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Risk factors and on-site simulation of environmental transmission of SARS-CoV-2 in the largest wholesale market of Beijing, China

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HIGHLIGHTS

• Field investigations and on-site simulations were conducted at Xinfadi Market after COVID-19 outbroke since June 11 of 2020.
• Transaction behaviors, physiological activities, and marketing activities could lead to viral spread.
• Low temperature and high humidity, poor ventilation, and insufficient hygiene facilities may contribute to viral transmission.
• Precautionary control strategies were proposed to reduce the clustering cases of COVID-19 in large-scale wholesale markets.

GRAPHICAL ABSTRACT

ABSTRACT

From June 11, 2020, a surge in new cases of coronavirus disease 2019 (COVID-19) in the largest wholesale market of Beijing, the Xinfadi Market, leading to a second wave of COVID-19 in Beijing, China. Understanding the transmission modes of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the personal behaviors and environmental factors contributing to viral transmission is of utmost important to curb COVID-19 rise. However, currently these are largely unknown in food markets. To this end, we completed field investigations and on-site simulations in areas with relatively high infection rates of COVID-19 at Xinfadi Market. We found that if goods were tainted or personnel in market was infected, normal transaction behaviors between sellers and customers, daily physiological activities, and marketing activities could lead to viral contamination and spread to the surroundings via fomite, droplet or aerosol routes. Environmental factors such as low temperature and high humidity, poor ventilation, and insufficient hygiene facilities and disinfection practices may contribute to viral transmission in Xinfadi Market. In addition, precautionary control strategies were also proposed to effectively reduce the clustering cases of COVID-19 in large-scale wholesale markets.
1. Introduction

On June 11, 2020, after 56 days without any new diagnoses of COVID-19 cases, a second wave suddenly sparked in Beijing, China that gripped the whole world’s attention (Owen, 2020; Wu, 2020). The center of this outbreak, Xinfadi Wholesale Market, is the largest food market in Asia, and was thus suspended immediately, and strict control measures (e.g., closed the market, locked down certain residential compounds, closed all schools, and canceled hundreds of flights) were quickly implemented to contain the spread. Between June 15 and July 10, individuals deemed as high-risk (e.g., close contacts of confirmed cases and residents living nearby to the market) were tested by nucleic acid test using qRT-PCR, with over 10 million personnel and 5342 environmental samples tested overall. A total of 401 confirmed cases and asymptomatic cases had been reported, of which 368 cases [169 (46.0%) employees and 103 (28.0%) visitors of Xinfadi Market as well as 96 (26.2%) contacted with these infected employees or visitors] were in Beijing, and 33 cases were in other provinces linked with this outbreak, suggesting a single outbreak source in Beijing. On August 6, the last hospitalized patient was discharged from hospital in Beijing, and no death had occurred.

This coronavirus resurgence was linked to a food market, which is reminiscent of the first outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that was identified at a Huanan Seafood Wholesale Market in Wuhan, Hubei Province. Cluster epidemics have also been reported within seafood industries (Radio, N.P. 2020) and meat processing facilities (Dyal et al., 2020) worldwide (e.g., in the United States, Germany, Australia and France). Previous epidemiological investigations and viral genome sequencing of Xinfadi strains speculated that the reemergent epidemic in Beijing was likely to be initiated by an environment-to-human transmission originated from the contaminated imported food via cold-chain transportation, and no evidence was found for alternative initial transmission source to date (Pang et al., 2020). A common thread among the outbreaks of Wuhan, Beijing, and recent Dalian seafood industries, are high humidity with chilled air, and adverse conditions such as drying out is not permitted for the food integrity. If the virus contaminated frozen, refrigerated or fresh goods (e.g., fish) or its packaging, the surrounding environment enables the virus to thrive for a relative long period as a potential hotbed to facilitate the subsequent transmission to personnel during their daily activities and behaviors, particularly when there is overcrowding, lack of efficient personal protection, disinfection (e.g., UV light), and/or adequate ventilation, and shouting in close proximity due to high ambient noises (Waltenburg et al., 2020).

