Vital Surveillances

Surveillance of SARS-CoV-2 Contamination in Frozen Food-Related Samples — China, July 2020 – July 2021

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ABSTRACT

Introduction: Current evidence shows that coronavirus disease 2019 (COVID-19) is neither a food safety issue nor a foodborne disease. However, the outbreaks of this disease in workers of meat- or poultry-processing plants and food markets have been reported in many countries. Systematic reports on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) contamination in food-related samples worldwide are lacking so far. This study aimed to survey and monitor SARS-CoV-2 contamination in samples of foods or their packaging, storage environment, and employees, as well as explore the possible potential for virus transmission via frozen foods.

Methods: Swabs of frozen food-related samples were collected between July 2020 and July 2021 in 31 provincial-level administrative divisions (PLADs) and Xinjiang Construction Corps in China. The SARS-CoV-2 RNAs were extracted and analyzed by real-time quantitative polymerase chain reaction using the commercially available SARS-CoV-2 nucleic acid test kit.

Results: More than 55.83 million samples were analyzed, and 1,455 (0.26 per 10,000) were found to be positive for SARS-CoV-2 nucleic acid. Among the virus-positive samples, 96.41% (1,398/1,450) and 3.59% (52/1,450) were food/food packaging materials and environment, respectively. As for 1,398 SARS-CoV-2-positive food and food packaging materials, 99.50%, (1,391/1,398) were imported and 7 were domestic. The outer packaging of food was frequently contaminated by the virus 78.75% (1,101/1,398).

Conclusions: Our study supported speculation that cold-chain foods might act as the SARS-CoV-2 carrier, and food handlers/operators were at high risk of exposure to the virus. It is necessary to carry out a comprehensive mass testing for SARS-CoV-2 nuclei acid, along with contact tracing and symptom screening in cold-chain food handlers and processors so as to identify high proportions of asymptomatic or pre-symptomatic infections. Meanwhile, research and development of effective self-protection equipment available at a temperature below –18 ℃ is urgent.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an enveloped, positive-sense, single-stranded ribonucleic acid (RNA) virus with approximately a 30-kb genome. It is classified within the genus *Betacoronavirus* (subgenus *Sarbecovirus*) of the family *Coronaviridae* (*1*). COVID-19 is a zoonotic respiratory epidemic that has been declared by the World Health Organization (WHO) as a global public health emergency. SARS-CoV-2 is transmitted from person to person, mainly via respiratory droplets generated during normal activities such as coughing, sneezing, heavy breathing, singing, and talking (*2*). These droplets fall quickly on any surface, and people can become infected by breathing or by touching a contaminated surface followed by touching their eyes, nose, or mouth without washing their contaminated hands (fomite transmission) (*3*). Additionally, airborne transmission has been reported depending on the situational context (*4*). The risk of resurgence caused by other routes of virus introduction and transmission remains unclear.

Fomite transmission has aroused extensive attention with the detection of SARS-CoV-2 on imported frozen foods and their packaging materials, which was linked to re-emergent outbreaks of COVID-19 in Beijing, China (*5*). In particular, it has been hypothesized that contaminated cold-chain food sources may act as a virus carrier and present a risk of SARS-CoV-2 transmission between countries and regions (*6*). Thereafter, SARS-CoV-2 RNA contamination of the outer packaging of frozen food imported from countries or regions that suffered COVID-19

outbreaks has been much more frequently reported prior to goods clearance at customs in China. However, no direct links were established between SARS-CoV-2 infection and environment-to-human transmission until the infectious virus from the cold-chain products linked to two dock workers who were asymptomatic COVID-19 cases in Qingdao City, Shandong Province, in September 2020 was isolated by cell culture, and typical SARS-CoV-2 particles were observed under an electron microscope (*7*). Since then, the COVID-19 pandemic has had a dramatic impact on the global food system with direct and indirect consequences, causing widespread concern and economic hardship for consumers, businesses, and communities across the globe, although it is neither a food safety issue nor a foodborne disease. For these reasons, China has launched a nationwide program for the systematic screening of SARS-CoV-2 contamination on packaged frozen food produced, either domestic or imported, since July 2020.

