Prolonged SARS-CoV-2 RNA Shedding from Therapy Cat after Cluster Outbreak in Retirement Home

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We report a therapy cat in a nursing home in Germany infected with severe acute respiratory syndrome coronavirus 2 during a cluster outbreak in the home residents. Although we confirmed prolonged presence of virus RNA in the asymptomatic cat, genome sequencing showed no further role of the cat in human infections on site.

Cats are susceptible to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and can transmit the virus to other cats (*1–3*). However, the pathophysiology and epidemiologic impact of SARS-CoV-2 infection of pets remain poorly understood (*4*). We report 3 therapy cats living in a retirement home in Germany for which evidence indicated naturally occurring human-to-cat transmission during SARS-CoV-2 outbreaks.

A total of 21 confirmed human SARS-CoV-2 infections occurred in the outbreaks, including 3 deaths. Six infected care and administrative personnel showed mild or no symptoms; 15 infected residents showed typical signs of coronavirus disease, including fever and severe respiratory disease (cough, pneumonia, and dyspnea). The first outbreak occurred on the home's ground floor at the end of March 2020 (Appendix Figure 1, panel A, https://wwwnc.cdc.gov/

EID/article/27/7/20-4670-App1.pdf); it is assumed that the virus was introduced through care personnel. One SARS-CoV-2–positive resident (90 years of age, given a diagnosis on April 4, 2020), already bedridden, died on April 12. He had been in close contact with cat K8, which snuggled in his face.

A strict hygienic plan was implemented to contain the initial outbreak, including using separate personnel for each floor. No visitors were allowed. All residents were kept in their rooms without social contact between them. Despite isolation, the cats still had access to all areas and to the outside.

At the end of April, residents of the first floor showed typical COVID-19 symptoms. We tested oropharyngeal swab specimens from the cats on April 29 (surveillance day 1) (Appendix Figure 1, panel A). Although 2 cats (K4 and K9) showed negative results, 1 (K8) showed positive results for SARS-CoV-2 RNA by quantitative reverse transcription PCR specific for partial envelope protein gene (Table; Appendix Figure 1, panel C).

Because of epidemiologic connections, we speculated whether K8 could have been involved in spreading SARS-CoV-2 to the first floor. We isolated the cats in a Biosafety Level 3 facility for surveillance (Appendix Figure 1, panel A) and tested them again on May 4. K8 was positive for SARS-CoV-2 RNA and had lower quantification cycle values (Table; Appendix Figure 1, panels B, C). Cats were housed in single cages during the first 4 days of quarantine (surveillance days 6–10), then moved into 1 combined cage system (surveillance day 11). After 15 days (surveillance day 21), cats were transferred to floor housing under Biosafety Level 3 conditions and permitted free movement and contact. Testing at regular intervals of conjunctival, fecal, and oropharyngeal swab specimens showed that K4 and K9 remained negative, whereas K8 was positive for SARS-CoV-2 RNA until day 21 of surveillance. K8 also had positive quantification cycle values (range 26.3–38.5; values <40 were considered positive) (Appendix Figure 1, panel B) and ≈5.7 ×10^4 to 5.0 ×10^3 RNA copies/mL (Appendix Figure 1, panel C).

Subsequently, we detected no viral RNA in swab samples through day 73.

These PCR results demonstrated an extended period of SARS-CoV-2 infection of the positive cat. When serum samples were analyzed for SARS-CoV-2–neutralizing antibodies (*5*), K8 showed a positive titer (range 1:20–1:52) (Appendix Figure 1, panel D). Multispecies ELISA results showed serum antibodies against the receptor-binding domain (*5*). Titers peaked by day 35 and decreased but remained positive until the end of surveillance (Appendix Figure 1,

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Table. Overview of cat swab specimen and blood sampling regimen for prolonged SARS-CoV-2 RNA shedding from therapy cat after cluster outbreak in retirement home, Germany*

