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Cold-chain transportation in the frozen food industry may have caused a recurrence of COVID-19 cases in destination: Successful isolation of SARS-CoV-2 virus from the imported frozen cod package surface

Peipei Liu ^{a,1}, Mengjie Yang ^{a,1}, Xiang Zhao ^{a,1}, Yuanyuan Guo ^{d,1}, Liang Wang ^b, Jing Zhang ^a, Wenwen Lei ^a, Weifang Han ^a, Fachun Jiang ^c, William J. Liu ^a, George F. Gao ^{a,b}, Guizhen Wu ^{a,*}

^a NHC Key Laboratory of Biosafety, National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing 102206, China

^b CAS Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences (CAS), Beijing 100101, China

^c Qingdao Center for Disease Control and Prevention, Qingdao 266011, China

^d School of Pharmaceutical Sciences, Nanjing Tech University, No. 30 South Puzhu Road, Nanjing 211816, China

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ABSTRACT

Coronavirus disease 2019 (COVID-19) pandemic has spread in 220 countries/regions to wreak havoc to human beings around the world. At present, the second wave of COVID-19 has begun in many European countries. The complete control of COVID-19 is very urgent. Although China quickly brought the virus under control, there have been eight sporadic outbreaks in China since then. Both in Xinfadi of Beijing and Dalian outbreak of COVID-19, environmental swab samples related to imported cold chain food were tested nucleic acid positive for SARS-CoV-2. In this outbreak in Qingdao, we directly isolated SARS-CoV-2 from the cod outer package's surface swab samples. This is the first time worldwide, SARS-CoV-2 were isolated from the imported frozen cod outer package's surface, which showed that imported frozen food industry could import SARS-CoV-2 virus.

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Since the outbreak in December 2019, the coronavirus disease 2019 (COVID-19) has spread in 219 countries (regions) worldwide. 45,678,440 confirmed cases and 1,189,945 deaths caused by the COVID-19 global pandemic have brought a massive disaster to human beings. At present, as the northern hemisphere enters autumn and winter, which may indicate a high incidence of respiratory infectious diseases, many European countries have started a second wave of the COVID epidemic. After the COVID-19 outbreak, China quickly took adequate measures to bring the outbreak under control in Wuhan and across the country. Since then, there have been eight sporadic and localized outbreaks in Suifenhe, Harbin [1], Shulan [2], Beijing, Urumqi [3], Dalian [4], and most recently Qingdao and Kashgar, respectively.

On September 24, 2020, during the routine nucleic acid inspection of the personnel in Qingdao Port, two stevedores were found to be SARS-CoV-2 positive and were identified as asymptomatic infection. The Qingdao Center for Disease Control and Prevention responded quickly by conducting an extensive epidemiological investigation to identify the source of infection. It was revealed that both cases had no COVID-19 case contact history and no

foreign personnel contact history. However, both carried out loading and unloading of frozen cod in bulk on September 19, 2020.

Subsequently, the surface swab samples of the frozen cod outer package were collected and then tested. Out of 421 surface samples, 50 were tested SARS-CoV-2 nucleic acid positive. After that, the whole-genome sequencing and virus isolation were performed on the throat swab samples taken from the two workers and the frozen cod outer package's surface swab samples. Full-length genome sequences were successfully achieved for seven surface swab samples and two nasopharyngeal swab samples. After inoculation on Vero-E6 cells, cytopathic effects (CPE) were not observed during six days of culture; however, the supernatants of one surface sample and one nasopharyngeal swab sample were tested positive for SARS-CoV-2. Then the two supernatants were inoculated on the new surface layers of Vero-E6 cells for continuing virus isolation. Six days after the passage, two infections, CPE were observed, and the supernatants were tested positive for SARS-CoV-2 nucleic acid (Fig. 1). Meanwhile, we got the whole viral genome sequence using the Illumina MiSeq platform successfully. For the two cell-cultured samples, genome sequence alignments also confirmed the positive results of SARS-CoV-2 isolation.

A total of 16 nucleotide variations for these viruses were observed compared with the reference sequence (NC 045512), isolated from Wuhan City in Hubei Province on January 7, 2020. Phylogenetic analysis indicated that the SARS-CoV-2 (hCoV-19/Qingdao/IVDC-QD-11-2P2/

* Corresponding author: NHC Key Laboratory of Biosafety, National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing 102206, China.

E-mail address: wugz@ivdc.chinacdc.cn (Guizhen Wu).

¹ These authors contributed equally to this work.

