5. Arons MM, Hatfield KM, Reddy SC, Kimball A, James A, Jacobs JR, et al. Presymptomatic SARS-CoV-2 infections and transmission in a skilled nursing facility. N Engl J Med. 2020 Apr 24 [Epub ahead of print]. https://doi.org/10.1056/NEJMoa2008457
6. Bourouiba L. Turbulent gas clouds and respiratory pathogen emission: potential implications for reducing transmission of COVID-19. JAMA. 2020 Mar 26 [Epub ahead of print]. https://doi.org/10.1001/jama.2020.4756
7. van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, et al. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. N Engl J Med. 2020;382:1564–7. https://doi.org/10.1056/NEJMc2004973
8. Andrade A, Dominski FH, Pereira ML, de Liz CM, Buonanno G. Infection risk in gyms during physical exercise. Environ Sci Pollut Res Int. 2018;25:19675–86. https://doi.org/10.1007/s11356-018-1822-8
9. Musher DM. How contagious are common respiratory tract infections? N Engl J Med. 2003;348:1256–66. https://doi.org/10.1056/NEJMra021771
10. Ebrahim SH, Memish ZA. COVID-19 - the role of mass gatherings. Travel Med Infect Dis. 2020 Mar 9 [Epub ahead of print]. https://doi.org/10.1016/j.tmaid.2020.101617

Address for correspondence: Ji-Young Rhee, Division of Infectious Diseases, Department of Medicine, Dankook University Hospital, Dankook University College of Medicine, 201 Manghang-ro, Dongnam-ku, Chungcheongnam-do, South Korea; email: pluripotent@naver.com

Infectious SARS-CoV-2 in Feces of Patient with Severe COVID-19

Fei Xiao,[1] Jing Sun,[1] Yonghao Xu,[1] Fang Li,[1] Xiaofang Huang,[1] Heying Li, Jingxian Zhao, Jicheng Huang, Jincun Zhao

Author affiliations: Sun Yat-sen University, Zhuhai, China (F. Xiao); Guangzhou Medical University, Guangzhou, China (J. Sun, Y. Xu, F. Li, X. Huang, Jingxian Zhao, Jincun Zhao); Chinese Academy of Sciences, Guangzhou (H. Li); Guangzhou Customs District Technology Center, Guangzhou (J. Huang)

DOI: https://doi.org/10.3201/eid2608.200681

[1]These authors contributed equally to this article.

Severe acute respiratory syndrome coronavirus 2 was isolated from feces of a patient in China with coronavirus disease who died. Confirmation of infectious virus in feces affirms the potential for fecal–oral or fecal–respiratory transmission and warrants further study.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) recently emerged in China, causing a major outbreak of severe pneumonia and spreading to >200 other countries (*1*). As of May 5, 2020, a total of 3,517,345 cases of coronavirus disease (COVID-2019) and 243,401 deaths had been reported to the World Health Organization (https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200505covid-19-sitrep-106.pdf?sfvrsn=47090f63_2). The virus is believed to be spread by direct contact, fomites, respiratory droplets, and possibly aerosols (*2*). Viral RNA has been detected in feces and urine of some patients (*3*–*7*). Infectious virus was also isolated from urine of a patient with severe COVID-19 (*8*). However, it is unclear whether the virus in feces is infectious and might be an additional source for transmission.

This study was approved by the Health Commission of Guangdong Province and the Ethics Committees of Guangzhou Medical University to use patient and healthy donor sample specimens. On January 17, 2020, a 78-year-old man who had a history of recent travel to Wuhan, China, was admitted to the Fifth Affiliated Hospital of Sun Yat-Sen University because of a cough for 7 days and intermittent fever (Appendix Figure 1, panel A, https://wwwnc.cdc.gov/EID/article/26/8/20-0681-App1.pdf). Computed tomography of his chest showed multiple, ground-glass opacities (Appendix Figure 2). Nasopharyngeal and oropharyngeal swab specimens were positive for SARS-CoV-2 RNA by quantitative reverse transcription PCR (qRT-PCR).

On January 22, the patient's condition deteriorated and he was intubated. Ventilator-assisted breathing was instituted. The first feces specimen was collected on January 27 and was positive for viral RNA by qRT-PCR. Serial feces samples were collected on January 29, February 1, and February 7. All samples were positive for viral RNA (Appendix Figure 1, panel A). Viral antigen was also detected in gastrointestinal epithelial cells of a biopsy sample, as reported (*9*). The patient died on February 20.