Day of surveillance	Sample type	Cq values by RdRp/RdRp gene screen	Cq values by E/E/S gene screen
1	OPS	ND/ND	ND/35.56/36.15†
6	OPS	ND/ND	ND/26.34/27.06†
7	OPS	29.66/31.40	30.52/ND
9	OPS, CS, FS, BS	37.48/no Cq	36.23/ND
11	OPS, CS, FS	30.95/34.80	34.66/ND
13	OPS, CS, FS, BS	32.63/35.50	35.98/ND
15	OPS, CS, FS	31.17/35.10	36.96/ND
17	OPS, CS, FS, BS	33.12/36.50	35.92/ND
19	OPS, CS, FS	No Cq/no Cq	No Cq/ND
21	OPS, CS, FS, BS	No Cq/38.80	No Cq/ND
28	OPS, CS, FS, **BS**	No Cq/no Cq	ND/ND
31	OPS, CS, FS	No Cq/no Cq	No Cq/ND
35	OPS, CS, FS, BS	No Cq/no Cq	ND/ND
38	OPS, CS, FS	No Cq/no Cq	ND/ND
42	OPS, CS, FS, BS	No Cq/no Cq	ND/ND
45	OPS, CS, FS	No Cq/no Cq	ND/ND
49	OPS, CS, FS, BS	No Cq/no Cq	ND/ND
69	BS	No Cq/no Cq	ND/ND
73	OPS, CS, FS, BS	No Cq/no Cq	ND/ND

*Results are given for virus-positive cat K8. At day 28, serum samples for analysis of blood were collected (in bold). PCRs were performed between day 1 and day 6 by using the RealStar SARS-CoV-2 RT-PCR Kit 1.0 (Altona Diagnostics, https://www.altona-diagnostics.com) for initial diagnosis (E gene screen and SARS-CoV-2 specific spike gene), and from day 7 by using an RdRP gene SARS-2-IP4 quantitative real-time PCR (World Health Organization–recommended assay) and an E gene-specific PCR (see details in Appendix, https://wwwnc.cdc.gov/EID/article/27/7/20-4670-App1.pdf). The 2 results in the RdRp and E gene columns indicate that these assays were performed independently in 2 different laboratories (University of Veterinary Medicine Hannover and Friedrich-Loeffler Institut). BS, blood sample; CS, conjunctival swab; Cq, quantification cycle; E, envelope; FS, fecal swab; ND, not done; OPS, oropharyngeal swab; RdRP, RNA-dependent RNA polymerase; S, spike protein; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
†Indicates a third test result for the S gene.

panel E). K4 and K9 remained SARS-CoV-2 seronegative (Appendix Figure 1, panels D, E).

To examine the effects of potential co-infections, we analyzed common feline viral infections. All cats were negative for feline leukemia virus. However, K8 was positive for feline immunodeficiency virus (FIV)–specific antibodies, and K4 and K9 were positive for feline coronavirus–specific antibodies. The marginal serologic reactivity of K8 indicated that this cat was not previously infected with feline coronavirus (Appendix Figure 2).

SARS-CoV-2 genome sequences obtained from K8 and related human cases in the retirement home (1 from the first outbreak and 3 from the second outbreak) differed from each other by 3 ambiguous sites, indicating low-frequency variants within K8, leading to viral quasispecies. Sequences from the second outbreak included a constant C→T change (Appendix Figure, panel F). These data support direct human-to-cat-transmission during the first outbreak but not zoonotic SARS-CoV-2 transmission from K8 because of the constant viral sequence difference within the second outbreak series.

Our data showed human-to-cat SARS-CoV-2 transmission in a community-acquired cluster outbreak that had multiple infection events. We demonstrated prolonged shedding of SARS-CoV-2 RNA up to day 21 after the first detection, in contrast to a recent study in a naturally infected cat (RNA-positive for 11 days) (6). We hypothesize a longer period of RNA shedding (>21 days) because we do not know the day

of infection before the start of cat surveillance. Prolonged SARS-CoV-2 RNA shedding could be related to immune status of individual animals or co-infections or immunosuppression as reported for humans (7–9).

Our sequencing data do not suggest zoonotic spillback from the SARS-CoV-2–infected cat to humans, as reported elsewhere (3,10). However, reinfections, prolonged virus replication, and transmission events in cats cannot be excluded, in particular if one considers emergence of SARS-CoV-2 variants that have potentially increased host range or ability to escape preexisting immunity. Thus, cats should be considered in surveillance and control measures.

About the Author

Dr. Schulz is a postdoctoral researcher at University of Veterinary Medicine Hanover, Hanover, Germany. Her primary research interests are the pathogenesis and epidemiology of emerging and vector-borne diseases in the wildlife–livestock and human–animal interfaces.

