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SARS-CoV-2 transmission via maritime cold chains: A statistical analysis of nucleic acid detection results of cold chain food imported from Fuzhou ports

Yuxiang Chen [a,1], Shuai Zhao [b,1], Yiyuan Xu [a], Mingzhi Cai [c], Guanbin Zhang [a,d,e,*]

[a] Fujian CapitalBio Medical Laboratory, Fuzhou, 350108, China
[b] Department of Breast Surgery, Fujian Provincial Maternity and Children's Hospital, Affiliated Hospital of Fujian Medical University, Fuzhou, 350001, China
[c] Zhangzhou Affiliated Hospital of Fujian Medical University, Zhangzhou, 363000, China
[d] National Engineering Research Center for Beijing Biochip Technology, Beijing, 102206, China
[e] Department of Laboratory Medicine, Fujian Medical University, Fuzhou, 350122, China

A R T I C L E I N F O

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A B S T R A C T

Numerous epidemic outbreaks related to cold chains have occurred since the coronavirus disease 2019 (COVID-19) outbreak, suggesting the potential danger of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transmission through cold chain foods (CCFs). By analyzing SARS-CoV-2 RNA contamination of CCFs imported from Fuzhou ports, this study evaluated the contamination and transmission of SARS-CoV-2 RNA via maritime cold chains, with the aim of provide suggestions for CCFs supervision and public health management. The statistical analysis included 131,385 samples. The majority of the CCFs imported into Fuzhou ports was aquatic raw food that originated in Southeast Asia (57.08 %), South America (19.87 %), and South Asia (11.22 %). South Asia had the highest positivity rate of 0.37 %, followed by Southeast Asia (0.21 %) and South America (0.08 %). The positivity rate showed that the outer packaging of CCFs was the most easily contaminated, accounting for 81.33 % of all positive samples. This suggested that CCFs storage and loading processes were the weak links vulnerable to SARS-CoV-2 contamination. The positivity rates in outer packaging, inner packaging, and content of raw food were 0.48 %, 0.08 %, and 0.05 %, respectively, which were obviously higher than those of processed and refined food. This indicated that increasing the mechanization of factories and implementing sensible worker management practices may decrease viral contamination. The monthly positivity rates varied widely from 0 % (March 2021) to 0.40 % (January 2021), with an average of 0.19 %. The positivity rates in outer packaging, inner packaging and content of crustaceans from Southeast Asia were 2.47 %, 0.41 %, and 0.69 %, which were approximately 5–14 times higher than those of fish and cephalopods. Meanwhile, the monthly detection number show that SARS-CoV-2 epidemic prevention strategies affected the trade of imported CCFs.

* Corresponding author. National Engineering Research Center for Beijing Biochip Technology, Beijing, 102206, China.
E-mail address: gbzhang@capitalbio.com (G. Zhang).

[1] The authors contribute equally.

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1. Introduction

Since the outbreak of the coronavirus disease 2019 (COVID-19) pandemic, the transmission route of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has received considerable attention. Airborne transmission, such as respiratory droplets and aerosols, was believed to be the main route of SARS-CoV-2 infection until several outbreaks were epidemiologically traced to contaminated cold chain foods (CCFs) [1–4]. This indicates that SARS-CoV-2 contamination in CCFs may represent a public health threat and requires further investigation.

Several studies have tested the viability and stability of SARS-CoV-2 in various environments. Van et al. reported that SARS-CoV-2 can survive for 3–4 days on plastic and stainless-steel surfaces and for 24 h on paper surfaces at room temperature [5]. Another study showed that SARS-CoV-2 RNA became undetectable on glass and banknotes by the fourth day, on stainless steel and plastic by the seventh day but remained detectable on surgical masks after seven days [6]. Coronaviruses usually survive longer in cold temperatures [7,8].

Laboratory studies have confirmed that SARS-CoV-2 can survive on the surface of chilled foods for a long time. The coronavirus survives for more than 24 h on chicken, salmon, shrimp, and spinach in 4 °C temperature [9]. Dai found that the virus on salmon remained stable for more than a week at 4 °C [10]. Feng et al. contaminated pork, beef, and salmon meat with SARS-CoV-2 close to the actual concentration in respiratory secretions, and found that the coronavirus on the meat surface could remain infectious for 3 days at 4 °C and for 7 days at −20 °C [11]. Jia et al. concluded that the survival time of SARS-CoV-2 could be maintained for up to 21 days in cooked food and meat with high protein, fat, and moisture under the refrigeration condition of 4 °C [12].

From June 2020 to December 2020, there were seven outbreaks of CCFs in China, and the first cases involved CCFs logistic laborers who had direct or indirect contact with imported CCFs [1,13]. In June 2020, an epidemiological investigation revealed that the infectious source in Beijing Xinfadi market was likely the imported frozen salmon [4]. The low ambient temperature and poor ventilation conditions in Xinfadi were thought to contribute to the spread of COVID-19 [14]. In the Dalian epidemic (July 2020), the dockworker was presumed to be infected by contact with CCFs. This hypothesis was substantiated by high-throughput sequencing of the virus present in frozen cod packaging, which exhibited a significant degree of homology with a virus isolated from an infected patient [3]. During an outbreak in Qingdao in September 2020, infectious SARS-CoV-2 was isolated from outer packaging for the first time worldwide [2]. The November 2020 pandemic in Tianjin was linked to contaminated frozen pork from Germany [15]. Collectively, these cases indicate that SARS-CoV-2 RNA can be easily detected on cold chain packaging, suggesting that the transportation and storage conditions of cold chain products provide a favorable environment for viral persistence, and infections among cold chain workers suggested the potential for cold chain packaging-mediated transmission [1].

In the food supply chain, cold chain logistics provide the low temperatures required for harvesting, processing, storage, delivery, and retail of food, thereby reducing nutritional losses and maintaining food safety [16]. With the acceleration of globalization and urbanization, CCFs consumption is increased. CCFs have become an important component of the food supply chain [17]. In the context of transnational food trade, the controlled and low-temperature environment facilitated by cold chain logistics creates favorable conditions for viral survival, thereby making it possible for enabling the transmission of SARS-CoV-2 from ports to various countries [18].

Zhang et al. assessed the risk of SARS-CoV-2 transmission using a complex network analysis based on CCFs consumption and trade matrix data. In the CCFs trade network constructed in this study, there were 198 nodes and 11,924 lines, with China among the top 20

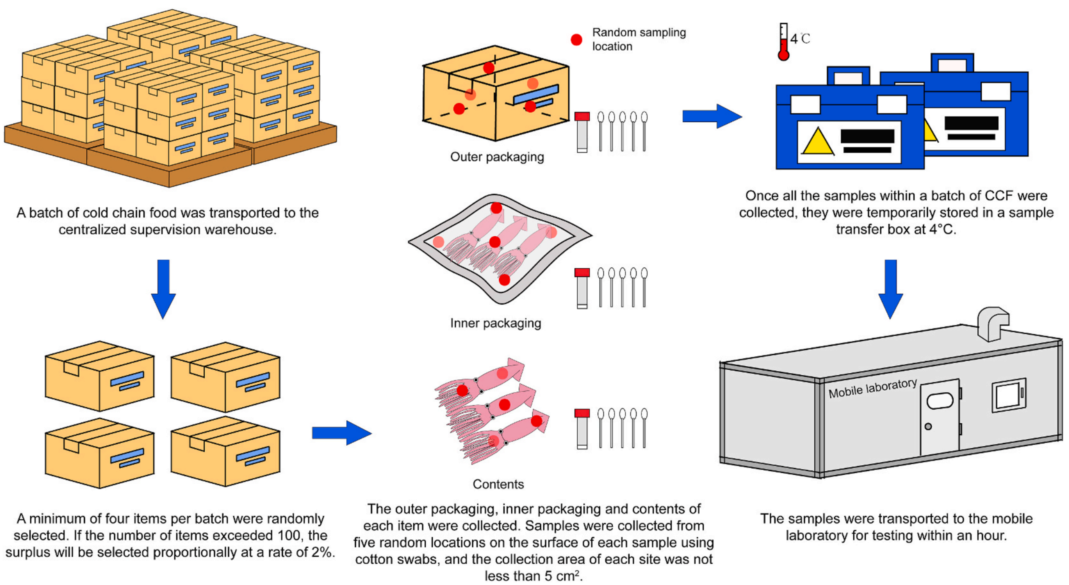

Fig. 1. Sample collection methods for CCFs.

