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1 # New detection of SARS-CoV-2 in two cats height months after COVID-19
2 # outbreak appearance in France
3
4 ## New detection of SARS-CoV-2 in two cats in France

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31 **Abstract**

32 Although there are several reports in the literature of SARS-CoV-2 infection in cats, few SARS-CoV-
33 2 sequences from infected cats have been published. In this report, SARS-CoV-2 infection was
34 evaluated in two cats by clinical observation, molecular biology (qPCR and NGS), and serology
35 (Microsphere immunoassay and seroneutralization). Following the observation of symptomatic SARS-
36 CoV-2-infection in two cats, infection status was confirmed by RT-qPCR and, in one cat, serological
37 analysis for antibodies against N-protein and S-protein, as well as neutralizing antibodies.
38 Comparative analysis of five SARS-CoV-2 sequence-fragments obtained from one of the cats showed
39 that this infection was not with one of the three recently emerged variants of SARS-CoV-2. This study
40 provides additional information on the clinical, molecular, and serological aspects of SARS-CoV-2
41 infection in cats.

42 **Keywords:** Cats, mild-respiratory symptoms, NGS, One Health, SARS-CoV-2, Serology

43

44 **Introduction**

45 SARS-CoV-2 has shown relatively generalist capacities by infecting many animal species, making it a
46 good-model in One Health research (MacLean et al., 2021). Indeed, SARS-CoV-2 infections have
47 been detected in numerous animal species living in close contact with infected humans. Based on
48 RNA detection, serological studies, and experimental infections, numerous animal species have
49 proven susceptibility to SARS-CoV-2 (Shi et al., 2020), including *Mustelidae* (ferret, mink) (Oude
50 Munnink et al., 2020), *Canidae* (dog) (Sit et al., 2020), *Felidae* (cat, tiger, lion) (Sailleau et al., 2020)
51 and *Cricetidae* (hamster, rat, mouse) (Sia et al., 2020) as well as by the highly transmissible British
52 variant (B.1.1.7) (Ferasin et al., 2021) . On March 24th, the World Organisation for Animal Health
53 reports cases of SARS-CoV-2 infections in cats in 17 countries (USA, China, Belgium, Germany,
54 Spain, France, Russia, United-Kingdom, Japan, Italy, Chile, Brazil, Greece, Canada, Argentina,
55 Switzerland, and Latvia). In the medical and scientific literature, we found 23 papers examining
56 natural SARS-CoV-2 infection in a total of 2242 cats (Barrs et al., 2020; Carlos et al., 2021; Chen,
57 Huang, Zhang, Zhang, & Jin, 2020; Deng et al., 2020; Ferasin et al., 2021; Fritz et al., 2021;
58 Garigliany et al., 2020; Hamer et al., 2020; Hosie et al., 2020; Klaus et al., 2021; Michelitsch,

