Stability of SARS-CoV-2 on Cold-chain Food

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Short report

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Abstract

Although close exposure to respiratory droplets from an infected patient is the main transmission route of SARS-CoV-2, touching contaminated surfaces and objects might also contribute to transmission of this virus. There are increasing reports that cold-chain food and food packages have been tested with SARS-CoV-2, raising concerns that importation of contaminated food could be a source for transmission of SARS-CoV-2. The survival of SARS-CoV-2 on cold-chain food was investigated. We found SARS-CoV-2 remained viable for more than three weeks on both beef and mutton at 4°C. Furthermore, at freezing temperature, SARS-CoV-2 can easily survive for more than eight weeks. Our study showed the ability of SARS-CoV-2 to survive for a long time on cold-chain food at low temperatures.

Main Text

Since the outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) at the end of 2019, the world is facing an exceptional and unprecedented pandemic threat. Strong evidence from the case and cluster reports indicated that respiratory droplet was the dominant transmission route of SARS-CoV-2(1). Additionally, World Health Organization (WHO) advised that touching fomites such as contaminated surfaces and objects might contribute to the transmission of this virus. We previously reported the stability of SARS-CoV-2 on environmental surfaces and in human excreta(2). At room temperature, SARS-CoV-2 was stable on environmental surfaces and remained viable for 7 days on smooth surfaces. This virus could survive for several hours in feces and 3–4 days in urine. Moreover, in the past few months, cold-chain food and food packages tested with SARS-CoV-2 have been reported, raising concerns that importation of contaminated food could be a source for transmission of SARS-CoV-2. Here, we report the survival of SARS-CoV-2 on cold-chain food.

SARS-CoV-2 strain BetaCoV/Beijing/AMMS01/2020 was originally isolated from the throat swab specimen of a COVID-19 patient. Viral stocks were prepared and titrated on Vero cells as previously described, which were grown in essential Dulbecco's modified Eagle's medium (DMEM) containing 2% fetal bovine serum (FBS), 100 U/ml penicillin and 100 µg/ml streptomycin in 5% CO₂ at 37°C. Cold-chain foods used in this study included beef and mutton. Both the beef and mutton were cut into small pieces with the size about 0.5×0.5cm, and all the meat pieces were put into tubes. Fifty microliters of virus stock with the infectious titer of 10⁴ 50% tissue culture infectious dose (TCID₅₀) per milliliter was added into each tube. The samples were stored at refrigerated (4°C) or freezing (-20°C) temperature, and the viruses were recovered by adding 1ml of DMEM every week. The infectivity of residual virus was titrated in quadruplicate on 96-well plates containing Vero cells. The plates were incubated in 5% CO₂ at 37°C. On the fifth day, the cytopathic effect (CPE) was observed, and the TCID₅₀ for each sample at a different time was calculated with the Reed-Muench method. Three replicate experiments were performed. All statistical analyses were performed using SPSS version 22.0, and a P-value < 0.05 was considered statistically significant.

As shown in Fig. 1A and 1B, the titer of SARS-CoV-2 declined slowly on both beef and mutton at 4°C. At refrigerated temperature, SARS-CoV-2 remained viable for more than three weeks on both beef and mutton, and no infectious virus could be recovered from beef and mutton after four weeks. Figure 1C and 1D show the duration of SARS-CoV-2 survival on beef and mutton at -20°C. At freezing temperature, SARS-CoV-2 can easily survive for more than eight weeks. The virus titers were more stable at -20°C than 4°C on both beef and mutton. Furthermore, the titer of SARS-CoV-2 remained constant at -20°C for the duration of the experiment. For example, its TCID₅₀/ml decreased from 10^{2.94} at time zero to 10^{2.44} at week 8 on beef, which was only a 0.5 log₁₀ reduction from the original inoculum. Meanwhile, there was no significant difference in virus survival between beef and mutton at 4°C or -20°C (p = 0.62, p = 0.87, respectively).

Before our study, two research teams had reported the stability of SARS-CoV-2 on food surfaces at low temperatures. One study reported by Fisher et al found that SARS-CoV-2 remained constant and viable in salmon, chicken and pork samples for the duration of the experiment (3 weeks) at 4°C, -20°C and -80°C(3). Dai and colleagues demonstrated salmon-attached SARS-CoV-2 can survive for 8 and 2 days at 4°C and 25°C, respectively(4). Compared with the above two studies, we set the samples at 4°C and -20°C which are the standard refrigerated and freezing temperatures in cold-chain transportation. Our study, coupled with the above two studies, showed the ability of SARS-CoV-2 to survive for a long time on cold-chain food at low temperatures.

The continuously rapid growth of the COVID-19 pandemic indicates the great difficulty in controlling and curbing the spread of this disease. More strikingly, even in the region where the epidemic has ended, there has been a resurgence of COVID-19 cases. It was speculated that contaminated cold-chain food sources initiated the COVID-19 resurgence. For example, an outbreak of SARS-CoV-2 in Xinfadi Wholesale Market in Beijing occurred 56 days after the last local confirmed case(5). All confirmed cases were associated with Xinfadi Wholesale Market, and SARS-CoV-2 RNA was also detected in environmental samples taken from the market. The complete genome sequences of the SARS-CoV-2 were closely related to the L-lineage from Europe and different from the S-lineage in China(6). Subsequently, SARS-CoV-2 were isolated from the imported frozen cold outer package's surface in Qingdao, China, which indicated contaminated food could be a source for international transmission of SARS-CoV-2(7). Cold-chain environments, such as seafood markets and logistics stations, are comparatively confined and crowded, facilitating the easy transmission of viruses. The loading and unloading process is also a challenge for epidemic prevention because workers come in direct contact with cold-chain products. Although there have not been reports of COVID-19 cases caused by eating cold chain products so far, epidemic prevention on the cold supply chain needs to be brought to the forefront.

Conclusions

Taken together, our study showed the ability of SARS-CoV-2 to survive for a long time on cold-chain food at low temperatures. Epidemic prevention on the cold supply chain needs to be brought to the forefront.

Abbreviations

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; DMEM: Dulbecco's modified Eagle's medium; TCID₅₀: 50% tissue culture infectious dose; FBS: fetal bovine serum; CPE: cytopathic effect.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

JYL and LL conceived the study. YJL, TYL and YLG performed most of the experiments. HPL, LJ, DZ, XLW and JWH analyzed the data. YJL wrote the paper. All authors read and approved the final manuscript.

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Availability of data and materials

The data used or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

Stability of SARS-CoV-2 on beef and mutton at 4°C and -20°C. (A), (B) Survival time of SARS-CoV-2 on beef and mutton at 4°C. (C), (D) Survival time of SARS-CoV-2 on beef and mutton at -20°C. All experimental measurements are expressed as means across three replicates. The Limit of Detection (LOD) for the assays was 101.5 TCID₅₀/ml.