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Effects of COVID-19 Vaccination Timing and Risk Prioritization on Mortality Rates, United States

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During rollout of coronavirus disease vaccination, policymakers have faced critical trade-offs. Using a mathematical model of transmission, we found that timing of vaccination rollout would be expected to have a substantially greater effect on mortality rate than risk-based prioritization and uptake and that prioritizing first doses over second doses may be lifesaving.

In December 2020, the US government issued emergency use authorization for two 2-dose severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccines, both estimated to be >94% efficacious in preventing symptomatic coronavirus disease (COVID-19) (1–3). The Advisory Committee on Immunization Practices immediately recommended the prioritization of frontline workers and high-risk subgroups (4). As of February 14, 2021, ≈52 million doses have been administered (5). We used a mathematical model of COVID-19 transmission to evaluate the effects of vaccine timing, risk prioritization, number of doses administered, and uptake rates on population-level mortality rates (Figure).

Focusing on Austin, Texas, USA, we projected COVID-19 deaths over 8 months for both an infection-blocking vaccine that prevents infection upon exposure (assuming 95% reduction in susceptibility in vaccinated persons) and a symptom-blocking vaccine that prevents symptoms upon infection (assuming 95% reduction in symptomatic ratio in vaccinated persons). Vaccination would begin on January 15 or February 15, with 10,000 vaccines administered weekly and allocated to cities pro rata. We compare 3 strategies: no priority groups; 1 of 3 priority groups vaccinated before the general public (adults >65 years of age, adults who have high-risk underlying

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Appendix

Detailed Description of COVID-19 Cluster Outbreak in Retirement Home

At the end of March 2020 (March 26–29), the first severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections were diagnosed in a retirement home in southern Germany. There were 21 laboratory-confirmed SARS-CoV-2–positive persons over a period of 10 weeks (Appendix Figure 1, panel A). On April 1, a 90-year-old woman who had been given a diagnosis of coronavirus disease (COVID-19) on March 30, 2020, died after showing severe clinical symptoms. A second patient (78-year-old man) who had been confirmed as being SARS-CoV-2 positive on April 4 died on April 8. There was still not a single case of infection on the first floor. On April 12, another male SARS-CoV-2 patient (90 years of age) who had been given a diagnosis on April 4 died because of COVID-19 (Appendix Figure 1, panel A). Because of underlying health conditions, this patient had already been bed-ridden before his disease, and had typical COVID-19 symptoms. This patient (owner of cat K8) had been in close contact with cat K8 for the entire period of the COVID-19 outbreak. There was no evidence for COVID-19 disease events on the first floor.

Protective clothing and rigorous isolation were used to further contain the initial outbreak. This included cohort isolation of the positive cases in one wing of the floor, including installing a gate and restricting access to only a few allocated care personnel who took care of the virus-positive patients. No visitors were allowed. In doing so, the first floor appeared to be spared from any SARS-CoV-2 infection. Despite the strict isolation, the cats still had access to all areas and to the outside. Because of the specific protective measures, all residents and staff were tested for SARS-CoV-2 at specific time intervals, which were set by the local health authority. It seemed that the initial SARS-CoV-2 outbreak could be stopped. However, second outbreak started at the end of April, when the first residents of the first floor showed typical

COVID-19 symptoms, including fever (Appendix Figure 1, panel A). On April 22, a 92-year-old woman was hospitalized. She was given a diagnosis of infection with SARS-CoV-2 on April 23. Up until April 27, all residents and staff had been tested for SARS-CoV-2.

In the context of the epidemiologic investigation of this second outbreak, the 3 therapy cats were also tested for SARS-CoV-2 by using oropharyngeal swab specimens. Cat K8, the close companion of the deceased bed-ridden COVID-19 patient from April 12, was confirmed to be SARS-CoV-2 positive by quantitative real-time quantitative reverse transcription PCR (qRT-PCR) on April 29 (Table; Appendix Figure 1, panel B). At this stage, 5 additional residents from the first floor were given diagnoses of COVID-19.

According to the SARS-CoV-2 genome information we could obtain for cat K8 and from 1 and 3 residents from the first and second outbreaks, respectively, we assume a separate entering of SARS-CoV-2 for the second outbreak in the care facility (on the basis of a nucleotide difference at sequence position 21157; Appendix Figure 1, panel F). Cat-specific mutations could not be identified for comparing nucleotide sequence of cat K8 with a cat sequence from the National Center for Biotechnology Information (Bethesda, MD, USA) NCBI (accession no. MT747438) that does not show any similar sequence variants.