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59 Hoffmann, Wernike, & Beer, 2020; Musso et al., 2020; Neira et al., 2020; Newman et al., 2020;
60 Pagani et al., 2021; Patterson et al., 2020; Ruiz-Arrondo et al., 2020; Sailleau et al., 2020; Segalés et
61 al., 2020; Stevanovic et al., 2020; Temmam et al., 2020; Villanueva-Saz et al., 2021; Zhang et al.,
62 2020). Among these cats, only 94 were positive for ongoing or previous SARS-CoV-2 infection, as
63 detected by qPCR or by serology. Additionally, the U.S Department of Agriculture has reported 67
64 cases of SARS-CoV-2 infection in cats identified by at least one of these assays. Viral RNA detected
65 in 25 cats suggested that COVID-19 infections were transmitted from infected owners. These few
66 sequences are available (31 on Global Initiative on Sharing All Influenza Data (Gisaid) and 18 on
67 Genbank) (https://www.gisaid.org/ ; https://www.ncbi.nlm.nih.gov/genbank/). While most cases of
68 SARS- CoV-2 infection in cats were asymptomatic, some cats (14/94) experienced lethargy, mild
69 respiratory or digestive symptoms (sneezing, coughing, ocular discharge, vomiting, and anorexia), and
70 two studies reported severe respiratory problems in two cats (Garigliany et al., 2020; Musso et al.,
71 2020). Recently, a study has shown association between B.1.1.7 infection and clinical signs of
72 myocarditis in cats (Ferasin et al., 2021). Moreover, several experimental studies have shown that
73 SARS-CoV-2 can be transmitted between cats (Bosco-Lauth et al., 2020; Gaudreault et al., 2020;
74 Halfmann et al., 2020). As yet, there is no evidence of cat-to-human transmission. Here we present a
75 clinical and biological investigation of SARS-CoV-2 infection of two cats that presented with mildly
76 symptomatic disease, and that came from households with confirmed cases of COVID-19 sampled
77 during the second wave (October-November 2020) of infections in France.
78
79 **Materials and methods**
80 **First line diagnostic: Veterinary diagnostic laboratory (VEBIO)**
81 **RNA extraction**
82 RNA extraction from nasopharyngeal and rectal swabs was done using QIAamp Viral RNA Mini Kit
83 (QIAGEN). Swabs were resuspended in 200µl of ATL + 20µl of proteinase K then heated at 70°C for
84 10 min. 200µl of ATL + 20µl of proteinase K was then added, and again heated to 70°C. Finally,
85 200µl of ethanol was added and the entire volume (640µl) transferred to a column. Subsequent steps
86 proceeded according to the manufacturer's protocol.

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87 **Real-time reverse-transcription PCR**

88 Viral RNA was quantified using real the Genesig® Real Time PCR Coronavirus COVID-19 (CE IVD)
89 kit (Primer Design) using the manufacturer's protocol and QuantStudio 5 Real-Time PCR System
90 thermocycler (Thermo Fisher Scientific)

91 **Second-line diagnostic: Virology laboratory of Caen Hospital**

92 **RNA extraction and Removal of genomic DNA**

93 Nasopharyngeal and rectal swabs were resuspended in 500µl of PBS, of which 140µl was used for
94 RNA extraction using the EZ1 RNA Tissue Mini kit (QIAGEN). The homogenate was then treated
95 with the Turbo DNA-free kit (Thermo Fisher Scientific), according to the manufacturer's protocol, in
96 order to remove genomic DNA.

97 **Ribosomal RNA Depletion and Real-time reverse-transcription PCR**

98 Critical to enable cost-effective sequencing of RNA samples, we depleted the eukaryotic rRNA using
99 the NEBNext rRNA Depletion Kit (Human/Mouse/Rat). After rRNA depletion, double-stranded
100 cDNA was synthesized by real-time reverse-transcription (RT-PCR) using Superscript III platinium
101 One-step Quantitative RT-PCR System (Invitrogen) as described previously with minor modifications
102 (Corman et al., 2020). Briefly, a 25 µL reaction contained 5 µL of RNA, 12.5 µL of 2 × reaction
103 buffer, 1 µL of reverse transcriptase/ Taq mixture, 0.4 µL of a 50 mM magnesium sulphate solution
104 (Invitrogen), 1µl of a 10µM primer, and 0.5l µl of a 10µM probe. Thermal cycling was performed at
105 50 °C for 15 min for reverse transcription, followed by 95 °C for 2 min and then 45 cycles of 95 °C
106 for 15 s and 60 °C for 30 s using a Light Cycler 480 (Roche).
107 To increase the amount of genetic material available for sequencing, whole transcriptome
108 amplification (WTA) was performed with QuantiTect Whole Transcriptome kit (QIAGEN) according
109 to the manufacturer's instructions. Amplified DNA reactions was then purified using AMPure XP
110 beads and quantified using Qbit dsDNA BR Assay Kit on a Qubit 3.0 fluorimeter (Invitrogen).
111 In complement to the *de novo* approach, we also used an adapted version of the published protocol
112 from the ARTIC Network using ARTIC (Quick, 2020) primer scheme version 3 which produces ~400
113 bp overlapping amplicons over the SARS-CoV-2 genome.

