

Perspectives

Contamination and Transmission of SARS-CoV-2 Variants in Cold-Chain Food and Food Packaging

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INTRODUCTION

The emergence of coronavirus disease 2019 (COVID-19) has posed a significant threat to global health and well-being. Vaccination serves as a vital strategy in preventing and mitigating the severity of clinical symptoms. However, due to natural selection, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has evolved, resulting in various mutations (1). Currently, the World Health Organization (WHO) has identified five variants of concern, including Alpha, Beta, Gamma, Delta, and Omicron (2). Of these, only Omicron remains in circulation and has already produced nearly one thousand sub-lineages or subvariants. These mutating variants demonstrate increased infectivity and vaccine breakthrough rates, as well as more pronounced antibody escape rates (3).

COVID-19 is primarily a respiratory illness, with its main transmission routes being direct contact and the spread of droplets or aerosols (4). The Food and Agriculture Organization of the United Nations (FAO) has reported that the likelihood of SARS-CoV-2 transmission via food or food packaging is low, as the virus cannot multiply on such surfaces. In addition, upon exposure to environmental factors, viral particles degrade and become less infectious (5). Despite this, emerging epidemiological evidence suggests that imported cold-chain foods and their packaging may contribute to outbreak occurrences.

According to the literature, there have been seven reported outbreaks in China associated with exposure to cold-chain food and food packaging contaminated with SARS-CoV-2. For instance, viral strain analysis revealed that the virus isolated from the outer packaging of imported cod was linked to the SARS-CoV-2 infections found among dock workers during the 2020 Qingdao outbreak (6). Moreover, several COVID-19 outbreaks have occurred in meat processing facilities overseas, with a diagnosis rate of

18.2% among workers in some states of the United States (7). Individuals working in cold, humid, and crowded environments are at an increased risk for both contracting and transmitting the virus.

Cold-chain food and packaging may become contaminated through two primary means: 1) viral shedding from hands coming into contact with food and packaging surfaces, and 2) expelled respiratory particles generated from talking, coughing, sneezing, and singing (8). In the investigation of the COVID-19 outbreak in Qingdao, live SARS-CoV-2 strains were successfully isolated and cultured from imported frozen seafood packaging (9). This finding suggests that SARS-CoV-2 can survive at low temperatures for several weeks, thereby enabling its spread across borders. Consumers may then transfer infectious particles from contaminated food surfaces and packaging to their eyes, noses, and mouths (10). Even during periods of strict control measures, the potential spread of SARS-CoV-2 through frozen food items and packaging, particularly imported frozen food and packaging, warrants close attention.

SARS-CoV-2 VARIANTS CONTAMINATION IN COLD-CHAIN FOOD AND FOOD PACKAGING

The overall contamination level of cold-chain food and food packaging appears to be low. Between May 5, 2020, and September 10, 2020, the Supervision Bureau of Consumer Rights and Citizen Safety Protection of the Russian Federation examined a total of 1,677 cold-chain samples, primarily consisting of vegetables (40%), meat and meat products (26%), and fruits (22%). All samples tested negative for SARS-CoV-2 (11). During November 2020 through January 2021, the Food Quality and Safety Administration's Medical Department of the Ministry of Public Health in Thailand randomly tested 117 samples of food (mainly seafood) and food packaging (cans, cartons, etc.), with no SARS-CoV-2 detected in any samples

(12).

In 2020, China Customs collected and tested 1,295,692 samples, of which 47 yielded positive results for nucleic acid (0.35/10,000), and the remaining samples tested negative (13). Furthermore, over 55.83 million swabs of frozen food-related samples were collected in China, including 31 provincial-level administrative divisions (PLADs) and the Xinjiang Production and Construction Corps (XPCC). More than 20.51 million swabs were related to cold-chain food and packaging materials. Among these, 1,455 (0.26/10,000) swabs tested positive. As shown in Table 1 (14), seafood displayed the highest levels of pollution among all food types. The risk of carrying viruses is higher for outer packaging than inner packaging, and the positive sample detection ratio of imported cold-chain food exceeded that of domestic cold-chain food. Imported cold-chain foods might be a potential source for COVID-19 outbreaks related to the cold chain in China.