Material and Methods

Ethics for Use of Animals

All animal studies were conducted in compliance with the German Regulations for Animal Experimentation (#20A522, Animal Welfare Act, approved by the Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit (Lower Saxony, Germany). All animal and laboratory work was performed in a Biosafety level 3 (BSL-3) laboratory or a BSL-2 laboratory and facilities at the Research Center for Emerging Infections and Zoonoses, University of Veterinary Medicine, Hannover.

The 3 therapy cats monitored in this study (K4, female, ≈14 years of age; K8, female, ≈6 years of age; K9, male, ≈10 years of age) were obtained from a home for the elderly. In agreement with the management team of the senior resident's home and the responsible veterinary authority, the cats were transferred to the BSL-3 animal facility at the University of Veterinary Medicine Hannover for detailed follow-up. Cats had free access to water and food.

Cats were monitored daily for well-being, health constitution, and clinical signs, such as body temperature, anorexia, diarrhea/loose stool, vomiting, lethargy/depression, and respiratory symptoms. Weights of all cats were checked daily. After repeated PCR confirmation that no SARS-CoV-2 RNA was excreted from any of the 3 cats, they were housed under BSL-2 conditions for another 14 days. After all cats were confirmed negative for SAR-CoV-2 by using PCR analysis, and they were discharged from the animal facility and placed again in private homes.

Ethics for Use of Human Samples

All participants and respective authorized person provided written informed consent. The study design was reviewed and approved by the institutional Ethics Review Board at the Hannover Medical School (#9350_BO_K2020).

Cat Sampling

Cats were sampled by using oropharyngeal swab specimens until day 7 of surveillance. From day 7 on, surveillance cats were sampled every other day (Table, Appendix Figure 1, panels B–E) by using oropharyngeal, rectal, and conjunctival swab specimens until day 21. After day 21 of surveillance, the cats were sampled by using oropharyngeal, rectal, and conjunctival swab specimens twice a week. After day 49 of surveillance, sampling was curtailed to a final sampling by oropharyngeal, rectal and conjunctival swab specimens at day 73 of surveillance. Swab specimens were placed immediately into a sterile transport tube containing 2 mL of Opti-MEM (Opti-MEM I Reduced Serum Medium, GlutaMAX Supplement, #51985026; ThermoFisher, https://www.thermofisher.com) containing 1% penicillin/streptomycin and stored at −80°C within 4 hours after collection until further analysis. Blood samples were taken at most twice a week from the cephalica or saphena vein. Serum samples were collected on days 9, 13, 17, 21, 28, 35, 42, 49, 69, and 73 of surveillance. EDTA was added to blood collected at day 28 of surveillance for blood cell analysis.

Human Sampling

Samples from humans were collected and tested by qRT-PCR as part of routine clinical care. Humans were sampled by using naso-oropharyngeal swab specimens from the nostril parallel to the palate to the nasopharynx with a gentle rub and roll. Swab specimens were immediately placed into sterile transport tubes containing 2–3 mL of virus transport medium.

Detection of Feline Leukemia Virus IgG

IgG for this virus was detected by using a qualitative ELISA (IDEXX Laboratories, https://www.idexx.com).

Detection of Feline Immunodeficiency Virus IgG

IgG for this virus was detected by using a qualitative ELISA (IDEXX Laboratories, https://www.idexx.com).

ELISA for Detection of Feline Coronavirus

Antigen-specific IgG responses for this virus were analyzed by using a qualitative ELISA (IDEXX Laboratories). For evaluation of test results, we calculated a quotient to determine specific cutoff values, and positive and negative results.

SARS-CoV-2 Neutralization Test and Indirect Multispecies ELISA

Serum samples were tested for SARS-CoV-2 neutralizing antibodies by using a virus neutralization assay (*1*). In brief, 50 μL of medium containing $10^{3.3}$ mean (50%) tissue culture infectious doses//mL of SARS-CoV-2 were mixed with 50 μL of diluted cat serum. Each sample was tested in triplicate. After 1 h of incubation at 37°C, the virus-serum mixture was transferred to confluent Vero E6 cells in a 96-well plate. Viral replication was assessed after incubation for 5 days at 37°C in an atmosphere of 5% CO2 by detection of a cytopathic effect.