This study aimed to carry out the surveillance and monitoring of SARS-CoV-2 RNA contamination on samples linked to frozen foods/food packaging at storage and retail levels in China. The findings might help formulate recommendations and strategies for both ensuring a safer food supply chain and facilitating livestock farmers, slaughterhouse workers, food processors and traders, and policymakers to combat COVID-19 effectively along the food chain.

METHODS

Sample Collection

Sample collection was performed following the description in both *Guidance on Strengthening the Detection of SARS-CoV-2 in the Cold-Food Chain and the Technical Specifications for SARS-CoV-2 Monitoring in Environment of the Agricultural Trade Market* (*8*–*9*). The samples of the imported and domestic cold-chain foods and their corresponding outer or inner packaging during slaughter, production and processing, storage, transportation, and retail in all 31 provincial-level administrative divisions (PLADs) and Xinjiang Production and Construction Corps in China were collected by surface swabbing every 2 weeks between July 2020 and July 2021. The sampling was performed simultaneously by at least two staff members with effective self-protection; all processes were controlled by video recording. The number of samples was increased for cold-chain foods from areas with a medium or high prevalence of COVID-19. Meanwhile, environmental samples from all steps mentioned earlier were also collected. In particular, sampling from the environment of wholesale and retail stores, chopping boards, surface of utensils, refrigerators, inner surface of the public cold storage, transportation vehicles, sewage, toilets, and the surface of the sinks were strongly strengthened. Additionally, oropharyngeal swab samples from employees working in cold and frozen meat facilities, aquatic production and processing enterprises, centralized trading markets, refrigerated and frozen warehouses, shopping malls, supermarkets and catering service units, cold-chain logistics, and takeaway express delivery units were taken regularly by staff members of CDC and intensified for both sample re-checking and SARS-CoV-2 nucleic acid detection following the requirements of the Chinese government. Information including, but not limited to, sampling time, sampling location, name of agricultural trade markets (or supermarkets, stores), number of booths, sample type, sample code, sampler, and so forth was recorded.

Sample Analysis

All samples stored at 4 ℃ in a transfer box special for high-risk biohazards were transported to the local designated agencies by a dedicated person to detect SARS-CoV-2 nucleic acid within 24 h. The samples that could not be analyzed within 24 h were stored at −70 ℃ or below. The sewage samples were first pretreated by centrifuging at 4,654 ×*g* for 30 min to remove the impurities, followed by concentrating the supernatant via membrane absorption or ultrafiltration. SARS-CoV-2 RNA in swabs were extracted either in an automated nucleic acid extraction system coupled with a nucleic acid extraction kit (Xi'an Tianlong Science and Technology, Xi'an, China, or Roche, Switzerland), or with a manual viral RNA kit (Qiagen, Germany) following the manufacturer's instructions. The extracted sample RNAs were divided into separate packages and analyzed by real-time quantitative polymerase chain reaction (RT-qPCR) using a commercially available SARS-CoV-2 nucleic acid test kit (BioGerm, Shanghai, China). A TaqMan probe-based kit (BioGerm, Shanghai, China) was designed to detect the ORF1ab and N genes of SARS-CoV-2 in one reaction following the procedure described by the Joint Prevention and Control

Mechanism of the State Council of the People's Republic of China (*8*). All test kits for either SARS-CoV-2 nucleic acid extraction or RT-qPCR analysis were approved by the State Food and Drug Administration of China. The viral copy number was determined according to the certified reference material of the COVID-19 virus ribonucleic acid genome [No. GBW(E)091099] obtained from the National Institute of Metrology using RT-qPCR. For each RT-qPCR assay, the negative control, positive control, and blank control were employed. A specific cycle threshold of ORF1ab and N gene targets less than or equal to 40 was used for determining positivity. The samples were defined as positive in the presence of at least a single N gene and/or ORF1ab. All analytical results were requested to be submitted to the National Health Commission for statistics.

