Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Dried SARS-CoV-2 virus maintains infectivity to Vero E6 cells for up to 48 h

Hyesun Jang a, Ted M. Ross a, b, * 

a Center for Vaccines and Immunology, University of Georgia, Athens, GA, USA 
b Department of Infectious Diseases, University of Georgia, Athens, GA, USA

ARTICLE INFO

Keywords:
SARS-CoV-2 
Stability of dried enveloped viruses 
Infectivity of SARS-CoV-2 
Prevention of COVID-19

ABSTRACT

Severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) is a great concern on both public and veterinary health. Multiple studies showed that the SARS-CoV-2 can persist for few days in wet condition, but it has not been clear whether the virus can maintain the infectivity in dry condition. Thus, we measured the infectious titer of dried SARS-CoV-2 (10⁴ pfu/25 μL droplet) at 0, 0.5, 1, 2, 3, 24, and 48 h. Strikingly, the dried SARS-CoV-2 virus did not lose the infectivity to Vero E6 cells for up to 48 h. Our findings warrants that the drying cannot replace the surface disinfection to prevent transmission via common vehicle or nosocomial infection. Future studies can apply our experimental setting to test the efficacy of diverse disinfecting procedures.

The world is experiencing an intensive pandemic (COVID-19) caused by a novel zoonotic severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2). The SARS-CoV-2 appear to be originally derived from bat, and pangolins might serve as a intermediate host to deliver the virus into human (Yoo and Yoo, 2020; Zhou et al., 2020). Also, multiple studies confirmed that the SARS-CoV-2 can cause the reverse-zoonotic infection of pet animals, such as cats, dogs, hamsters and minks (Molenaar et al., 2020; Stout et al., 2020; Yoo and Yoo, 2020). Thus, control of SARS-CoV2 is a great issue to both public and veterinary health.

The world-wide lockdown and quarantine procedures could not stop the spread of SARS-CoV-2. Multiple factors may contribute to the rapid spreading of this virus, such as viral shedding from asymptomatic patients, lack of pre-existing immunity, and high stability of the virus.

Several studies have reported that SARS-CoV-2 does not lose its infectivity in diverse environments for days, especially on glass surfaces (World Health Organization, 2020). According to an early report from the World Health Organization, SARS-CoV-2 collected from sterilized stool was infectious for up to four days on glass slides. In a different setting, the half-life of free SARS-CoV-2 was 1.2 h on a glass surface, which is similar to its half-life on a mask (1.0–1.4 h) or a plastic surface (1.6 h) but much longer than its half-life on stainless steel (0.3 h) or a banknote (Chin et al., 2020). Pastorino et al. verified that the presence of BSA could substantial prolong the infectivity of SARS-CoV-2 on glass, plastic, and aluminum surface (Pastorino et al., 2020). As the presence of BSA mimics the environment of body fluids, such as cough droplets, sputum, and airway mucus secretions, the study result warrants that the SARS-CoV-2 virus contained in body fluid could persist longer than 100 h (Pastorino et al., 2020).

These early reports emphasize the need for surface decontamination to prevent the spreading of SARS-CoV-2. However, those studies were conducted on viruses in liquid, and might not truly represent dry environment where nosocomial or contact transmission occurs.

Table 1

| Time   | Virus titer<br>(TCID₅₀/mL) | Previous report (Chin et al) Log<br>(TCID₅₀/mL) |
|--------|-----------------------------|-----------------------------------------------|
|        | Log (TCID₅₀/mL) | Log (PFU/mL) | Mean ± SD | Mean ± SD | Mean ± SD |
| Stock  | 6.1 0          | 5.60³ –      | –7.8 –    |          |          |
| .5 hr  | 5.23 0.18      | 4.00 0.17    | 5.83 0.04 |          |          |
| 1 hr   | 5.10³ –        | N.D. N.D. N.D. | N.D. N.D. | N.D. N.D. |
| 2 hrs  | 4.48 0.18      | 3.22 0.12    | 3.48 0.37 |          |          |
| 3 hrs  | 4.23 0.18      | 3.48 0.09    | 3.14 0.05 |          |          |
| 24 hrs | 3.10 0.35      | 3.48 0.12    | 2.44 0.19 |          |          |
| 48 hrs | 1.23 0.18      | N.D. N.D. N.D. |          |          |

¹All the virus titers were titrated using Vero-E6 cells. All TCID₅₀/mL was calculated in this study is based on two independent quadruplicates unless indicated otherwise. The PFU/mL was based on one experiment with triplicates.

