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About the Author

Dr. Vetter is an infectious diseases physician at Geneva University Hospitals and Geneva Centre for Emerging Viral Diseases, Geneva. Her research interests include emerging viral diseases.

References

1. Manigold T, Vial P. Human hantavirus infections: epidemiology, clinical features, pathogenesis and immunology. Swiss Med Wkly. 2014;144:w13937. https://doi.org/10.4414/smw.2014.13937
2. Tkachenko EA, Ishmukhametov AA, Dzagurova TK, Bernshtein AD, Morozov VG, Siniugina AA, et al. Hemorrhagic fever with renal syndrome, Russia. Emerg Infect Dis. 2019;25:2325–8. https://doi.org/10.3201/eid2512.181649
3. Kramski M, Meisel H, Klempa B, Krüger DH, Pauli G, Nitsche A. Detection and typing of human pathogenic hantaviruses by real-time reverse transcription-PCR and pyrosequencing. Clin Chem. 2007;53:1899–905. https://doi.org/10.1373/clinchem.2007.093245
4. World Health Organization. Clinical management of patients with haemorragic fever. 2016 [cited 2020 Sep 30]. https://apps.who.int/iris/bitstream/handle/10665/205570/9789241549608_eng.pdf
5. Alexeyev OA, Suzdaltsev AA, Verkhovtsev VN, Efratova ES, Roschupkin VI. A major outbreak of hemorrhagic fever with renal syndrome in the Samara region, European Russia. Infection. 1998;26:322. https://doi.org/10.1007/BF02962264
6. Fontana-Binard L, Schultze D, Rojanavisut BS, Krüger DH, Dollenmaier G, Zanetti G, et al. First case of nephropathia epidemica acquired in Switzerland [in French]. Rev Med Suisse. 2008;4:1572–5.
7. Garanina E, Martynova E, Davidyuk Y, Kabwe E, Ivanov K, Titova A, et al. Cytokine storm combined with humoral immune response defect in fatal hemorrhagic fever with renal syndrome case, Tatarstan, Russia. Viruses. 2019;11:601. https://doi.org/10.3390/v11070601
8. Mäkelä S, Mustonen J, Ala-Houhala I, Hurme M, Partanen J, Vapalahti O, et al. Human leukocyte antigen-B8-DR3 is a more important risk factor for severe Puumala hantavirus infection than the tumor necrosis factor-alpha(-308) G/A polymorphism. J Infect Dis. 2002;186:843–6. https://doi.org/10.1086/342413
9. Mustonen J, Partanen J, Kanerva M, Pietilä K, Vapalahti O, Pasternack A, et al. Association of HLA B27 with benign clinical course of nephropathia epidemica caused by Puumala hantavirus. Scand J Immunol. 1998;47:277–9. https://doi.org/10.1046/j.1365-3083.1998.00302.x
10. Malinin OV, Platonov AE. Insufficient efficacy and safety of intravenous ribavirin in treatment of haemorrhagic fever with renal syndrome caused by Puumala virus. Infect Dis (Lond). 2017;49:514–20. https://doi.org/10.1080/23744235.2017.1293841

Address for correspondence: Pauline Vetter, Geneva Center for Emerging Viral Diseases, Geneva University Hospitals, 4, rue Gabrielle-Perret Gentil, 1205 Geneva, Switzerland; email: pauline.vetter@hcuge.ch; Manuel Schibler, Division of Infectious Diseases, Geneva University Hospitals, 4, rue Gabrielle Perret-Gentil, 1205 Geneva, Switzerland; email: manuel.schibler@hcuge.ch

Protective Immunity and Persistent Lung Sequelae in Domestic Cats after SARS-CoV-2 Infection

Shiho Chiba, Peter J. Halfmann, Masato Hatta, Tadashi Maemura, Shufang Fan, Tammy Armbrust, Olivia M. Swartley, LaTasha K. Crawford, Yoshihiro Kawaoka

Author affiliations: University of Wisconsin–Madison School of Veterinary Medicine, Madison, Wisconsin, USA (S. Chiba, P.J. Halfmann, M. Hatta, T. Maemura, S. Fan, T. Armbrust, O.M. Swartley, L.K. Crawford, Y. Kawaoka,); University of Tokyo Institute of Medical Science, Tokyo, Japan (Y. Kawaoka)

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Severe acute respiratory syndrome coronavirus 2 readily transmits between domestic cats. We found that domestic cats that recover from an initial infection might be protected from reinfection. However, we found long-term persistence of inflammation and other lung lesions after infection, despite a lack of clinical symptoms and limited viral replication in the lungs.