We collected fecal specimens on January 29 to inoculate Vero E6 cells. Cycle threshold values for the fecal sample were 23.34 for the open reading frame 1lab gene and 20.82 for the nucleoprotein gene. A

cytopathic effect was visible in Vero E cells 2 days after a second-round passage (Appendix Figure 1, panel B). We extracted viral nucleic acid from virus culture supernatant by using the QIAamp Viral RNA Extraction Kit (QIAGEN, https://www.qiagen.com) and obtained full-length viral genome sequence (GenBank accession no. MT123292) by using next-generation sequencing. The sequenced showed 5 nt substitutions compared with the original Wuhan strain (GenBank accession no. NC045512.2) (Appendix Table).

We negatively stained culture supernatant and visualized by transmission electron microscopy. Viral particles that were visible were spherical and had distinct surface spike protein projections, consistent with a previously published SARS-CoV2 image (Appendix Figure, panel C) (*1*).

To estimate viral loads ($\log_{10}$ PFU equivalents/mL) in clinical samples from qRT-PCR cycle threshold values, we generated a standard curve from a serially diluted SARS-CoV-2 of known plaque titer. Viral loads quantified by using this method were viral RNA levels, not of infectious virus. The viral load was higher in feces than in respiratory specimens collected at multiple time points (17–28 days after symptom onset) (Appendix Figure, panel D). Isolation of virus from feces samples collected at later time points was not successful, although results for virus RNA remained positive, indicating only RNA fragments, not infectious virus, in feces of this patient collected at later time points of disease onset.

We collected feces specimens from 28 patients; 12, including the patient described in this report, were positive for viral RNA for ≥1 time point. We attempted to isolate SARS-CoV-2 virus from 3 of the viral RNA–positive patients. Results were successful for 2 of 3 patients, including the patient from this report, indicating that infectious virus in feces is a common manifestation of COVID-19.

The patient from this report had a high level of IgG against spike protein. Levels of nucleocapsid protein–specific antibodies were relatively lower. Spike protein (1,274 aa) is much larger than nucleoprotein protein (420 aa), which potentially contains more epitopes inducing specific antibody responses.

We also identified neutralization antibodies by using a focus reduction neutralization test. Neutralizing titers (50% focus reduction neutralization test) ranged from 1:1,065 to >1:4,860 at different time points (Appendix Figure, panel E). To show that isolated virus was infectious to susceptible cells, we tested fresh Vero E6 cells infected with the virus isolate by using indirect immunofluorescent assay and serum samples from the patient and a healthy donor. A positive reaction was only obtained with the patient serum (Appendix Figure 1, panel F).

Isolation of infectious SARS-CoV-2 in feces indicates the possibility of fecal–oral transmission or fecal–respiratory transmission through aerosolized feces. During the 2003 severe acute respiratory syndrome pandemic, 329 residents of a private housing estate in Hong Kong were infected; 42 died (*10*). Investigation of the building's structure showed that faulty sewage pipelines led to aerosolization of contaminated feces, which was believed to be the source of infection.

Our findings indicate the need for appropriate precautions to avoid potential transmission of SARS-CoV-2 from feces. Discharge and hospital cleaning practices should consider this possibility for critically ill patients or those who died who had high viral loads and are more likely to shed infectious virus.

Acknowledgments

We thank Nanshan Zhong and Malik Peiris for providing helpful discussions and critically reviewing the manuscript and the patient for participating in the study.

This study was supported by grants from The National Key Research and Development Program of China (2018YFC1200100), the National Science and Technology Major Project (2018ZX10301403), emergency grants for prevention and control of SARS-CoV-2 from the Ministry of Science and Technology (2020YFC0841400) and Guangdong Province (2020B111108001 and 2018B020207013).

About the Author

Dr. Xiao is a research scientist in the Department of Infectious Diseases, Guangdong Provincial Key Laboratory of Biomedical Imaging, Guangdong Provincial Engineering Research Center of Molecular Imaging, The Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, Guangdong, China. His research interests focus on immunopathogenesis of infectious diseases in humans.

References

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al.; China Novel Coronavirus Investigating and Research Team. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020;382:727–33. https://doi.org/10.1056/NEJMoa2001017
2. Chan JF, Yuan S, Kok KH, To KK, Chu H, Yang J, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet. 2020;395:514–23. https://doi.org/10.1016/S0140-6736(20)30154-9
3. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al.; China Medical Treatment Expert Group for Covid-19. Clinical characteristics of coronavirus disease 2019 in China.