RESULTS

SARS-CoV-2 contamination results in cold-chain food, packaging materials, and cold food storage environment at the storage and retail level, as well as the test results of nasopharyngeal swabs of people whose work was related to cold-chain food, between July 2020 and July 2021, are shown in Table 1.

Among more than 55.83 million swabs surveyed and monitored, more than 20.51 million were cold-chain food and packaging materials, and the rest were environmental and nasopharyngeal swabs. A total of 1,455 samples (0.26 per 10,000) were positive for SARS-CoV-2 nucleic acid. In terms of the virus-positive samples, 96.41% (1,398/1,450) and 3.59% (52/1,450) samples were swabs of food and food packaging materials, and environment, respectively. As for 1,398 SARS-CoV-2-positive samples of food and food packaging material, 99.50% (1,391/1,398) were imported, and 0.50% (7/1,398) were domestic. Regarding the SARS-CoV-2 positive food and packaging samples, 18.60% (260/1,398), 2.65% (37/1,398), and 78.75% (1,101/1,398) were food, inner packaging, and outer packaging, respectively. Additionally, 53.86% (753/1,398), 37.91% (530/1,398), and 8.23% (115/1,398) SARS-CoV-2-positive swabs were from seafood, poultry meat, and other foods, respectively (Table 2). Hence, aquatic foods were at the highest risk of SARS-CoV-2 contamination, followed by poultry meat. However, the outer packaging contamination by SARS-CoV-2 nucleic acid was much more frequent and serious than that of the inner packaging and food itself. The top-ranked PLADs with SARS-CoV-2 detection frequency higher than 2 per 10,000 were Tianjin (7.03 per 10,000), Yunnan (6.38 per 10,000), Zhejiang (3.41 per 10,000), and Fujian (2.85 per 10,000) (Table 3), which were either located on the border (Yunnan), or the most important ports of entry for foods in the regions of Bohai Rim (Tianjin), the Yangtze River

TABLE 1. SARS-CoV-2 contamination in cold-chain food-related samples collected between July 2020 and July 2021 in China.

Category	No. of samples	No. of positive samples (%, packaging material)	Percentage of total positive samples (%)
Food and food packaging materials	20,517,959	260 (18.6, cold-chain foods) 37 (2.6, inner packaging material) 1,101 (78.8, outer packaging material)	96.41
Environment	15,656,958	52	3.59
Total	55,832,289	1,450	100

Abbreviation: SARS-CoV-2=severe acute respiratory syndrome coronavirus 2.

TABLE 2. Contamination of SARS-CoV-2 in different cold-chain food-related samples between July 2020 and July 2021 in China.

Food categories	No. of positive samples	Percentage of total positive samples (%)
Seafood	753	53.86
Poultry meat	530	37.91
Other foods	115	8.23
Total	1,398	100

Abbreviation: SARS-CoV-2=severe acute respiratory syndrome coronavirus 2.

Delta (Zhejiang), and the Southeast coast (Fujian) of China, respectively.

Regarding the 1,391 SARS-CoV-2-positive imported cold-chain foods and food packaging samples, the exporter of 99.07% (1,378/1,391) samples was confirmed. Of these, 46.66% (643/1,378) were from 11 European countries, 27.50% (379/1,378) from 6 South American countries, 16.04% (221/1,378) from 9 Asian countries, 5.81% (80/1,378) from 2 North American countries, and 3.99% (55/1,378) from 2 African countries (Table 4). These results suggested that SARS-CoV-2-

TABLE 3. SARS-CoV-2 contamination in cold-chain food-related samples detected from some PLADs of China between July 2020 and July 2021.