countries. They concluded that China has a high risk of external inputs and comprehensive transmission [19]. Between July 2020 and July 2021, the Chinese Center for Disease Control and Prevention conducted over than 55.83 million CCFs-related SARS-CoV-2 RNA samples nationwide. The findings indicated that the outer packaging of CCFs was the most susceptible to detection, with aquatic food exhibiting the highest positivity rate [20]. The detection of SARS-CoV-2 RNA in CCFs and their packaging highlighted the potential for transmission of COVID-19 through contaminated CCFs, indicating a systemic risk [21].

Many studies have shown that CCFs-mediated transmission carry the risk of epidemic transmission. However, current studies have only researched individual epidemic cases or laboratory samples and large-scale and long-term detection and analysis of CCFs are lacking [1,15,22]. Therefore, macroscopic understanding of the mediated transmission of SARS-CoV-2 through CCFs is limited.

Furthermore, traditional CCFs management does not include procedures for SARS-CoV-2 related precautions and environmental controls [23]. In response, the Chinese government issued a series of guidelines to prevent COVID-19 spread through CCFs-mediated transmission, and these had been widely applied in epidemic prevention and control [21]. The centralized supervision warehouse for imported cold chain food in Tingjiang Town, Mawei District, Fuzhou City was established in December 2020, with the mandate to detect and disinfected SARS-CoV-2 from imported CCFs entering through the Fuzhou port or circulating within the local market.

The current study analyzed the factors that may cause SARS-CoV-2 RNA contamination of CCFs imported into China through statistical analysis of the RNA detection results of samples collected from imported CCFs in a Fuzhou supervised warehouse by Reverse transcription quantitative polymerase chain reaction (RT-qPCR).

2. Methods

2.1. Sample collection

Samples were collected according to the descriptions in Technical Specification for Environmental Monitoring of SARS-CoV-2 in Farmers' (Fair) Markets and Technical Guide for SARS-CoV-2 prevention and Control in Cold Chain Food Production and Operation [24,25]. Fig. 1 shows how CCFs were sampled. After CCFs were transferred to the centralized supervision warehouse, a minimum of four items per batch were randomly selected (if the number of items exceeded 100, the surplus will be selected proportionally at a rate of 2 %) for testing for SARS-CoV-2 RNA in the outer packaging, inner packaging, and contents. Therefore, at least 12 samples were tested for each CCFs batch. Between December 2020 and May 2022, 5,352 batches of CCFs were sampled, resulting in a total of 131, 585 samples.

During sampling, a disposable swab (Changgeng, Fuzhou, China) was fully immersed in the sample preservation solution in an inactivated virus sampling tube (Changgeng, Fuzhou, China). The swab was then used to evenly wipe an area of at least 5 cm^2 at a random location on the sample surface while being rotated to ensure the full collection of material. This was repeated five times for each sample, and all swabs used in the five collections were subsequently combined into a single inactivated-type virus sampling tube.

Once all the samples within a batch of CCFs were collected, they were temporarily stored at 4 °C and transported to the laboratory for testing within an hour.

2.2. RNA extraction

Before extraction, the virus sampling tubes were vigorously agitated using a vortex oscillator to ensure the complete release of SARS-CoV-2 particles that might have adhered to the swab into the sample-holding solution. A total of 300 μl of each sample was added to the 96-well deep well plate that had been pre-loaded with the nucleic acid extraction kit (Sansure Biotech, Hunan, China), and then the deep well plate was placed in a supporting automatic nucleic acid extraction instrument Natch 96A (Sansure Biotech, Hunan, China) for automatic extraction. In the end, approximately 80 μl RNA was obtained for RT-qPCR detection.

2.3. RT-qPCR detection

RT-qPCR was performed for virus detection. The extracted RNA was amplified using the 2019-nCoV Nucleic Acid Amplification Kit (Sansure Biotech, Hunan, China) and ran on an Applied Biosystems 7500 Real-Time PCR system (Thermo Fisher Scientific, CA, USA) to detect the presence of the *N* and *ORF1ab* target genes in the sample. No replicates were used for each sample. Each experiment (96-well plate) contained three negative quality controls (kit attached quality control, nuclease-free water, nuclease-free water exposed to the environment) and two positive quality controls (kit attached quality control, 2019-nCoV-RNA liquid internal quality control (Guangzhou BDS Biological Technology, Guangzhou, China). The amplification procedure was 50 °C 30 min (1 cycle), 95 °C 1 min (1 cycle), 95 °C 15 s, 60 °C 30 s (45 cycles), 25 °C 10 s (1 cycle).

Ct value were calculated for target genes with auto-baseline and auto-threshold. Samples with Ct values > 40 for both genes or those with no amplification curves were classified as negative. If the Ct value of any gene in *N* or *ORF1ab* was ≤ 40 or if the Ct value of both genes was ≤ 40 and the result remained consistent after re-testing (re-extraction and re-amplification) twice, the sample was considered positive for SARS-CoV-2 RNA.

2.4. Statistical analyses

For a comprehensive analysis, we divided the data into groups according to their origin, sample type, food processing degree, and food type, and calculated the detection number and corresponding positive rate for each group. Overall, the monthly changes in the detection number and positivity rates were analyzed in three major import regions: Southeast Asia, South Asia, and South America. The factors influencing SARS-CoV-2 RNA contamination in imported CCFs were simultaneously assessed by comparing the detection in various groups. The Pearson correlation coefficient was used to establish the correlation between the number of enterprises that triggered circuit breaks and the monthly detection number in each region. All statistical analyses were performed using IBM SPSS Statistics 26.0 (IBM, New York, USA). Statistical significance was set at a P value of <0.05.

3. Results

3.1. Classification of imported CCFs

From December 2020 to May 2022, the imported CCFs tested in the Fuzhou Imported Cold Chain Food Centralized Supervision Warehouse were from 46 countries and regions worldwide. The top eight countries of origin of the imported CCFs (Fig. 2A) were Indonesia (31.01 %), Malaysia (13.97 %), Ecuador (10.78 %), Vietnam (8.43 %), India (8.16 %), Peru (3.36 %), Thailand (3.34 %) and Pakistan (2.07 %). With respect to the geographical division (Fig. 2B), the imported CCFs mainly came from Southeast Asia (57.08 %), South America (19.87 %), and South Asia (11.22 %).

With respect to the category (Fig. 2C), fish products such as frozen hairtail were the most common CCFs (61.32 %), followed by cephalopod products such as frozen squid (18.59 %), crustacean products such as frozen shrimp (14.32 %), shellfish products such as frozen snails (1.89 %), soy products such as fried tofu skin rolls (1.81 %), fruit products such as coconut (1.69 %), and other types of products (0.37 %). More than 95 % of these food products were frozen products, and only some of the bean products and fruit products were chilled products.