N Engl J Med. 2020;382:1708–20. https://doi.org/10.1056/NEJMoa2002032
4. Pan Y, Zhang D, Yang P, Poon LL, Wang Q. Viral load of SARS-CoV-2 in clinical samples. Lancet Infect Dis. 2020; 20:411–2. https://doi.org/10.1016/S1473-3099(20)30113-4
5. Wu Y, Guo C, Tang L, Hong Z, Zhou J, Dong X, et al. Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. Lancet Gastroenterol Hepatol. 2020;5:434–5. https://doi.org/10.1016/S2468-1253(20)30083-2
6. Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, et al. Detection of SARS-CoV-2 in different types of clinical specimens. JAMA. 2020. https://doi.org/10.1001/jama.2020.3786
7. Zheng S, Fan J, Yu F, Feng B, Lou B, Zou Q, et al. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January–March 2020: retrospective cohort study. BMJ. 2020;369:m1443. https://doi.org/10.1136/bmj.m1443
8. Sun J, Zhu A, Li H, Zheng K, Zhuang Z, Chen Z, et al. Isolation of infectious SARS-CoV-2 from urine of a COVID-19 patient. Emerg Microbes Infect. 2020;Apr 28:1–8. https://doi.org/10.1080/22221751.2020.1760144
9. Xiao F, Tang M, Zheng X, Liu Y, Li X, Shan H. Evidence for gastrointestinal infection of SARS-CoV-2. Gastroenterology. 2020;158:1831–1833.e3. https://doi.org/10.1053/j.gastro.2020.02.055
10. Yu IT, Li Y, Wong TW, Tam W, Chan AT, Lee JH, et al. Evidence of airborne transmission of the severe acute respiratory syndrome virus. N Engl J Med. 2004;350:1731–9. https://doi.org/10.1056/NEJMoa032867

Address for correspondence: Jincun Zhao, State Key Laboratory of Respiratory Disease, National Clinical Research Center for Respiratory Disease, Guangzhou Institute of Respiratory Health, the First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510182, China, and Institute of Infectious Disease, Guangzhou Eighth People's Hospital of Guangzhou Medical University, Guangzhou 510060, China; email: zhaojincun@gird.cn or jczhao@gzhmu.edu.cn

Estimation of Coronavirus Disease Case-Fatality Risk in Real Time

Yang Ge, Shengzhi Sun

Author affiliations: The University of Georgia, Athens, Georgia, USA (Y. Ge); Boston University School of Public Health, Boston, Massachusetts, USA (S. Sun)

DOI: https://doi.org/10.3201/eid2608.201096

We ran a simulation comparing 3 methods to calculate case-fatality risk for coronavirus disease using parameters described in previous studies. Case-fatality risk calculated from these methods all are biased at the early stage of the epidemic. When comparing real-time case-fatality risk, the current trajectory of the epidemic should be considered.

We read with interest the research letter on estimating case-fatality risk for coronavirus disease (COVID-19) by Wilson, et al. (*1*). In their analyses, the authors estimated the case-fatality risk adjusted to a fixed lag time to death. They acknowledged that the calculated adjusted case-fatality risk (aCFR) might be influenced by residual uncertainties from undiagnosed mild COVID-19 cases and a shortage of medical resources. However, we believe the time-varying number of cumulative cases and deaths also should be considered in the epidemic profile.

Because of the exponential growth curve of the COVID-19 outbreak, the numbers of cumulative cases and cumulative deaths have been relatively close to each other in the early stages of the outbreak, leading to a much higher aCFR. As the outbreak progresses, the ratio of the cumulative cases and deaths declines, which reduces the aCFR. Thus, a higher aCFR does not necessarily indicate increased disease severity.

To test our hypothesis, we performed a simulation study by using a susceptible-infectious-recovered–death model and parameters set according to prior studies. We set the infectious period as 10 days (*2*); case-fatality risk as 3% (*3*); basic reproductive ratio (R_0) as 2.5 (*4*); recovery rate as 1/13 day (*5*), that is, 13 days from illness onset to recovery; and the population size as 1 million. We compared crude case-fatality risk, aCFR per Wilson et al.'s method, and aCFR per Mizumoto et al.'s method (*6*). Although the case-fatality risk calculated from these methods all are biased at the early stage of the epidemic, case-

