# Long-term survival of SARS-CoV-2 on salmon as a source for international

## transmission

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Correspondence : M. Liao, Professor, College of Veterinary Medicine, South China Agricultural University, Guangzhou, China ; Institute of Animal Health, Guangdong Academy of Agricultural Sciences, Guangzhou, China. Email: mliao@scau.edu.cn (M.L.) To THE EDITOR-In the recent article, Ratnesar-Shumate et al reported that sunlight could be a potential disinfectant for contaminated nonporous materials [1]. They found that simulated sunlight rapidly inactivated SARS-CoV-2 suspended in either simulated saliva or culture media and dried on stainless steel coupons. Although the surface stability of SARS-CoV-2 in different environmental conditions was reported, we are curious about the survival time of SARS-CoV-2 on meat and fish products.

The first outbreak of COVID-19 in late 2019 and early 2020 was associated with the Huanan Seafood Market in Wuhan, China, while the second outbreak of COVID-19 in June of 2020 was associated with the Xinfadi Seafood Market in Beijing, Chinan [2]. Most recently, China has found several frozen seafood products tainted with COVID-19 [3], raising concerns that imported fish attached with SARS-CoV-2 could be a source of international transmission. Therefore, it is essential to determine the survival time of SARS-CoV-2 in the low-temperature environments of seafood markets.

We studied the titer (50% tissue culture infectious dose/mL, TCID<sub>50</sub>/mL) of viable SARS-CoV-2 attached on salmon or untreated SARS-CoV-2 in culture medium stored at 4°C, the temperature in refrigerators or cold rooms for the temporary storage of fish, or 25°C, the regular room temperature, respectively, using an end-point titration assay on Vero E6 cells, as described previously [4]. The detection limit of the typical TCID<sub>50</sub> assay used in this study was 10<sup>2</sup> TCID<sub>50</sub> / mL [5]. SARS-CoV-2 GDPCC-nCOV4 strain used in this experiment was isolated and provided by Guangdong Provincial Center for Disease Control and Prevention. As shown in Figures A and B, salmon-attached SARS-CoV-2 remained viable at 4°C and 25°C for 8 and 2 days, respectively, while the untreated SARS-CoV-2 in culture medium remained infectious at 4°C and 25°C for more than 8 days. SARS-CoV-2 attached on salmon or suspended in culture medium stored at 4°C remained viable for at least 8 days, while these stored at 25°C resulted in attenuated infectivity very quickly. The result from the experiment on samples stored at 25°C is consistent with that reported by van

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Doremalen et al. (4). They showed that SARS-CoV-2 remained viable in aerosols, or on the surface of copper, cardboard, stainless steel, and plastic, at  $21^23^{\circ}$ C and  $40^{\circ}$  relative humidity for  $3^{\circ}24$  hours, confirming that the loss of SARS-CoV-2 viability is associated with increased temperature.

Imported and exported fish must be transported under a low-temperature (e.g., 0~4°C) environment. Under such condition, SARS-CoV-2-contaminated fish from one country can be easily transported to another country within one week and, thus, may serve as a source for international transmission of SARS-CoV-2. Different from many other food products, fish have to be transported, stored and sold under a low-temperature environment. Fish are generally sold in quarters having temperatures much lower than regular room temperatures. This means that virus attached on fish and sold in seafood markets might survive for a long time. It was recently reported that SARS-CoV-2 could productively infect human gut enterocytes [6]. Such virus on fresh raw salmon fillet, a popular food in many countries, could be transmitted via oral route.

In conclusion, fish-attached SARS-CoV-2 can survive for more than one week at 4°C, the temperature of refrigerators, cold rooms, or transport carriers for storage of fish before selling in markets. This calls for strict inspection and detection of SARS-CoV-2 as an important new protocol in fish importation and exportation.

### Notes

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**Author contributions.** M.D., H.L., Y.N., J. H., L.Z., S.X., and J.W performed the experiment; M. D. and H.L. analyzed the data and wrote the manuscript; S.J., C.P., and M.L. conceived the idea, supervised the study, and revised the manuscript. Besides, the first two authors contributed equally. And the last three authors contributed equally.

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#### Fiugre A and B.

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Viability of salmon-attached and untreated SARS-CoV-2 in culture medium at 4°C and 25°C. Panel A is the overview of the study design and experimental procedure. Briefly, the individual salmon cubes (5x5x5 mm) were mixed with 13 mL liquid of SARS-CoV-2 at  $3.16 \times 10^6$  TCID<sub>50</sub>/mL by inverting 5 times, transfered into the 10 cm dishes, incubated for 15 seconds at room temperature , and then put on filter paper to remove excess virus liquid. After that, the salmon cubes were transferred to 1.5 mL freezing tubes and stored at 4°C and 25°C for later treatment. On day 1, 2, 3, 4, 5, 6, 7, 8, 10, and 12, freezing tubes was taken out and 1 mL DMEM culture medium was added, oscillated for 5 seconds, and then centrifuged at 6,000 rpm for 5 minutes at 4°C. The liquid was used for virus titer detection. On day 1, 3, and 8, the virus in culture medium kept at 4°C or 25°C was detected. In panel B, the titer of SARS-CoV-2 was quantified by end-point titration on Vero E6 cells and expressed as log<sub>10</sub> TCID<sub>50</sub>/mL. Plots show the means of data from two or three samples. The dashed lines indicate the limit of detection, which were  $10^2$  TCID<sub>50</sub>/mL.

### Figure A

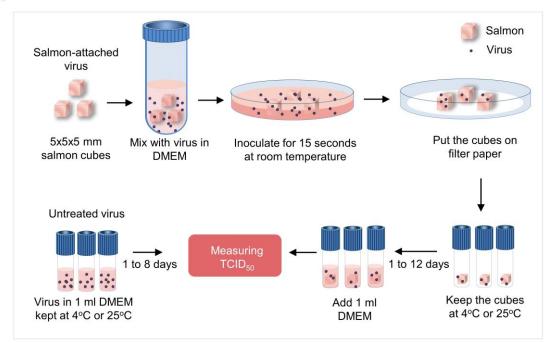


Figure B