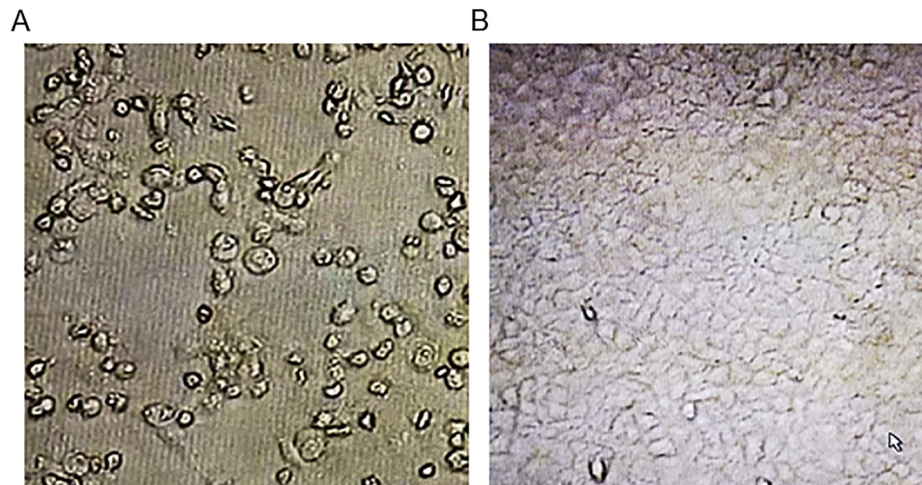


Fig. 1. Cytopathic effects in Vero-E6 cell cultures after inoculation with cod outer package surface swab samples. A. Six days after the passage 2 in Vero-E6 cell, cytopathic effects were observed with a 10× objective lens; B. negative control were observed with a 10× objective lens.

2020 and hCoV-19/Qingdao/IVDC-QD-G2020QD80P2/2020 isolated from cod package surface and a worker's throat swab sample, respectively) resulted in the outbreak in Qingdao City fell in a European branch (L lineage B.1.1), which originated in Europe (Fig. 2). The virus' full genome sequence isolated from the patient's nasopharyngeal swab has a 100% similarity to that of the original sample. In contrast, the virus' genome sequence is highly homologous to the original piece from the frozen cod outer package, with two distinct nucleotides (Table 1).

The first time worldwide, we isolated SARS-CoV-2 from the imported frozen cod outer package's surface. Together with the epidemiological

data, we concluded that the COVID-19 outbreak of Qingdao was probably caused by SARS-CoV-2 contamination of cod outer package during production or cold-chain transportation. To our knowledge, when the temperature reaches -18°C during cold-chain transportation, viruses can survive for more than 21 days under the low-temperature condition [5]. Once the destination is reached, the SARS-CoV-2 virus may infect stevedores or other staff working in the port through direct contact with the goods (Fig. 3). The salmon surface was nucleic acid positive for SARS-CoV-2 in the COVID-19 epidemic of Beijing's Xinfadi market. However, the live virus has not been isolated due to the low nucleic acid concentration of the