Previous studies have demonstrated the transmissibility of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) by direct or indirect contact between domestic cats (*1*,*2*). Given the

close relationship between cats and humans, further characterization of the biology of SARS-CoV-2 in cats is warranted.

We inoculated domestic cats with SARS-CoV-2, and on postinfection days 3, 6, and 10, sampled organs to titrate virus (Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/27/2/20-3884-App1.pdf). In plaque-forming assays in VeroE6/TMPRSS2 cells, infectious viruses were detected in the nasal turbinates and trachea of all animals on day 3, and most on day 6, whereas virus detection in the lungs was limited on day 3 and absent on day 6 (Appendix Figure 2, panel A). These results suggest that the virus replicated efficiently in upper respiratory organs, which might contribute to its high transmissibility among cats. Infectious virus was cleared from the upper and lower respiratory organs by day 10 (Appendix Figure 2, panel A). No animal showed any signs of respiratory illness during the study (Appendix Figure 3). Infectious virus was not detected (detection limit 10 pfu/g of tissue) in other examined organs (e.g., brain, liver, spleen, kidney, small and large intestine, heart, and eyelids). Viral antigen was detected in nasal turbinates and trachea but was sparse within the lungs at day 3 (Appendix Figure 4).

We conducted histopathologic examination of the lungs, trachea, and nasal turbinates. Lymphocytic inflammation within the tracheal submucosa was present on days 3 to 10, whereas lymphocytic to mixed inflammation in the nasal cavity was more severe on days 3 and 6 but minimal on day 10. In lungs, moderate lesions persisted despite clearance of virus. On day 3, we observed mild bronchitis with lymphoid hyperplasia, moderate to severe histiocytic bronchiolitis with partial to complete occlusion of lumina, and moderate to severe thickening of alveolar septa (Appendix Figure 2, panel B; Appendix Figures 4, 5). Interstitial inflammatory infiltrate decreased significantly over time ($p = 0.0012$, $F = 34.70$, by 1-way analysis of variance) (Appendix Figure 2, panel C); however, by day 10, alveolar septa remained thickened (Appendix Figure 5). Bronchiolitis remained with partial occlusion of bronchioles, even in regions with minimal alveolar lesions (Appendix Figure 2, panel B).

Because SARS-CoV-2 did not cause acute lethal respiratory disease in the cats in our study, cats are a compelling animal model for studying the long-term effects of nonfatal infections. Cats were infected with SARS-CoV-2 and euthanized at postinfection day 28 (Appendix Figure 6, 7). Persistent lung lesions were observed 28 days after infection, including histiocytic bronchiolitis with luminal plugs and thickened alveolar septa, similar to lesions observed on day 10 but with more chronic features such as peribronchiolar fibrosis and vascular