According to the previously thorough epidemiological investigations (Pang et al., 2020), 72.2% (122/169) of the infected employees worked in the Basement One of the Xinfadi trading hall, and highly clustered cases were found in aquatic products (51.5%, 52/101) and soy products (28%, 14/50) areas (Fig. 1A). Salmon was the only imported commodity sold at Booth S14 in aquatic products area, leading to the viral transmission patterns and the high positive rates of surface samples from the objects and goods collected in these two areas. It is known that in addition to contact and droplet spread, the transmission of SARS-CoV-2 via aerosols is plausible under certain conditions (Santarpia et al., 2020). To explore the potential viral transmission routes, approaches including epidemiological investigations, environmental samplings and analysis, and simulation experiments are widely adopted in some high-risk settings such as hospital wards and restaurants (Zhou, 2020; Lu et al., 2020; Guo et al., 2020). However, to date, the viral transmission patterns and the personal behaviors and environmental factors affecting environment-to-human or human-to-human transmissions that have profound implications for effective control measures and public safety are largely unknown in food markets.

We aimed to (1) explore the potential transmission routes (e.g., fomite, droplet and aerosol) of SARS-CoV-2 in Xinfadi, (2) mimic and identify the key personal behaviors and the associated environmental conditions that may contribute to viral spread, and (3) propose precautionary control strategies to inform prevention of viral transmission in similar markets for policymaking. To this end, we completed field investigations and on-site simulations in areas with relatively high infection rates of COVID-19 at Xinfadi Wholesale Market. More specifically, we (1) investigated the layout floor structure (e.g., water supply and drainage distribution, sewage drainage channels, air conditioning and ventilation systems, and sanitation facilities) on-site and the behavior patterns of sellers and customers, (2) determined the dissemination of fluorescent microsphere as a surrogate for SARS-CoV-2 under different behaviors and interactions between sellers and customers (e.g., trading activities in the aquatic products and soy products areas, activities in the toilets, and washing the ground with a pressure washer gun), and (3) collected and analyzed the samples from the simulation experiments.

2. Material and methods

2.1. Study site

The site of this investigation and simulation experiment was the Basement One of Xinfadi Wholesale Market, Fengtai District, Beijing where most of cases (122/169, 72.2%) among Xinfadi employees was found. Basement One is a sunken underground space with an area of approximately 10,000 square meters and 5 m height surrounded by circular corridor. The entrance is located in the north and south of Basement One. There are several hundreds of business booths selling aquatic products, soy products, beef, lamb, chicken, etc. Each booth has a unified counter, and drainage channel is located around the counter. Two toilets (around 55m²) for male and female are on the south. According to the findings of epidemiological investigation during outbreak (Pang et al., 2020), seller cases were mainly distributed on the west side of Basement One with the incidence rates of 51.5% and 28.0% in aquatic and soy products areas, respectively (Wu, 2020; Pang et al., 2020). Before the experiments, on-site investigation was conducted to understand the layout floor design (e.g., floor plans, air-conditioning system, ventilation routes, drainage system, sanitation facilities, and toilet) of Basement One. The aquatic and soy products areas plus high-risk toilet were finally chosen for the following simulation experiments (Fig. 1A).

2.2. Materials preparation

Fluorescent powders (Beijing Cypress Chemical Co., LTD., China) with diameter 48 μm are colorless under natural light but showed red fluorescence under blacklight (365 nm). This powder did not exist in nature and were therefore used as a tracer in on-site experiments. Fluorescent polystyrene latex microspheres with a diameter 2 μm were provided by Section of Ecological Environment and Energy Resources at Beijing Institute of Metrology. It showed silver-gray under scanning electron microscope (SEM) and green under blacklight or fluorescence microscope. As a proxy for SARS-CoV-2, SARS-CoV-2 spike pseudovirus, which has no autonomous replication ability, was purchased (Sino Biological Inc., Beijing, China).
2.3. Laboratory experiments

Before the on-site experiments, the dispersion differences between SARS-CoV-2 spike pseudovirus and fluorescent microsphere were compared in water, aerosol and object surface at laboratory. For water experiment, a same amount of pseudovirus (10^9 copy/mL) and fluorescent microsphere (10^6 balls/mL) was respectively prepared in tap water and then serial diluted with tap water. For aerosol and object surface experiments, pseudovirus and fluorescent microsphere were respectively placed in a micro-aerosol generator to simulate a sneezing and then contaminated the plates. The sprayed aerosols were immediately collected, while the plates were exposed to aerosols for 30 min and then smeared for analysis.