Receptor-binding domain antibodies were detected by using a multispecies ELISA described by Wernike et al. (*2*). In brief, receptor-binding domain antigen–coated plates were incubated at 4°C overnight and blocked for 1 h at 37°C with 5% skim milk in phosphate-buffered saline. Serum samples were added and incubated for 1 h at room temperature, and a multispecies conjugate (SBVMILK; IDvet, https://www.id-vet.com) France) was added for 1 h at room temperature. Plates were washed 3 times after each step. After adding 3,3′,5,5′-tetramethylbenzidine substrate (IDEXX Laboratories), the ELISA result was read at a wavelength of 450 nm. Adsorbance was calculated by subtracting the optical density of uncoated wells from those of protein-coated wells for the respective sample (*2*).

Quantitative Real-Time Reverse Transcription PCR

Initial SARS-CoV-2 diagnosis for cats (days 1–6 of surveillance) and humans was performed by using the RealStar SARS-CoV-2 RT-PCR Kit 1.0 (#821005; Altona Diagnostics, https://www.altona-diagnostics.com) using the envelope (E) gene screening (lineage B

betacoronavirus) and SARS-CoV-2 specific (spike gene) assays. During cat monitoring at the containment animal facility at the University of Veterinary Medicine Hanover, nasal, oropharyngeal, and rectal swab specimens were stored in Opti-MEM (Opti-MEM I Reduced Serum Medium, GlutaMAX Supplement; #51985026; ThermoFisher) containing 1% penicillin/streptomycin at −80°C within 4 hours after collection until further analysis. RNA was extracted from a 140-µL sample by using the Qiamp Viral RNA Mini Kit (QIAGEN, https://www.qiagen.com) and eluted in 60 µL of AVE buffer according to the manufacturer's protocol. Furthermore, an internal control system and negative controls were used according to Jendrny et al. (*3*), Hoffmann (*4*), and Hoffmann et al. (*5*).

For SARS-CoV-2 RNA amplification, the AgPath-ID One-Step RT-PCR Kit (ThermoFisher) was used. SARS-CoV-2 RNA isolated from cell culture supernatants (kindly provided by Sven Reiche, Friedrich-Loeffler-Institut, Insel Riems, Germany) served as a positive control. SARS-CoV-2 RNA was amplified with the AriaMx Real-Time PCR (qPCR) Instrument (Agilent, https://www.agilent.com) by using a temperature profile of 10 min at 45°C and 10 min at 95°C, and 42 repetition cycles of 15 s at 95°C, 20 s at 57°C, and 30 s at 72°C described by Hoffmann (*4*). Therefore, the qRT-PCR specific for the RNA-dependent RNA polymerase gene of SARS-CoV-2 (SARS-2-IP4 assay, Institute Pasteur, 2020, assay recommended by the World Health Organization (*6*) was conducted as described by Jendrny et al. (*3*) and Hoffmann (*4*). Samples were analyzed collaterally in 2 independent laboratories (Friedrich-Loeffler-Institut, Insel Riems, Germany and University of Veterinary Medicine, Hanover, Germany). In addition, positive samples were analyzed and quantified for SARS-CoV-2 RNA copies/mL in another independent laboratory at the Institute for Virology, University of Veterinary Medicine Hanover, for confirmation and quantification of SARS-CoV-2 RNA. A qRT-PCR assay specific for the envelope (E) gene (E_Sarbeco assay) according to Corman et al. (*7*) was used.

For quantification, an RNA copy standard was used. In brief, SARS-CoV-2 RNA isolated from cell culture supernatants (kindly provided by Sven Reiche, Friedrich-Loeffler-Institut, Insel Riems, Germany) served to synthesize cDNA with random primers and Moloney Murine Leukemia Virus Reverse Transcription (ThermoFisher). Complementary DNA served to generate a 113 bp–spanning amplicon of the E gene by using Taq polymerase (ALLin HS Red Taq Mastermix; HighQu, https://www.highqu.com). The PCR product was TOPO cloned into the vector pCR2.1 under the transcriptional control of the T7 promoter (pCR2.1.SARS-COV-2-

E). Plasmid DNA integrity was confirmed by Sanger sequencing (LGS Genomics, https://shop.lgcgenomics.com). RNA transcripts were produced by using T7RNA polymerase and purified (MEGAscript T7 Transcription Kit and MEGAclear Clean-Up Kit, ThermoFisher). The synthetic RNAs were used to generate a standard dilution series for the qRT-PCR (10^6–10^1 copies). E gene-specific qRT-PCR E_Sarbeco assay (*7*) detected SARS-CoV-2 RNA with high sensitivity. Up to 10 RNA copies per reaction were successfully detected. RNA samples and RNA dilutions were run in parallel, and genome equivalents were calculated on the basis of Cq values obtained by amplification of the RNA copy standard.