Article DOI: https://doi.org/10.3201/eid2608.200681

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Appendix

Methods

Virus Isolation and Transmission Electron Microscopy

Vero E6 cells were used for virus isolation. Cells were cultured in Dulbecco modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS). A quantitative reverse transcription PCR (qRT-PCR)–positive fecal swab specimen was saved in viral transport media (DMEM containing 1% bovine serum albumin, 15 μg/mL amphotericin, 100 units/mL penicillin G, and 100 μg/mL streptomycin). Before virus isolation, the sample was filtered with 0.45-μm strainer and diluted 1:10 with DMEM containing 2% FBS and antimicrobial drugs. Cells were infected at 37°C for 1 h. The inoculum was removed and replaced with fresh culture medium. The cells were incubated at 37°C and observed daily for a cytopathic effect. If there was no obvious cytopathic effect until day 6 postinfection, the cells and supernatant were scraped up, freeze-thawed once, and overlaid to new cells for second-round passage. Culture supernatant was negatively stained and visualized by transmission electron microscopy.

qRT-PCR

Viral RNA from respiratory and fecal swab specimens was extracted by using the Nucleic Acid Isolation Kit (Zybio Inc., https://m.zybio.com). A real-time PCR assay kit targeting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) open reading frame 11ab and nucleoprotein gene regions was provided by Zybio Inc. Serial sampling of nasopharyngeal swab, oropharyngeal swab, and fecal swab specimens were used to monitor viral shedding during SARS-CoV-2 infection. To estimate viral loads in clinical samples from qRT-PCR cycle threshold values, a standard curve was generated from a serially diluted SARS-CoV-2 standard of known plaque titer. Viral loads in clinical samples were then calculated as $\log_{10}$ PFU equivalents/mL.

Complete Genome Sequencing

Full-length viral genome sequence was obtained by using next-generation sequencing. Viral RNA was extracted from virus culture supernatant by using the QIAamp Viral RNA Extraction Kit (QIAGEN, https://www.qiagen.com) according to the manufacturer's instructions. The concentration of RNA was determined by using a Qubit 4 Fluorometer (ThermoFisher Scientific, https://www.thermofisher.com), and 50–1,000 ng RNA were mixed with rRNA deletion probes for rRNA depletion. Library construction was then completed by using the RNA library construction kit (Vision Medicals, http://m.visionmedicals.com), which including fragmentation, the synthesis of first and second strands, end repairment, and adaptor ligation. The amplified libraries of each sample were pooled and sequenced on an Illumina Nextseq Sequencer (https://www.illumina.com) for clinical metagenomic analysis. A minimum of 10 million single-end 75-bp reads were obtained per sample. Sequence analysis was performed by using the Vision Medicals IDseqTM commercial bioinformatic pipeline. In brief, low-quality and short (length < 35 bp) reads and reads that mapped to human genome and plasmids were removed. The remaining reads were taxonomically classified by aligning curated microbial database consisting of viruses, bacteria, fungi, and parasites. The taxonomic references were downloaded from National Center Biotechnology Information (Bethesda, MD, USA). Upon identification of critical pathogen, the identified species-specific sequences were further confirmed by using blastn (https://blast.ncbi.nlm.nih.gov) for further accuracy validation. Complete genome was aligned to SARS-CoV-2 reference genome NC045512.2.

SARS-CoV-2 Spike- and Nucleoprotein-Specific IgG ELISAs

Serum samples were collected and used to analyze SARS-CoV-2–specific IgG against SARS-CoV-2 spike and nucleocapsid proteins in ELISAs. Spike and nucleocapsid proteins were coated on ELISA plates at a concentration of 50 ng/well overnight at 4°C. After blocking in Dulbecco phosphate-buffered saline and 10% FBS, 100 μL diluted plasma (1:100) were added, and plates were incubated at 37°C for 1 h. After washing, plates were incubated with 100 μL of horseradish peroxidase–conjugated mouse anti-human IgG (heavy plus light chain) (Jackson ImmunoResearch, https://www.jacksonimmuno.com) at 37°C for 1 h. Reactions were visualized by adding 50 μL of 3,3',5,5'-tetramethylbenzidine substrate solution (Biohao Biotechnology Co., Ltd., http://www.biohao.com. Optical densities at 450 nm were then read. A serum sample from a healthy donor was used as a negative control, and a serum sample from a confirmed coronavirus disease patient was used as a positive control.

