Rapid monitoring of SARS-CoV-2 on fruits and vegetables using reverse-transcription loop-mediated isothermal amplification assay

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COVID-infected people handling fruits and vegetables may spread the virus to healthy people on contact. Here we examined if SARS-CoV-2 was detectable on the fruits, vegetables, hand gloves and packaging materials collected from the open markets and pack houses in India. During the study (2021–22), swabs from 748 samples representing the majorly traded items were tested using RT-LAMP assay. The sensitivity and specificity of the kit were found to be equivalent to the RT-PCR assay. All test samples were found negative for SARS-CoV-2. Thus, it can be concluded that the virus is unlikely to spread to foods and packaging materials through human contact.

Keywords: COVID-19 pandemic, fresh fruits and vegetables, open markets and pack houses, packaging materials, transmission and monitoring.

SINCE its discovery in Wuhan, China, at the end of 2019, the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has infected millions of people worldwide. On 30 January 2020, the World Health Organization (WHO), Geneva, declared SARS-CoV-2 a global public health emergency[1]. As a respiratory disease, SARS-CoV-2 is transmitted through direct contact with respiratory droplets produced by coughing or sneezing[2]. Although evidences suggest that in the beginning this disease was linked with the food being consumed, researchers have indicated that the human digestive system is a potential route of infection[3–5]. According to epidemiological studies, numerous initial cases were associated with a wet market, the Huanan South Seafood Wholesale Market in Wuhan[6]. Foodborne transmission was proposed as a potential risk factor in early COVID-19 discussions, and precautionary measures were recommended for the staff involved in food preparation and distribution. SARS-CoV-2 was detected on a frozen chicken wing sample imported from Brazil on 12 August 2020, marking its presence for the first time in actual food samples[7–9]. All these necessitate a rigorous scientific study on the possibility of this deadly virus surviving on foods.

To prevent the spread of COVID-19, the WHO recommended social isolation, resulting in the global lockdown of businesses, educational institutions and public transportation. The coupled effect of border closure and stay-at-home had increased food losses and export costs, particularly for vegetables and perishable goods. According to Ceylan *et al.*[10], viruses can contaminate food in three ways: contamination of water in which shellfish grow or the water used for washing fruits after harvest, poor hand-hygiene practices, and consumption of animal-based products containing zoonotic viruses. Compared to frozen or wet food items, studies on fruits and vegetables that are commonly purchased commodities are scarce. During the peak of the COVID-19 pandemic, Shah *et al.*[11] monitored SARS-CoV-2 on fruits and vegetables in Philadelphia and neighbouring Pennsylvania, New Jersey and Delaware in the US. They found that the spread of SARS-CoV-2 through fresh foods was unlikely. A concern for India was ensuring food safety during and after the pandemic, as there are numerous open markets here too, where the risk of tactile contamination is high. A large number of workers performing a variety of pre- and post-harvest tasks on site are at risk of contracting the disease[12,13]. Hence, thorough hand washing after handling raw food is essential, although the risk is less for raw items carried at ambient, frozen or refrigerated temperatures over several days[14,15]. However, Telang *et al.*[15] confirmed that this virus could scarcely contaminate fruits and vegetables while drawing their samples from Indian markets, but its long-term survival on fruits and vegetables is limited. Thus, the present study was conducted to examine SARS-CoV-2 infectivity on different fruits, vegetables and packaging materials that are sold in open markets and supermarkets in different parts of India.

Similar to other SARS-CoV and MERS coronaviruses, studies for SARS-CoV-2 showed that the virus is highly stable at 4°C; it is expected to remain infectious at –20°C

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for up to two years like its predecessors[16]. Coronaviruses are thermolabile; SARS-CoV-2 was found to be inactive after 5 min of incubation at 70°C (ref 17). These findings indicate that standard cooking temperatures (>70°C) are sufficient for viral inactivation, although transmission from frozen foods has been a concern. The frequent detection of SARS-CoV-2 in frozen foods suggests that these are not isolated incidents but interlinked. The detection of SARS-CoV-2 in frozen foods, including their packaging materials and storage environments, revealed food transmission evidence in China in early July 2020. Since then, at least nine incidents of food contamination have been reported in China, with SARS-CoV-2 detected on imported foods, mostly on their packaging materials. The virus was found on a salmon cutting board at Beijing's Xinfadi wholesale market. In most of these cases, SARS-CoV-2 was found in frozen shrimps and on their packaging materials and shipping containers imported from Ecuador, South America, indicating that the virus can survive on the packaging surface of cold-chain seafood for at least 20 days[18–20]. Thus, the farm-to-table process favours exposure to food workers, and ambient environments add to the contamination risk.

