SARS-CoV-2 Natural Transmission from Human to Cat, Belgium, March 2020

Mutien Garigliany, Anne-Sophie Van Laere, Cécile Clercx, Didier Giet, Nicolas Escriou, Christèle Huon, Sylvie van der Werf, Marc Eloit, Daniel Desmecht

Author affiliations: University of Liège, Liège, Belgium (M. Garigliany, A.-S. Van Laere, C. Clercx, D. Giet, D. Desmecht); Institut Pasteur, Paris, France (N. Escriou, C. Huon, S. van der Werf, M. Eloit); Alfort National Veterinary School, Maisons Alfort, France (M. Eloit)

In March 2020, a severe respiratory syndrome developed in a cat, 1 week after its owner received positive test results for severe acute respiratory syndrome coronavirus 2. Viral RNA was detected in the cat's nasopharyngeal swab samples and vomitus or feces; immunoglobulin against the virus was found in convalescent-phase serum. Human-to-cat transmission is suspected.

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We report the investigation of illness and infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in a household cat in Belgium (*1*). The cat was a female domestic shorthair, ≈15 years of age, that had been adopted 2 years earlier. The owner considered the cat to have been healthy since adoption, although it had never been assessed by a veterinarian. In February 2020, the owner took part in a 7-day tour to a mountain resort in Lombardy, Italy. The day after returning home, March 2, the owner felt suddenly too short of breath to conduct normal activities. As a precautionary measure, the family doctor decided to take a deep oropharyngeal swab sample and asked the patient to remain at home until the test result was reported. Over the next 10 days, the patient experienced a series of general, respiratory, and then digestive symptoms consistent with the clinical signs associated with coronavirus disease (COVID-19) (Figure). On March 6, the swab sample was declared positive for the SARS-CoV-2 genome, and home quarantine was extended until the end of March.

During that time, the patient's household cat was asymptomatic (Video 1, https://wwwnc.cdc.gov/EID/article/26/12/20-2223-V1.htm). However, 1 week later, the cat suddenly demonstrated clinical signs; the cat was found prostrated and vomiting in her litter, then showed pronounced lethargy, poor appetite to anorexia, vomiting, and diarrhea (Figure). Several days later, the clinical signs worsened. The cat demonstrated sneezing (Video 2, https://wwwnc.cdc.gov/EID/article/26/12/20-2223-V2.htm; Video 3, https://wwwnc.cdc.gov/EID/article/26/12/20-2223-V3.htm); a harsh, productive cough several times a day; episodes of paroxysmal reverse sneezing (Video 4 https://wwwnc.cdc.gov/EID/article/26/12/20-2223-V4.htm; Video 5, https://wwwnc.cdc.gov/EID/article/26/12/20-2223-V5.htm); labored breathing with increased respiratory effort and frequency; and emaciation (Video 6, https://wwwnc.cdc.gov/EID/article/26/12/20-2223-V6.htm). The clinical impression at this time was that of a restrictive breathing pattern suggestive of substantial involvement of parenchyma, pleura, or both. The cat's condition then gradually improved; she became less lethargic, vomiting stopped, feces resumed normal consistency, episodes of cough became less frequent, and appetite quickly improved. The cat recovered completely within <2 weeks.

A series of laboratory analyses were then conducted (Appendix, https://wwwnc.cdc.gov/EID/article/26/12/20-2223-App1.pdf). The cat's owner collected 26 swab samples according to instructions received by telephone; 16 samples contained varying amounts of the SARS-CoV-2 genome (Table). Overall, positive samples were detected March 11–24. The cat was examined by veterinarians at the time of blood sampling on day 22 after onset of first symptoms. Clinical examination of the cat was unremarkable at that time, and auscultation of the thorax revealed no abnormalities. Results of a complete blood count and a serum biochemistry panel were within reference ranges. Presence of serum IgG was first sought by Western blotting of mock-exposed and SARS-CoV-2–exposed Vero E6 cells lysates. In convalescent-phase serum, 5 protein bands that were simultaneously absent from mock-exposed Vero E6 cell lysates were identified (Appendix Figure). Furthermore, the convalescent-phase serum was positive by double-epitope sandwich ELISA and for 2 of the 3 antigens tested by double-epitope luciferase assay (Table; Appendix Table). Whereas serum samples from 30 control cats and 10 control humans were negative by virus neutralization assay, the convalescent-phase serum samples from the cat and her owner were positive; endpoints were 1:512 for the cat and 1:128 for the human.