In conclusion, by analyzing the results of SARS-CoV-2 nucleic acid testing of cold-chain food and food packaging both domestically and internationally, it can be demonstrated that the risk of cold-chain food contamination by SARS-CoV-2 is relatively low.

Persistence of SARS-CoV-2 Variants on the Surfaces of Cold-Chain Food and Materials

The persistence of SARS-CoV-2 variants on various surfaces of cold-chain food products is influenced by the specific food substrate. Table 2 demonstrates that fresh agricultural products, such as grapes and tomatoes, as well as deli products like turkey and cheese, can maintain the infectivity of SARS-CoV-2 variants for up to 21 days at 4 °C. In contrast, avocado shells, avocado pulp, and salami have been found to

exhibit antiviral effects (15). Certain meats, including salmon, beef, pork, and chicken, can support the survival of SARS-CoV-2 surrogates for 30 days at both 4 °C and -20 °C (16).

Additionally, the persistence of SARS-CoV-2 variants on different surfaces of cold-chain food products is affected by the temperature of cold-chain transmission. One study revealed that the persistence of SARS-CoV-2 variants on frozen meat (-20 °C) is longer compared to that on freshly stored meat (4 °C) (17). Therefore, SARS-CoV-2 demonstrates a strong survival capacity under refrigerated or frozen conditions. Low-temperature environments during the storage and transportation of cold-chain food products provide favorable conditions for the survival of SARS-CoV-2.

When the cold-chain transmission is maintained and materials are consistently kept at the same temperature, the persistence of SARS-CoV-2 mutations does not increase. One study demonstrated that SARS-CoV-2 variants, specifically Alpha and Delta, can survive on stainless steel surfaces for up to 10 days at 4 °C (18). Another study observed consistent persistence levels for three distinct SARS-CoV-2 variants on the same material surfaces and at the same temperature (19). As presented in Table 2, persistence levels of the strains SARS-CoV-2L, SARS-CoV-2S, and 229E at 4 °C differ across various materials. The persistence of SARS-CoV-2 variants on porous surfaces like kraft and parchment paper is lower compared to non-porous surfaces such as low-density polyethylene. The persistence of SARS-CoV-2 variants on both kraft and parchment paper is longer at -20 °C than at 4 °C. Lower temperatures are more conducive to the survival of SARS-CoV-2. Furthermore, the persistence and infectivity of the virus can be extended in refrigerated and frozen conditions when materials are exposed to cold-chain transmission in the food industry.

TABLE 1. Contamination of the SARS-CoV-2 variants in cold-chain food and food packaging in China.

Date (region)	Food	No. of positive samples	Percentage of total positive samples (%)
2020.07–2021.07 (31 PLADs and XPCC)	Seafood	753	53.86
	Poultry meat	530	37.91
	Other foods	115	8.23
	Inner packaging material	37	3.25
	Outer packaging material	1,101	96.75
	Imported food and packaging	1,391	99.50
	Domestic food and packaging	7	0.50

Abbreviation: SARS-CoV-2=severe acute respiratory syndrome coronavirus 2; PLADs=provincial-level administrative divisions; XPCC=Xinjiang Production and Construction Corps.

TABLE 2. Persistence of the SARS-CoV-2 variants on cold-chain food and material surfaces.

