https://doi.org/10.3181/0903-MR-94.

Wu, F., Zhao, S., Yu, B., Chen, Y.M., Wang, W., Song, Z.G., Hu, Y., Tao, Z.W., Tian, J.H., Pei, Y.Y., Yuan, M.L., Zhang, Y.L., Dai, F.H., Liu, Y., Wang, Q.M., Zheng, J.J., Xu, L., Holmes, E.C., Zhang, Y.Z., 2020. A new coronavirus associated with human respiratory disease in China. Nature 579, 265–269. https://doi.org/10.1038/s41586-020-2008-3.

Zhang, Q., Zhang, H., Huang, K., Yang, Y., Hui, X., Gao, J., Jin, M., 2020. SARS-CoV-2 Neutralizing Serum Antibodies in Cats: A Serological Investigation. bioRxiv. https://doi.org/10.1101/2020.04.01.021196.

Zhou, P., Yang, X.L., Wang, X.G., Hu, B., Zhang, L., Zhang, W., Si, H.R., Zhu, Y., Li, B., Huang, C.L., Chen, H.D., Chen, J., Luo, Y., Guo, H., Jiang, R.D., Liu, M.Q., Chen, Y., Shen, X.R., Wang, X., Zheng, X.S., et al., 2020. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579, 270–273. https://doi.org/10.1038/s41586-020-2012-7.