RT-PCR (Rotor-Gene Q, QIAGEN, MD, USA) was used to measure viral loads in all collected samples. Fluorescent probe method was used to detect the copy number of the WPRE gene of pseudovirus, and the obtained CT value was converted to obtain the copy number in each sample. Qiagen's QIAamp® Viral RNA Mini Kit was used to extract the genome, which serves as a template to reverse-transcribe the RNA using the cDNA first-strand synthesis kit (Sino Biological Inc., Beijing, China). 1 μL cDNA was taken for RT-PCR detection with a detection limit of 7.97E+03 virus copies/mL.

Fluorescence microscope (Nikon Corporation, Tokyo, Japan) were used to measure the number of fluorescent microspheres in all collected samples. Our results indicated that the dispersions of pseudovirus and fluorescent microspheres in water, aerosols and object surfaces were consistent and insignificant (Fig. S1). Therefore, fluorescent microspheres could replace viruses with a consistent transmission dynamic and better traceability in the following simulation experiments.

2.4. On-site simulation experiments

The market was strictly controlled after the outbreak, and we were allowed to complete the experiments in a day and could not recruit real sellers. Therefore, both buyers and sellers were the members of our CDC team, who have well learnt the behaviors of sellers and buyers by observing, consulting and mimicking from real sellers and buyers in other food markets. After the completion of terminal disinfection of the market, on-site simulation experiments were carried out on July 8, 2020. During the experiments, the indoor air-temperature was 19–20 °C and the relative humidity was 55.5%, the atmospheric pressure was 100.4 kPa, and the central air-conditioning system was turned on. All the CDC staffs wore full personal protective equipment (PPE), namely an N95 respirator, goggles, gloves, shoe covers, cap, and protective apparel. The stands of vegetables, fruits and bakery were designed as the origin of this outbreak is given, and the positive rates of employees and surface samples of environment and goods among different regions are also shown (Pang et al., 2020). A total of 584 employees (20.5% positive) and 2467 environmental samples (5.5% positive) were tested in every booth at the Basement One. (B) The technical roadmap for the present study is also shown.
repeated twice and lasted for 20 min at the same area within the seller's booth. After the business hours, the ground was washed with pressure washer gun daily by the market cleaner. Hence, this process was mimicked in both aquatic products and soy products areas and lasted for 10 mins.

For the toilet, a semi-solid solution of 0.8% agar broth mixed with fluorescence solution was used to simulate "tainted feces" from an infected person. The activities at toilet, including waiting, flushing, walking around, and leaving, were simulated. All the processes were repeated twice and lasted 30 mins.

Fig. 2. Schematic diagrams of representative sampling points and positive points in the aquatic products area (A), soy products area (B), and the toilet (C). Smear samplings for several items (e.g., knife, calculator and platform scale button) are not shown. Each counter is numbered as A# and S# for aquatic products and soy products areas, respectively. For the toilet, each cubicle is numbered as T#. A window and hand washing facilities are also shown. Bright gray and dark gray indicate the counters/toilet/cubicle where the simulation experiment was conducted, respectively. Blue strips around the counters indicate the drainage channels. The points stuck on the side of the counters indicate that samples were smeared or collected between the countertop and the ground. (D–F) Representative photos of fluorescent microspheres tracked by different sampling methods in different areas. (D) After sales transactions in aquatic products area, fluorescent powders (red) and fluorescent microspheres (green) were detected on the button of platform scale under fluorescence microscopy (×400). (E) After washing the ground in soy products area, fluorescent microspheres were detected in an air sample (Counter S2 with 1 m height) using natural sedimentation under fluorescence microscopy (×1000). (F) After flushing the toilet, fluorescent microspheres were detected in quartz filter of an air sample (1.2 m above the squat) using PM10 samplers (10 L/min) under scanning electron microscopy (SEM).
2.5. Environmental samplings

For all the three simulated areas, long handle cotton swabs (Copan Medical Equipment Co., LTD, Shanghai, China) were used to collect surface samples (e.g., chopping board, knife handle, blade, fishing net, calculator, countertop, door handle, ground, and PPEs) before (as blank control) and after the experiments (e.g., sales transactions, washing the countertop, and washing the ground). The smear sampling area was 5 cm × 5 cm and repeated three times. After collection, they were immediately stored in sealed plastic bags and kept away from light, and then transferred to the laboratory for further analysis. Before, during or after the simulation experiments, the sterile plates with 9 cm diameter (Jindian Biochemical Equipment Co. LTD, Qingdao, China) were placed at different distances and heights within 15 m of operation sites according to the droplet diffusion distances of larger droplets and aerosols, and each simulation scenario was collected 20 mins–2 h for the particles (droplets) precipitation in air (Kutter et al., 2018). After sampling, the plates were sealed and stored away from light and transferred to laboratory for analysis.