We classified foods into three categories based on the degree of processing: raw, rough, and refined (Fig. 2D). Raw food (RF) referred to food that had not been processed, was frozen directly, or remained intact (such as frozen whole fish). Rough processed food (RPF) was defined as food that has only undergone minor processing (e.g., cutting) that did not significantly change the food properties (e.g., frozen fish fillets). Refined food (RFF) referred to food that underwent visible changes in its properties as a result of complex processing (e.g., frozen surimi products). Most of the imported CCFs were RF (79.92 %), and only a few are RPF (17.26 %) and RFF (2.86 %).

3.2. SARS-CoV-2 RNA detection results of imported CCFs

Among the 131,385 imported CCF samples were collected and tested, 241 samples tested positive for SARS-CoV-2 RNA, yielding an overall positive rate of 0.18 % (Table 1). The positivity rate of the outer packaging reached 0.44 %, markedly higher than that of the inner packaging (0.06 %) and content (0.04 %), accounting for 81.33 % of the total positive detections. This indicated that the outer

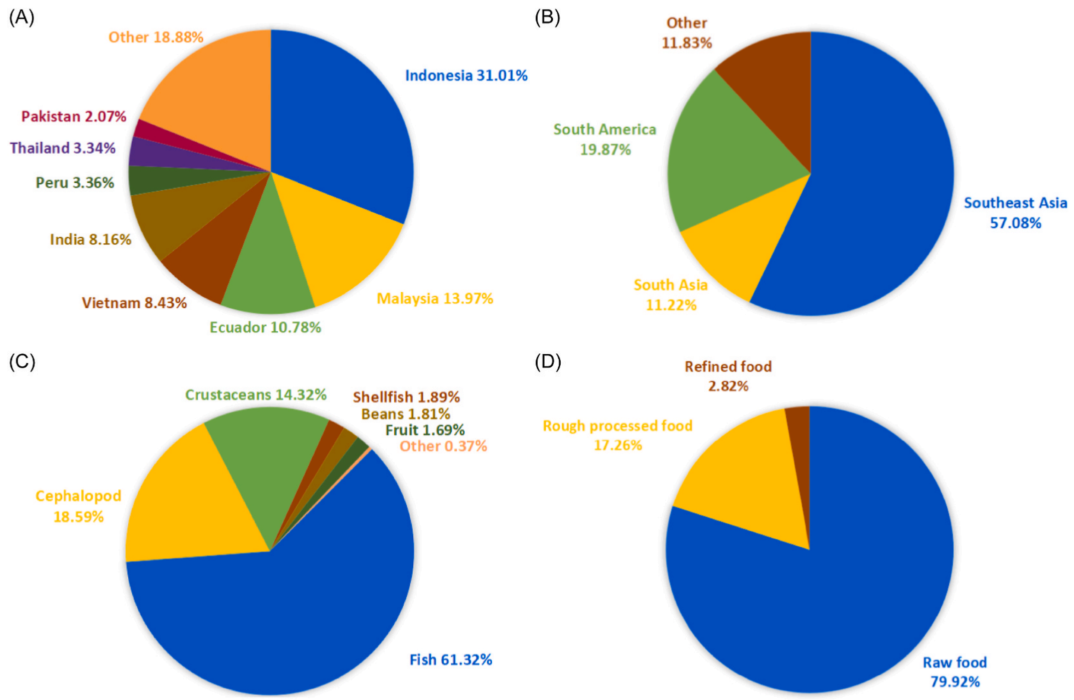

Fig. 2. Statistics on imported CCFs according to various classifications. (A) By country of origin. (B) By geographical region. (C) By food type. (D) By food processing degree.

Table 1
An overview of the imported CCFs SARS-Cov-2 RNA detection results from December 2020 to May 2022 at the Fuzhou Imported Cold Chain Food Centralized Supervision Warehouse.

Sample type	Number of samples	Number of positive samples	Positivity rate	Proportion of positive samples
Outer packaging	44,120	196	0.44 %	81.33 %
Internal packaging	43,626	28	0.06 %	11.62 %
Content	43,639	17	0.04 %	7.05 %
Total	131,385	241	0.18 %	100 %

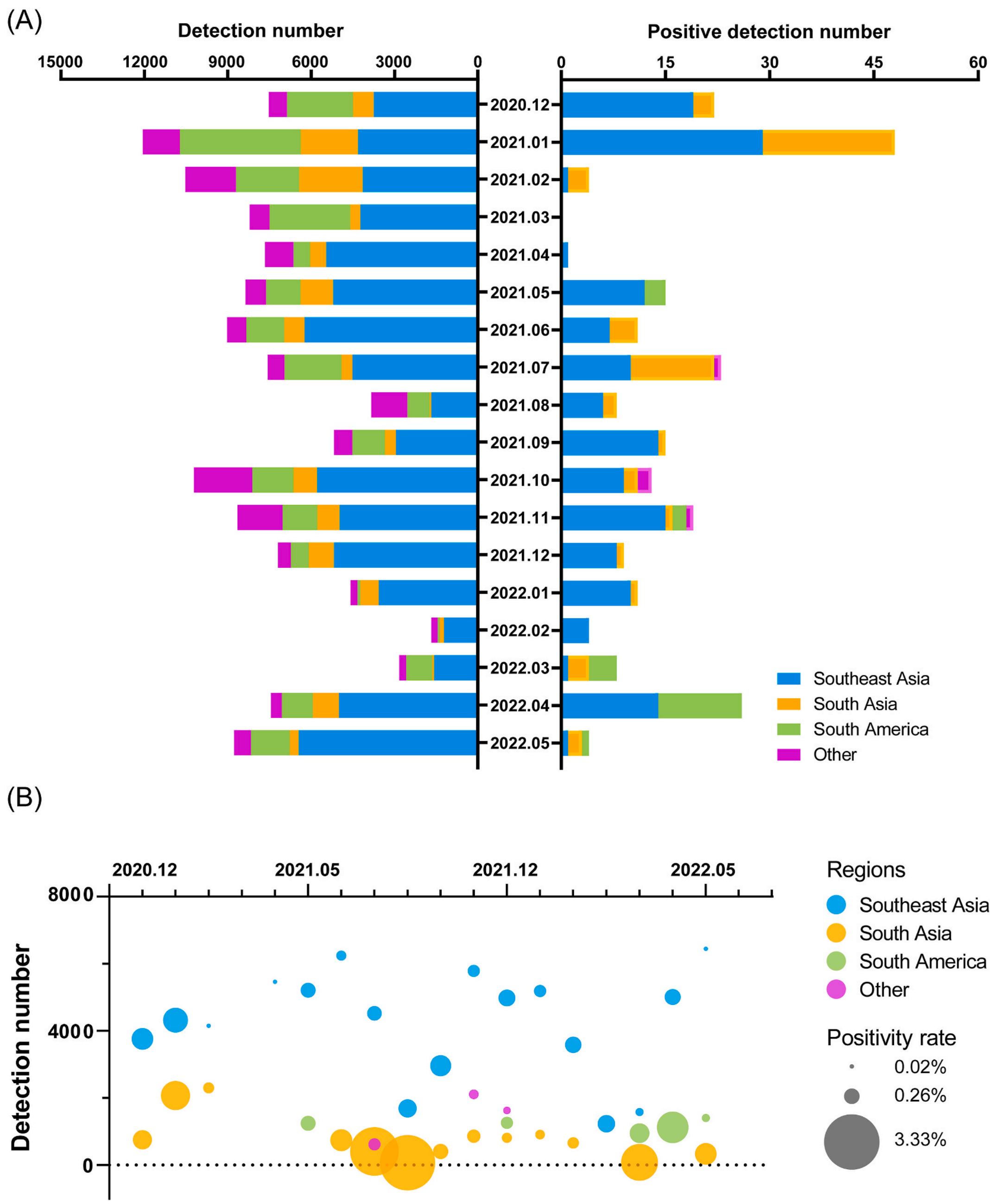

Fig. 3. Monthly results of SARS-CoV-2 RNA detection of imported CCFs imported from various geographical regions. (A) Monthly total detection number and monthly positive detection number for each region. (B) Monthly positive rate of imported CCFs from each region.

packaging was more frequently and more easily contaminated by SARS-CoV-2 RNA compared to the inner packaging and content. Given that the outer packaging is the most exterior layer of packaging, the highest contamination rate suggested that CCFs may be more susceptible to viral RNA contamination during storage and transportation than food production and packaging.