The cat at first showed general signs, then gastrointestinal signs, and finally respiratory signs, similar to those observed in humans. Subsequently examined samples from the cat revealed viral RNA persisting for about 10 days. With the exception of a vomitus fluid sample collected on March 13, the amounts of viral RNA were relatively low. For this reason, and

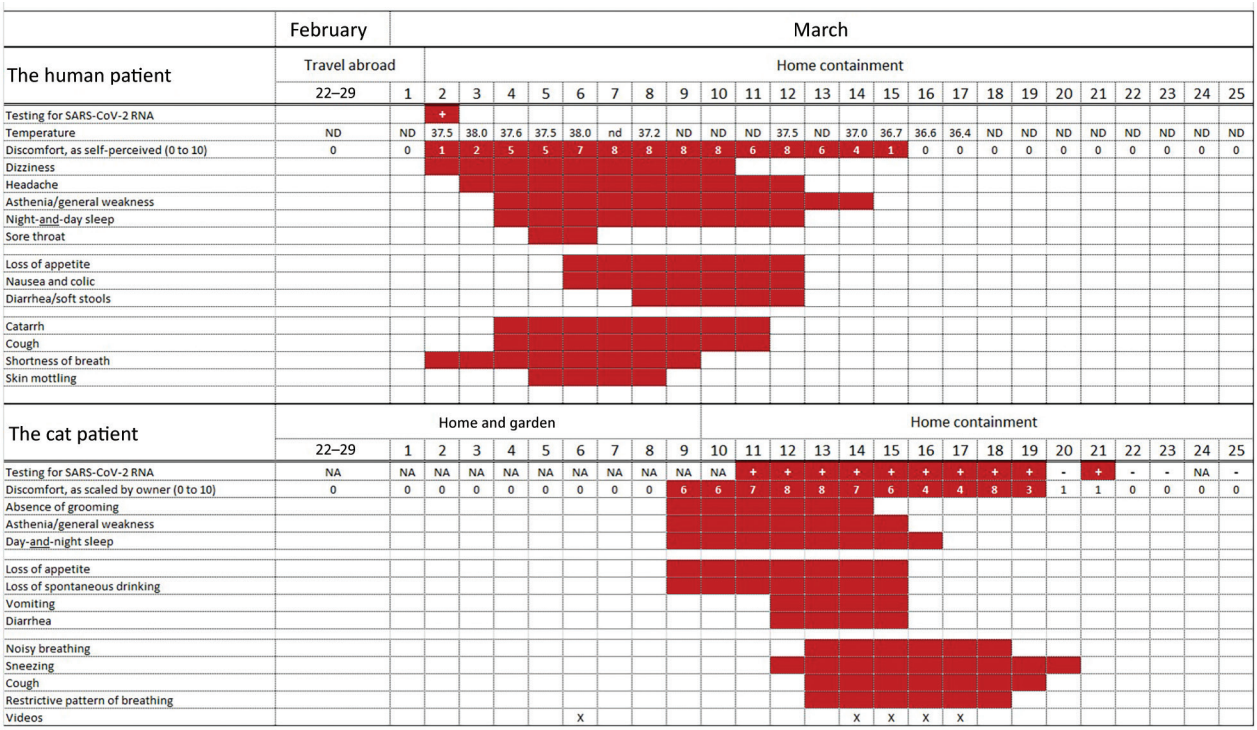

Figure. Timeline of disease course for human and cat with SARS-CoV-2 infection, by days from illness onset according to the cat owner, Belgium, February 22–March 25, 2020. NA, not available; ND, not determined; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

despite the simultaneous presence of a compatible clinical syndrome and a suggestive chronology of events, we cannot automatically rule out passive contamination of the cat's samples by its owner.