In the aquatic products area, one total suspended particulate sampler (TSP, 100 L/min, TH-150C, Wuhan Tianhong Instrument Company, China) with a diameter of 90 mm quartz filter, two PM_{10}/PM_{2.5} two-stage samplers (10 L/min, SKC Inc., PA, USA) with a diameter of 37 mm/47 mm quartz filters, and two bioaerosol samplers (12.5 L/min, with 20 mL water) were all placed around the counter. The sampling time were 79 min for TSP during the whole experiments, 72 min for PM_{10}/PM_{2.5} and water, respectively, including 40 min during sales transactions, washing the countertop and 32 min during washing the ground and simulation experiments. In the soy products area, two PM_{10}/PM_{2.5} two-stage samplers and two bioaerosol samplers were placed around the counter, and the sampling time for PM_{10}/PM_{2.5} and water were 40 min during sales transactions and for water were 14 min during washing the ground. In the toilet, two PM_{10}/PM_{2.5} two-stage samplers and two bioaerosol samplers were placed near the squat toilet (<0.5 m) or 1.5 m away, and the sampling time were 19 min for PM_{10}/PM_{2.5} and water during the simulation experiments. After sampling, all the filters were sealed individually in membrane cartridges and sampling water were placed in 50 mL centrifuge tubes, and all samples were analyzed within 3 days.

After washing the ground, 10 mL sewage from drainage channels around the counter were collected following the washing direction within 15 m away and transferred to the laboratory for further analysis. All the samples were collected by well-trained CDC staffs with sampling experiences before. In total, we collected 106 surface samples, 113 air samples, 35 sewage samples, and 70 PPE samples, including blank controls.

2.6. Laboratory measurements

The sampling swabs were wetted with 75% ethanol and repeatedly smeared on the surface of plates that collected precipitation particles (droplets) in the air. The swabs were then smeared on the clean slides. Water samples were centrifuged to remove impurities and then filtered through a 0.1 μm-pore-diam membrane filter (Millipore Corporation, MA, U.S.A.), and the membrane filters were pasted onto another clean slide for observation. All the slides were observed to check the red (for fluorescent powder) or green (for fluorescent microspheres) fluorescence under a fluorescence microscope with ≥400 and ≥1000, respectively. A scanning electron microscope (ZEISS SEM, Sigma 300 Field Emission Scanning Electron Microscope, Oberkochen, Germany) was used to observe the fluorescent microspheres on filters. The excitation and emission wavelengths were 266 nm and 531 nm, respectively.

2.7. Statistics

Non-parametric Wilcoxon test was conducted to determine whether there is a statistically significant difference among different groups using R software version 3.3.1 (R Development Core Team, 2006). A p value less than 0.05 was considered as statistically significance. Bar plot was visualized using ggplot2 package in R.

3. Results

3.1. On-site investigation

The high COVID-19 infection rates of 51.5% and 28% were found for employees from aquatic products and soy products areas in the Basement One of Xinfadi Market, respectively (Fig. 1A) (Pang et al., 2020). This is further supported by the high positive rates (7.9% and 15.2%, respectively) for SARS-CoV-2 of surface samples from objects and goods in these two areas (Fig. 1A). Fan-coil air conditioners with fresh air were used in Basement One during the outbreak. Since the counters of business booth are open, the end air outlets and the air return inlets can circulate air between the nearby counters (Figs. S2). There were four air-conditioning units to exchange the air of Basement One. However, the inward air came from the circular corridor instead of outdoor fresh air, and hence the fresh air exchange rate was low. It was not only poorly ventilated, but it also caused unsterilized and unfiltered air to circulate in nearby counters. The drainage channels were opened to the air around all the counters, which was only about 15 cm wide and 5 cm deep. Objects (e.g., fish scales and debris) often blocked these shallow drainage channels, resulting in a poor drainage and humid environment. There were one entrance and one window in the toilets with 8 cubicles and two hand-washing taps, and no any hand hygiene tools (e.g., soap and sanitizer) and disinfection (e.g., UV light) were available.