3.3. Total number of SARS-CoV-2 RNA detections of imported CCFs from different regions

The origin of CCFs varied widely, but they were mainly from South Asia, Southeast Asia, and South America, and less frequently from Africa, North America, Oceania, and other regions in Asia. Fig. 3A shows the monthly SARS-CoV-2 RNA test results for CCFs from South Asia, Southeast Asia, South America, and other regions. From December 2020 to May 2022, an average of 7,300 CCFs were detected monthly in the warehouse. Nevertheless, because of the influence of policies, local epidemic situation of origin, market demand and other factors, the monthly detection number fluctuated.

Analyzing the composition of the monthly detection, the CCFs from Southeast Asia constituted the primary focus of detection in the supervisory warehouse, accounting for 35.74 %–76.37 % of the total monthly inspection volume (Table 2). The monthly detection number of CCFs from South Asia ranged from 7.87 % to 22.00 %, peaking at 2316 in February 2021 before taking a sharp decrease to approximately 640. The monthly detection number for South American CCFs ranged from 2.35 % to 36.00 %. A significant number of detections were conducted from December 2020 to March 2021, peaking at 4,344 tests in January 2021. The number of monthly detections had decreased to approximately 1,000 since April 2021.

3.4. Positive SARS-CoV-2 RNA detections in imported CCFs from various regions

CCFs from Southeast Asia had the most positive samples (161 cases), followed by those from South Asia (54 cases), and South America (22 cases). In total, 95.34 % of all positive samples came from these three regions. According to the monthly statistics (Fig. 3A and Table 3), 48 positive cases were detected in January 2021, the month with the highest positive cases. Particularly, the volume of SARS-CoV-2 RNA tests was high from February to April 2021 and May 2022, despite maintaining low positive detection rates, especially in the absence of positive samples in March 2021. The positive rates exhibited a marginal decrease of no more than 0.04 % during the periods from February 2021 to April 2021 and May 2022.

The average monthly positivity rate was 0.19 %. The positivity rate was the highest (0.40 %) in January 2021 and the lowest (0 %) in March 2021.The positivity rates of different regions were analyzed separately, and South Asia had the highest positive rate (0.37 %), followed by Southeast Asia (0.21 %) and South America (0.08 %).

The monthly positivity rates for each region are depicted in Fig. 3B. Southeast Asia and South Asia had positive detections in most months. In contrast, South Asia experienced a stronger and more concentrated positive rate change trend, with higher positivity rates concentrated during the three periods of December 2020 to February 2021, July 2021 to August 2021, and March 2022 to May 2022. In July and August 2021, South Asia had a very high positive rate at 9–10 times higher than that in Southeast Asia during the same period. In contrast, the positive detections in South America mainly occurred after March 2022, and only a small number were detected sporadically in 2021. The results showed that between December 2020 and May 2022, South America's COVID-19 prevention and control in CCFs were was better than those of Asia.

Table 2
Monthly detection number of each region and its proportion in the total detection number.

Month	Southeast Asia		South Asia		South America		Other areas	
	Detection number	Proportion	Detection number	Proportion	Detection number	Proportion	Detection number	Proportion
2020.12	3,753	49.83 %	745	9.89 %	2,368	31.44 %	666	8.84 %
2021.01	4,311	35.74 %	2,064	17.11 %	4,344	36.01 %	1,344	11.14 %
2021.02	4,119	39.13 %	2,316	22.00 %	2,268	21.54 %	1,824	17.33 %
2021.03	4,134	50.33 %	456	5.55 %	2,904	35.35 %	720	8.77 %
2021.04	5,316	69.33 %	720	9.39 %	600	7.82 %	1,032	13.46 %
2021.05	5,064	60.55 %	1,320	15.78 %	1,236	14.78 %	744	8.90 %
2021.06	6,204	68.75 %	768	8.51 %	1,359	15.06 %	693	7.68 %
2021.07	4,443	58.70 %	468	6.18 %	2,049	27.07 %	609	8.05 %
2021.08	1,680	43.72 %	60	1.56 %	795	20.69 %	1,308	34.04 %
2021.09	2,941	56.75 %	408	7.87 %	1,167	22.52 %	666	12.85 %
2021.10	5,592	54.73 %	1,044	10.22 %	1,482	14.50 %	2,100	20.55 %
2021.11	4,892	56.55 %	888	10.26 %	1,251	14.46 %	1,620	18.73 %
2021.12	5,041	70.04 %	1,044	14.51 %	645	8.96 %	467	6.49 %
2022.01	3,504	76.37 %	724	15.78 %	108	2.35 %	252	5.49 %
2022.02	1,224	72.86 %	144	8.57 %	72	4.29 %	240	14.29 %
2022.03	1,440	50.74 %	204	7.19 %	942	33.19 %	252	8.88 %
2022.04	4,968	66.67 %	972	13.04 %	1,116	14.98 %	396	5.31 %
2022.05	6,372	72.62 %	396	4.51 %	1,395	15.90 %	612	6.97 %

Table 3
Overall detection conditions on a monthly basis and the related positivity rate.

Month	Detection number	Positive samples	Positivity rate
2020.12	7,532	22	0.29 %
2021.01	12,063	48	0.40 %
2021.02	10,527	4	0.04 %
2021.03	8,214	0	0.00 %
2021.04	7,668	1	0.01 %
2021.05	8,364	15	0.18 %
2021.06	9,024	11	0.12 %
2021.07	7,569	23	0.30 %
2021.08	3,843	8	0.21 %
2021.09	5,182	15	0.29 %
2021.10	10,218	13	0.13 %
2021.11	8,651	19	0.22 %
2021.12	7,197	9	0.13 %
2022.01	4,588	11	0.24 %
2022.02	1,680	4	0.24 %
2022.03	2,838	8	0.28 %
2022.04	7,452	26	0.35 %
2022.05	8,775	4	0.05 %
Total	131,385	241	0.19 %

3.5. SARS-CoV-2 RNA contamination analysis of various types of imported CCFs

To positivity rate of samples from different regions, classified based on the degree of processing and food type, are presented in Tables 4 and 5. As shown in Table 4, the CCFs imported from Fuzhou port were mainly RF, accounting for approximately 80 %. RFF had the lowest imports and were all from Southeast Asia. A comparison of the positivity rates of RF, RPF, and RFF showed that the contamination of food gradually declined with increasing degree of processing. All the RFF tested negative. In the case of RPF and RF, the positivity rates of their outer packaging, inner packaging and contents gradually decreased, suggesting that CCFs were more vulnerable to viral contamination in the logistics links. Notably, the positivity rates of outer packaging and inner packaging of RF from South Asia (0.84 % and 0.28 %, respectively) were significantly higher than those of outer packaging and inner packaging of RF from other regions.