114 **ONT library preparation and MinION sequencing**

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115 Libraries were prepared without shearing to maximize sequencing read length. The Oxford Nanopore
116 Technology (ONT) protocol for native barcoding genomic DNA sequencing was followed using the
117 barcoded ligation sequencing kit SQK-LSK108 and the EXP-NBD104 Native Barcoding kit.
118 Sequencing libraries were constructed, and sequencing performed according to manufacturer's
119 instructions, as briefly described below. First, the NEBNext Ultra II End Repair/dATailing module
120 (E7546S, NEB, USA) was used to prepare 1000 ng DNA samples. End-prepared DNA was ligated
121 with native barcode adapters NBD04 using Blunt/TA Ligase Master Mix (M0367S, NEB, USA).
122 Following the barcode ligation reaction, the DNA was cleaned with AMPure XP beads. The two
123 samples were then pooled to produce a 54 µl equimass pool used for adapter ligation with the 'Native
124 Barcoding Adapter Mix (BAM). The final library was loaded onto an R9.4 flowcell (FLO-MIN106,
125 Oxford Nanopore Technologies, UK), and the run was performed on a MinION Mk1B device (ONT).

126 **Genome assembly**

127 Following the MinION run, reads generated were basecalled and subsequently demultiplexed using
128 Guppy GPU basecaller and barecoder (Oxford Nanopore Technologies). Reads were then mapped
129 against a custom reference of SARS-CoV-2 genome comprising four Chinese and 70 early French
130 sequences using Bowtie2 (Langmead & Salzberg, 2012) and minimap2 (Heng Li, 2018). Finally, a
131 consensus genome sequence based on mapped reads was generated with bcftools consensus (H. Li,
132 2011). SARS-CoV-2 sequences were deposited on GISAID (EPI_ISL_1328819;EPI_ISL_1328821;
133 EPI_ISL_1328824; EPI_ISL_1328826).

134 **Microsphere immunoassay (MIA)**

135 MIA was proceeded in Maladies Infectieuses et vecteurs: Ecologie, Génétique, Evolution et Contrôle
136 (MIVEGEC). Cat serum samples were tested units using a multiplex Microsphere immunoassay
137 (MIA). 10µg of three recombinant SARS-CoV-2 antigens: nucleoprotein (N), receptor-binding
138 domain (RBD), and trimeric spike (tri-S) were used to capture specific serum antibodies whereas a
139 human protein (O6-methylguanine DNA methyltransferase) was used as a control antigen in the assay.
140 Distinct MagPlex microsphere sets (Luminex Corp) were respectively coupled to viral antigens using
141 the amine coupling kit (Bio-Rad Laboratories) according to manufacturer's instructions. The MIA
142 procedure was performed as described previously (Fritz et al., 2021). Briefly, microsphere mixtures

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143 were successively incubated with serum samples (1:400) biotinylated protein A and biotinylated
144 protein G (4 µg/ml each) (Thermo Fisher Scientific), and Streptavidin-R-Phycoerythrin (4 µg/ml)
145 (Life technologies) on an orbital shaker and protected from the light. Measurements were performed
146 using a Magpix instrument (Luminex). To account for nonspecific binding of antibodies to beads,
147 Relative Fluorescence Intensities (RFI) were calculated for each sample by dividing the Mean
148 Fluorescence Intensity (MFI) signal measured for the antigen-coated microspheres by the MFI signal
149 obtained for the control microspheres. Specific seropositivity cut-off values for each antigen were set
150 at three standard deviations above the mean RFI of the 18 dogs and 14 cat serum samples sampled
151 before 2019. Based on a pre-pandemic population, MIA specificity was set at 100% for dogs and cats.