3.2. On-site simulation experiments

Before the simulation experiments, smear and sedimentation samples were all negative from the aquatic products area, soy products area, and toilet. Detailed results are shown in Supplementary Appendix Tables S1-S3.

For the aquatic products area, in brief, after the trading processes, all of touchable items (e.g., fishnets, knife, chopping board, platform scale button, calculator, etc.) were positive, and fluorescent microspheres could be detected in smear samples with a positive rate of 65.0% (13/20), but most of sedimentation samples were negative (Fig. 2A and Table S1). After the washing the countertop, the positive rate of the sedimentation samples was 62.5% (5/8), which were detected on counters A1 and A3, but not the opposite counter (A7). Fluorescent microspheres were captured beside the counter A1 (1.1 m away and 1.3 m height) on the PM_{10} quartz filter film (Fig. 2D). After ground washing, fluorescent microspheres were found in water samples of the ground and the drainage channels around the counter A1–1. The sewage samples taken 9 m away from pollution point were also positive. After the completion of simulation experiment, the positive rate of sedimentation samples was 40.0% (4/10), and it was still positive at 5 m away from counter A1–1.

For the soy products area, similarly, fluorescent microspheres were detected in 80.0% (8/10) of touchable items after sales transactions. 70.0% (7/10) of the smear samples of counter and ground were positive (Fig. 2B and Table S2), particularly in the operating area counter S1 and opposite counter S3. The positive rate of air sedimentation samples was 36.4% (4/11) with the furthest distance is 0.5 m away from the operating point. When washing the ground, the positive rate of sedimentation samples from 0.5 m and 1 m above the ground were 80.0% (4/5) and 33.3% (1/3), respectively. 20 min after washing the ground, the positive rates of sedimentation samples were 50.0% (2/4) and 25.0% (2/8) from 0.5 m and 1 m above the ground. All of the ground smear samples were positive, and the positive sewage samples were 55.6% (5/9) in the
drainage channels with a maximum of 10 m away from the pollution point.

After flushing the toilet, in the cubicle of simulated area, smear samples from the ground next to squat toilet, wastebasket in cubicle T2, and some surfaces of water tank were positive, whereas the surrounding wall and the wall above the water tank were negative (Fig. 2C and Table S3). The positive rates of sedimentation samples were 71.4% (5/7). Fluorescent microspheres were captured at 0.5 m and 1 m above the squat position, which were on the PM_{10} quartz filter film and in the absorption liquid. Outside the simulated area, the ground and the tap smear samples were also positive. By contrast, both the sedimentation samples and aerosol samples were negative. After the simulation experiment, some sampling plates were left in the operation area for 2 h. Fluorescence microspheres can be detected at 0.5 m and 1 m above the ground in the cubicle, but not at 1.5 m. Samples were also positive at 0.5 m of adjacent cubicle, while the samples from higher and more distant were negative.

In addition, a total of 21 CDC staffs participated in on-site experiments, and fluorescent microspheres were detected on the surface of breathing area of all the N95 masks (100%, Fig. 3 and Tables S4–5). 100% PPEs of the sellers was positive, and among 18 PPE samples from the customer, 15 of them was positive (83.3%). Fluorescence microspheres can also be tracked on the soles of seller’s and customer’s shoes, which are in accordance with the high positive rate all over the ground after sales transaction, washing the ground, and flushing the toilet (Tables S1–S4).

4. Discussion

To the best of our knowledge, this is the first on-site simulation experiment attempting to explain the potential spread patterns of SARS-CoV-2 in a real clustering epidemic center using fluorescent powders and microspheres as tracers. Through on-site investigations and simulating common behaviors and activities (e.g., sales transactions, fish slaughter, countertop washing, ground washing, defecating, toilet flushing, and walking around) that may cause viral spread in the market, samples of environment, object surfaces and PPEs were collected to explore the potential routes and environmental factors of SARS-CoV-2 transmission in Xinfadi market. In general, the results indicated that if goods were tainted or personnel in the market were infected by SARS-CoV-2, normal sales transaction behaviors between sellers and customers (e.g., scaling, fish slaughter, weighing, delivering, payment, etc.), daily physiological activities (e.g., using toilet, talking, walking, etc.), and marketing activities (e.g., countertop-washing, ground-washing, etc.) could easily contribute contamination and spread to the surroundings through fomite, droplet or aerosol routes.