The positivity rates of samples from different regions, categorized by sample and food types are shown in Table 5. All positive foods were aquatic products and were mainly crustaceans, fish, and cephalopods and rarely shellfish. Crustaceans exhibited the highest positivity rate. The total positivity rate of the outer packaging of crustaceans were 0.71 %, which was 1.58 and 1.68 times higher than that of fish and cephalopods, respectively. The positivity rate of contents in crustaceans was 0.08 %, higher than that in fish (0.04 %) and cephalopods (0.01 %). The positivity rates of outer packaging, inner packaging, and contents of crustacean from Southeast Asia were elevated (2.47 %, 0.41 %, and 0.69 %, respectively), representing approximately 5–14 times of those of fish and cephalopods. These data suggested that harvesting, processing, storage, and delivery of crustacean CCFs products in Southeast Asia have increased contamination events.

3.6. Comprehensive analysis of positive SARS-CoV-2 RNA detections of imported CCFs from various countries

The heat map in Fig. 4 illustrates the CCFs items with positive samples, which have been thoroughly analyzed based on factors such as country of origin, sample types, levels of food processing, food types, and positivity rates.

The significantly high positivity rate of raw crustacean food from Indonesia had a considerable impact on the total positivity rate because Indonesia was the primary supplier of CCFs in Southeast Asia. Similar to Indonesia, positive testing of raw crustacean foods from India was prevalent. However, in contrast to Indonesia, positive detections were primarily found on the outer packaging, rather than in the contents of CCFs from India. In addition, the outer packaging of fish and cephalopods from Thailand, Vietnam, and Pakistan also displayed a high positivity rate, indicating potential widespread infection among local logisticians.

4. Discussion

Fuzhou is the largest distribution center of imported aquatic products in Southeast Asia. The import of cold chain aquatic products from Fuzhou ports accounts for 30%–40 % of the total cold chain aquatic imports in China. For the duration of this study, imported CCFs were required to pass SARS-CoV-2 RNA detection at Fuzhou Imported Cold Chain Food Centralized Supervision Warehouse before entering the market. Therefore, through the statistical analysis of SARS-CoV-2 RNA detection results of imported CCFs detected in this institution, we can describe the SARS-CoV-2 RNA contamination status of CCFs imported from Fuzhou ports.

In this study, SARS-CoV-2 RNA is detected across geographical regions, diverse cold chain products, and within layers of packaging. In terms of origin, the positive cases were predominantly detected in CCFs originating from Southeast Asia and South Asia. With regards to processing level, the positive detection was concentrated in raw food items. As for the biological categories, crustaceans, fish and cephalopods constituted a majority of positive detection CCFs. Finally, concerning packaging layers, the outer packaging had a

Table 4
Number and positivity rate of CCFs by degree of processing.

Sample type	Processing stage	Southeast Asia		South Asia		South America		Other regions		Total	
		Detection number	Positivity rate	Detection number	Positivity rate	Detection number	Positivity rate	Detection number	Positivity rate	Detection number	Positivity rate
Outer packaging	RF	19,936	0.55 %	4,693	0.83 %	6,997	0.29 %	3,665	0.08 %	35,291	0.48 %
	RPF	4,009	0.55 %	260	0.00 %	1,790	0.11 %	1,530	0.07 %	7,589	0.33 %
	RFF	1,240	0.00 %	0	/	0	/	0	/	1,240	0.00 %
	Total	**25,185**	**0.52 %**	**4,953**	**0.79 %**	**7,315**	**0.30 %**	**5,195**	**0.08 %**	**44,120**	**0.44 %**
Inner packaging	RF	19,695	0.07 %	4,642	0.28 %	6,868	0.00 %	3,647	0.00 %	34,852	0.08 %
	RPF	3,972	0.03 %	252	0.00 %	1,789	0.00 %	1,528	0.00 %	7,541	0.01 %
	RFF	1,233	0.00 %	0	/	0	/	0	/	1,233	0.00 %
	Total	**24,900**	**0.06 %**	**4,894**	**0.27 %**	**8,657**	**0.00 %**	**5,175**	**0.00 %**	**43,626**	**0.06 %**
Content	RF	19,707	0.07 %	4,642	0.04 %	6,868	0.00 %	3,647	0.00 %	34,864	0.05 %
	RPF	3,973	0.03 %	252	0.00 %	1,789	0.00 %	1,528	0.00 %	7,542	0.01 %
	RFF	1,233	0.00 %	0	/	0	/	0	/	1,233	0.00 %
	Total	**24,913**	**0.06 %**	**4,894**	**0.04 %**	**8,657**	**0.00 %**	**5,175**	**0.00 %**	**43,639**	**0.04 %**

RF: Raw food; RPF: Rough processed food; RRF: Refined food.

Table 5
Number and positivity rate of CCFs by food type.

Sample type	Food type	Southeast Asia		South Asia		South America		Other regions		Total	
		Detection number	Positivity rate	Detection number	Positivity rate	Detection number	Positivity rate	Detection number	Positivity rate	Detection number	Positivity rate
Outer packaging	Crustaceans	730	2.47 %	432	1.62 %	5,082	0.39 %	118	0.00 %	6,362	0.71 %
	Fish	17,652	0.47 %	3,521	0.77 %	1,942	0.00 %	3,955	0.08 %	27,070	0.42 %
	Cephalopod	5,069	0.59 %	736	0.68 %	1,759	0.11 %	592	0.00 %	8,156	0.45 %
	Shellfish	148	0.00 %	264	0.00 %	0	/	418	0.24 %	830	0.12 %
	Beans	791	0.00 %	0	/	0	/	0	/	791	0.00 %
	Fruit	748	0.00 %	0	/	0	/	0	/	748	0.00 %
	Other	47	0.00 %	0	/	4	0.00 %	112	0.00 %	163	0.00 %
Inner packaging	Crustaceans	729	0.41 %	424	0.24 %	4,957	0.00 %	118	0.00 %	6228	0.06 %
	Fish	17,402	0.05 %	3,473	0.29 %	1,937	0.00 %	3,937	0.00 %	26,749	0.07 %
	Cephalopod	5,046	0.06 %	733	0.27 %	1,759	0.00 %	592	0.00 %	8,130	0.06 %
	Shellfish	147	0.00 %	264	0.00 %	0	/	416	0.00 %	827	0.00 %
	Beans	791	0.00 %	0	/	0	/	0	/	791	0.00 %
	Fruit	738	0.00 %	0	/	0	/	0	/	738	0.00 %
	Other	47	0.00 %	0	/	4	0.00 %	112	0.00 %	163	0.00 %
Content	Crustaceans	729	0.69 %	424	0.00 %	4,957	0.00 %	118	0.00 %	6,228	0.08 %
	Fish	17,403	0.05 %	3,473	0.06 %	1,937	0.00 %	3,937	0.00 %	26,750	0.04 %
	Cephalopod	5,058	0.02 %	733	0.00 %	1,759	0.00 %	592	0.00 %	8,142	0.01 %
	Shellfish	147	0.00 %	264	0.00 %	0	/	416	0.00 %	827	0.00 %
	Beans	791	0.00 %	0	/	0	/	0	/	791	0.00 %
	Fruit	738	0.00 %	0	/	0	/	0	/	738	0.00 %
	Other	47	0.00 %	0	/	4	0.00 %	112	0.00 %	163	0.00 %
Total		**74,998**	**0.21 %**	**14,741**	**0.37 %**	**26,101**	**0.08 %**	**15,545**	**0.03 %**	**131,385**	**0.18 %**