152 **Neutralization activity measurement**

153 Neutralization activity measurement was proceeded in Virus enveloppés, vecteurs et immunothérapie
154 (EVIR) team from CIRI. An MLV-based pseudoparticle carrying a GFP reporter pseudotyped with
155 SARS-CoV-2 spike protein (SARS-CoV-2pp) was used to measure the neutralizing antibody activity
156 in cat sera. Each SARS-CoV-2 positive sample detected by MIA was processed according to a
157 neutralization procedure as previously described (Legros et al., 2021). The level of infectivity is
158 expressed as the percentage of GFP-positive cells and compared to cells infected with SARS-CoV-2pp
159 incubated without serum. Pre-pandemic sera from France was used as negative controls, and anti-
160 SARS-CoV-2 RBD antibody was used as a positive control.

161 **Results**

162 Cat 1 was a solitary and sedentary 5-year-old female European whose only contact was with her
163 owner. Her last vaccination was 3 years prior and she was presented with no previous medical history.
164 On October 24th 2020, 10 days after her owner developed symptoms and tested positive for SARS-
165 CoV-2 infection, Cat 1 developed sneezing with non-purulent nasal secretions but no digestive trouble
166 or others notable symptoms. Five days later, on October 29, clinical examination of the cat showed
167 pink mucous membranes, a heart's rate of 175 bpm (110bpm-200bpm), a rectal temperature of 38.4°C
168 (38°C-39°C), slight dehydration, a urea seric concentration of 0.49g/l (0.4g/l-0.6g/l), and a creatinine
169 seric concentration of 12.7mg/l (10mg/l-20mg/l). A 10-days treatment of 1 Doxybactin tablet in the

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170 evening and a daily dose of Meloxoral taken over 3 days with the morning meal was then initiated. An
171 improvement of the symptoms was observed in this cat three days after the onset of the treatment.
172 Based on the owner's positive SARS-CoV-2 diagnosis and the cat's symptoms, a blood sample and
173 two oropharyngeal and rectal swabs were collected for further SARS-CoV-2 testing. Swabs were
174 submitted to VEBIO for RNA SARS-CoV-2 detection. The oropharyngeal swab tested positive by
175 RT-qPCR targeting the ORF1ab with a ct value of 20.41. No viral RNA was detected in the rectal
176 swabs. The virology laboratory of Caen Hospital confirmed the diagnosis following detection of viral
177 RNA in the second ororpharyngeal swab by RT-qPCR targeting gene E with a ct value of 21.43 (Table
178 1). We did not obtain a SARS-CoV-2 sequence due to the sample's poor conservation condition prior
179 to its arrival at the lab. To detect anti-SARS-CoV-2 IgG antibodies, the cat 1 serum was analysed by
180 the MIVEGEC and EVIR teams, using MIA and retrovirus-based pseudoparticle assay. Antibodies
181 against N, RBD, and tri-S SARS-CoV-2 proteins as well as neutralizing antibodies were detected,
182 confirming productive infection in cat 1 (Table 1).
183
184 Cat 2 was a 13-year-old male European with chronic rhinitis and living with two other cats. The cat's
185 owner, who had recently tested positive for SARS-CoV-2, reported an acute deterioration in his three
186 cats' general condition, without others details. A November 20th, 2020 clinical investigation reported
187 retro-mandibular adenopathy, and no other symptoms was observed. As with Cat 1, oropharyngeal and
188 rectal swabs were collected but with no blood sample. Similarly, VEBIO and Caen laboratories
189 detected viral RNA in both oropharyngeal swabs, with ct-values of 20.55 and 23.44, respectively
190 (Table 1). No viral RNA was detected in the rectal swabs. Again due to poor conservation of the
191 swabs prior to their arrival in our laboratory, only 5 partial fragments of the SARS-CoV-2 genome
192 could be obtained by high-throughput sequencing on RNA derived from the oropharyngeal swabs. In
193 fragment 2, we did not observe the 11288-11296 deletion characterizing 3 variants of concern (English
194 B1.1.7, South African B.1.351, and Brazilian P.1) (Table 2), indicating that this cat was not infected
195 by any of these three novel variants. Compared to the original Wuhan_Hu-1 reference sequence, we
196 observed, in fragment 4, only one single nucleotide polymorphism (SNP) -G25563T- leading to the
197 amino acid change Q57H. This H57 mutation is widely distributed around the world and was present

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198 in about 70% of the sequences reported in France between October and December 2020, according to
199 Nextstrain (https://nextstrain.org/).