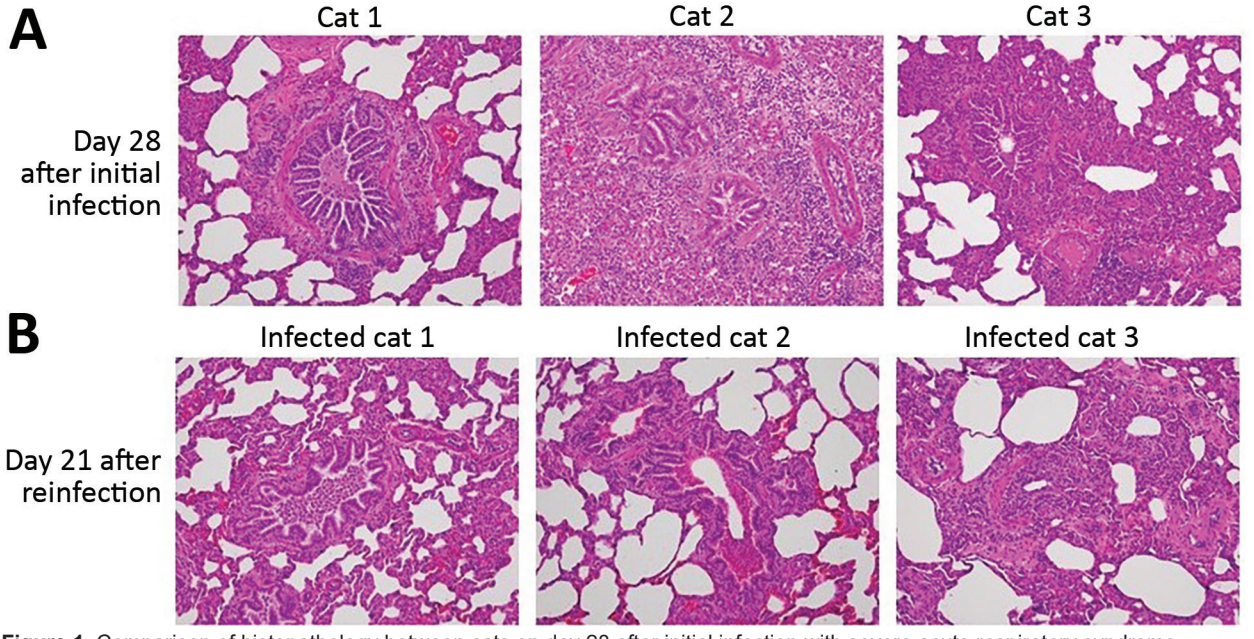
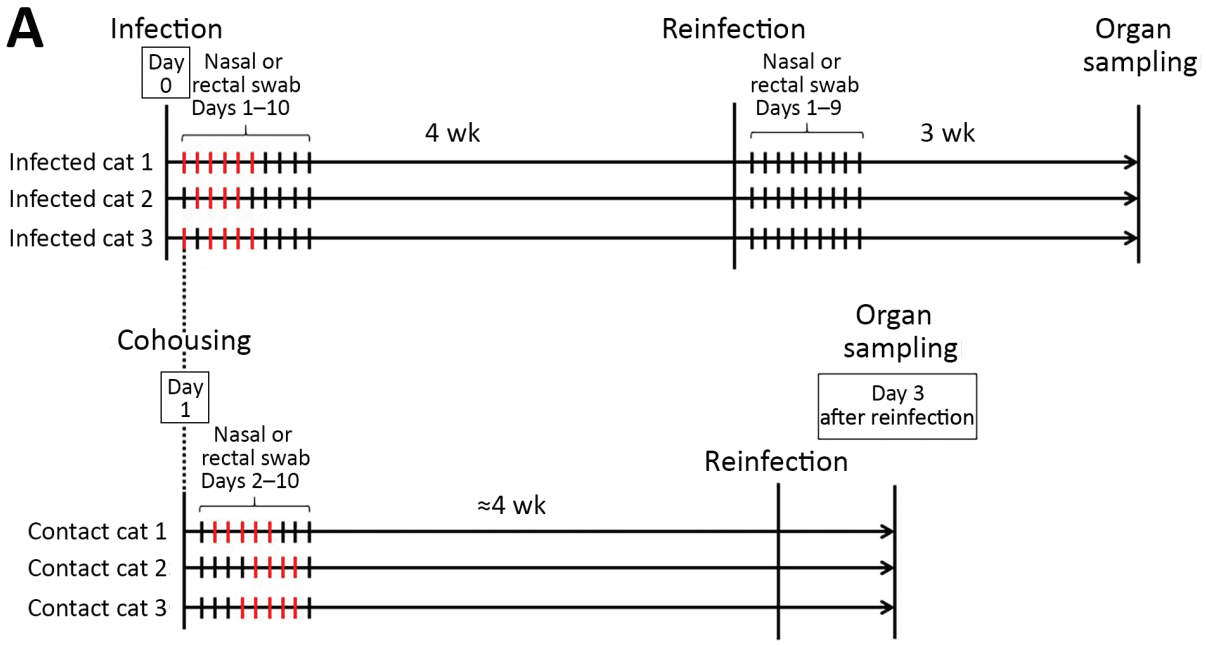