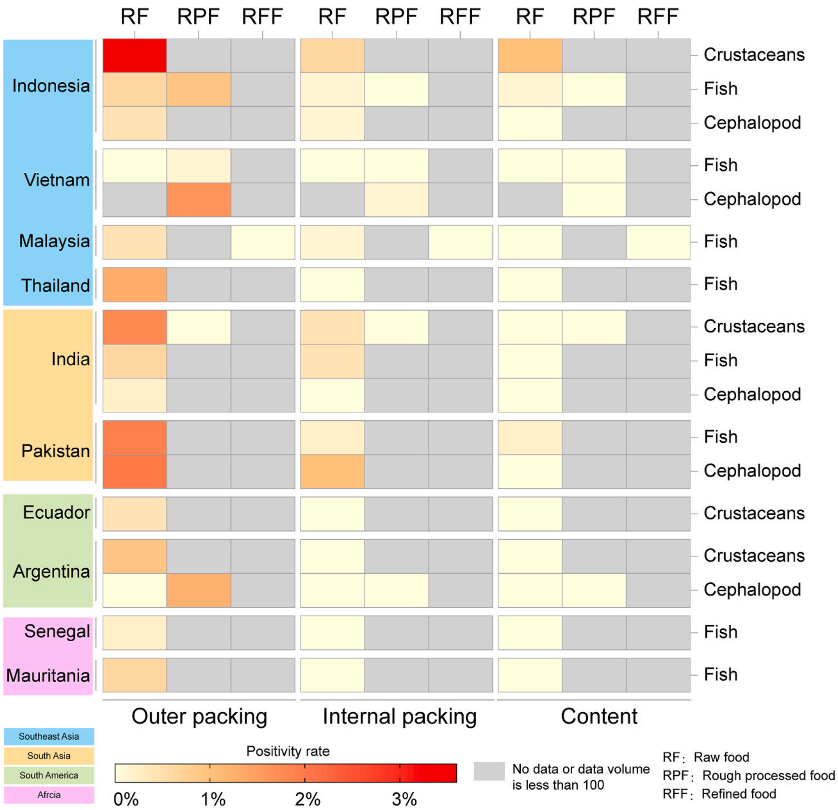

Fig. 4. The heatmap based on a comprehensive analysis of origin, sample types, food processing degrees, food types, and the positivity rate.

higher incidence of positive detections. These trends can effectively assist us in proposing targeted measures for epidemic prevention of CCFs in the future.

4.1. The weak links in CCFs storage and transportation require attention

As previously mentioned, our study revealed a higher positivity rate of the outer packaging of CCFs compared to that of the inner packaging and contents. In general, cross-border transport of CCFs typically includes packaging products into containers by overseas manufacturers, transporting them to the destination using cold chain vehicles, and then storing, distributing and retailing them [26]. During transportation, the CCFs container in a relatively closed environment, which makes contamination difficult. Based on China's stringent epidemic prevention policy, workers are required to have negative test results on daily COVID-19 nucleic acid testing before they are permitted to commence work. Meanwhile, personnel who require direct contact with CCFs products must wear adequate personal protective equipment (such as masks, gloves, and protective clothing) to ensure that safety measures are met. This made it unlikely that the CCFs would be contaminated by workers infected with COVID-19 once they arrived in China [25,27]. According to a study that utilized viral genome sequence alignment, the SARS-CoV-2 strains responsible for the six confirmed CCFs-related outbreaks in China were not linked to previous localized COVID-19 outbreaks. In contrast, certain strains exhibit high homology with those present in the regions of origin of the CCFs, which also supports this opinion [28]. Therefore, we hypothesized that the primary occurrence of SARS-CoV-2 RNA contamination in CCFs packaging was likely during their transfer to local warehouses or loading into containers by workers infected with COVID-19 in the exporting country. This implies that CCFs are more susceptible to SARS-CoV-2 RNA contamination during storage and transportation rather than processing, as well as a significant probability of facilitated cross-border transmission of SARS-CoV-2 RNA through the cold chain. Consequently, this potential vulnerability within the CCFs transportation process should be addressed in future studies.

The resolution of this issue necessitates concerted efforts from both exporting and importing nations. The most effective approach is to enhance the prevention and control measures for individuals engaged in cold chain transportation, while also elevating the level of mechanization in cold chain logistics. This will effectively minimize the likelihood of infected individuals serving as potential sources of contamination coming into contact with cold chain food. Furthermore, if conditions allow, countries engaged in the import and export of cold chain food should consider implementing disinfection treatments for CCFs, especially the outer packaging. With the development of disinfection technologies, cold plasma and electron beam irradiation have been demonstrated effective disinfection capabilities at low temperatures and normal atmospheric pressure conditions [29]. Simultaneously, they exhibited a certain level of penetration in terms of sterilization efficacy and possess the potential to disinfect internal packaging and contents of CCF without necessitating the opening of the outer packaging [30,31], making them a favorable choice for CCFs disinfection. The aforementioned characteristics may render them advantageous for the disinfection of CCFs during storage and transportation. If the government considers the cost of fully disinfecting cold chain food to be prohibitive, it should at minimum implement a robust sampling program to mitigate the spread of contaminated products across borders. In addition, we recommend that the import and export sides can achieve the mutual recognition of sampling inspection information and disinfection information for cold chain food, thereby reducing the need for repeated testing the efficiency of importing and exporting the same batch of products.

4.2. Regulations and laws of importing/exporting countries impact the CCFs industry

The CCFs industry is affected by the supervision and legislation of the nations where the CCFs are imported or exported. The CCFs market in Fuzhou can be forecast by analyzing the monthly detection number of a centralized supervision warehouse. When examining the factors that affect the monthly detection number, we considered three crucial factors.

Firstly, Chinese government's import-related policies affected the monthly detection number. According to Regulation No.103 in 2020 issued by the General Administration of Customs of the People's Republic of China (GACC), which was still in effect during the statistical period, the GACC would take circuit breaker measures against overseas export enterprises in which CCFs had been detected positive for SARS-CoV-2 RNA. The GACC will require customs nationwide to suspend the acceptance of import declaration by the enterprises for at least 1 week [32]. For example, in an April 2022 announcement, an Ecuadorian company was suspended 28 weeks due to the detection of SARS-CoV-2 RNA in eight outer packaging samples from seven batches of CCFs [33]. Un-avoidably, this affected the monthly detection number.

We compared the number of enterprises notified of circuit breakers on the GACC website (www.customs.gov.cn/) with the monthly detection numbers in Southeast Asia, South Asia, and South America. The results are shown in Fig. 5A–C. The correlation coefficients and *p*-values for Southeast Asia ($r = -0.100$, $p = 0.693$), South Asia ($r = -0.298$, $p = 0.229$), and South America ($r = -0.147$, $p = 0.561$) were calculated. It showed that the number of enterprises triggered circuit breaker and the monthly detection number presented a contrary trend, but the correlation between them was not significant. It may pertain to the implementation of circuit breaker mechanism by GACC. The list of enterprises which triggered circuit breakers was applied to all national customs, but some enterprises on the list did not export CCFs to Fuzhou, leading to discrepancies in the data. Meanwhile, different overseas exporters were affected differently by circuit breaker measures due to differences in the number of CCFs they exported to China as well as the length of the import restriction.

