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

Fengqin Li; Jiahui Wang; Zhaoping Liu; Ning Li

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 nucleic 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 °C 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 °C 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 °C 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. 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>
<thead>
<tr>
<th>TABLE 1. SARS-CoV-2 contamination in cold-chain food-related samples collected between July 2020 and July 2021 in China.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Food and food packaging materials</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Environment</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>TABLE 2. Contamination of SARS-CoV-2 in different cold-chain food-related samples between July 2020 and July 2021 in China.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food categories</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Seafood</td>
</tr>
<tr>
<td>Poultry meat</td>
</tr>
<tr>
<td>Other foods</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

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.

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

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.

<table>
<thead>
<tr>
<th>Regions</th>
<th>No. of positive samples</th>
<th>Percentage of total positive samples (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Europe</td>
<td>643</td>
<td>46.66</td>
</tr>
<tr>
<td>South America</td>
<td>379</td>
<td>27.50</td>
</tr>
<tr>
<td>Asia</td>
<td>221</td>
<td>16.04</td>
</tr>
<tr>
<td>North America</td>
<td>80</td>
<td>5.81</td>
</tr>
<tr>
<td>Africa</td>
<td>55</td>
<td>3.99</td>
</tr>
<tr>
<td>Total</td>
<td>1,378</td>
<td>100</td>
</tr>
</tbody>
</table>

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.

Acknowledgments: Members of the risk assessment and response to the cold-chain food and SARS-COV-2 transmission working group of the China National Health Commission: Jianxin Tian, Tairan Li, Zhaoping Liu, Bo Chen, Ting Fu, Ruiming Zhang, Dongqun Xu, Nan Cai, Ye Tian, Yanping Zhang, Dajin Yang, Hongwei Han, Laiying He, Yunchang Guo, Shufeng Song, Yeru Wang, Hongqiu Li, Ge Guo, Rui Wang, Jin Shen, Cao Chen, Hong Wang, Zhiyuan Jia, Daoyuan Yang, and Xueli Lyu.

doi: 10.46234/ccdcw2022.105

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Submitted: December 17, 2021; Accepted: May 31, 2022

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**Vital Surveillances**

**Vibrio parahaemolyticus O10:K4: An Emergent Serotype with Pandemic Virulence Traits as Predominant Clone Detected by Whole-Genome Sequence Analysis — Beijing Municipality, China, 2021**

Ying Huang; Bing Lyu; Xin Zhang; Yi Tian; Changying Lin; Lingyu Shen; Hanqiu Yan; Daitao Zhang; Lei Jia; Mei Qu; Quanyi Wang

**ABSTRACT**

**Introduction:** *Vibrio parahaemolyticus* (*V. parahaemolyticus*) is a common foodborne pathogen which causes gastroenteritis in humans, especially the O3:K6 pandemic clone which is still a prominent serotype in Beijing, China. In this study, we observed a novel serotype O10:K4 isolated from clinical diarrhea cases, which became the most prevalent clone in 2021.

**Methods:** 73 clinical isolates were collected through sentinel hospitals’ surveillance in 2021. Serum agglutination testing and antimicrobial susceptibility testing were conducted. Whole genome sequencing was applied to characterize 73 *V. parahaemolyticus* strains and complete phylogenetic analysis.

**Results:** Seven serotypes were identified among 73 strains. O10:K4 was the most common serotype (83.6%), followed by O2:KUT, O4:KUT, and O1:KUT. Multilocus sequence typing divided the 73 isolates into 10 sequence types (STs) with ST3 as the most prevalent, which covered all O10:K4 strains. Most isolates were sensitive to common antimicrobial agents apart from colistin. All the O10:K4 isolates were positive for the thermostable direct hemolysin gene, toxRS/new, and orf8, and negative for the TDH-related hemolysin gene. The whole genome sequencing-single nucleotide polymorphism phylogenetic analysis revealed O10:K4 strains formed a main genetic lineage, which was genetically distinct from other serotypes. We also demonstrated the presence of two type III secretion system genes (T3SS1 and T3SS2) and β lactamase resistance gene blaCARB-22 in all O10:K4 strains.

**Conclusions:** The study confirmed the emergence of *V. parahaemolyticus* O10:K4 possessing virulence factors similar to the O3:K6 pandemic clone, which may have enabled them to become prevalent in Beijing, China.
elucidate their genetic characteristics, pathogenicity and transmission.

METHODS

Study Design and Population
Hospital-based active surveillance has been conducted since 2010 in Beijing, China. The sentinel hospitals affiliated with 16 different districts enrolled outpatients with acute diarrhea. The average monthly enrollment number was around 20–40 patients per district. A total of 5,337 cases were collected from January to December 2021. Enrollment was subject to obtaining informed verbal consent. All specimens were collected on the day of presentation by rectal swabs in Cary-Blair transport media and were immediately transported to the laboratory of the District Center for Disease Prevention and Control (CDC) for processing within 24 hours.

Detection of Bacteria and Serotyping
For selective enrichment of *Vibrio* spp., swabs were inoculated on peptone water containing 3% NaCl, pH 8, incubated at 37 °C overnight, then inoculated on CHROMagar *Vibrio* media (CHROMagar Co., Paris, France), and incubated for 16–24 h. After culturing, at least three suspected colonies were picked out for further identification. The systematic identification was confirmed with the VITEK 2 Compact instrument (bioMérieux, Marcyl’Etoile, France). Finally, serologic identification was performed by a slide agglutination test with 11 O (lipopolysaccharide) and 65 K (capsule) antisera (Denka Seiken Ltd., Tokyo, Japan). One serotype was defined as a unique combination of O and K serogroups.