4.1. Multiple transmission routes confirmed in Xinfadi Market

Most confirmed cases and positive environmental samples in Xinfadi Market appeared along the aisles, which connect aquatic products area and soy products area (Fig. 1A). Multiple transmission routes of fluorescent microspheres were observed. Smear samples of countertop, toilet door-handle, faucet surface, and ground that were frequently touched were positive, indicating virus may transmit through direct contact and/or fomite route by infected people, objects or surfaces. Over half of sedimentation samples detected were positive (62.5% in aquatic products area, 36.4% in soy products area, and 33.3% in toilet), which are in line with previous researches showing the transmission of SARS-CoV-2 was mostly through droplet or fomite route (Santarpia et al., 2020; Lu et al., 2020; Tang et al., 2020). In addition, after the...
activities of sales transactions (e.g., killing fish and scraping fish scales), washing the ground or flushing the toilet in simulated areas, the rate of positivity was relatively high for ground swab samples, perhaps because of virus splash, gravity, or air flow causing droplets or aerosols to float to the ground. The virus could thus be transported all over the ground as personnel walking around the market. Furthermore, the smear samples from the soles of seller’s and customer’s shoes were positive, indicating the soles of shoes might function as carriers of the SARS-CoV-2. Our finding is in line with a previous study in hospital wards (Guo et al., 2020), and it would therefore be advisable to keep the ground and the shoes clean in the market.

WHO recently have acknowledged aerosol transmission of SARS-CoV-2, especially in closed indoor settings. Small virus-laden aerosols have been found in many reports displaced by airflows and deposited on vents (Ong et al., 2020), or in patients’ rooms (Chia et al., 2020). The peak concentrations of SARS-CoV-2 RNA in hospital air appear in two distinct size ranges of 0.25–1.0 μm and > 2.5 μm aerodynamic diameter (Lu et al., 2020). Another study found SARS-CoV-2 particles with sizes > 4 μm and 1–4 μm in two AIRRs rooms (Chia et al., 2020). In the present study, the particle sizes of fluorescent microspheres used were about 2 μm. Results indicated that these particles could mimic virus-containing aerosols, and are small enough to remain suspended in air for a long period of time and be inhaled (Lu et al., 2020).

It is worth noting that positive sedimentation samples were detected away from experiment site in aquatic area and as long as 2 h after the completion of experiment in toilet. Besides, fluorescent microspheres were detected in aerosol samples collected after counter-washing, ground washing, and toilet-flushing. Previously several studies have also reported that SARS-CoV-2 were found on toilets and in the air in settings such as hospitals and apartment used by COVID-19 patients (Santarpia et al., 2020; Lu et al., 2020; Chia et al., 2020; Ding et al., 2020). SARS-CoV-2 has been frequently detected in patient’s rooms (Casanova et al., 2020). The initial Salmon-to-human transmission at Booth#14 in aquatic product area led to the viral spread in the Xinfadi Market (Pang et al., 2020). The closer distance between aquatic product and soy product areas as well as the characteristics of higher density of population (e.g., buyers, visitors), low temperature, high humidity, poor ventilation and hygiene, and lack of disinfection may contribute to the highly cluster cases in these two areas. This is not unique in Xinfadi Market since cluster epidemics have also been reported within other seafood markets (e.g., Huanan Seafood Wholesale Market in Wuhan), seafood industries (Radio, N.P. 2020) and meat processing facilities globally (Dyal et al., 2020). Therefore, wholesale markets with these characteristics may face outbreak risks.