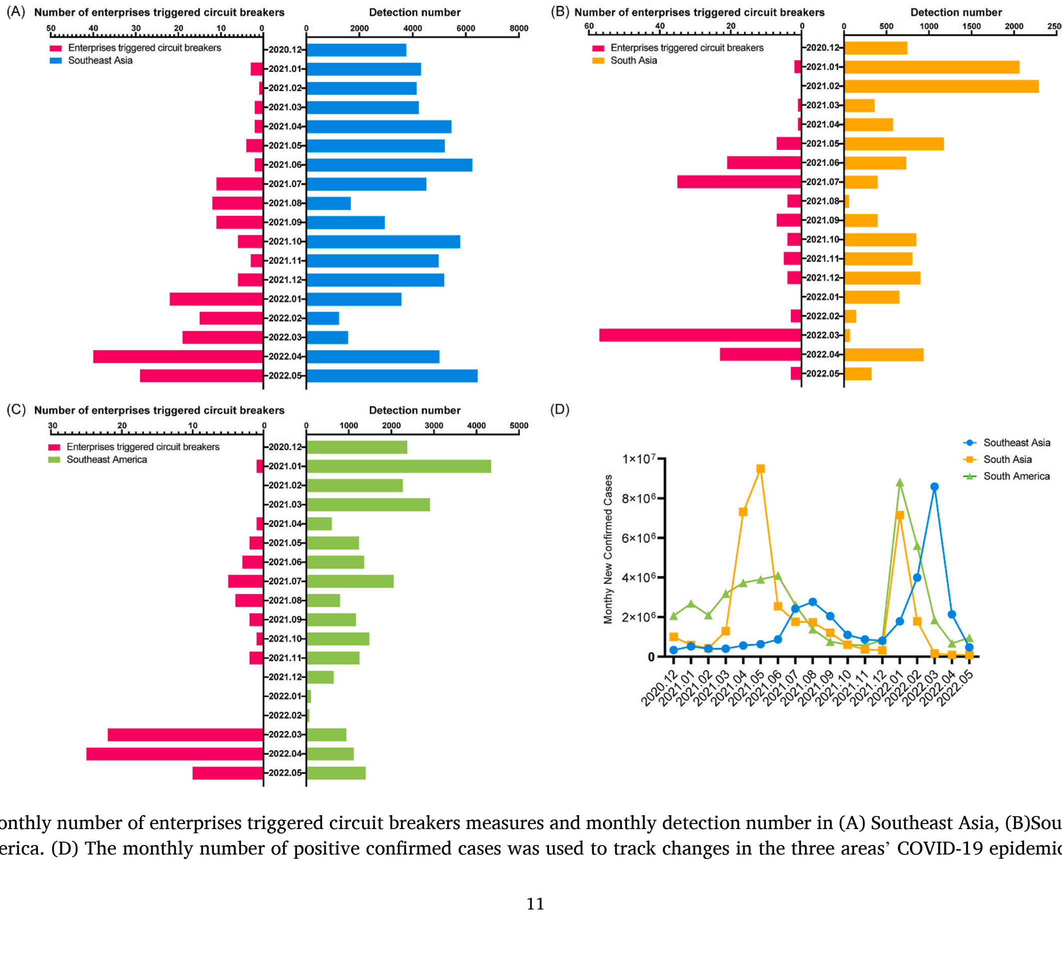

Fig. 5. Monthly number of enterprises triggered circuit breakers measures and monthly detection number in (A) Southeast Asia, (B)South Asia, (C) South America. (D) The monthly number of positive confirmed cases was used to track changes in the three areas' COVID-19 epidemic condition.

Second, the local SARS-CoV-2 pandemic situation and epidemic prevention policies of the country of origin had an impact on the detection number each month. CCFs are more susceptible to SARS-CoV-2 RNA contamination when the COVID-19 outbreak strikes the country of origin. This increases the probability that CCFs imported into China will trigger circuit breaker measures. The number of newly confirmed positive cases per month provided by the World Health Organization (covid 19.who.int) was used to assess the COVID-19 epidemic situation in Southeast Asia, South Asia, and South America. The results are shown in Fig. 5D. The detection number in these regions declined within or after the months an of serious local epidemic conditions.

For example, in Vietnam, the government implemented Prime Minister Directive No. 16 to contain the COVID-19 epidemic that broke out in the summer of 2021 [34]. According to news reports, Vietnam's aquatic exports declined by more than 30 % in the first half of August as a result of the strict implementation of social isolation measures, which caused delays in the transportation of imported and exported raw materials and the shutdown of a large number of enterprises [35,36]. At that time, the epidemic spread to Indonesia and Malaysia. Indonesia implemented Pemberlakuan Pembatasan Kegiatan Masyarakat [37] and Malaysia implemented Pelan Pemulihan Negara [38] to combat the disease. Both policies aimed to slow the transmission of the pandemic by reducing social mobility, similar to the measures implemented in Vietnam.

Third, the monthly detection number was also influenced by domestic CCFs importer demand. The Fuzhou centralized supervision warehouse primarily detects CCFs entering the wholesale and retail markets in Fuzhou. Whether the CCFs enters the centralized supervision warehouse for detection is largely determined by local market demand in Fuzhou. The detection number of the corresponding products changes with the market demand. If the imported CCFs were found to be SARS-CoV-2 RNA positive, it would be detained at the port for an extended period or returned to overseas exporters, causing economic losses to the importer. The Measures of the People's Republic of China for the Administration of Import and Export Food Safety stipulate that food importers should be accountable for the safety of imported CCFs in their marking production and should establish an examination and verification system for overseas exporters and overseas production enterprises [39]. Consequently, food importers may reduce the order of CCFs in the regions with a widespread epidemic to avoid economic and legal risks.

Ensuring the safety of CCFs while minimizing the impact on CCFs import trade is an issue that national governments must consider when formulating relevant epidemic prevention measures. One study suggested that strict monitoring and prevention policies could effectively reduce the risk of SARS-CoV-2 RNA contamination of CCFs [40]. Based on two surveys on Chinese consumers' intentions to purchase CCFs, although the COVID-19 outbreak has had a negative impact on imported CCFs sales, stringent supervision can effectively enhance consumer confidence in CCFs [41,42]. The GACC is currently cancelling the circuit breakers mechanism, emphasizing source control, and adopting more scientific and accurate management methods [43].

4.3. Higher mechanization in CCFs processing may help to reduce virus residues

We found that RF had a higher positive rate than processed food and this was true for outer packaging, inner packaging, and contents. Processed food may undergo washing, chopping, heating, and other processes depending on the degree of processing, while RF can be frozen and packaged directly. These processes reduce SARS-CoV-2 retention on food surfaces. By contrast, facilities that produce more processed foods tend to have higher levels of mechanization. A high level of mechanization reduces worker density and mobility in factories, and stringent management measures are implemented in these factories. These practices can reduce contact between workers, thereby lowering the risk of epidemics and food contamination in the factory [44,45]. This could explain the lower positivity rates for inner packaging and content in processed food than those in RF. Similarly, higher mechanization and management levels reduce the likelihood of the outer packaging of processed food being contaminated by workers during the transportation. The positivity rates for processed food were generally lower than those for RF. Therefore, enhancing the level of mechanization in CCFs processing may contribute to a reduction in virus residues.