PLADs	No. of samples	No. of positive samples	Positive rate (‰)
Tianjin	112,314	79	7.03
Yunnan	89,288	57	6.38
Zhejiang	5,860	2	3.41
Fujian	231,933	66	2.84
Henan	385,672	73	1.89
Jilin	265,465	48	1.81
Guizhou	75,624	13	1.72
Qinghai	41,607	6	1.44
Shandong	4,441,257	630	1.42
Jiangsu	536,112	66	1.23
Guangdong	1,648,965	184	1.11
Shaanxi	784,633	64	0.81
Liaoning	81,505	6	0.73
Shanxi	383,436	15	0.39
Jiangxi	230,370	7	0.30
Anhui	1,648,214	36	0.21
Gansu	182,087	3	0.21
Hubei	2,030,209	14	0.16
Hebei	893,506	7	0.08
Sichuan	552,323	6	0.07
Heilongjiang	152,943	1	0.07
Hunan	205,012	1	0.05
Beijing	1,304,293	5	0.04
Inner Mongolia	664,049	9	0.14

Abbreviations: SARS-CoV-2=severe acute respiratory syndrome coronavirus 2; PLADs=provincial-level administrative divisions.

TABLE 4. SARS-CoV-2 contamination in the imported cold-chain food-related samples from some representative regions between July 2020 and July 2021 in China.

Regions	No. of positive samples	Percentage of total positive samples (%)
Europe	643	46.66
South America	379	27.50
Asia	221	16.04
North America	80	5.81
Africa	55	3.99
Total	1,378	100

Abbreviation: SARS-CoV-2=severe acute respiratory syndrome coronavirus 2.

contaminated cold/food chain products and their containers might be a potential source of SARS-CoV-2 infection by workers who contact with them followed by acting as a trigger of COVID-19 outbreak.

CONCLUSIONS

SARS-CoV-2 transmission was initially proposed to originate from bats and infect humans following a direct contact with intermediate host animals (*10*). As the epidemic progressed to a pandemic, the spread via direct contact between humans was recognized as the main route of exposure. China has experienced several outbreaks related to imported frozen foods since 2020, at that time, SARS-CoV-2 was nearly eliminated in this country. The initial detection of SARS-CoV-2 on imported frozen foods and their packaging in Beijing (June of 2020) and Dalian (July of 2020) illustrated the possibility of fomite the contamination of SARS-CoV-2 nucleic acid in the outer packaging of imported frozen foods was frequently reported nationwide. All batches except for one of chicken wings were frozen aquatic products. Therefore, the potential for SARS-CoV-2 introduction via cold/food chain products was considered, as described in the report of *WHO-convened Global Study of Origins of SARS-CoV-2: China Part*, jointly made by the international expert team from WHO, Food and Agricultural Organization, World Organization for Animal Health (OIE), and China at the beginning of 2021 (*11*). Hence, frozen food as a source for infection and the cold chain as an introduction pathway of SARS-CoV-2 might present a risk for transmission between countries and regions. Additionally, the infectious virus has been found in the feces of some infected people, further suggesting the possibility of fecal-oral transmission via contaminated vehicles such as food (*12*–*13*). This indicates that the initial introduction of the SARS-CoV-2 virus through frozen foods into Huanan Wholesale Seafood Market in Wuhan, Hubei, China, in late 2019, leading to the secondary big epidemic, cannot be ruled out.

This was the first report on the yearly surveillance of SARS-CoV-2 contamination in swabbing samples of frozen foods and their packaging, as well as samples from the environment and workers' nasopharynx. The survival and recovery of SARS-CoV-2 in certain foods highlighted the importance of safe food handling practices in mitigating any public health concerns related to SARS-CoV-2-contaminated foods. Further studies are needed on the presence and persistence of infectious SARS-CoV-2 and/or its RNA in frozen food environmental compartments, sample conditions, and interventional strategies for reducing the virus infection.

Conflicts of interest: No conflicts of interest.

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