To confirm the hypothesis of a productive infection of the cat, we conducted a series of serologic analyses by using 4 different testing approaches and targeting distinct viral protein targets. All procedures

Table. Severe acute respiratory syndrome coronavirus 2 genome loads measured by qRT-PCR in a series of consecutive swab samples from cat, Belgium, March 2020*

	Oropharyngeal swab samples		Vomitus		Feces	
Date	β-actin gene	N gene	β–actin gene	N gene	β-actin gene	N gene
11	NS	NS	26.95	23.5 ± 0.1	35.5	33.3 ± 0.2
12	33.4	38.2 ± 0.5	NS	NS	32.9	34.8 ± 0.0
13	37.8	37.9 ± 0	ND	34.9 ± 0.1	34.4	37,6 ± 0,1
14	25.1	39.3 ± 0.1	NS	NS	30.7	35.1 ±0.1
15	35.9	35.7 ± 0.1	NS	NS	27.8	33.2 ± 0.1
16	38.2	Negative	NS	NS	26.0	35.1 ± 0
17	27.1	38.2 ± 0	NS	NS	28.7	Negative
18	26.7	Negative	NS	NS	36.1	37.9 ± 0
19	NS	NS	NS	NS	27.9	39.0 ± 0
20	NS	NS	NS	NS	30.1	Negative
21	NS	NS	29.9	33.8 ± 0.1	32.8	Negative
22	37.9	Negative	NS	NS	31.7	Negative
23	NS	NS	NS	NS	33.8	Negative
25	NS	NS	34.9	Negative	35.0	Negative

*Numbers reported are defined as the number of cycles required for the real-time PCR assay fluorescent signal curve to intersect with a threshold line that exceeds background level (mean ± SD). It is a relative measure of the concentration of the genomic target in the qRT-PCR reaction (the severe acute respiratory syndrome coronavirus 2 N gene or cat β-actin gene); values >40 are considered negative. All samples with qRT-PCR values <40 were analyzed further by a standard gel RT-PCR targeting the coding sequence of the virus spike protein gene followed by Sanger sequencing of the correctly sized amplicon retrieved (≈370 bp). Only samples positive for all 3 tests were defined as positive, which was the case for all samples with a value <40 for the N gene aggregated in this table. NS, no sample available; negative, RT-qPCR and/or gel PCR and/or sequencing test failed; qRT-PCR, quantitative reverse transcription PCR.

converged toward the same result: the convalescent-phase serum from the cat contained immunoglobulins against SARS-CoV-2, which were absent from the serum from control cats. These antibodies target several distinct viral proteins, and they caused a total neutralizing effect up to a much higher dilution than those from the owner's serum. This household cat was therefore productively infected with the SARS-CoV-2 virus excreted by its owner, and the infection caused a nonfatal but nevertheless severe disease, mainly of the respiratory system (Videos 2–6).

Public health officials are still learning about SARS-CoV-2, but no current evidence indicates that pets play a role in spreading the virus. Therefore, taking measures against companion animals that may compromise their welfare is not justified.

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About the Author

Prof. Garigliany is a senior researcher and professor of General and Molecular Pathology at Liège University, Belgium. His primary research interests include host–pathogen interactions between animals and RNA viruses.

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Address for correspondence: Daniel Desmecht, University of Liège, Faculty of Veterinary Medicine, Department of Pathology, Sart Tilman B43, 4000 Liège, Belgium; email: daniel.desmecht@uliege.be

SARS-CoV-2 in Quarantined Domestic Cats from COVID-19 Households or Close Contacts, Hong Kong, China

Vanessa R. Barrs,[1] Malik Peiris,[1] Karina W.S. Tam, Pierra Y.T. Law, Christopher J. Brackman, Esther M.W. To, Veronica Y.T. Yu, Daniel K.W. Chu, Ranawaka A.P.M. Perera, Thomas H.C. Sit

Author affiliations: City University of Hong Kong, Hong Kong, China (V.R. Barrs); The University of Hong Kong, Hong Kong (M. Peiris, D.K.W. Chu, R.A.P.M. Perera); Government of the Hong Kong Special Administrative Region, Hong Kong (K.W.S. Tam, P.Y.T. Law, C.J. Brackman, E.M.W. To, V.Y.T. Yu, T.H.C. Sit)

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We tested 50 cats from coronavirus disease households or close contacts in Hong Kong, China, for severe acute respiratory syndrome coronavirus 2 RNA in respiratory and fecal samples. We found 6 cases of apparent human-to-feline transmission involving healthy cats. Virus genomes sequenced from 1 cat and its owner were identical.