²Value obtained from one trial N.D.: not done.

* Corresponding author at: Center for Vaccines and Immunology, University of Georgia, Athens, GA, USA.
E-mail address: tedross@uga.edu (T.M. Ross).

https://doi.org/10.1016/j.vetmic.2020.108907

Received 18 August 2020; Accepted 20 October 2020
Available online 23 October 2020
0378-1135/© 2020 Elsevier B.V. All rights reserved.
We investigated the stability of SARS-CoV-2 on a dry surface, particularly on glass. As a non-porous material, glass is commonly used as bowl for feed or water, and caging which can act as a vehicle for the transmission of SARS-CoV-2. The SARS-CoV-2 infectivity was maintained in glass for longer than that in other types of surfaces, such as aluminum. Further, a glass surface was more appropriate for the development of a standardized methodology by simply using glass slides that are commonly used for histology analyses.

Briefly, prior to the experiment, the backside of a glass slide was divided into four equal sections (about 35 mm by 7.5 mm) using ImmEdge Hydrophobic Barrier PAP Pen (Vector Laboratories, USA). The SARS-CoV-2 stock solution from passage 2 was diluted to $10^4$ pfu/25 μL in 1 % FBS-containing DMEM, and a 25-μL droplet of the diluted virus suspension was placed on one section of the prepared and previously sterilized glass slide (approximately 10 mm × 35 mm; Fig. S1). To facilitate drying, the droplet was spread within the pre-defined space. The thin layer of diluted virus suspension was left to completely dry inside the biosafety cabinet for about 40 min under the conventional florescence lamp. The glass slide was then moved onto a table in the biosafety-level-3 laboratory. The room temperature and humidity were maintained at around 20 °C and 25 %, respectively, and the air change was approximately 30 times/h. At 0, 0.5, 1.2, 3, 24, and 48 h, the dried virus layer was resuspended in 100 μL 1 % FBS-containing DMEM. To calculate the median tissue culture infectious dose (TCID$_{50}$), the resuspended virus was diluted 10-fold and inoculated onto 5 × 10^4 Vero E6 cells seeded in 96-well cell culture plates. All time points were assessed in quadruplicate and all the procedures were repeated twice. In addition, we repeated the same procedure at 0, 3, and 24 h to measure the plaque-forming unit.

We found that SARS-CoV-2 maintained its infectivity for up to 2 days, despite the dry condition (Table 1). The virus titer right after drying (at 0 h) was only one log lower to that in the original stock solution for (Table 1). The titer was maintained as around $10^5$ TCID$_{50}$/mL for 1 h, and only one log reduction was observed after 3 h. By plaque forming unit, no reduction was observed for up to 3 h. At 24 h, the virus titer was around $10^7$ TCID$_{50}$/mL or pfu/mL (Fig. 1). Even after 48 h, the virus titer was still detectable. The half-life of dried SARS-CoV-2 was calculated as 1.331 h using PRISM GraphPad Software (San Diego, CA, USA) and high relative humidity (>95 %). The viral stability was not significantly influenced by the high relative humidity at a low temperature, or the high temperature at a relatively lower humidity.