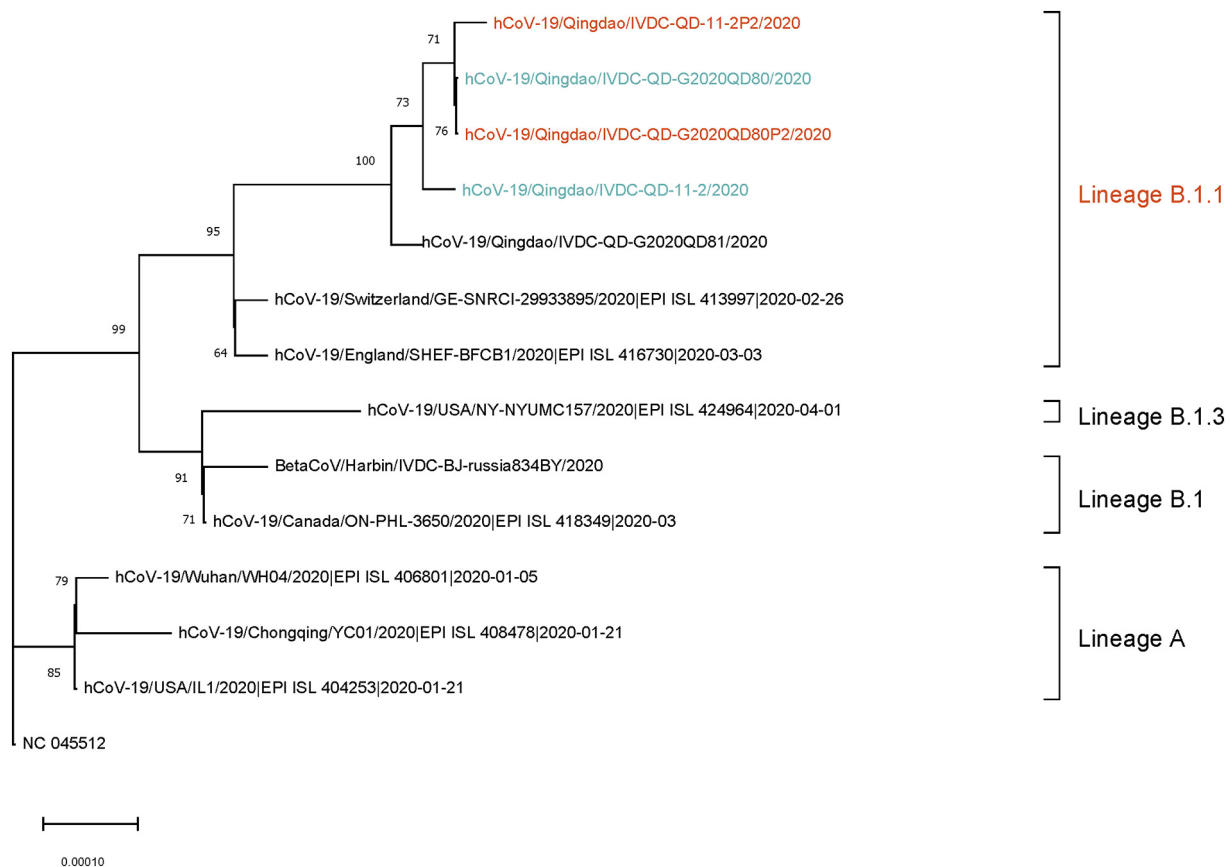


Fig. 2. Phylogeny of isolated SARS-CoV-2 and corresponding original samples from Qingdao based on the full-length genome sequences of the COVID-19 virus. The virus isolated from workers and cod package surface along with the corresponding original samples are indicated with red and cyan characters, respectively.

Table 1
Nucleotide variations for distinct SARS-CoV derived from Qingdao samples.

Position	Ref ^a	Alt ^b	11-2	11-2P2	G2020QD80	G2020QD81	QD-80P2
241	C	T	1	1	1	1	1
1,282	C	T	1	1	1	0	1
2,523	C	T	0	0	0	1	0
3,037	C	T	1	1	1	1	1
3,743	C	T	1	1	1	1	1
3,773	C	T	1	1	1	1	1
5,144	C	T	0	1	1	0	1
5,170	C	T	1	1	1	1	1
14,408	C	T	1	1	1	1	1
23,299	A	G	1	1	1	1	1
23,403	A	G	1	1	1	1	1
23,755	G	T	1	1	1	1	1
28,881	G	A	1	1	1	1	1
28,882	G	A	1	1	1	1	1
28,883	G	C	1	1	1	1	1
29,868	G	T,*,C	3	1	0	2	0

^a Ref means reference sequence.

^b Alt means alteration compared with the reference sequence.