Figure 1. Comparison of histopathology between cats on day 28 after initial infection with severe acute respiratory syndrome coronavirus 2 and on day 21 after reinfection. Bronchioles and alveoli of cats (cats 1–3 in Appendix Figure 6; https://wwwnc.cdc.gov/EID/article/27/1/20-3884-App1.pdf) on day 28 after initial infection (A) and those of cats (infected cats 1–3 in Appendix Figure 6, upper half) on day 21 after reinfection (49 days after the initial infection) (B); original magnification 20 ×. Cats from both groups showed histiocytic bronchiolitis with occlusive plugs, peribronchiolar fibrosis, and thickening of alveolar septa. Mild acute hemorrhage was detected in affected and less affected regions of the lung on day 21 after reinfection, with a trend toward an increase compared with day 28 (severity score 1.8 ± SEM 0.8 on day 21 vs. 0.3 ± SEM 0.2 on day 28; $p = 0.187$ by unpaired *t*-test).

proliferation within the thickened interstitium. We observed a notable dearth of fibrosis within alveolar septa, in contrast to what has been reported for humans with severe acute respiratory syndrome or Middle East respiratory syndrome (*3*,*4*). One cat had severe pneumonia with fibrin in alveolar spaces and endothelialitis (Appendix Figure 8), similar to what has been reported in humans with fatal coronavirus disease (*5*), although this cat did not show any respiratory signs.

To determine whether previous infection provides protection from future potential infection by SARS-CoV-2, we performed a reinfection study with 2 groups of cats. We previously reported that SARS-CoV-2 was transmitted from cats inoculated with the virus to cohoused, naive cats (*1*). In the previous study, the 3 cats that had been inoculated with SARS-CoV-2, whose nasal swabs were virus-negative on day 6 or 7 after the initial infection (*1*), were reinoculated with the same virus 4 weeks after the initial infection (Figure 1; Figure 2, panel A). No infectious virus was detected in the nasal or rectal swabs after reinfection, suggesting that the animals were protected from reinfection. These cats were euthanized at 21 days after reinfection (49 days after the initial infection), and tissue was submitted for histopathologic examination. The reinfection group showed lesions that were comparable with lung lesions observed on day 28 but with less severe thickening of alveolar septa ($p = 0.041$, by unpaired *t*-test) (Figure 1; Figure 2 panel B). The 3 cats in the other group, which recovered from infection that was transmitted by contact with virus-inoculated cats, were reinfected with the virus at ≈4 weeks (29–32 days) after transmission. On day 3 after reinfection, organs were harvested; infectious virus was not detected (detection limit 10 pfu/g of tissue) in respiratory organs or other organs