Naturally occurring human-to-animal transmission of severe acute respiratory syndrome (SARS) coronavirus was reported during 2003 when viral RNA was detected in oropharyngeal and rectal swab specimens from healthy domestic cats in a housing estate at the center of a large SARS cluster in Hong Kong, China; infections were confirmed serologically (*1*). Susceptibility of cats to infection with this virus and transmission between cats were demonstrated experimentally (*2*). Pulmonary pathologic changes, similar to those for humans with SARS, developed in infected cats, but the cats remained asymptomatic (*2*,*3*).

These findings informed the current precautionary strategy of the Agriculture, Fisheries and Conservation Department of Hong Kong to quarantine mammalian pets from households with confirmed human coronavirus disease (COVID-19) or their close contacts (defined as a person who had face-to-face contact for >15 min with a person who had confirmed SARS Coronavirus-2 [SARS-CoV-2] infection) in a holding facility, when alternative care was unavailable. Pets are swabbed for SARS-CoV-2 testing and confined until reverse transcription PCR (RT-PCR) results are negative on 2 consecutive oc-

[1]These authors contributed equally to this article.

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Appendix

Materials & Methods related to the case study

Clinical data

Due to the strict confinement imposed to the owner, it would have been inconsistent to ask a veterinarian to enter the house to conduct a clinical examination of the animal and to collect samples. The clinical signs were therefore aggregated indirectly by questioning the owner on the phone and by visualizing the daily videos she was asked to send. For the samples, instructions were given to perform deep oro-pharyngeal mucosal swabs, vomit swabs and faeces swabs. The swabs were then stored at 4°C in a tube for up to 24 hours and deposited on the front door step of the house where an attendant collected them every morning. On March 31, seven days after the last positive SARS-CoV-2 sample, a jugular blood sample was collected under general anaesthesia, centrifuged and the corresponding serum stored at -20°C until serological testing was performed.

Virological diagnosis

The presence of the SARS-CoV-2 genome was first ascertained by reverse transcriptase quantitative PCR (RT-qPCR) using the β-actin transcript as internal control. The primer-probe set was selected based on sequence information from the Centers for Disease Control and Prevention (USA, reagent label 2019-nCoV_N1). Each swab was immersed and agitated in 500 µL of PBS for 30 sec. and the RNA was isolated using the Nucleo Spin RNA® Set for NucleoZOL according to the manufacturer's instructions (Macherey Nagel). RT-qPCR detection and quantitation of target viral N1 sequence was made using the Luna® Universal Probe qPCR Master Mix (New England Biolabs) and the StepOnePlus real time PCR instrument (ThermoFisher). Cycle threshold (Ct) values above 40 were considered negative. Samples proved positive twice by RT-qPCR were then confirmed further by a standard gel RT-PCR targeting the RBS coding sequence of the spike protein gene (using in-house primers RBD-F 5′-GCAAACTGGAAAGATTGCTGA-3′ and RBD-R 5′-ACCAAATTAGTAGACTTTTTAGGTCCA-3′) followed by subsequent Sanger’s sequencing of

the correctly sized amplicon retrieved (≈370 bp). Only samples positive for all three tests were considered positive.

Serological diagnosis

Four methods were used to detect possible anti-SARS-CoV-2 antibodies. Three months after the pandemic emergence, no serodiagnostic tool specifically validated for the feline species was available. In this context, we opted for the use of very different tools, implemented by 3 independent laboratories, and targeting different viral targets. The cat sample was anonymised and aggregated with 1-5 reference samples taken before December 2019 to allow blind testing.