Antimicrobial Resistance Testing
Antimicrobial susceptibility testing (AST) of *V. parahaemolyticus* strains was assessed using the broth microdilution method following the guidelines of the Clinical and Laboratory Standards Institute document (CLSI M100-S29:2019). *Escherichia coli* ATCC 25922 was included in the test as a quality control strain. Seventeen antimicrobial agents (Shanghai Xingbai Co) were used for AST: chloramphenicol, trimethoprim-sulfamethoxazole, colistin, ertapenem, meropenem, cefotaxime, cefazidime, cefazidime-avibactam, tetracycline, tigecycline, ciprofloxacin, nalidixic acid, aztreonam, amikacin, streptomycin, ampicillin, and ampicillin-sulbactam.

DNA Extraction and WGS
DNA was extracted using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Quantification of extracted genomic DNA (gDNA) was determined by agarose gel electrophoresis and fluorometric analysis (Qubit 2.0). Whole genome sequencing (WGS) was conducted using an Illumina PE150 platform with 200x coverage (Novogene Technology Co., Ltd., Beijing, China). Raw sequencing data was checked for quality, trimmed, and assembled de novo into contigs. Whole genome sequencing-single nucleotide polymorphism (WGS-SNP) analysis for all draft genomes was performed using parsnp software with the reference strain sequence GCF_000196095.1 available from NCBI’s genome database. The phylogenetic tree was finally visualized using the online tool iTOL (http://itol.embl.de/).

MLST, ARGs, and VGs
The genomic analysis was based on the Center for Genomic Epidemiology’s web server (https://cge.cbs.dtu.dk/services/cge/). Multilocus sequence typing (MLST) 2.0 was performed using seven housekeeping genes (*dnaE*, *gyrB*, *recA*, *ddS*, *pntA*, *pyrC*, and *tnaA*) to characterize sequence type (ST) of *V. parahaemolyticus* isolates. The new STs were submitted to PubMLST (tted to PubMLST (https://pubmlst.org/organisms/vibrio-parahaemolyticus). ResFinder 4.1 was used for screening antimicrobial resistant genes (ARGs). The virulence-associated genes (VGs) were found using virulence factor database (VFDB) (http://www.mgc.ac.cn/cgi-bin/VFs/v5/main.cgi).

RESULTS

Serotypes
73 out of 5,337 (1.4%) diarrheal outpatients were positive for *V. parahaemolyticus* in 2021. Serological analysis of the 73 *V. parahaemolyticus* isolates revealed a total of 7 serovars with 3 defined serotypes (O10:K4, O3:K6, and O6:K18) and 4 kinds of untypeable K antigens. O10:K4 (83.6%, 61/73) was the most common one, followed by O2:KUT (5.4%, 4/73), O4:KUT (4.1%, 3/73), O1:KUT (2.7%, 2/73) and O3:K6, O6:18, and O10:KUT each (1.4%, 1/73) (Table 1). These results indicated the emerging serotype O10:K4 had replaced O3:K6, which accounted for 67.7% of clinical isolates during the period of 2010–2019 (8), becoming the predominant
serotype in 2021.

Antibiotic Resistance Profile and Resistance Genes

The antimicrobial susceptibilities of 73 V. parahaemolyticus strains were listed in Table 2. All isolates were sensitive to the following 14 antimicrobial agents such as ampicillin-sulbactam, ceftazidime-avibactam, cefotaxime, ceftazidime, ertapenem, meropenem, amikacin, tetracycline, aztreonam, tigecycline, nalidixic acid, ciprofloxacin, trimethoprim-sulfamethoxazole and chloramphenicol. Only 2.7% of 73 isolates were resistant to colistin and 97.3% demonstrated intermediate resistance to colistin. Additionally, the sensitivity rates of 73 isolates to ampicillin and streptomycin were 89.0% and 67.1%, respectively, and the intermediate resistance rates were 11.0% and 32.9%, respectively. The ARGs analysis showed that all 73 strains carried at least one of the 9 kinds of \( \beta \) lactamase resistance genes (\( \text{blaCARB-18, blaCARB-20, blaCARB-22, blaCARB-24,} \)).

<table>
<thead>
<tr>
<th>Serovars</th>
<th>No. of isolate (s)</th>
<th>ST</th>
<th>Virulence genes</th>
<th>Pandemic markers</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>( \text{tdh} )</td>
<td>( \text{trh} )</td>
</tr>
<tr>
<td>O10:K4</td>
<td>61</td>
<td>ST3</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>O3:K6</td>
<td>1</td>
<td>ST3</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>O6:K18</td>
<td>1</td>
<td>ST1490</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>O1:KUT</td>
<td>1</td>
<td>ST3</td>
<td>+</td>
<td>–</td>
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<tr>
<td></td>
<td>1</td>
<td>ST2620</td>
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</tr>
<tr>
<td>O2:KUT</td>
<td>4</td>
<td>ST2781, ST2894, ST2895, ST2896</td>
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<tr>
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<td>ST499</td>
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<td>–</td>
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<tr>
<td></td>
<td>2</td>
<td>ST2516</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>O10:KUT</td>
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<td>ST2897</td>
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Abbreviations: ST=sequence type; V. parahaemolyticus=Vibrio parahaemolyticus.