200 **Discussion**

201 Here we report SARS-CoV-2 infection in two cats, diagnosed by molecular and serological assays,
202 and with mild clinical manifestations, including sneezing for one cat. Although we cannot definitively
203 rule out infection of the cats by an individual outside the household, the information given by
204 COVID19+ owners, including the exclusive and unique contact with its owner for cat 1 and the
205 general deterioration in the condition of all cats of the cat 2 owner, strongly suggests a transmission
206 from owners to cats.

207 Human-to-cat transmission is now widely reported worldwide, and while certainly not insignificant in
208 COVID19+ households, precise estimates of the frequency of such transmission has not been
209 established (Fritz et al., 2021; Hamer et al., 2020). To our knowledge, among the 23 papers
210 investigating possible SARS-CoV-2 infection in cats, only 14 reported PCR results confirming
211 infection in 27 cats. The present study is only the second showing molecular results of cat infection in
212 France, eight months after the first case report by Saillau *et al.* (Sailleau et al., 2020). It appears that
213 the majority of infections in cats are asymptomatic, but can, at times, lead to lethargy, mild respiratory
214 or digestive disease and, rarely, acute respiratory clinical signs (as observed in two cats). We report
215 here one case of mild respiratory symptoms (sneezing) in a cat, an observation consistent with
216 symptomatic infections sporadically observed in other studies. We did not observe any digestive
217 symptoms, as previously reported in a French cat. In accordance with other recent studies, our results
218 suggest that since the beginning of SARS-CoV-2 pandemic, almost one year after the first report of
219 infection in a cat, the pathology in cats has not changed globally, with only a relatively small
220 proportion of cases reported as a result of clinical investigation by a veterinarian. This low
221 pathogenicity can explain the paucity of studies reporting SARS-CoV-2 infection in cats in the
222 absence of a global pet detection policy.

223 The two cats in the study were sampled during the second wave of infection in France and at the
224 beginning of the ongoing emergence of multiple novel variants. Although we did not find evidence of
225 infection by one of the three novel variants in cat 2, the emergence of these new variants raises the

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226 question of potential changes in pathogenicity or transmissibility in domestic animals. This question
227 will become rapidly crucial in a very near future as the British variant, known to be much more
228 infectious, is currently removing the ancestral variant of SARS-CoV-2 in France as well in other
229 countries of Europe. Therefore, it is becoming more and more important to implement a One Health
230 approach to face SARS-CoV-2 epidemic that takes into account infection and viral circulation in pets.

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237 **Conflict of Interest statement**

238 None of the authors have any conflict of interest (financial or personal) in this study.

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246 **Data Availability Statement**

247 The data that support the findings of this study are available from the corresponding author upon
248 reasonable request.

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253 **Table1.** Characteristics of cats sampled during the second wave of SARS-CoV2 infections in France

Cat ID	Age	Gender	Severity of cat's symptoms	Nasopharyngeal swab	Rectal swab	Serology (MIA)	Serology (Seroneutralisation)
Cat 1	5	F	Mild	Positive	Negative	Positive	Positive
Cat 2	13	M	Mild	Positive	Negative	ND	ND

254
255 **Table 2**: Details of the five SARS-CoV2 genome fragments obtained from cat 2

Sequence Fragments	Position (length)	Gene	Mutation[†]	AA Change[†]	GISAID accession number
Fragment 1	3427-3623 (197)	ORF1a/NSP3	No	No	EPI_ISL_1328819
Fragment 2	11163-11484 (322)	ORF1a/NSP6	No	No	EPI_ISL_1328821
Fragment 3	18883-19002 (120)	ORF1ab	No	No	EPI_ISL_1328824
Fragment 4	25513-25667 (155)	ORF3a	G25563T	Q57H	EPI_ISL_1328826
Fragment 5	26769-26863 (95)	Membrane	No	No	Not available because <100pb

256
257 [†]as compared to the Wuhan_Hu-1 reference sequence
258
259 **References:**
260