4.4. Municipal sewage may contaminate cold chain aquatic products

Surprisingly, the positive rates of the outer packaging, inner packaging and contents of crustacean CCFs were higher than those of fish and cephalopods in Southeast Asia. Contamination from infected workers cannot explain why crustacean produce has such a high positivity rate. Although there is evidence that SARS-CoV-2 can infect some mammals [46,47], aquatic organisms are generally not thought to be infected with SARS-CoV-2 [48,49]. Considering the inherent inability of crustaceans to harbor a substantial viral load due to SARS-CoV-2 infection, the source of contamination is probably from the surrounding aquatic environment [50]. SARS-CoV-2 can be introduced into sewage via patients' fecal matter, and the viral load in sewage exhibited a strong correlation with local epidemics [51,52].

The direct discharge of municipal sewage into the sea can result in contamination of the surrounding aquatic environment. Notably, the types of crustacean CCFs detected in the supervision warehouse were unitary, mainly a species of prawn (*Penaeus vannamei*). *Penaeus vannamei* has been reported to live mainly in estuaries and near-coastal waters [53]. They are more vulnerable to contamination than most fish and cephalopods that live farther out to sea. These prawns are primarily exported to China as RF, where they can be directly packaged and frozen in a factory after fishing without the need for further processing. In addition, water contaminated by SARS-CoV-2 might enter the factory with the prawns and lead to the factory environment pollution, thereby resulting in contamination of both the inner and outer packaging. This may explain the higher positivity rate of crustaceans in the outer packaging, inner packaging, and contents of crustaceans than those in fish and cephalopods in Southeast Asia. The implication is that the presence of SARS-CoV-2 RNA in raw materials could potentially contaminate the entire food processing chain within a food plant. However, this hypothesis remains speculative at present, necessitating further research for its validation.

4.5. Limitations

This study had several limitations. First, additional studies warranted to evaluate the actual risk of transmission associated with cold chain SARS-CoV-2 RNA contaminated packaging. On one hand, the lack of sequence analysis of presumed positive SARS-CoV-2 RNA samples hindered a comprehensive investigation of the associations between detected positive samples within the packaging layers, batches of samples, or across regions. On the other hand, because viral infectivity was not evaluated, there are no available data to support the potential transmission dynamics associated with these CCFs products. Second, due to limited information access, we could not obtain adequate data to analyze in detail the specific reasons for changes in the positive detection rate of SARS-CoV-2 RNA in different countries during the study period and can only put forward a more general hypothesis. Finally, the CCFs detected in the supervision warehouse were primarily from Southeast Asia, South Asia, and South America, and the CCFs category was highly concentrated to aquatic products. This may not fully reflect the actual situation of SARS-CoV-2 RNA contamination in CCFs imported throughout the country.

The reason for exclusively employing RT-qPCR to detect SARS-CoV-2 RNA in this study is that, as a government-funded project, it is essential to ensure cost-effective detection and rapid issuance of test reports in order to meet the high-throughput demand for goods in centralized supervision warehouses. Concurrently, all CCFs entering centrally supervised warehouses must undergo comprehensive preventive disinfection before departure, regardless of RNA test results [54]. Therefore, it was not feasible to confirm the infectivity of the SARS-CoV-2 strains on a case-by-case basis under the circumstances.

Despite these limitations, this study presents compelling evidence of SARS-CoV-2 RNA contamination in a range of products imported from various regions worldwide, showing the trends in product types and packaging locations.

4.6. Future perspectives

As a result of urbanization, the size and vigor of the food industry have expanded. Moreover, advancements in transportation and globalization have fostered a thriving trade within an intricate web of international routes for food products. However, these opportunities often present challenges, potentially accelerating the transmission of infectious diseases [55].

The food safety of CCFs has emerged as a prominent global concern in the era of globalization. The transmission of foodborne pathogens along the cold chain is a frequent occurrence. A statistical investigation of the microbial safety test data for domestic and imported fish products in the United States from 1998 to 2018 revealed that *Listeria* was the most frequent cause of fish product recalls whereas *Salmonella* was the main bacterial cause of fish-related foodborne infections [56]. Researchers suggested that several foodborne viruses, including cyclic virus and hepatitis A virus, can be bacterial biofilms on food surfaces [57]. This markedly enhanced adherence of foodborne viruses to food surfaces. A review found that frozen food was mostly responsible for hepatitis A epidemics in developed countries [58]. Additionally, there have been numerous cases of individuals becoming diarrheal after consuming frozen berries contaminated with norovirus [59,60]. The aforementioned evidences highlight the detrimental impact of foodborne pathogenic microorganisms on the safety of CCFs.

The emergence of SARS-CoV-2 redirected our attention to an issue that has previously received less focus: Although not the primary mode of transmission for SARS-CoV-2, individuals can contract the virus by touching a contaminated surface and subsequently touching their eyes, nose, or mouth [61,62]. And the cold temperature environment of the cold chain facilitates the preservation of viral infectiousness on the surface of CCFs, thereby facilitating the cross-regional transmission of Covid-19 [8,63]. The findings of our study demonstrated a severe presence of SARS-CoV-2 RNA contamination in CCFs. Certainly, further investigations such as sequencing or cell-culture infectivity analysis should be conducted to evaluate the actual transmission risk associated with cold chain SARS-CoV-2 contaminated packaging. But it reminds us that ensuring the safety of cold chain food requires attention not only to the contamination of foodborne pathogens but potential risks posed by other severe non-foodborne infectious diseases.

Except for SARS-CoV-2, no other respiratory viruses have been documented to disseminate via the CCFs. However, there remains a potential for epidemic outbreaks via indirect routes, as respiratory viruses such as rhinoviruses, influenza viruses, and other varieties of coronaviruses have been demonstrated to survive on the surfaces of objects [64–66]. With an increase in the population and range of human activities, the likelihood of genetic mutations in pathogens has also increased. Since the beginning of 20th century, epidemics of SARS, H1N1, MERS, Zika, Ebola, COVID-19, monkeypox and other diseases have broken out on a global scale. The fight against pathogens is expected to intensify. Thereby, we need to be more aware of the possibility of extensive transmission of infections by pathogens through CCFs. Only through the comprehensive enhancement of preventive and control measures can we ensure food safety of CCFs and safeguard the health and well-being of the general public.

With the Chinese government has readjusted COVID-19 to be managed as Class B infectious diseases, the importance of SARS-CoV-2 RNA testing on CCFs has diminished. Our forthcoming research will focus on investigating the prevalent pathogenic microorganisms present on the CCFs, while also devising efficient detection protocols to further enhance the food safety of CCFs.

5. Conclusions

Through the 18-month testing period at the Fuzhou Imported Cold Chain Food Centralized Supervision Warehouse, "outer packaging", "raw food" and "aquatic products", particularly "crustaceans", were the hot words closely associated with the positive detection of SARS-CoV-2 RNA.

In view of these trends, we hold the conviction that strengthening the preventive and control measures for individuals involved in the CCFs supply chain, enhancing the mechanization level of the food supply appropriate disinfection measures at each stage contamination sources that CCFs may encounter throughout the entire process. This approach serves as a crucial strategy to mitigate contamination risks. Simultaneously, it is imperative for the government to fulfill its supervisory role and supervise enterprises to rigorously implement corresponding measures in order to ensure the efficacy of prevention and control protocols.

Ethics declarations

Review and/or approval by an ethics committee was not needed for this study because the study exclusively focused on cold chain foods without involving any human or live animal subjects.

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Data availability statement

Data associated with this study has not been deposited into a publicly available repository. Data will be made available on request.

CRediT authorship contribution statement

Yuxiang Chen: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Shuai Zhao:** Writing – original draft, Validation, Methodology, Formal analysis. **Yiyuan Xu:** Writing – review & editing, Validation, Resources, Methodology, Conceptualization. **Mingzhi Cai:** Writing – original draft. **Guanbin Zhang:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no competing interests.

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