Classification	Food	Virus	Initial viral load	Temperature	Persistence (day)	Test method	Viral load	Reference	
Fresh agricultural product	Avocado shell or pulp	USA-WA1/2020, NR-52281	3.9 log PFU/mL	4 °C	7	qRT-PCR	LOD	(15)	
	Grape, Tomato	USA-WA1/2020, NR-52281	3.9 log PFU/mL	4 °C	21	qRT-PCR	+	(15)	
Deli	Turkey, Cheese	USA-WA1/2020, NR-52281	3.9 log PFU/mL	4 °C	21	qRT-PCR	+	(15)	
	Salami	USA-WA1/2020, NR-52281	3.9 log PFU/mL	4 °C	14	qRT-PCR	LOD	(15)	
	Oyster	USA-WA1/2020, NR-52281	3.9 log PFU/mL	4 °C	21	qRT-PCR	LOD	(15)	
Meat	Salmon	Phi6 (COVID-19 surrogates)	9.0 log PFU/mL	4 °C, -20 °C	30	Plaque assay	+	(16)	
	Beef steak, Ground beef	IVCAS6.7512; NMDCN000HUI	10 ⁴ TCID ₅₀ /mL	4 °C	9	qRT-PCR	+	(17)	
	Beef	USA-WA1/2020, NR-52281	3.9 log PFU/mL	-20 °C	20	LOD	LOD	(17)	
	Beef	Phi6 (COVID-19 surrogates)	9.0 log PFU/mL	4 °C, -20 °C	30	Plaque assay	+	(16)	
Meat	Beef	IVCAS 6.7512; NMDCN000HUI	10 ⁴ TCID ₅₀ /mL	4 °C	9	qRT-PCR	+	(17)	
	Ground pork, Pork chop	USA-WA1/2020, NR-52281	3.9 log PFU/mL	-20 °C	20	LOD	LOD	(17)	
	Pork	Phi6 (COVID-19 surrogates)	9.0 log PFU/mL	4 °C	21	qRT-PCR	+	(15)	
	Pork	Phi6 (COVID-19 surrogates)	9.0 log PFU/mL	4 °C, -20 °C	30	Plaque assay	+	(16)	
Material	Chicken	IVCAS 6.7512; NMDCN000HUI	10 ⁴ TCID ₅₀ /mL	4 °C	9	qRT-PCR	+	(17)	
	Chicken	Phi6 (COVID-19 surrogates)	9.0 log PFU/mL	-20 °C	20	LOD	LOD	(17)	
	SS	Phi6 (COVID-19 surrogates)	9.0 log PFU/mL	4 °C, -20 °C	30	Plaque assay	+	(16)	
	SS	Alpha variant	6.20×10 ⁴ PFU/mL	4 °C	10	Plaque assay	LOD	(18)	
	SS	Delta variant	1.56×10 ⁴ PFU/mL	4 °C	10	Plaque assay	LOD	(18)	
	Kraft	SARS-CoV-2 L;	SARS-CoV-2 S; 229E	4.77±0.04; 4.82±0.05; 4.84±0.05 log TCID ₅₀ /mL	4 °C	2	TCID ₅₀ assay	LOD	(19)
		SARS-CoV-2 S; 229E			-20 °C	5	+	+	(19)
	Parchment	SARS-CoV-2 L;	SARS-CoV-2 S; 229E	4.77±0.04; 4.82±0.05; 4.84±0.05 log TCID ₅₀ /mL	4 °C	4	TCID ₅₀ assay	LOD	(19)
		SARS-CoV-2 S; 229E			-20 °C	5	+	+	(19)
	LDPE	SARS-CoV-2 L;	SARS-CoV-2 S; 229E	4.77±0.04; 4.82±0.00; 4.84±0.05 log TCID ₅₀ /mL	4 °C	5	TCID ₅₀ assay	+	(19)
SARS-CoV-2 S; 229E				-20 °C	5	+	+	(19)	

Abbreviation: SARS-CoV-2=severe acute respiratory syndrome coronavirus 2; COVID-19=coronavirus disease 2019; RT-PCR=reverse transcription-polymerase chain reaction; + = be detected; LOD=limit of detection; SS=stainless steel; LDPE=low-density polyethylene.