261 Barrs, V., Peiris, M., Tam, K. W. S., Law, P. Y. T., Brackman, C., To, E. M. W., . . . Sit, T.
262 H. C. (2020). SARS-CoV-2 in Quarantined Domestic Cats from COVID-19
263 Households or Close Contacts, Hong Kong, China. *Emerging Infectious Disease*
264 *journal, 26*(12), 3071. doi:10.3201/eid2612.202786
265 Bosco-Lauth, A. M., Hartwig, A. E., Porter, S. M., Gordy, P. W., Nehring, M., Byas, A. D., . .
266 . Bowen, R. A. (2020). Experimental infection of domestic dogs and cats with SARS-
267 CoV-2: Pathogenesis, transmission, and response to reexposure in cats. *Proceedings of*
268 *the National Academy of Sciences, 117*(42), 26382-26388.
269 doi:10.1073/pnas.2013102117
270 Carlos, R. S. A., Mariano, A. P. M., Maciel, B. M., Gadelha, S. R., de Melo Silva, M.,
271 Belitardo, E. M. M. A., . . . Albuquerque, G. R. (2021). First genome sequencing of
272 SARS-CoV-2 recovered from an infected cat and its owner in Latin America.
273 *Transbound Emerg Dis, n/a*(n/a). doi:https://doi.org/10.1111/tbed.13984
274 Chen, J., Huang, C., Zhang, Y., Zhang, S., & Jin, M. (2020). Severe Acute Respiratory
275 Syndrome Coronavirus 2-Specific Antibodies in Pets in Wuhan, China. *J Infect, 81*(3),
276 e68-e69. doi:10.1016/j.jinf.2020.06.045
277 Corman, V. M., Landt, O., Kaiser, M., Molenkamp, R., Meijer, A., Chu, D. K., . . . Drosten,
278 C. (2020). Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR.
279 *Euro Surveill, 25*(3). doi:10.2807/1560-7917.Es.2020.25.3.2000045