<table>
<thead>
<tr>
<th>Antimicrobial class</th>
<th>Antimicrobial agent</th>
<th>Susceptible ( n ) (%)</th>
<th>Intermediate ( n ) (%)</th>
<th>Resistant ( n ) (%)</th>
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<td>71(97.3)</td>
<td>2(2.7)</td>
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</table>

Abbreviation: V. parahaemolyticus=Vibrio parahaemolyticus.

TABLE 1. Serotypes, ST and virulence factors of 73 clinical V. parahaemolyticus strains in Beijing, 2021.

blaCARB-29, blaCARB-30, blaCARB-33, blaCARB-34, and blaCARB-46) (Figure 1). Two strains had the quinolone resistance gene qnrC. Interestingly, all 61 O10:K4 strains carried blaCARB-22.

**Distribution of Virulence-associated Genes**

All of the 73 strains had the tdh gene, but none had the trh gene (Figure 1). 64 isolates (87.7%) were positive for the tdh gene, of which 61 strains carried the orf8 gene. The serotypes of these 64 tdh+ strains included O10:K4 (n=61), O4:KUT (n=2), and O1:KUT (n=1) (Table 1). In addition, all 64 tdh+ strains were pandemic clones with gene marker tdh+ trh toxRS/new+. The other 9 tdh- strains belonged to serotypes O3:K6 (n=1), O6:K18 (n=1), O1:KUT (n=1), O2:KUT (n=4), O4:KUT (n=1), and O10:KUT (n=1). All 73 strains contained multivalent adhesion molecules encoding the VP1611 gene and nearly all 39 T3SS1 genes except for vopB and vseC. The 64 tdh+ strains carried all 25 T3SS2 genes, but the 9 tdh- strains were negative for the 25 T3SS2 genes (Figure 1).

**MLST Analysis**

A total of 73 V. parahaemolyticus strains were categorized into 10 STs. Four new STs (ST2894, ST2895, ST2896, and ST2897) were identified. The most frequently observed ST was ST3 (63/73, O10:K4 n=61, O3:K6 n=1, and O1:KUT n=1). The 64 pandemic strains (tdh+ trh toxRS/new+) belonged to ST3 (O10:K4 n=61 and O1:KUT n=1) and ST2516 (O4:KUT n=2) (Table 1). All 61 O10:K4 isolates had the characteristic of tdh+ trh toxRS/new orf8+ ST3, which was also characteristic of most strains from diarrhea patients.

**Phylogenetic Analysis**

The phylogenetic analysis of the 73 strains was evaluated using a WGS-SNP analysis with the reference sequence GCF_000196095.1. All of the 61 O10:K4 strains with ST3 formed the main lineage.

FIGURE 1. Distributions of serotype, STs, antibiotic resistance genes, virulence genes, and pandemic markers among 73 clinical V. parahaemolyticus strains in Beijing in 2021.

Note: The color strips indicate areas corresponding to the isolates. Pink colored cells represent the presence of pandemic markers and white cells represent the absence of the pandemic markers; Lilac colored cells represent the presence of antibiotic resistance genes and white cells represent the absence of the antibiotic resistance genes; Light blue cells represent the presence of virulence-associated genes and white cells represent the absence of the virulence-associated genes.

Abbreviations: ST=sequence type; V. parahaemolyticus=Vibrio parahaemolyticus.
FIGURE 2. Phylogenetic tree of 73 clinical *V. parahaemolyticus* strains by WGS-SNP analysis in Beijing in 2021.

Note: The 61 genomes from O10:K4 strains with ST3 were indicated within the blue ring lineage. The two genomes from the other ST3 strains (2021VP046 belonging to O1:KUT and 2021VP010 belonging to O3:K6) were indicated within the yellow ring lineage. The other 10 genomes from 5 serotypes (O6:K18, O1:KUT, O2:KUT, O4:KUT, and O10:KUT) and 9 different STs, were indicated within the green ring lineage.

Abbreviations: *V. parahaemolyticus*; WGS=whole genome sequence; SNP=single nucleotide polymorphism; ST=sequence type.

(Figure 2), which was close to the other two ST3 strains (2021VP046 belonging to O1:KUT and 2021VP010 belonging to O3:K6). The other 10 strains belonging to 5 serotypes (O6:K18, O1:KUT, O2:KUT, O4:KUT, and O10:KUT) and 9 different STs, formed the individual branches.

**DISCUSSION**

*V. parahaemolyticus* serotype O3:K6 with pandemic makers (*tdh*+ *trh*+ *toxRS/new*+ *orf8*) has been widespread in many countries including China since 1996 (1,4). In this study, only one of 73 clinical *V. parahaemolyticus* isolates was identified as O3:K6 in Beijing in 2021, which was much lower than our previous study reporting of 67.7% over the previous 10 years from 2010 to 2019 (8) and 48% of the clinical isolates of *V. parahaemolyticus* collected in Guangdong Province from 2007 to 2011 (9). Moreover, this O3:K6 isolate was neither a pandemic nor a pathogenic strain. Above all, 61 O10:K4 strains (83.6%) with pandemic traits (*tdh*+ *trh*+ *toxRS/new*+ *orf8*) were found for the first time and became the dominant clone instead of O3:K6 in 2021. The emergence of pathogenic and pandemic *V. parahaemolyticus* O10:K4 strains presented in this
study should be a matter of concern for public health authorities, as the risk of outbreak rises. Recent studies have shown that at least 21 non-O3:K6 serotypes such as O4:K8, O4:KUT, and O3:K8 exhibited pandemic markers, and most likely originated from the same clones as O3:K6 (4,10). These findings suggest that the O and K antigen encoding loci are subject to exceptionally high rates of recombination (11). Serovar conversion through mutation or horizontal gene transfer of the O and K antigen encoding genes may be one means for *V. parahaemolyticus* to adapt to environmental changes and human immune responses (12).