4.3. Precautionary control strategies to wholesale markets

Based on what we have found, precautionary control strategies that are important for public health protection are needed in wholesale markets during the pandemic of COVID-19 to limit future transmission of infectious viruses (West et al., 2020). (1) Efforts to avert the outbreak risk must begin at the source. Strict strategies of inspection, monitor, and quarantine for both international travelers and imported goods are essential to prevent the potential outbreaks during the virus circulating worldwide. (2) Ventilation should be supplied adequately and properly. Wholesale markets with the selling of frozen, chilled and fresh products should be operated in well natural ventilation place or in above-ground space. All fresh air should be taken from outside and operated with maximum exchange volume. In case of air recirculation, air filtration and disinfection devices (e.g., ultraviolet, ionization units, HEPA or antimicrobial filters) should be added to ensure the safety of air supply. Local exhaust devices could be added to the counters with aerosol-
generation processes and discharged directly to the outdoor. (3) To avoid crowding, wholesales and retails should be better separated, and strict management should be applied. During the pandemic, medium and high-risk areas should shorten business hours and limit the number of customers. Education and training of sellers and staffs is necessary to encourage adherence guidelines and precautions. (4) Environment should be kept clean and dry as well as increase the frequency of routine disinfection practices. Ground should be cleaned thoroughly and properly by a professional after business hours. Mechanical floor-washing vehicles or manual wet towning should be used instead of using pressure washer guns. Alcohol or chlorine-containing disinfectants could be used to keep floors or surfaces (e.g., door handles and buttons) clean that may also help reduce secondary transmission. After cleaning the ground, indoor ventilation should be increased to increase the air exchange and to dry the ground as soon as possible. If the toilet seat is equipped with a lid, it is recommended to close the lid before flushing the toilet to reduce aerosolization of virus and contamination of surrounding surfaces. (5) Personnel should be aware of personal hygiene. Surgical masks should be worn properly in conjunction with strict adherence to hand hygiene (e.g., hand washing, using alcohol hand rub or hand sanitizer) and social distance to avoid potential infection (Wang et al., 2020). Hand sanitizers or quick drying hand disinfectants should be available for people entering the markets.

5. Limitations and conclusions

There are limitations in this study. First of all, we were not able to use actual virus or pseudovirus on-site, and the particle sizes of SARS-CoV-2 and fluorescent microspheres are different, which in this case may lead to certain bias from real conditions. Our results could not quantify the viral load or check virus viability as well since the infection should be dose-dependent on the load of viable virus in the contaminated environment. Secondly, behaviors and activities mimicked by us were may have been different from real scenarios of the market. Our experiments only described the possibility of the spread, and that is not proof of what happened, and the distinction has thus to be clearly made between what could happen and what did happen. Finally, the cumulative effects of viral transmission were limited by the number of people, time, and the frequency of behaviors and activities.

Taken together, the present study implies that the semi-underground, fresh and frozen products markets with poor ventilation and insufficient hygiene facilities may increase the risks of infectious disease outbreaks. The findings also pointed towards personal behaviors and activities which may contribute to the virus spread via fomite, droplet or aerosol routes in large-scale wholesale markets. Future studies regarding the transmission dynamics, simulation models of transmission, and quantitative microbial risk assessment (QMRA) of SARS-CoV-2 in wholesale markets are highly needed to mitigate the potentially devastating effects of COVID-19.

CRediT authorship contribution statement

DX, ZF and ZW proposed the research hypotheses. DX, ST and BY designed the present study. ZF and ZW provided the surveillance data of outbreak. DX, ST, XL, PD, CD, QW, WG, YM and CRM drafted the manuscript. DX, ST, XL, PD, YM, CD, YW, QW, JW, BY and LP drafted field investigation proposal. DX, ST, QW, and BY organized the field implementation. XL, PD and YM conducted laboratory pre-experiments. DX, ST, XL, YC, YM, WG, PD, CD, YW, BW, LP, QW, CW, JC, YL, CM, JZ and ZT conducted on-site simulation experiments and sample collections. XL, PD, ZX, QW, YW, CD, YM, YC and LL involved in laboratory analysis of samples. XL, PD, YC, FD, QW, ST and DX analyzed the data and drafted the figures and tables. All of the authors approved the submitted version and have agreed to be personally accountable for their own contributions.

Declaration of competing interest

The authors declared that they have no known competing financial interests or personal relationships that could have appeared to influence this study.

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Appendix A. Supplementary data

The Supporting Information includes additional information about details of samples collected in aquatic, soy and toilet area and PPE, comparison between pseudovirus and fluorescent microscope, and representative photo of air conditioner of the market. Supplementary data to this article can be found online at doi: https://doi.org/10.1016/j.scitotenv.2021.146040.

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