Next-Generation Sequencing

For next-generation sequencing, RNA was extracted from naso-oropharyngeal swab specimens from the second outbreak and cat K8 by using the RNAdvance Viral Kit (Beckman Coulter, https://www.beckman.com). After cDNA synthesis, sequencing was performed with SARS-COV-2 amplificons generated according to the ARTIC nCoV-2019 sequencing protocol v2 with primer version V3 (https://github.com) and subsequent library preparation with the Nextera XT Kit on the MiSeq (Illumina, https://www.ilumina.com) with 2×200 bp PE reads. Human samples from the second outbreak and a sample from cat K8 was sequenced by using high-throughput sequencing procedures and the Ion Torrent S5TM XL Instrument (ThermoFisher) (*8*) and subsequent enrichment by using RNA baits for SARS-CoV 2 (*9*). Sequence datasets were analyzed by using reference mapping with the Genome Sequencer Software Suite (version 2.6; Roche, https://roche.com), default software settings for quality filtering and mapping, and 2019_nCoV_Muc_IMB1 (accession no. LR824570) as a reference. Resulting SARS-CoV-2 contigs were mapped against the reference genome by using the software tool Geneious Prime (2019.2.3; https://assets.geneious.com) and gaps were filled with N to obtain full-length sequences. To identify potential single nucleotide polymorphisms in the read data and for acquisition of variant frequencies for each variant, the variant analysis tool integrated in Geneious Prime (2019.2.3) was applied (default settings, minimum variant frequency 0.02). Genome sequences assembled from cat K8 and affected residents are available in the European Nucleotide Archive Study (accession no. PRJEB41549).