Immunoblotting — The SARS-CoV-2 isolate Belgium/Sart Tilman/2020/01 was derived from a positively-testing nasopharyngeal swab in the University Hospital. Infection of Vero E6 cells was carried out in phosphate-buffered saline (PBS) containing 2% fetal calf serum. The inoculum was added to the cells for 1 h at 37°C, after which cells were washed twice with PBS and maintained in Dulbecco's Modified Eagle's Medium (Lonza) with 2% FCS, 2mM glutamine and antibiotics for 48 h. Protein extracts (PE) from SARS-CoV-2-infected and uninfected Vero E6 cell monolayers were prepared in RIPA buffer. Protein extract (9 µg), NuPAGE LDS Sample Buffer (6,25 µL) and reducing agent (2,5 µL) were mixed, heated at 70°C for 10 min and, along with a protein ladder (5 µL, Precision Plus Protein™ Dual Xtra Prestained Protein Standards, Biorad), loaded in a 4-12% Bis-Tris Protein Gel. Gels were run at 200 V for 1 h using the XCell SureLock™ mini-cell electrophoresis system. Migrated proteins were transferred onto polyvinylidene difluoride membranes, nonspecific binding sites were blocked with skim milk (5% in PBST) for 2 h at RT and membranes were probed overnight at 4°C with feline or human serum diluted 1/100 in PBST/skim milk (2,5%). Blots were washed with PBST and incubated with an HRP-conjugated goat anti-feline (ab112801, from Abcam) or anti-human (ab97225, from Abcam) IgGs antibody (diluted 1/10000 v/v in PBS-Tween/skim milk) for 1 h at RT. Membranes were washed 3 times with PBST on a plate shaker for 5 min, then in deionized water for 2 min. Membranes were revealed using the chemiluminescent substrate Novex® ECL (Invitrogen) according to the manufacturer's protocol. Visualisation was made using the imaging system ImageQuant™ LAS 4000 and ImageJ software (3 min exposition). After, membranes were washed in PBST, incubated 1 h at RT with an HRP-conjugated anti-b-actin antibody (ab49900, from Abcam) diluted 1/25000 v/v in PBST/skim milk, then revealed and vizualised as described.

Double-epitope sandwich enzyme immunoassay — Platelia™ SARS-CoV-2 Total Ab is a one-step assay for the semi-quantitative detection of antibodies IgM, IgA and IgG to the

SARS-CoV-2 nucleocapsid in human serum using EIA technology (ref. #72710, from Bio-Rad). Briefly, the test uses a nucleocapsid protein in two forms: either coated on the bottom of the wells or conjugated to horseradish peroxidase. The conjugate is added to the serum sample and the mixture is then incubated for one hour at 37°C in the sensitised well. During this incubation step, if IgM and/or IgG and/or IgA antibodies are present in the sample, an immune complex is formed by aggregation of the recombinant nucleocapsid proteins deposited on the surface of the well, the specific antibodies and the recombinant nucleocapsid proteins coupled to peroxidase. The presence of the immune complexes is then demonstrated in a conventional manner by the distribution of a chromogenic solution inducing a colour reaction read with the spectrophotometer at 450/620 nm ; the presence of specific antibodies in a sample being demonstrated by performing a ratio between the optical density of the sample and that of the threshold control. This tool had been initially developed for the detection of anti-SARS-CoV-1 antibodies in human serum/plasma and it proved insensitive to antibody cross-reactions against seasonal human coronaviruses. It appears that this assay very reliably detects the serological response to SARS-CoV-2 infections, presumably due to the phylogenetic proximity between the two nucleoproteins. Further, we hypothesized that the format of this technology, in which specific ligands are sandwiched between two copies of their target, should in principle work to detect specific antibodies produced by other species.

Luciferase immunoprecipitation assay — Schematically, this assay is based on the use of recombinant NanoLuc (Nluc) luciferase-antigen fusions (*1*,*2*). The assay is initiated by incubating crude Nluc-antigen containing cell extracts with patient/animal sera in microtiter wells. The immune complexes are then precipitated onto a filter plate by protein A/G-coated beads. After washing, antibody-bound Nluc-antigen is measured by the addition of coelenterazine substrate and light units are measured with a luminometer. Three specific Nluc-antigen fusions were produced and validated by the Pasteur Institute for profiling antibody responses against SARS-CoV-2 : Nluc-full_S1_subunit_(residues 1-698), Nluc-full_S2_subunit_(686-1208) and Nluc-full_N_(1-419) (L. Grzelak et al., unpub. data, https://www.medrxiv.org/content/10.1101/2020.04.21.20068858v1). These novel reagents were used to test the cat sera.