In addition, this study revealed that T3SS1 genes were present in all *V. parahaemolyticus* strains and T3SS2 genes were predominantly present in the pathogenic and pandemic strains, indicating that the T3SS2 region may enhance virulence when present. Our results are consistent with the recent finding in an experimental animal model, which demonstrated that T3SS2 is necessary for pathogen colonization and the development of gastroenteritis (13). In this study, all the O10:K4 strains carried the entire T3SS1 and T3SS2 genes, which confirmed that the O10:K4 strains had a similar virulence as the O3:K6 pandemic clone. The 9 tdh* trh* T3SS2* strains were isolated from diarrheal patients, which also suggested that *V. parahaemolyticus* might harbor other virulence factors responsible for diarrhea. Therefore, the understanding of pathogenicity is still incomplete and it is necessary to discover more reliable predictors of virulence.

Among 10 STs analyzed in this study, ST3 was the most common one, which matched the findings of previous studies that ST3 was representative of pandemic clones on a global scale (14). The correlation between different serogroups and STs has been observed and some STs contained several different serogroups, such as ST3 (O1 and O3) (9,15). This phenomenon was observed in this study, that all the ST3 strains belonging to different serotypes O10:K4, O1:KUT, and O3:K6 formed a cluster, different from other STs which broke into individual branches in the phylogenetic analysis. Moreover, all the O10:K4 strains and the genetic variant O3:K6 (tdh* trh* toxRS/new* orfB*) were placed in the same cluster, suggesting a possibility of transfer of the pandemic clone.

To the best of our knowledge, this was the first report of O10:K4 associated with diarrhea cases in China. The whole-genome sequence analysis indicated that it belonged to ST3 lineage which has the capacity to spread rapidly and the potential to replace native strains. However, it was unclear where this novel clone originated from and how it entered Beijing. Therefore, it is necessary to track the source of O10:K4 strains and to strengthen monitoring of their spread and epidemic trends through the continuous surveillance of *V. parahaemolyticus* in the future.

**Conflicts of Interest:** No conflicts of interest.

**Acknowledgements:** Staff of District CDCs of Beijing Municipality.

**doi:** 10.46234/ccdcw2022.106

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Submitted: April 12, 2022; Accepted: May 31, 2022

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Global Food Safety Strategies: Need to Develop Roadmap of Implementation in China

Yongning Wu

The Updated Global Strategy for Food Safety (GSFS)

The Seventy-Fifth Session of World Health Assembly (75th WHA) has adopted the updated Global Strategy for Food Safety (2022–2030): towards stronger food safety system and global cooperation with the recommendations from the 150th Executive Board meeting (1–2). This response to the WHA 73.5 resolution on "Strengthening efforts on food safety" and Member States are requested to update GSFS to respond to current and emerging challenges. Developed by the World Health Organization (WHO) Secretariat with the advice of the Technical Advisory Group (TAG) on Food Safety: Safer Food for Better Health (Figure 1), the vision of the updated strategy is to ensure that all people, everywhere, consume safe and healthy food to reduce the burden of foodborne diseases. The updated strategy aims to guide and support Member States in their efforts to prioritize, plan, implement, monitor, and regularly evaluate their actions towards reducing the burden of foodborne diseases by continuously strengthening food safety systems and promoting global cooperation. WHO will publish and translate the final document; develop guidance to assist Member States to implement the updated strategy and develop work plans (tools, investment case, map of stakeholders, baseline surveys, etc). It is important to have alignment between the Food Safety TAG and the Foodborne Disease Burden Epidemiology Reference Group (FERG) in WHO for impact measurement. There is an opportunity to promote the updated strategy during the World Food Safety Day (WFSD) events celebrated annually on June 7. The theme of WFSD this year is “Safer food, better health” which is the same as the theme of the updated strategy. World Food Safety Day affords the opportunity to inspire global participation. The WFSD campaigns and the updated strategy stress the need to transform food systems to deliver better health in a sustainable manner in order to prevent most foodborne diseases.

GSFS Need Member States to Develop Roadmap of Implementation

The 75th WHA has adopted the updated strategy with the recommendations from the 150th Executive Board meeting (1) as follows: to call on each Member State to develop a national roadmap of implementation and to make appropriate financial resources available to support such work; and to request the WHO Director-General to report back on progress in the updated strategy implementation to the 77th WHA in 2024 and thereafter every two years until 2030 (Figure 2). The implementation of the updated strategy will support the realization of food safety commitments emanating from the United Nations Food Systems Summit (UNFSS) convened as part of the Decade of Action to achieve the Sustainable Development Goals by 2030. It will particularly support the WHO initiatives to support safe healthy diets, school meals, and One Health agendas.