Focus Reduction Neutralization Test

A SARS-CoV-2 focus reduction neutralization test was performed in a certified Biosafety Level 3 Laboratory. Plasma samples (75 μL/sample) were serial diluted, mixed with 75 μL of SARS-CoV-2 (8×10^3 FFU/mL) in 96-well microwell plates, and incubated for at 37°C for 1 h. Mixtures were then transferred to 96-well plates seeded with Vero E6 cells and incubated (to enable absorption) at 37°C for 1 h. Inoculums were then removed before adding the overlay media (100 μL minimum Eagle medium containing 1.6% carboxymethylcellulose. The plates were then incubated at 37°C for 24 h. Cells were fixed with 4% paraformaldehyde solution for 30 min, and overlays were removed. Cells were permeabilized with 0.2% Triton X-100 and incubated with cross-reactive rabbit anti-SARS-CoV-N IgG (Sino Biological, Inc., https://www.sinobiological.com) for 1 h at room temperature before adding horseradish peroxidase–conjugated–conjugated goat anti-rabbit IgG (heavy plus light chain) (Jackson ImmunoResearch). Cells were further incubated at room temperature. The reactions were developed with KPL TrueBlue Peroxidase substrates (Sera care Life Sciences Inc., https://www.seracare.com). The numbers of SARS-CoV-2 foci were calculated by using an EliSpot reader (Cellular Technology Ltd.; http://www.immunospot.com).

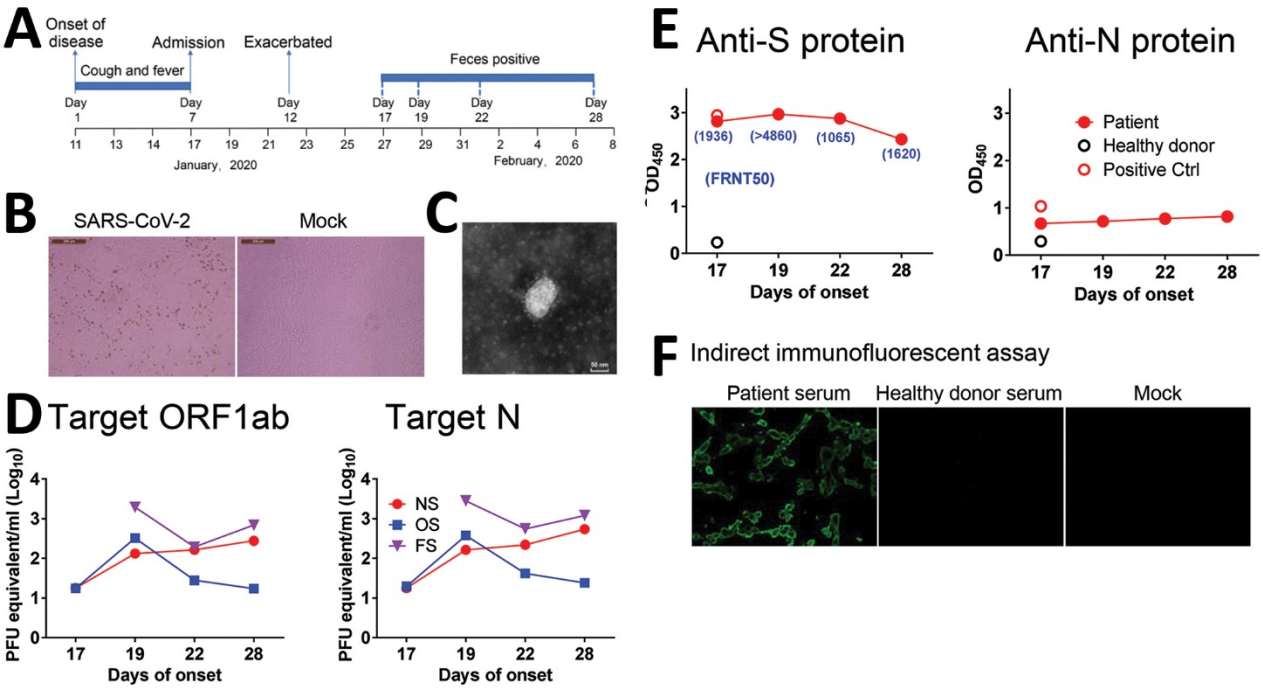
Indirect Immunofluorescence Assay

An indirect immunofluorescence assay IFA was performed by using Vero E6 cells grown on cover slips that were infected with the virus isolate at a multiplicity of infection of 0.05 for 24 h. Viral antigens were detected by using patient serum as primary antibody and Alexa Fluor 488 dye–labeled goat anti-human IgG as secondary antibody. Nuclei were counterstained with 4′,6-diamidino-2-phenylindole. Serum from a healthy donor was used as a negative control. The slips were observed by using a laser scanning confocal microscope (LSM 880; Zeiss, https://www.zeiss.com).

