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Possible processes and origin-tracing methods of “human-to-item” contamination and “item-to-human” infection with SARS-CoV-2



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1. Introduction

The first wave of coronavirus disease 2019 (COVID-19) outbreak in mainland China was declared controlled after April 2020. Since then, the origins of all local outbreaks were imported cases of COVID-19 infection or imported articles contaminated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1–3]. The volume of import and export trade in China is enormous, and the entry of SARS-CoV-2-contaminated articles into China is inevitable. Additionally, SARS-CoV-2 can survive on the surface of noncold-chain products for prolonged periods in cold environments during fall and winter [4–6]. Consequently, human contact with contaminated items may result in infection. Although incidental, infection can lead to local COVID-19 outbreaks and even multiple transmission chains caused by different SARS-CoV-2 strains circulating within the same area.

Thus, it should be noted that performing SARS-CoV-2 origin-tracing on items suspected of being contaminated presents several difficulties. First, the number of articles or environmental samples is very large compared to the number of infected individuals, and sampling process may not target contaminated areas, resulting in failure to detect contamination. Second, the viral load in articles or environmental samples is low, and whole genomes are not easily obtained for whole genome sequencing (WGS). Third, even when WGS results from article samples are available, data analysis and interpretation are complex. Therefore, this study aimed to explore the possible causes of local outbreaks caused by articles contaminated with SARS-CoV-2 and share “item-to-human” origin-tracing methods.

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2. Possible processes of “human-to-item” contamination

The production, storage, and circulation of articles (goods or commodities) before entry into the consumer market will be inevitably exposed to unspecified groups of people. If this population is considered an epidemiological study object, the disease distribution (time/region/population) would be complex. The most significant factor in population distribution is occupation, which may include production workers, packaging workers, fishers, warehouse workers, porters, drivers, and quality inspectors. The means of exposure to contaminated articles in this population is through their work. This population has an extensive regional distribution, which means these workers may come from different cities, provinces, or even countries. In the context of global trade and the COVID-19 pandemic, imported commodities are likely to have been in contact with COVID-19 cases in various regions in multinational trade before customs entry. Also, because SARS-CoV-2 continues to evolve in the human population, the virus on articles contaminated by cases at different time points may belong to different virus generations.

Therefore, the same batch of articles may be contaminated with different SARS-CoV-2 strains carried by different infected individuals in various regions, and their genetic characteristics may vary dramatically in temporal and spatial dimensions. The “human-to-item” contamination process is similar to making a pizza (Fig. 1). A clean pizza crust represents an uncontaminated item in the figure, and different food ingredients represent different SARS-CoV-2 strains. When the first infected individual makes contact with the clean article, the SARS-CoV-2 strain carried by that individual (tomato in the figure) remains on the article’s surface. When the second, third, and fourth infected individuals successively make contact with the article, the corresponding SARS-CoV-2 strain carried by each individual (green pepper/bacon/olive) remains, which mixes on the object’s surface, making it difficult to determine the order of contamination. This is a possible example of the “human-to-item” process in which multiple SARS-CoV-2 strains contaminate the same item.