**Fig. 1. Reduction of plaque forming unit of dried SARS-CoV2 droplet (25 μl before dry) by time. Representative plaque plates. Dried SARS-CoV-2 virus (~$10^4$pfu) was resuspended with 100 μl of 1 % FBS containing DMEM at 0, 3, and 24 h. The virus suspension was 10-fold diluted and used for the plaque assay performed on monolayer of Vero E6 cells seeded on 12 well cell culture plate (~5 × 10^4/well).**

As a common belief, the enveloped viruses will decay just by drying process. Our findings emphasizes the importance of frequent surface decontamination. For our knowledge, the stability of dried SARS-CoV-2 has not previously investigated. The most similar study was conducted in 2010, with SARS-CoV-1 virus (Chan et al., 2011). The SARS-CoV dried on plastic surface (representing a nonporous surface) showed only one log reduction during incubation at 22~25 °C at relative humidity of 40~50 % for five days (Chan et al., 2011). Interestingly, the viral infectivity dropped faster at high temperature high temperature (38 °C) and high relative humidity (~95 %). The viral stability was not significantly influenced by the high relative humidity at a low temperature, or the high temperature at a relatively lower humidity.

**Funding sources**

This work was funded, in part, by the University of Georgia (UGA) (UGA-001). In addition, TMR is supported by the Georgia Research Alliance as an Eminent Scholar.

**Declaration of Competing Interest**

The authors report no declarations of interest.

**Acknowledgments**

Influenza viruses were provided by BEI resources (Manassas, VA, USA) and by Dr. Jeff Hogan (Athens, GA, USA). The authors would also like to thank the University of Georgia Animal Resource staff, technicians, and veterinarians for animal care and the staff of the Animal Health Research Center (AHRC) Biosafety Level 3 laboratories for providing biosafety and animal care.

**Appendix A. Supplementary data**

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetmic.2020.108907.

**References**

Chan, K.H., Peiris, J.S., Lam, S.Y., Poon, L.L., Yuen, K.Y., Seto, W.H., 2011. The effects of temperature and relative humidity on the viability of the SARS coronavirus. Adv. Virol. 2011, 734690.

Chin, A.W.H., Chu, J.T.S., Perrera, M.R.A., Hui, K.P.Y., Yen, H.-L., Chan, M.C.W., Peiris, M., Poon, L.L.M., 2020. Stability of SARS-CoV-2 in different environmental conditions. Lancet Microbe 1, e10.

Firquet, S., Beauchard, S., Lobert, P.-E., Sané, F., Caloone, D., Izard, D., Hober, D., 2015. Survival of enveloped and non-enveloped viruses on inanimate surfaces. Microbes Environ. 30, 140–144.
Molenaar, R.J., Vreman, S., Hakze-van der Honing, R.W., Zwart, R., de Rond, J., Wessendorp, E., Smits, L.A.M., Koopmans, M., Bouwstra, R., Stegeman, A., van der Poel, W.H.M., 2020. Clinical and pathological findings in SARS-CoV-2 disease outbreaks in farmed mink (Neovison vison). Vet. Pathol. 57, 653–657.

Pastorino, B., Touret, F., Gilles, M., de Lamballerie, X., Charrel, R.N., 2020. Prolonged infectivity of SARS-CoV-2 in Fomites. Emerg. Infect. Dis. 26, 2256–2257.

Stout, A.E., André, N.M., Jaimes, J.A., Millet, J.K., Whittaker, G.R., 2020. Coronaviruses in cats and other companion animals: Where does SARS-CoV-2/COVID-19 fit? Vet. Microbiol. 247, 108777.

World Health Organization, 2020. First Data on Stability and Resistance of SARS Coronavirus Compiled by Members of WHO Laboratory Network. https://www.who.int/csr/sars/survival_2003_05_04/en/.

Yoo, H.S., Yoo, D., 2020. COVID-19 and veterinarians for one health, zoonotic- and reverse-zoonotic transmissions. J. Vet. Sci. 21 e51–e51.

Zhou, H., Chen, X., Hu, T., Li, J., Song, H., Liu, Y., Wang, P., Liu, D., Yang, J., Holmes, E.C., Hughes, A.C., Bi, Y., Shi, W., 2020. A novel bat coronavirus closely related to SARS-CoV-2 contains natural insertions at the S1/S2 cleavage site of the spike protein. Curr. Biol. 30, 2196-2203.e2193.