Member States are at different stages regarding their national food safety systems and there is a need for a tailored approach to the implementation of the strategy. Adopting a One Health approach to food safety will allow Member States to detect, prevent and respond to emerging diseases at the human-animal-environment interface and to more effectively address food-related public health issues. The use of existing WHO, Food and Agriculture Organization of the United Nations (FAO), and World Organisation for Animal Health (OIE) tools and programs will support the implementation of the updated strategy and highlight areas where there is a need for new resources. The existing FAO/WHO Assessment Tool and FAO/WHO Diagnostic Tool (CTF) can be used to develop a baseline that will guide the GSFS implementation. Then TAG will update and detail the TAG work plan (2022–2023). The Member States can use the WHO Regional Advisors in the implementation of the updated strategy to assist in aligning with existing regional frameworks and identifying the regional priorities.
FIGURE 1. The overall process for the update of the WHO Global Strategy for Food Safety.
Notes: The TAG refers to the technical advisory group on food safety: safety for better health in the World Health Organization.
Abbreviations: TAG=Technical Advisory Group; EB=Executive Board; WHO=World Health Organization; WHA=World Health Assembly; MS=Member States.

China’s National Strategy for Food Safety and Roadmap of Implementation

The China national strategy for food safety, proposed in 2016, marks the foundation of a unique Chinese framework in food safety management system with a core goal to ensure Chinese people “eat at ease and safely”. As a concrete response to the WHO updated GSFS, the Chinese government has proposed its own timetable and roadmap for its domestic food safety strategy which includes: 1) A zero tolerance of systemic food safety risks by 2020 and constantly improving the level of food safety assurance; 2) Establishing a strict, high-efficient, and socially governed food safety governance system by 2027; 3) Fundamentally achieving the modernization of food safety governance and oversight of the food chain by 2035; 4) Achieving universal modernization of food safety governance throughout China, and be one of the leading counties in the world for food safety standards and governance by 2050 (3).

WHO devolved the updated GSFS to guide and support Member States to prioritize, plan, implement, monitor and regularly evaluate actions towards the reduction of the incidence of foodborne diseases by continuously strengthening food safety systems and promoting global cooperation. The updated GSFS also reflects, and is complementary to, existing WHO health programmes, such as nutrition and non-communicable diseases (e.g., salt reduction), antimicrobial resistance, public health emergency and emerging diseases, climate change, environmental health, water and sanitation, and neglected tropical diseases.

China will be an essential stakeholder in food safety to promote, support and protect public health and reduce the burden of foodborne diseases under the Healthy China framework. The updated WHO GSFS provides the five strategic priorities (SP) for concerted global action that will underline both the importance of food safety as a public health priority and the need to enhance its critical role as a public health component in food systems (Figure 3). Strengthening national food safety systems begins with establishing or improving infrastructure and components of food control systems as described in SP 1. For example, developing framework food legislation, standards and guidelines, laboratory capacity and surveillance, food control activities and programmes, and emergency preparedness capacity. In addition to establishing a national food control system, four important characteristics/principles are being considered and adopted for the system to be fully operational. Global targets and identifying indicators of success have been established, i.e., what gets measured gets done. In the case of the updated strategy, this means that regular

![Figure 3](image-url)

**FIGURE 3.** Conceptual framework for strategic priorities. Abbreviation: SP=strategic priority.
measurement and reporting will keep a focus on priorities and objectives and will facilitate continuous improvement. Effective management of food control programmes involves monitoring to ensure proper implementation, efficient operation, and continuous improvement. Identifying expected outcomes and setting appropriate objectives which are communicated and being achieved, are all key to success. A part of the management of any programme is to select indicators and set targets to ensure progress. These simplify performance management by allowing all participants to understand not just their roles, but those of others. Indicators provide information about progress towards an objective and targets, and also support decision-making at all levels of an organization so that appropriate actions can be taken and remedial actions if required. Indicators are important for achieving the objectives of the national food safety systems because they keep the objectives at the centre of decision-making. The possible indicators are the followings: 1) Foodborne diarrheal disease incidence per 100,000 population; 2) Surveillance system in place for the detection and monitoring of foodborne disease and food contamination; 3) Multisectorial collaboration mechanism for food safety incidents.

Indicator 1 (foodborne diarrheal diseases) is an example of the health outcome indicator while Indicators 2 and 3 are process indicators. In setting targets and indicators, it is important to ensure that they are realistic and achievable. In China there is a lack of background data for disease burden of foodborne illness and foodborne diarrheal diseases. Enhanced Surveillance system and multi-sectorial coordination are now in place for the detection and monitoring of foodborne diseases and food contamination.

One of the challenges associated with using foodborne diarrheal disease as a health outcome indicator is that not all national surveillance systems are at the same stage of development. China sounded a word of caution about the interpretation of surveillance data, for example, the temptation of using the collected data to compare countries as this may be a reflection of the surveillance systems and laboratory capability rather than the true incidence and prevalence of the disease. TAG members suggested that foodborne disease outbreaks might be a better indicator to consider although there remain issues concerning comparability between countries due to differences in epidemiological investigative competencies and surveillance systems. The collection of data on the incidence of foodborne diarrheal disease is quite challenging, even for high-income countries. Additionally, using this indicator would overlook data on chemical contamination of food, which impacts diet-related diseases, such as cancer and chronic diseases.