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280 Deng, J., Jin, Y., Liu, Y., Sun, J., Hao, L., Bai, J., . . . Tian, K. (2020). Serological survey of
281 SARS-CoV-2 for experimental, domestic, companion and wild animals excludes
282 intermediate hosts of 35 different species of animals. *Transbound Emerg Dis, 67*(4),
283 1745-1749. doi:10.1111/tbed.13577
284 Ferasin, L., Fritz, M., Ferasin, H., Becquart, P., Legros, V., & Leroy, E. M. (2021).
285 Myocarditis in naturally infected pets with the British variant of COVID-19. *bioRxiv*,
286 2021.2003.2018.435945. doi:10.1101/2021.03.18.435945
287 Fritz, M., Rosolen, B., Krafft, E., Becquart, P., Elguero, E., Vratskikh, O., . . . Leroy, E. M.
288 (2021). High prevalence of SARS-CoV-2 antibodies in pets from COVID-19+
289 households. *One health (Amsterdam, Netherlands), 11*, 100192.
290 doi:10.1016/j.onehlt.2020.100192
291 Garigliany, M., Van Laere, A. S., Clercx, C., Giet, D., Escriou, N., Huon, C., . . . Desmecht,
292 D. (2020). SARS-CoV-2 Natural Transmission from Human to Cat, Belgium, March
293 2020. *Emerg Infect Dis, 26*(12), 3069-3071. doi:10.3201/eid2612.202223
294 Gaudreault, N. N., Trujillo, J. D., Carossino, M., Meekins, D. A., Morozov, I., Madden, D.
295 W., . . . Richt, J. A. (2020). SARS-CoV-2 infection, disease and transmission in
296 domestic cats. *Emerg Microbes Infect, 9*(1), 2322-2332.
297 doi:10.1080/22221751.2020.1833687
298 Halfmann, P. J., Hatta, M., Chiba, S., Maemura, T., Fan, S., Takeda, M., . . . Kawaoka, Y.
299 (2020). Transmission of SARS-CoV-2 in Domestic Cats. *N Engl J Med, 383*(6), 592-
300 594. doi:10.1056/NEJMc2013400
301 Hamer, S. A., Pauvolid-Corrêa, A., Zecca, I. B., Davila, E., Auckland, L. D., Roundy, C. M., .
302 . . Hamer, G. L. (2020). Natural SARS-CoV-2 infections, including virus isolation,
303 among serially tested cats and dogs in households with confirmed human COVID-19
304 cases in Texas, USA. *bioRxiv*, 2020.2012.2008.416339.
305 doi:10.1101/2020.12.08.416339
306 Hosie, M. J., Epifano, I., Herder, V., Orton, R. J., Stevenson, A., Johnson, N., . . . Weir, W.
307 (2020). Respiratory disease in cats associated with human-to-cat transmission of
308 SARS-CoV-2 in the UK. *bioRxiv*, 2020.2009.2023.309948.
309 doi:10.1101/2020.09.23.309948
310 Klaus, J., Meli, M. L., Willi, B., Nadeau, S., Beisel, C., Stadler, T., . . . Hofmann-Lehmann,
311 R. (2021). Detection and Genome Sequencing of SARS-CoV-2 in a Domestic Cat
312 with Respiratory Signs in Switzerland. *Viruses, 13*(3), 496. Retrieved from
313 https://www.mdpi.com/1999-4915/13/3/496
314 Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nat
315 Methods, 9*(4), 357-359. doi:10.1038/nmeth.1923
316 Legros, V., Denolly, S., Vogrig, M., Boson, B., Siret, E., Rigaill, J., . . . Pozzetto, B. (2021).
317 A longitudinal study of SARS-CoV-2-infected patients reveals a high correlation
318 between neutralizing antibodies and COVID-19 severity. *Cellular & Molecular
319 Immunology, 18*(2), 318-327. doi:10.1038/s41423-020-00588-2
320 Li, H. (2011). A statistical framework for SNP calling, mutation discovery, association
321 mapping and population genetical parameter estimation from sequencing data.
322 *Bioinformatics, 27*(21), 2987-2993. doi:10.1093/bioinformatics/btr509
323 Li, H. (2018). Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics,
324 34*(18), 3094-3100. doi:10.1093/bioinformatics/bty191
325 MacLean, O. A., Lytras, S., Weaver, S., Singer, J. B., Boni, M. F., Lemey, P., . . . Robertson,
326 D. L. (2021). Natural selection in the evolution of SARS-CoV-2 in bats created a
327 generalist virus and highly capable human pathogen. *PLOS Biology, 19*(3), e3001115.
328 doi:10.1371/journal.pbio.3001115