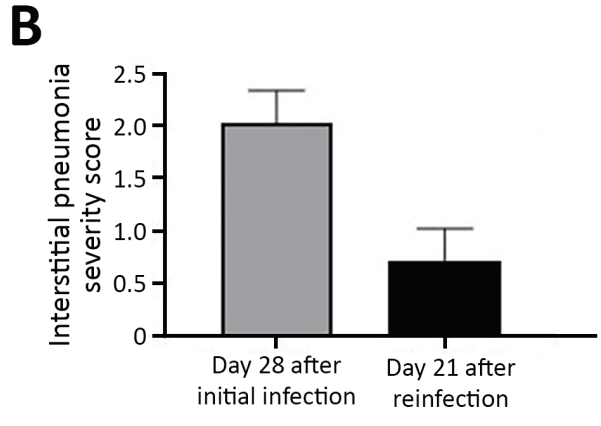


Figure 2. Timeline of severe acute respiratory syndrome coronavirus 2 infection and reinfection of cats and distribution of interstitial thickening. A) Timeline of infection and reinfection. As reported previously (*1*), a group of cats was inoculated with severe acute respiratory syndrome coronavirus 2 on day 0 (infected cats 1–3, upper half). A virus-naive cat was cohoused with each of the infected cats from day 1 (contact cats 1–3, lower half). The days on which infectious virus was detected in the nasal swabs are shown as red bars for each animal. In this study, we infected the cats with the same severe acute respiratory syndrome coronavirus 2 isolate at ≈4 weeks after initial infection or exposure to infected cats. After reinfection of the group shown in the upper half of the figure, no infectious virus was detected in the nasal swabs. The cats were confirmed to be seronegative before the initial infection or cohousing with infected cats, and seropositive before reinfection, on the basis of neutralization assay results. B) The distribution of interstitial thickening (interstitial pneumonia severity score) was decreased on day 21 after reinfection compared with day 28 ($p = 0.041$ by unpaired *t*-test).

analyzed (e.g., brain, liver, spleen, kidney, small and large intestine, heart, and eyelids). These results suggest that virus infection by natural transmission between cats, as well as by experimental inoculation, induces protective immunity against a second SARS-CoV-2 infection.

In conclusion, SARS-CoV-2 replicated effectively in the upper respiratory tract in cats, and infectious virus was cleared from the lungs within 6 days of infection; however, histopathologic examination demonstrated chronic lung sequelae in cats even a month after viral clearance. After initial infection with SARS-CoV-2, cats were protected from reinfection, with no virus replication in respiratory organs and no additional lung damage.

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About the Author

Dr. Chiba is a molecular virologist at the Influenza Research Institute at the University of Wisconsin–Madison, with a background in innate immunity studies and structural biology. Her primary research interests include mechanisms of virus infection, virus antigenicity, and host immune responses.

References

1. Halfmann PJ, Hatta M, Chiba S, Maemura T, Fan S, Takeda M, et al. Transmission of SARS-CoV-2 in domestic cats. N Engl J Med. 2020;383:592–4. https://doi.org/10.1056/NEJMc2013400
2. Shi J, Wen Z, Zhong G, Yang H, Wang C, Huang B, et al. Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. Science. 2020;368:1016–20. https://doi.org/10.1126/science.abb7015
3. Cheung OY, Chan JW, Ng CK, Koo CK. The spectrum of pathological changes in severe acute respiratory syndrome (SARS). Histopathology. 2004;45:119–24. https://doi.org/10.1111/j.1365-2559.2004.01926.x
4. Das KM, Lee EY, Singh R, Enani MA, Al Dossari K, Van Gorkom K, et al. Follow-up chest radiographic findings in patients with MERS-CoV after recovery. Indian J Radiol Imaging. 2017;27:342–9. https://doi.org/10.4103/ijri.IJRI_469_16
5. Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, et al. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. N Engl J Med. 2020;383:120–8. https://doi.org/10.1056/NEJMoa2015432

Address for correspondence: Yoshihiro Kawaoka, 575 Science Dr, Madison, Wisconsin 53711, USA; email: yoshihiro.kawaoka@wisc.edu; or LaTasha K. Crawford, 2015 Linden Dr, Madison, Wisconsin 53706, USA; email: lkcrawford@wisc.edu

Long-Term Humoral Immune Response in Persons with Asymptomatic or Mild SARS-CoV-2 Infection, Vietnam

Huynh Kim Mai, Nguyen Bao Trieu, Trinh Hoang Long, Hoang Tien Thanh, Nguyen Dinh Luong, Le Xuan Huy, Lam Anh Nguyet, Dinh Nguyen Huy Man, Danielle E. Anderson, Tran Tan Thanh, Nguyen Van Vinh Chau, Guy Thwaites, Lin-Fa Wang, Le Van Tan, Do Thai Hung

Author affiliations: Pasteur Institute, Nha Trang City, Vietnam (H.K. Mai, N.B. Trieu, T.H. Long, H.T. Thanh, N.D. Luong, L.X. Huy, D.T. Hung); Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam (L.A. Nguyet, T.T. Thanh, G. Thwaites, L.V. Tan); Hospital for Tropical Diseases, Ho Chi Minh City (D.N.H. Man, N.V.V. Chau); Duke-NUS Medical School, Singapore (D.E. Anderson, L.-F. Wang); Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, UK (G. Thwaites); SingHealth Duke-NUS Global Health Institute, Singapore (L.-F. Wang)

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