It was also suggested to consider including sub-indicators under the main indicators. A sub-indicator could focus on countries’ testing capacity, for example, whether the country is testing for certain foodborne pathogens, etc. In this way, these sub-indicators can also be used to identify gaps within a country’s surveillance system, which, if addressed, could further strengthen their food safety capacity. It should be noted that the lack of capacity is the main bottleneck for setting indicators and targets. The sensitivity of trade implications highlighted by the indicators and targets could also be a reason that countries might be reluctant to report data to international organizations.

The Global Foodborne Infections Network (GFN) is a very powerful resource to build countries’ capacity in foodborne diseases surveillance. Rather than looking at all foodborne pathogens, it might be good to focus at least initially on a few key foodborne pathogens, such as Salmonella, Campylobacter, etc. Another challenge is that the foodborne disease surveillance in many countries is managed by the health sector and there is no, or limited linkages, with food safety competent authorities and food testing laboratories. The challenge with setting targets is that there are limited baseline studies within countries, which means that the target-setting experience in the regions is very subjective. Another important issue is that countries have different capacities, so it might be more reasonable to set different targets for different countries or regions. The global strategy would require global targets. In the meantime, it should be possible to adapt global targets at regional and country levels. Setting global food safety indicators and targets within the strategy will encourage countries to invest more in their food safety systems and further strengthen their capacity.

China has made great advances in forensic microbiology, molecular diagnostics, surveillance systems and supporting IT infrastructure for both routine surveillance and early warning. Many of our initiatives are transferable to other member states and China can be a role model to help others. We live in a global village with food and ingredients globally distributed so the need for international co-operation has never been more important. No country can be complacent as often the safety of a Member State’s
citizens is dependent on standards in another jurisdiction from where they import food. Nobody will be safe from food borne disease until we are all safe.

doi: 10.46234/ccdcw2022.107

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Submitted: May 30, 2022; Accepted: June 02, 2022

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COVID-19 Outbreaks Linked to Imported Frozen Food in China: Status and Challenge

Jiahui Wang; Fengqin Li; Zhaoping Liu; Ning Li*

ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA contamination was reported on China’s imported frozen foods and packaging materials. However, there was no evidence of this disease initiated by environment-to-human transmission until the outbreak of coronavirus disease 2019 (COVID-19) in Beijing in June 2020. This article aimed to analyze and summarize COVID-19 outbreaks related to cold-chain foods to provide a scientific basis for tracing the epidemiological trajectory of the pandemic, providing risk assessments, and mitigation policies. Overall, 37 COVID-19 outbreaks and 5,741 infected cases were reported within the study period. It was found that 7 outbreaks and 689 cases were linked to imported frozen foods. The first index case among the 7 outbreaks was exposed to SARS-CoV-2-contaminated outer packaging of frozen food, triggering the subsequent community transmission. This study supported the speculation that cold-chain foods act as a pathway for SARS-CoV-2 and might present a risk for virus transmission between countries and regions. Handlers and processors exposed to the imported frozen foods should be effectively self-protected, daily monitored for clinical manifestations of COVID-19, and tested for SARS-CoV-2 nucleic acid at regular intervals.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (1). It was primarily thought to spread mostly person-to-person through respiratory droplets (2). COVID-19 infections can occur by touching a surface or object, including food or food packaging, containing the virus, and then touching the mouth, nose, or eyes. However, there was no evidence that food was associated with spreading SARS-CoV-2 until COVID-19 cases resurged in Xinfadi Market, Beijing, in June 2020. Fomite transmission has been proposed, and it has attracted extensive attention worldwide since then (3–4).

SARS-CoV-2 has been reported to persist in conditions similar to the ones found in frozen food, packaging, and cold-chain products. It was noted that the infectivity of SARS-CoV-2 on cold-chain products did not decrease after 21 days at 4 °C (refrigerated food) or at −20 °C (frozen food) (5). SARS-CoV-2 was isolated from the outer packaging of frozen cod in Qingdao City, Shandong Province (6), and the investigations of the outbreak in Dalian City, Liaoning Province, strongly suggested that the infection source was from a virus-contaminated packaging of frozen seafood (7), providing evidence that the contaminated cold-chain foods and packaging might be the vector in the virus transmission.

This article evaluated and reviewed COVID-19 outbreaks linked to cold-chain products as a source of SARS-CoV-2 infection and virus transmission from imported cold-chain products in China.

DATA COLLECTION

China has established the Joint Prevention and Control Mechanism of the State Council in early 2020 in response to SARS-CoV-2. It is a multi-ministerial coordination mechanism and work platform at the central level (or at the provincial and local levels). A total of 32 departments are involved. This joint mechanism is responsible for data collection and releasing information regarding the COVID-19 pandemic. It includes big data collection and mining, epidemiological investigation, tracing the origin of outbreaks and virus sources, vaccination, and press release.