Cold-Chain Food and Food Packaging Causing COVID-19 Outbreaks

Since the outbreak began three years ago, multiple epidemiological findings have indicated the spread of SARS-CoV-2 through imported cold-chain foods and food packaging. Existing literature has reported seven outbreaks in China linked to contact with SARS-CoV-2-contaminated cold-chain food and food packaging (Table 3) (6). In another study, viral genome sequence alignment analyses revealed that the SARS-CoV-2 strains causing six outbreaks in China were unrelated to previous local COVID-19 outbreaks. However, some of these strains exhibited high homology with circulating strains abroad, consistent with the countries of origin for imported cold-chain products from the outbreak area (20). These findings suggest that cold-chain foods and packaging can facilitate cross-border viral transmission.

The potential transmission of the virus from cold-chain products to humans may occur if workers handling these items do not properly wear personal protective equipment. Workers such as stevedores or wholesalers may experience higher risk for SARS-CoV-2 exposure due to their direct and frequent contact with cold-chain transported goods. For instance, during the 2020 Qingdao outbreak, the virus strain isolated from the outer packaging of imported cod was found to be a precursor of the strain infecting dock workers. The aforementioned traceability investigation and virology results imply that, under specific environmental conditions, the virus present on the surface of goods might infect high-risk populations

without adequate protection, such as cold-chain workers (21). For consumers, the risk may be lower as goods are often stored and distributed in well-ventilated environments.

In recent years, sporadic reports of COVID-19 outbreaks linked to cold-chain processes have emerged in China in media reports. As indicated in Table 3, two such outbreaks were associated with the SARS-CoV-2 Omicron BA.2 variant, which was traced back to the external packaging of cold-chain food and products. In contrast, the sources of contamination in other outbreaks, primarily in coastal cities, were not identified in relation to cold-chain food and packaging materials.

The Quantitative Microbial Risk Assessment Model for the Transmission of SARS-CoV-2

The risk of non-foodborne SARS-CoV-2 transmission through cold-chain food and food packaging has raised concerns about food facilities as high-risk settings. The transmission risk factors in these facilities include enclosed environments, frequently touched surfaces, and difficulty maintaining physical distancing. Sobolik et al. utilized a quantitative risk assessment model to assess SARS-CoV-2 transmission in enclosed food manufacturing facilities (28). Their findings demonstrated that workers are at elevated risk for SARS-CoV-2 infection through close contact (large droplets and small aerosol particles) compared with fomite transmission (cold-chain food and food

TABLE 3. COVID-19 outbreaks associated with cold-chain food and packaging.

Report	Date	Region	No. of cases	Source of infection	Reference
	2020.06	Beijing	402	Imported frozen food	
	2020.07	Dalian	135	Outer packaging of imported frozen food	
	2020.09	Qingdao	14	Outer packaging of imported frozen cod	
Literature	2020.11	Tianjin	2	Cold-chain food environment	(6)
	2020.11	Tianjin	10	Cold-chain food	
	2020.12	Dalian	83	Cold-chain food	
	2021.05	Liaoning, Anhui	43	Imported frozen cod	
	2020.12.01	Qingdao	1	–	(22)
	2022.05.27	Qingdao	1	–	(23)
	2022.05.16	Tianjin	28	Imported cold-chain food (Omicron BA.2)	(24)
Media	2022.06.30	Qingdao	13	Outer packaging of cold-chain goods (Omicron BA.2)	(25)
	2022.06.21	Jilin	10	–	(26)
	2022.08.07	Jilin	1	–	(27)

Abbreviation: COVID-19=coronavirus disease 2019.

packaging) and aerosol exposure alone.