Virus neutralization assay — Vero E6 cells were seeded in 96-well plates at 2.10^4 cells/well. The day after, 100 $TCID_{50}$ of virus (isolate Belgium/Sart Tilman/2020/01) were incubated with serial 2-fold dilutions of sera, starting from 1:10, in 100 µl of DMEM for 1 hour at 37°C. Mixes were then added to cells and incubated for 1 hour at 37°C. Virus/sera mixes were removed, 100µl of DMEM were added, and cells were incubated for 5 days at 37°C with 5%

CO_2. Virus inoculum was back titrated in each experiment. Neutralisation was assessed by CPE, the reading of which was performed by independent, blind direct observations under the inverted microscope and after cell coloration with crystal violet. All these operations were carried out in a level 3 biosafety laboratory. The neutralisation endpoint was determined as the serum highest dilution that inhibited 100% of the SARS-CoV-2 infection observed by CPE of inoculated cells.

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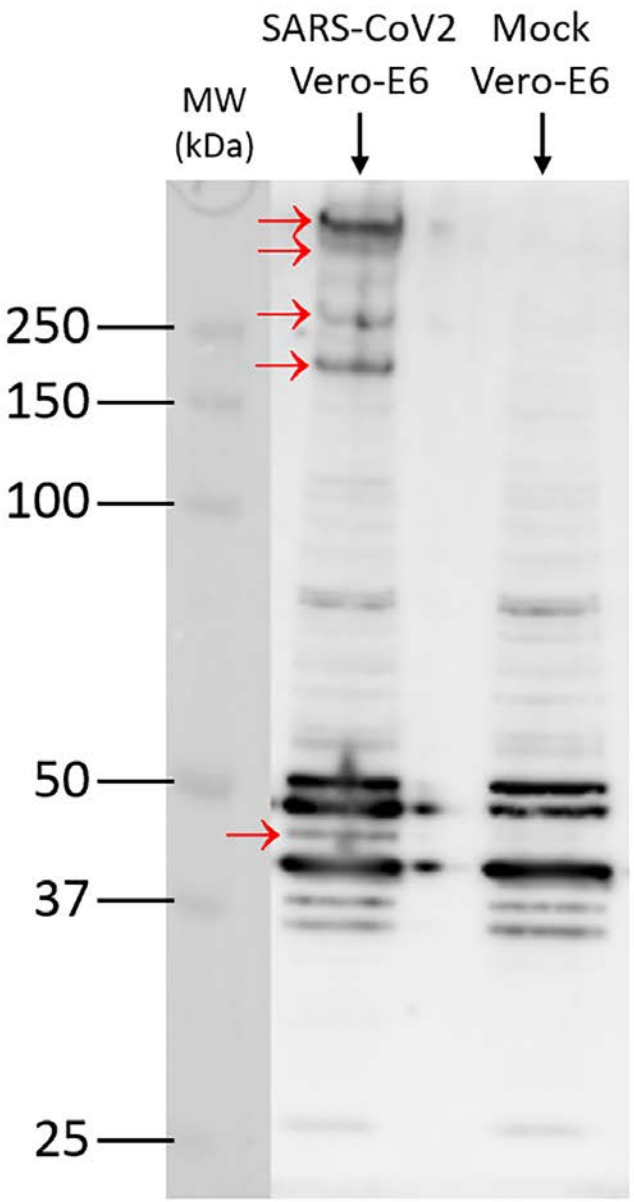
Appendix Table. Serologic data*

Testing tool/procedure	Cut-off value	Control cat serum	Case cat serum	Conclusion
Double-epitope sandwich ELISA	1,35	0,90	2,89	Positive
Luciferase immunoprecipitation				
S1 domain	0,52	0,02	1,29	Positive
S2 domain	1,20	0,21	7,07	Positive
C-term N protein	0,15	0,01	0,11	Dubious
Virus neutralization test	NA	<1:10	1:512	Positive

*Cut-off values have been established using a set of prepandemic and SARS-CoV-2-positive human sera. Values above the cut-off are considered positive. NA, not applicable. See text for detailed description of the different tools/procedures. Blood had been collected 3 weeks after first clinical signs appeared.



Appendix Figure. Western blot probing of mock- and SARS-CoV-2 infected Vero E6 cell lysates using cat serum. The strip was probed with convalescent cat serum diluted 1:100 and exposed for 3-min. The arrows indicate SARS-CoV-2 protein bands. The bands migrating at ca. 180 and 250 kDa probably represents two glycoforms of the spike and the band at ca. 45 kDa the nucleoprotein. The heavy bands at ca. 700 and 800 kda could represent the replicase. Probing with control cat sera did not display these bands. MW, molecular weight.