All data were obtained from the Joint Prevention and Control Mechanism of the State Council.
OUTBREAKS LINKED TO IMPORTED FROZEN FOOD DISTRIBUTION AND ANALYZE

Although COVID-19 has been effectively controlled in China since the Wuhan epidemic, several outbreaks or clusters of COVID-19 cases linked to either importation of cases or virus contaminated products occurred, as shown in Table 1. In China, 37 COVID-19 outbreaks and 5,741 cases were reported from June 2020 to July 15, 2021. Among them, 7 outbreaks and 689 infected cases were linked to imported cold-chain foods. Investigations demonstrated that all 7 outbreaks were suspected of having occurred due to handling or exposure to SARS-CoV-2-positive imported cold-chain foods, especially their outer packaging materials. In total, 368 people infected with SARS-CoV-2 were reported in Beijing in June 2020. These infected people are all directly or indirectly related to Xinfadi Market: 169 people were market staff, and 103 people were visitors to the market less than 14 days before the symptoms appeared. The remaining 96 people were in close contact with the abovementioned individuals. No other early independent transmission chain beyond the market was found, indicating that Xinfadi Market was the unique source of the outbreak. Notably, five surface-swabbing samples collected from salmon in the original sealed package in the company’s cold storage, a unique imported food supplier of Xinfadi Market located outside the market, were positive for SARS-CoV-2. The virus in the fish swab shared at least seven mutations with that of Xinfadi Market. This is the first time it has been proposed that environment-to-human transmission originated from contaminated imported food.

Thereafter, clusters of COVID-19 cases linked to cold-chain foods were frequently reported in China. The outbreak in Dalian, on July 22, 2020, was associated with imported cold-chain foods (7). The porter was first infected by having contact with the outer packaging of imported frozen cod contaminated with SARS-CoV-2. The virus was subsequently introduced to a local seafood company through the infected porter’s wife, and then spread further. However, active and infectious viruses were not successfully isolated from the samples of cold-chain food in Beijing and Dalian. Hence, the role of SARS-CoV-2-contaminated cold-chain foods in the spread of the COVID-19 epidemic could not be confirmed.

Afterwards, 2 infected dock workers who transported the imported frozen cod for 10 hours in 2 separate storage warehouses on the same freighter in Qingdao, were reported to be infected with the virus, although they had never been to any high-risk areas of COVID-19 and had no contact histories with either COVID-19 patients or overseas visitors. They took off their masks and smoked without washing their hands during work. However, the other 69 dockers who handled the frozen cod simultaneously, but did not take off their masks, were not infected. An active SARS-CoV-2 sample was successfully isolated from a virus-contaminated outer packaging sample of the imported frozen cod. A comparison of the virus gene sequence showed that the virus isolated from the infected port handlers was the progeny of that isolated from the outer packaging of frozen imported cod, further indicating that SARS-CoV-2-contaminated outer packaging of the imported frozen cod was the source of the COVID-19 epidemic in Qingdao (6). Therefore, SARS-CoV-2 posed a significant health risk to essential workers maintaining the cold-chain food supply.

The first infected cases of two COVID-19 outbreaks in Tianjin Municipality in November 2020 were all porters who were in close contact with the virus-contaminated outer packaging of imported frozen food, or exposed to the virus-contaminated environment during the handling of frozen foods from the cabin to the deck. The COVID-19 resurgence in Dalian, in December 2020, also originated from the infected dockers due to the handling of the imported cold-chain cargoes that further triggered large-scale community transmission.

The virus sequence that resulted in the Yingkou City, Liaoning Province, and Lian City, Anhui Province, COVID-19 outbreaks in May 2021 was highly homologous to the virus linked to the Dalian COVID-19 outbreak on July 22, 2020, mentioned above. Further epidemiological investigation illustrated that the virus on the outer packaging material of the imported frozen cod that caused the COVID-19 epidemic in Dalian in July 2020 was also the source of the Yingkou and Luan outbreaks. These SARS-CoV-2 contaminated frozen cod were stored in cold storage for nearly 11 months in Dalian since July 2020, but still infected the workers during handling. This further elucidates that SARS-CoV-2 can maintain its infectivity for a long time (at least 11 months) at a low temperature of −18 °C.
TABLE 1. Summary of COVID-19 outbreaks linked to the imported cold-chain food between June 2020 and May 2021 in China.

<table>
<thead>
<tr>
<th>Location &amp; time</th>
<th>Cases</th>
<th>Confirmed cases n, %</th>
<th>Asymptomatic patient n, %</th>
<th>Duration of epidemic</th>
<th>Age median &amp; range</th>
<th>Ratio of male to female</th>
<th>Source of infection</th>
<th>Path of infection</th>
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<tr>
<td>2020-6, Fengtai, Beijing</td>
<td>402</td>
<td>362, 90.0</td>
<td>0, 0</td>
<td>2020/6/5–7/9</td>
<td>35</td>
<td>42, 1–86</td>
<td>1:0.8</td>
<td>Virus-contaminated imported cold-chain food</td>
</tr>
<tr>
<td>2020-7, Dalian-1, Liaoning</td>
<td>135</td>
<td>99, 73.3</td>
<td>0, 0</td>
<td>2020/7/9–8/6</td>
<td>29</td>
<td>43, 0–73</td>
<td>1:1.2</td>
<td>Virus-contaminated outer packaging of imported frozen cod</td>
</tr>
<tr>
<td>2020-9, Qingdao, Shandong</td>
<td>14</td>
<td>12, 85.7</td>
<td>1, 7.1</td>
<td>2020/9/24–10/14</td>
<td>21</td>
<td>47.5, 23–83</td>
<td>1:0.8</td>
<td>Virus-contaminated outer packaging of the imported frozen cod</td>
</tr>
<tr>
<td>2020-11, Binhai, Tianjin-1</td>
<td>2</td>
<td>2, 100.0</td>
<td>0, 0</td>
<td>2020/11/5–11/10</td>
<td>6</td>
<td>48.5, 38–47</td>
<td>male only</td>
<td>Virus-contaminated cold-chain food or environment</td>
</tr>
<tr>
<td>2020-11, Binhai, Tianjin-2</td>
<td>10</td>
<td>7, 70.0</td>
<td>1, 10.0</td>
<td>2020/11/17–11/27</td>
<td>11</td>
<td>38.5, 6–61</td>
<td>1:0.4</td>
<td>Virus-contaminated cold-chain food</td>
</tr>
<tr>
<td>2020-12, Dalian-2, Liaoning</td>
<td>83</td>
<td>51, 61.5</td>
<td>0, 0</td>
<td>2020/12/15–2021/1/8</td>
<td>25</td>
<td>46, 0–88</td>
<td>1:1.8</td>
<td>Virus-contaminated cold-chain goods</td>
</tr>
<tr>
<td>2021-5, Liaoning-Anhui</td>
<td>43</td>
<td>24, 55.8</td>
<td>0, 0</td>
<td>2021/5/3–5/24</td>
<td>22</td>
<td>29, 2–82</td>
<td>1:2.3</td>
<td>Virus-contaminated imported frozen cod</td>
</tr>
</tbody>
</table>