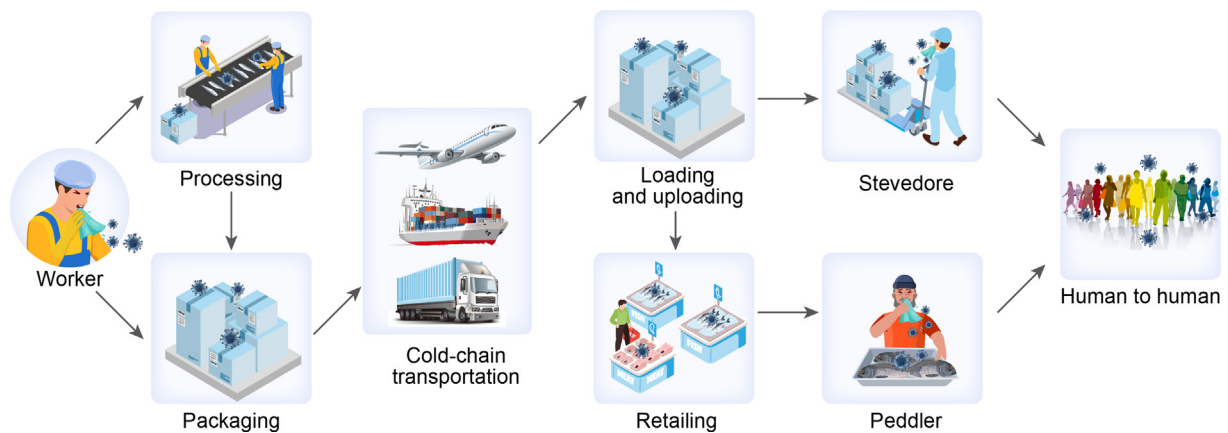


Fig. 3. Probable route of transmission for SARS-CoV-2 through cold-chain transportation in the frozen food industry. Frozen food and outer package were contaminated with SARS-CoV-2 during processing and packaging. Through cold-chain transportation with low temperature (such as -20°C), SARS-CoV-2 survived and then infected stevedores or peddlers after reaching the destination.

samples tested. The successful SARS-CoV-2 virus isolation from the seafood packaging surface proves that the imported virus that re-infected humans and caused the outbreaks through cold-chain transportation is all possible. In the future, we should strengthen the inspection and quarantine of imported cold-chain food and pay more attention to the personal protection of relevant workers to better prevent and control COVID-19.

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Conflict of interest statement

The authors declare that there are no conflicts of interest. Given their roles as Editorial Board Member, William J. Liu and Guizhen Wu had no involvement in the peer-review of this article and had no access to information regarding its peer-review. Full responsibility for the editorial process for this article was delegated to the editor Jianwei Wang.

Author contributions

Peipei Liu: Investigation, Methodology, Writing - original draft. **Mengjie Yang:** Investigation, Data curation. **Xiang Zhao:** Investigation,

Software. **Yuanyuan Guo:** Investigation. **Liang Wang:** Software, Visualization. **Jing Zhang:** Investigation. **Wenwen Lei:** Investigation. **Weifang Han:** Resources. **Fachun Wang:** Resources. **William J. Liu:** Writing - review & editing, Validation. **George F. Gao:** Supervision. **Guizhen Wu:** Conceptualization, Funding acquisition, Supervision, Project administration, Writing - review & editing.

References

- [1] X. Zhao, J. Xu, Y. Zhang, D. Wang, W. Dai, G. Jiao, W. Shiwen, S. Wang, J. Liu, C. Chen, S. Chen, S. Yan, H. Hua, G.F. Gao, W. Xu, G. Wu, A reemergent case of COVID-19 — Harbin City, Heilongjiang Province, China, April 9, 2020, *China CDC Weekly* 2 (25) (2020) 460–462, <https://doi.org/10.46234/ccdcw2020.127>.
- [2] C. Chen, X. Zhao, D. Wang, J. Li, A. Wang, D. Wu, Z. Chen, Y. Wu, Y. Meng, W. Shi, G.F. Gao, G. Wu, B. Huang, W. Xu, The initial case of COVID-19 — Shulan City, Jilin Province, China, May 8, 2020, *China CDC Weekly* 2 (25) (2020) 458–459, <https://doi.org/10.46234/ccdcw2020.115>.
- [3] C. Chen, H. Ma, Z. Jia, X. Zhao, D. Wang, J. Zhao, Z. Gao, P. Liu, Y. Song, Z. Chen, Y. Wu, Y. Meng, G. Wu, W. Xu, X. Fan, Y. Zhang, Reemergent cases of COVID-19 — Xinjiang Uygur autonomous region, China, July 16, 2020, *China CDC Weekly* 2 (39) (2020) 761–763, <https://doi.org/10.46234/ccdcw2020.206>.
- [4] Z. Bo, X. Zhao, L. Mao, J. Zhang, Y. Zhang, Y. Song, H. Wang, J. Wang, C. Chen, J. Xiao, T. Ji, Q. Yang, W. Xu, D. Wang, W. Yao, Reemergent cases of COVID-19 — Dalian City, Liaoning Province, China, July 22, 2020, *China CDC Weekly* 2 (34) (2020) 658–660, <https://doi.org/10.46234/ccdcw2020.182>.
- [5] D. Fisher, A. Reilly, A.K.E. Zheng, A.R. Cook, D.E. Anderson, Seeding outbreaks of COVID-19 by contaminated fresh and frozen food, *bioRxiv* (2020), 2020.2008.2017.255166. <http://dx.doi.org/10.1101/2020.08.17.255166>.