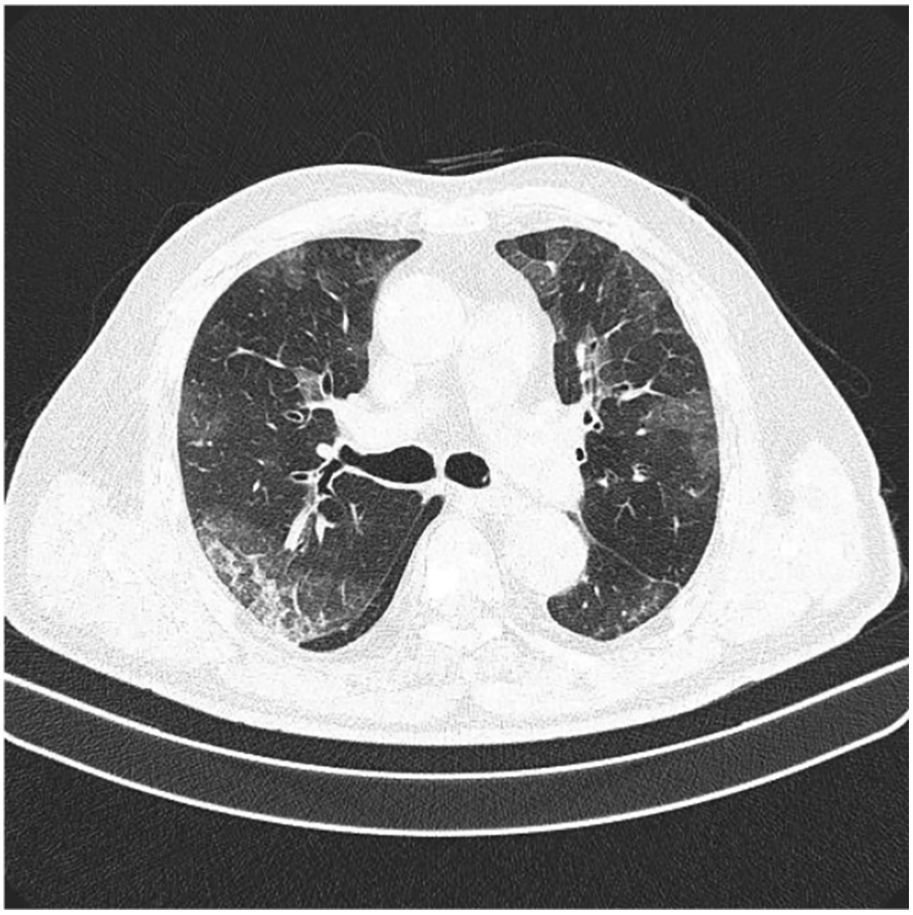
Appendix Table. Nucleotide and amino acid changes between original coronavirus virus strain (Wuhan-Hu-1 NC_045512.2) and virus isolated from the feces of the patient

Genome nucleotide position	Protein	Nucleotide changes		Amino acid changes
		NC_045512.2	MT123292	
654	ORF1ab	G	A	Gly > Glu
6819	ORF1ab	G	T	Ser > ILe
6996	ORF1ab	T	C	Ile > Thr
17373	ORF1ab	C	T	Synonymous
29527	N	G	A	Synonymous

*N, nucleoprotein; ORF, open reading frame.



Appendix Figure 1. Clinical information and isolation of SARS-CoV-2 from feces of a patient. A) Clinical events. B) Vero E6 cells infected with SARS-CoV-2 isolate for 72 hours. C) Detection of viral particles by using transmission electron microscopy (original magnification, ×98,000). D) Viral loads in respiratory and fecal specimens. NS, nasopharyngeal swab specimen; OS, oropharyngeal swab specimen; FS, fecal swab specimen. E) SARS-CoV-2–specific antibody against spike (S) and nucleoprotein (N) in patient and neutralizing antibody. F) Indirect immunofluorescent assay detection of SARS-CoV-2–infected Vero E6 cells by using patient serum collected on February 7, 2020 (28 days postonset of illness) (original magnification ×200). Ctrl, control; FRNT50, 50% focus reduction neutralization test; ORF, open reading frame; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.



Appendix Figure 2. Chest computed tomography of the patient infected with severe acute respiratory syndrome coronavirus 2.