It may be initially caused by the introduction of virus into Xinfadi Wholesale Market via the imported cold-chain foods contaminated with SARS-COV-2.

A dockworker exposed to SARS-CoV-2-contaminated outer packaging of the imported frozen cod during the inbound unloading transferred the infection to his wife who worked at a cold-chain product processing and storage company, resulting in a community spread of COVID-19.

A dockworker exposed to SARS-CoV-2-contaminated outer packaging of the imported frozen cod during inbound unloading and caused the subsequent community transmission via personal contact during his health check in hospital.

Porters were infected via exposure to the imported frozen food or environment contaminated by SARS-CoV-2, causing a community transmission.

Porters were infected via exposure to the imported frozen foods or environment contaminated by SARS-CoV-2, causing a community transmission.

Porters were infected by contact with cold-chain goods, causing a wide community transmission.

Porters were infected with SARS-CoV-2 during the handling of cold-chain foods in a cold storage, resulting in a local transmission of COVID-19 and spread to other areas.
DISCUSSION

The persistence of SARS-CoV-2 on the objects’ surfaces was different depending on the materials (5). A recent study showed that the titer of SARS-CoV-2 in artificially contaminated pieces of salmon, chicken, and pork with $3 \times 10^6$ TCID$_{50}$ (median tissue culture infectious dose) was stable at 4, −20 °C, and −80 °C over 24 hours (8). This indicated that for some countries that appear to have eradicated the virus, there is a potential fear of re-emergence of COVID-19. Clusters of COVID-19 cases related to cold-chain foods in China, as described in this study, suggested that SARS-CoV-2 can survive on the contaminated surface of foods and packaging materials and maintain its infectivity for at least 11 months at a temperature of −18 °C. Although the virus genome sequences from the cold-chain foods were identical to those of the cases, there is still no direct evidence that the virus caused the local epidemic. As Koch postulates, four criteria are needed to establish a causative relationship between a microbe and a disease. One of them states: “The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.” Nucleic-acid-based detection methods led to the revisions of Koch’s postulates, but these modifications were still controversial as they do not account well for established disease associations.

Additionally, even when no active virus was detected, the RNA extract of the package sample could cause infections in animals (data not published). Therefore, active virus isolation is complex and influenced by many factors. The outbreak of COVID-19 in Qingdao gave direct evidence that the virus could be transmitted from frozen food packaging material to humans after long-distance transportation across borders (4). Further studies are required to evaluate the presence and persistence of infectious SARS-CoV-2 and its RNA in frozen food compartments, sample conditions, and the intervention strategies for reducing the virus infection.

It is difficult to protect the workers efficiently when they work for several hours in cold storage or refrigerated cargo cabin at a temperature of −20 °C or below. Sweat and deep breathing deposit ice on workers’ masks, face shields, and goggles. This reduces the effect of protective facilities and makes workers take off their masks to breathe, increasing the probability of being infected by the virus on the outer packaging materials. In addition, many aerosols generated during frozen food handling, processing, or selling in a high-humidity, poorly ventilated environment facilitate COVID-19 transmission from person to person. Therefore, this study supports the speculation that the infected COVID-19 cases among cold-chain food handling and operating groups are likely related to occupational risk. China is a vast country, and the number of workers exposed to virus-contaminated cold-chain foods and their packaging is still significant, although the prevalence of SARS-CoV-2 contamination is very low. Therefore, it is essential to strengthen personal protective equipment development available for use in a cooling or freezing environment. All these elements mentioned above should be considered when implementing workplace interventions to ensure that communication and training are culturally and work-specifically tailored. Meanwhile, it is necessary to perform a comprehensive mass testing for SARS-CoV-2 nucleic acids, contact tracing, and symptom screening in the food of animal origin handling and processing facilities to identify high proportions of asymptomatic or pre-symptomatic infections.

Conflicts of interest: No conflicts of interest.

doi: 10.46234/ccdcw2022.072

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Submitted: January 13, 2022; Accepted: April 04, 2022

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