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329 Michelitsch, A., Hoffmann, D., Wernike, K., & Beer, M. (2020). Occurrence of Antibodies
330 against SARS-CoV-2 in the Domestic Cat Population of Germany. *Vaccines (Basel), 8*(4). doi:10.3390/vaccines8040772
332 Musso, N., Costantino, A., La Spina, S., Finocchiaro, A., Andronico, F., Stracquadanio, S., . . . 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. Retrieved from https://www.mdpi.com/2076-0817/9/9/746
336 Neira, V., Brito, B., Aguero, B., Berrios, F., Valdes, V., Gutierrez, A., . . . Medina, R. A. (2020). A household case evidences shorter shedding of SARS-CoV-2 in naturally infected cats compared to their human owners. *Emerg Microbes Infect*, 1-22. doi:10.1080/22221751.2020.1863132
340 Newman, A., Smith, D., Ghai, R. R., Wallace, R. M., Torchetti, M. K., Loiacono, C., . . . 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*(23), 710-713. doi:10.15585/mmwr.mm6923e3
344 Oude Munnink, B. B., Sikkema, R. S., Nieuwenhuijse, D. F., Molenaar, R. J., Munger, E., Molenkamp, R., . . . Koopmans, M. P. G. (2020). Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans. *Science*. doi:10.1126/science.abe5901
348 Pagani, G., Lai, A., Bergna, A., Rizzo, A., Stranieri, A., Giordano, A., . . . Zehender, G. (2021). Human-to-Cat SARS-CoV-2 Transmission: Case Report and Full-Genome Sequencing from an Infected Pet and Its Owner in Northern Italy. *Pathogens (Basel, Switzerland), 10*(2), 252. Retrieved from https://www.mdpi.com/2076-0817/10/2/252
352 Patterson, E. I., Elia, G., Grassi, A., Giordano, A., Desario, C., Medardo, M., . . . Decaro, N. (2020). Evidence of exposure to SARS-CoV-2 in cats and dogs from households in Italy. *Nature Communications, 11*(1), 6231. doi:10.1038/s41467-020-20097-0
355 Quick, J. (2020). nCoV-2019 sequencing protocol v3 (LoCost). *protocols.io*. Retrieved from https://protocols.io/view/ncov-2019-sequencing-protocol-v3-locost-bh42j8ye
357 Ruiz-Arrondo, I., Portillo, A., Palomar, A. M., Santibáñez, S., Santibáñez, P., Cervera, C., & Oteo, J. A. (2020). 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, n/a*(n/a). doi:https://doi.org/10.1111/tbed.13803
362 Sailleau, C., Dumarest, M., Vanhomwegen, J., Delaplace, M., Caro, V., Kwasiborski, A., . . . Le Poder, S. (2020). First detection and genome sequencing of SARS-CoV-2 in an infected cat in France. *Transbound Emerg Dis*. doi:10.1111/tbed.13659
365 Segalés, J., Puig, M., Rodon, J., Avila-Nieto, C., Carrillo, J., Cantero, G., . . . 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, 117*(40), 24790-24793. doi:10.1073/pnas.2010817117
369 Shi, J., Wen, Z., Zhong, G., Yang, H., Wang, C., Huang, B., . . . Bu, Z. (2020). Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. *Science, 368*(6494), 1016-1020. doi:10.1126/science.abb7015
372 Sia, S. F., Yan, L. M., Chin, A. W. H., Fung, K., Choy, K. T., Wong, A. Y. L., . . . Yen, H. L. (2020). Pathogenesis and transmission of SARS-CoV-2 in golden hamsters. *Nature, 583*(7818), 834-838. doi:10.1038/s41586-020-2342-5
375 Sit, T. H. C., Brackman, C. J., Ip, S. M., Tam, K. W. S., Law, P. Y. T., To, E. M. W., . . . Peiris, M. (2020). Infection of dogs with SARS-CoV-2. *Nature, 586*(7831), 776-778. doi:10.1038/s41586-020-2334-5

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378 Stevanovic, V., Vilibic-Cavlek, T., Tabain, I., Benvin, I., Kovac, S., Hruskar, Z., . . . Barbic,
379 L. (2020). Seroprevalence of SARS-CoV-2 infection among pet animals in Croatia
380 and potential public health impact. *Transbound Emerg Dis, n/a*(n/a).
381 doi:https://doi.org/10.1111/tbed.13924
382 Temmam, S., Barbarino, A., Maso, D., Behillil, S., Enouf, V., Huon, C., . . . Eloit, M. (2020).
383 Absence of SARS-CoV-2 infection in cats and dogs in close contact with a cluster of
384 COVID-19 patients in a veterinary campus. *One health (Amsterdam, Netherlands), 10*,
385 100164. doi:10.1016/j.onehlt.2020.100164
386 Villanueva-Saz, S., Giner, J., Tobajas, A. P., Pérez, M. D., González-Ramírez, A. M., Macías-
387 León, J., . . . Fernández, A. (2021). Serological evidence of SARS-CoV-2 and co-
388 infections in stray cats in Spain. *Transbound Emerg Dis, n/a*(n/a).
389 doi:https://doi.org/10.1111/tbed.14062
390 Zhang, Q., Zhang, H., Gao, J., Huang, K., Yang, Y., Hui, X., . . . Jin, M. (2020). A serological
391 survey of SARS-CoV-2 in cat in Wuhan. *Emerg Microbes Infect, 9*(1), 2013-2019.
392 doi:10.1080/22221751.2020.1817796

393