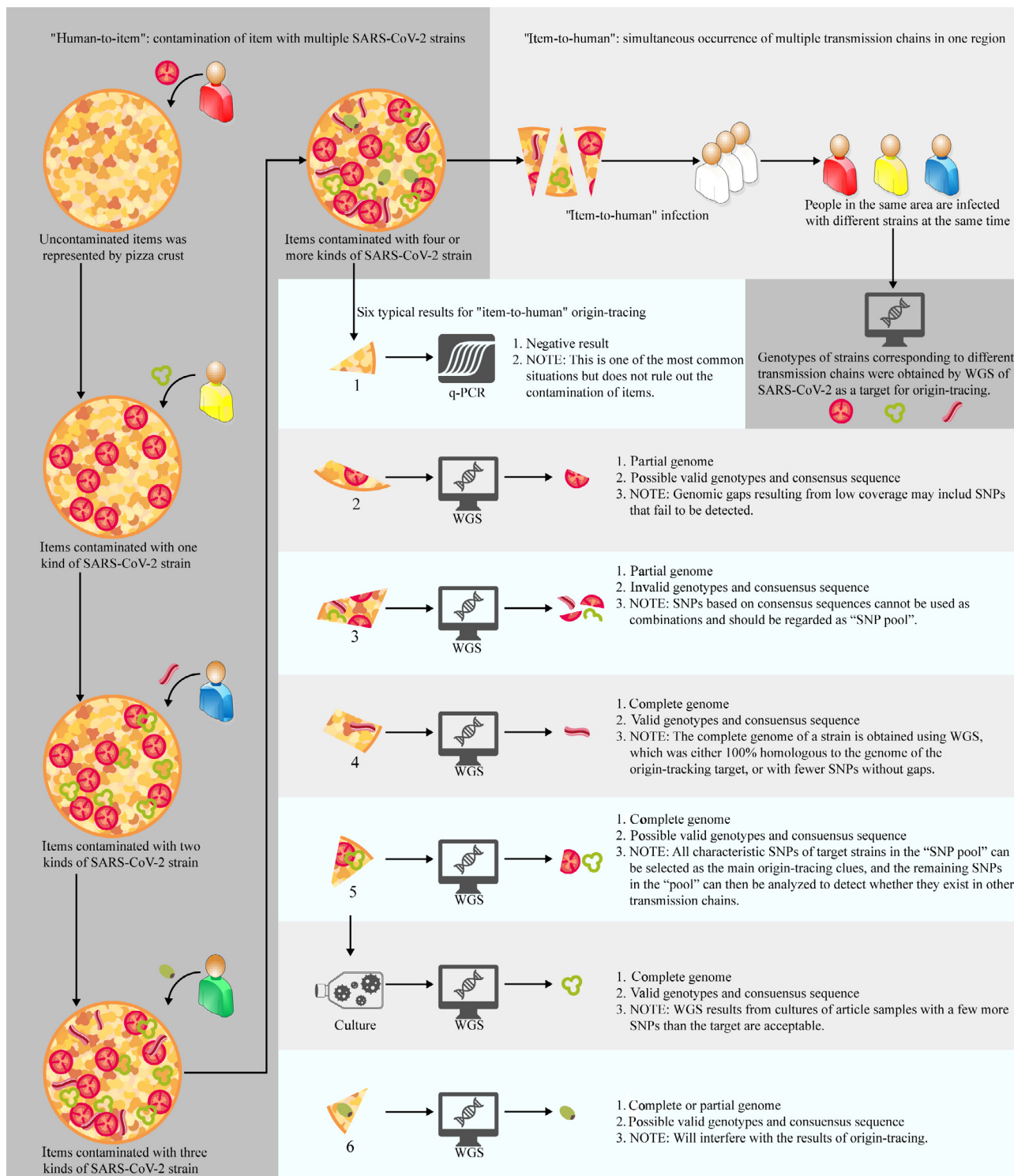


Fig. 1. Possible process of “human-to-item” contamination and “item-to-human” infection and the method for origin-tracing. A clean pizza crust represents an uncontaminated item; tomatoes, green peppers, bacon, and olives represent different severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) strains.

3. Possible processes of “item-to-human” infection causing multiple transmission chains

Items contaminated with multiple SARS-CoV-2 strains come into contact with a new population upon entering the consumer market. Unlike the epidemiological characteristics of the population in the “human-to-item” process, the exposure time and region of the second population are relatively concentrated, but with a diverse population

composition (not only mainly workers). This primarily depends on the characteristics of the items, such as food, parcels, or mail, which are more widely circulated than packed warehousing goods and may more likely to trigger local outbreaks via an “item-to-human” infection process. It is worth noting that cash and credit cards are special commodities that circulate more easily. Several studies have revealed that high viral loads of SARS-CoV-2 can survive for up to 21 days on banknotes, steel, and glass [7–10]. Therefore, we can speculate that the

increased use of electronic payment methods (no touching or exchange of items) is a possible reason for China's good epidemic prevention and control results. However, the risk of virus-contaminated cash entering China from the border is also worth considering.

Since the "item-to-human" phenomenon of SARS-CoV-2 has been confirmed by scientific methods [2], the possibility exists that the same batch of articles can simultaneously cause multiple transmission chains in the same area. This process can be illustrated by a group of people sharing a pizza (Fig. 1), with each person randomly eating a part of the pizza with a certain ingredient (tomato/green pepper/bacon). Similarly, different people exposed to articles contaminated with active viral particles will be infected with different virus strains. If a batch of goods contaminated with multiple strains is distributed or sold, different groups of people who are far apart will be infected by different strains simultaneously. Thus, the onset time of early cases belong to different transmission chains will be relatively close; however, there may be no significant epidemiological association between them, which further complicates the epidemiological investigation. Notably, WGS can be used to identify the genotypes of SARS-CoV-2 strains isolated from early cases with different transmission chains. The goal of origin-tracing in an epidemic is to find viruses with the identified genotypes (tomato/green pepper/bacon) in the environment or on articles. The epidemic caused by the entry of infected individuals is relatively easy to trace and is not discussed in this paper.