SARS-CoV-2 contamination in food and packaging primarily occurs via respiratory particle spray (droplets and aerosols) generated by workers, especially those who are latently infected or asymptomatic (29). Droplet transmission is characterized by close contact (less than 2 meters) exposure to large, virus-containing particles (greater than 100 µm diameter) that originate from coughing or sneezing and rapidly fall onto food or packaging surfaces (29). Aerosols consist of small particles that can be contacted at close distances and up to 9 meters away. Workers release aerosol particles when breathing, talking, singing, or laughing. Epidemiological studies have demonstrated viral accumulation and persistence in enclosed indoor spaces, with high levels of small aerosol particles in the

air leading to food and packaging contamination (30).

Moreover, workers involved in cold-chain food processing, packaging, and transportation may spread the virus via contaminated hands, which in turn could result in the contamination of food and packaging (31). Table 4 reveals that implementing measures such as increasing physical distancing among cold-chain practitioners, wearing N95 masks, enhancing air exchange rates, and handwashing can significantly reduce the risk of SARS-CoV-2 infection.

Additionally, Huang et al. successfully isolated the first monkeypox virus (MPXV) strain in China (32). A qualitative risk assessment of monkeypox transmission suggests that the virus can be spread through food (bushmeat), even though it is not classified as foodborne. However, heat treatment effectively

TABLE 4. Risk assessment models for the transmission of the SARS-CoV-2.

Virus	Transmission route	Simulation of situation	Risk	Reference	
SARS-CoV-2; MERS; Influenza A virus	Droplet and aerosol		0.96		
	Fomite	Enclosed food manufacturing facility (Exposure time 8 h)	0.26	(28)	
	Aerosol exposure alone		0.05		
SARS-CoV-2; MERS; Influenza A virus	Aerosol, droplet, and fomite	Enclosed food manufacturing facility (PD)	1 m	0.98	(28)
			2 m	0.15	
			3 m	0.09	
			Cloth	0.47	
SARS-CoV-2; MERS; Influenza A virus	Aerosol, droplet, and fomite	Enclosed food manufacturing facility (PD: 1 m)	Surgical	0.35	(28)
			Double mask	0.12	
			N95	0.01	
Recombinant SARS CoV-2 variants; Murine coronavirus strain	Aerosol	Indoor environment	100 cm ²	2.3×10 ⁻⁵	(34)
			400 cm ²	5.1×10 ⁻⁶	
SARS-CoV-2 particles	Aerosol	Indoor environment	0 ACH	0.13	(35)
			1 ACH	0.065	
SARS-CoV-2; MERS; Influenza A virus	Aerosol, droplet, and fomite	Enclosed food manufacturing facility (PD: 1 m; Double mask)	2 ACH	0.04	(28)
			6 ACH	0.02	
SARS-CoV-2	Fomite	Frozen food packaging facility	The absence of interventions, exposure to packaging under cold-chain conditions	<2.0×10 ⁻³	(36)
			No SARS-CoV-2 immunity from vaccination or prior infection; Without standard infection control interventions	1.5×10 ⁻³	
			Standard infection control interventions	1.2×10 ⁻³	
			Handwashing	3.9×10 ⁻⁵	
			Handwashing with mask-wearing	8.5×10 ⁻⁶	
			A fully vaccinated workforce, as well as handwashing and mask-wearing	1.4×10 ⁻⁶ –8.8×10 ⁻⁶	
			The susceptible worker with two doses of mRNA vaccine and hourly handwashing and mask-wearing	5.2×10 ⁻⁷	

Abbreviation: SARS-CoV-2=severe acute respiratory syndrome coronavirus 2; MERS=Middle East Respiratory Virus; ACH=air changes per hour; PD=physical distancing.

inactivates the monkeypox virus in food (33).

CONCLUSIONS

In China, COVID-19 has been reclassified as a Class B infectious disease and is managed accordingly, leading to significant adjustments in epidemic prevention and control strategies. While the relevance of SARS-CoV-2 transmission through frozen food and packaging has diminished in importance, this study remains highly pertinent for preventing other infectious diseases similar to COVID-19. In particular, it offers valuable insight into the transmission of emerging infectious diseases through cold-chain food and packaging.

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