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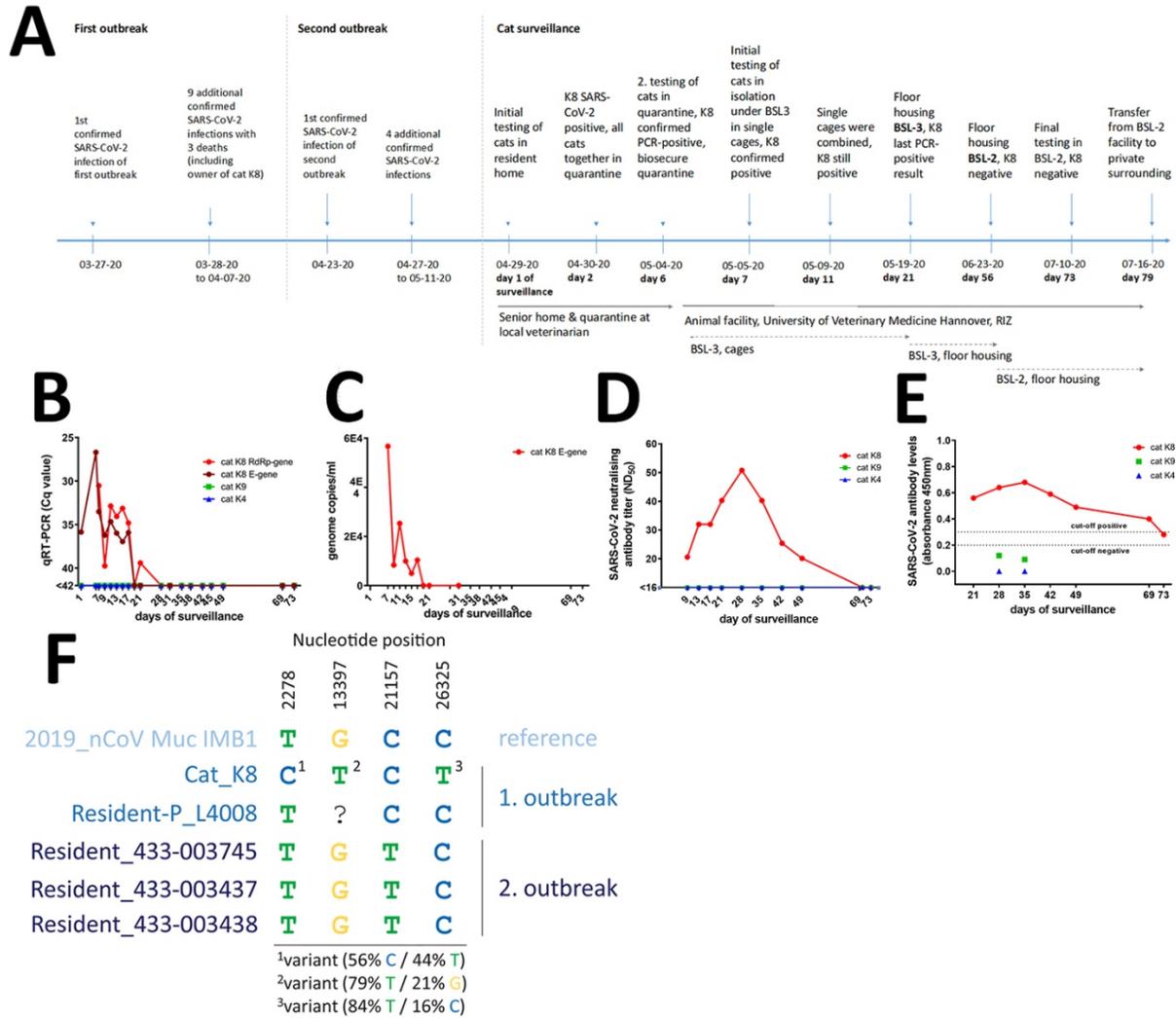
Appendix Figure 1. Human and feline monitoring after a SARS-CoV-2 cluster outbreak in a retirement home. A) Timeline of clinical events in the human SARS-CoV-2 infections, as well as virologic, serologic, and clinical surveillance of the 3 therapy cats, including their transfer for implementation of quarantine measures. BSL, biosafety laboratory; RIZ, Research Center for Emerging Infections and Zoonoses; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2. B) Cq values of oropharyngeal swab specimens collected from the 3 cats during surveillance as determined by using qRT-PCR targeting either the RdRp gene (IP4 assay) or the E gene (E_Sarbeco assay). Cq, quantification cycle; E, envelope; qRT-PCR, real-time quantitative reverse transcription PCR; RdRp, RNA-dependent RNA polymerase. C) Viral RNA copy numbers per milliliter of RNA eluate from cat K8 were calculated on the basis of quantified E gene–specific RNA. E, envelope. D) SARS-CoV-2 neutralizing antibody dynamics in serum samples from 3 cats by using a neutralization test. ND_{50}, 50% neutralizing dose. E) Progression of SARS-CoV-2 antibodies specific for the receptor binding domain in indirect multispecies ELISA. F) Genome sequences generated from oropharyngeal swab samples (day 7) from cat K8 and affected residents. Single nucleotide polymorphism sites relative to the SARS-CoV-2 genome of isolate 2019_nCoV_Muc_IMB1

(LR824570). Nucleotide positions 2278, 13397, and 21157 are located in the open reading frame 1ab gene, and 26325 is located in the envelope gene. The question mark indicates that there is no coverage of the genome sequence at this position, genome sequences from K8 and affected residents are available in the European Nucleotide Archive Study (accession PRJEB41549).
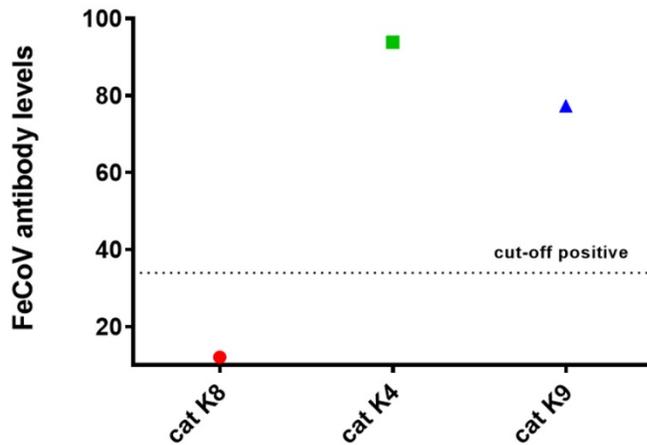
Appendix Figure 2. Prolonged SARS-CoV-2 RNA shedding from therapy cat after cluster outbreak in retirement home. Co-infection status of cats with FeCoV. FeCoV-specific IgG titers were analyzed by using a qualitative ELISA at day 28 of surveillance for the 3 cats. FeCoV, feline coronavirus SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.