4. Methods for origin-tracing of items or environmental samples based on WGS

Epidemiological investigation and WGS of SARS-CoV-2 are the primary methods used for origin-tracing in an outbreak. Additionally, sequencing platforms, such as Illumina, BGI, and Oxford Nanopore, have become increasingly common at provincial Centers for Disease Control and Prevention in China and play an essential role in origin-tracing. The origin-tracing method for items can be summarized as follows: Taking the viral genotypes of early cases in each transmission chain as the target, search for highly homologous viral genomes or fragments in article samples (such as surface wipes). This work is greatly affected by random factors, and the interpretation of the results is complex. Empirically, we summarized six possible situations of WGS based origin-tracing of items or environmental samples (Fig. 1).

- 4.1 There is no need for WGS because viral particles or nucleic acids are not collected during sampling, resulting in negative qPCR test results. This is one of the most common situations but does not rule out the contamination of items.
- 4.2 A partial genome fragments of a certain strain is obtained via WGS. The more complete the fragment, the more accurate the obtained consensus sequence and genotype, making it easier to find the target genome with the highest homology through alignment. Genomic gaps resulting from low coverage may include single-nucleotide polymorphisms (SNPs) that are not detected in items. For important events and key samples, specific amplification of gap positions alone can be attempted in the next round of sequencing to supplement the initial data.
- 4.3 Partial genome fragments of various strains are obtained by WGS, and the consensus sequence and genotype do not represent the real strains regardless of whether the fragments are complete. Therefore, SNPs based on consensus sequences cannot be used in combination and should be regarded as an "SNP pool" to independently analyze and judge the relationship between each locus and the origin-tracing target. Furthermore, SNPs may be homologous to the origin-tracing target in any transmission chain, and heterozygous positions with high depth

may indicate that multiple strains are simultaneously sequenced (systematic errors caused by experiments may exist in all six possible situations).

- 4.4 The complete genome of a strain is obtained using WGS, which was either 100% homologous to the genome of the origin-tracking target, or with fewer SNPs without gaps. Additionally, viral culture can be attempted for article samples with low Ct values to further strengthen the evidence of origin-tracing.
- 4.5 The complete genome of target strain (green papper) and partial genome fragments of non-target strains (tomato) are obtained using WGS. The more incomplete the genome fragment of the non-target strain, the more accurate the obtained consensus sequences and genotypes, and the easier it is to find the target genome with the highest homology through alignment. All characteristic SNPs of target strains (green papper) in the "SNP pool" can be selected as the main origin-tracing clues, and the remaining SNPs in the "pool" can then be analyzed to detect whether they exist in other transmission chains. If they exist in other transmission chains (part of tomato), it means that the item may be contaminated with multiple strains; otherwise, it is not enough to confirm that the item is the origin of the outbreak, and virus isolation of samples with low Ct values is needed to strengthen the evidence. Because the virus may develop new variations during culture, the WGS results from cultures of article samples with a few more SNPs than the target are acceptable.
- 4.6 A strain that contaminates an article but does not cause a new chain of transmission is excluded from the origin-tracing target. The characteristic SNPs of this strain will appear in the WGS results from article samples, and co-occurrence with any of the above conditions will interfere with the results of origin-tracing.

5. Discussion and conclusion

A report issued by the China–WHO Joint Expert Group on the origin-tracing of COVID-19 concluded that humans are "likely" to be exposed to SARS-CoV-2 via cold-chain foods. We have also repeatedly demonstrated that imported cold-chain products contaminated with SARS-CoV-2 could lead to local COVID-19 outbreaks [1–3,11,12]. Multinational studies have shown that coronaviruses have survival ability and carry a risk of causing infection via contact on noncold-chain foods, such as beverages, food packaging, metals, banknotes, and personal protective materials [7–10,13–16]. Because of the ongoing COVID-19 pandemic, medical and health authorities at all levels in China should consider screening articles suspected of being contaminated with SARS-CoV-2 for origin-tracing. In addition to focusing on contaminated imported items, various relationships between articles and environmental factors and the risk of SARS-CoV-2 transmission need to be further studied and investigated. The findings of our study can serve as a reference for determining the origins of local COVID-19 outbreaks to hastily cut off the origin of infection and block transmission.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

Author contributions

Ji Wang: Writing – Original Draft. **Wenbo Xu:** Project Administration, Writing – Review & Editing. **Xuejun Ma:** Supervision, Writing – Review & Editing.

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