Researchers have also studied the lifespan of human coronaviruses of inanimate surfaces or built environments. An analysis of the lifespan of human coronaviruses showed that their survival could vary between 2 h and 9 days[21]. Van Doremalen *et al.*[22] showed that the virus could remain viable up to 72 h on plastic and stainless steel, although no viable SARS-CoV-2 was detected after 4 and 24 h application on copper and cardboard respectively. Wei *et al.*[23] reported that frequently touched surfaces such as bedside tables, door handles, floor, pillows and bed sheets were positive for SARS-CoV-2. The presence of SARS-CoV-2 on the frequently handled surfaces of inanimate objects by asymptomatic COVID-19 patients was reported[23]. Recent findings have fuelled speculation that the novel coronavirus can also spread through food packages. Thus proper hygiene is necessary for the food supply chain during the COVID-19 pandemic[24].

The transmission of SARS-CoV-2 was closely monitored by the food safety authorities around the world, such as the European Food Safety Authority (EFSA) and the United States Food and Drug Administration (FDA). Our nodal governmental agency, the Food Safety and Standards Authority of India (FSSAI), has provided guidelines for properly cleaning fruits and vegetables to prevent the spread of COVID-19 through them[25]. It is mentioned that '… potential for foodborne transmission is a concern with every new emerging infection'. Also, '… while coronaviruses appear to be stable at low and freezing temperatures on food surfaces for a certain period; however food hygiene and good food safety practices can prevent their transmission through food'[25].

With the proliferation of COVID-19 cases, we need to develop accurate and rapid technologies for detecting SARS-CoV-2 in food and working environments. Different methodologies have been suggested, such as molecular detection assays based on RT-qPCR, enzyme-free immunosorbent assay and nano-ELISA[26–28]. Researchers have used the RT-qPCR method to detect the virus in fomite samples[29,30]. A few companies have developed commercially available kits for identifying SARS-CoV-2 in environmental swabs, although these findings are preliminary, while others have offered sampling kits for rather expensive surfaces, making it difficult for their broad applications in large facilities of the food sector[31–34]. The use of detection tools for SARS-CoV-2 is warranted in the food sector to ensure food safety and to prevent disruption of the food supply chain.

In order to avoid potential adverse health effects and SARS-CoV-2 transmission in the food supply chain during the COVID-19 pandemic, it is crucial to comprehend the food safety risk factors and test the food items, especially their packaging materials or the food surface. Studies have indicated that grocery stores, particularly supermarkets are high-risk environments for virus transmission due to a combination of risk factors, including enclosed environments, frequently touched surfaces, a large number of people, and consequently, difficulty in maintaining physical distance[25]. It is anticipated that the virus can be easily transmitted through droplets or aerosols from infected humans to buyers/sellers through touch in bazaars or mobile units that sell food items. As only a few studies have been conducted in this regard, we examined real-life samples of fruits, vegetables, hand gloves and packaging materials from open markets in India using the reverse-transcription loop-mediated isothermal amplification (RT-LAMP) kit (indigenously developed by the Indian Council of Medical Research-National Institute of Virology (ICMR-NIV), Mumbai) and licensed to Acrannolife Genomics Pvt Ltd, Chennai). The validity of the methodology was tested by spiking the samples with SARS-CoV-2-positive RNA. This study covers a range of fruits and vegetables in multiple locales on the foodborne transmission of SARS-CoV-2 in the Indian subcontinent.

Materials and methods

Sample collection

The samples of various fruits and vegetables were collected randomly from local markets of different Indian cities in Maharashtra, Gujarat, Telangana, Karnataka, Rajasthan and Tamil Nadu. The sampling locations included most of the agriculturally important regions of the country from where domestic sales and exports take place. Table 1 presents the list of samples and their respective locations. The samples were obtained between 1 October 2021 and 28 February 2022. According to the COVID-19 Dashboard of the Government of Maharashtra, the daily number of positive cases

Table 1. List of samples analysed for SARS-CoV-2 using RT-LAMP assay

Sample	Location (no. of samples)	Total no. of samples	Positive samples	Negative samples
Fruits				
Apple	Maharashtra: Shivane, Pune (3); Navi Mumbai (5); Theur, Pune (3); Mumbai market (5)	16	0	16
Avocado	Maharashtra: Hadapsar, Pune (2); Magarpatta, Pune (2); Thane (4) Karnataka: Hosur, Bengaluru (6)	14	0	14
Banana	Maharashtra: Baramati (7); Pandharpur (2); Nashik (8); Tamil Nadu: Chennai (4)	21	0	21
Coconut	Maharashtra: Chiplun, Ratnagiri (6); Pune (4); Solapur (3)	13	0	13
Fig	Maharashtra: Saswad, Pune (3); Satara (4); Theur, Pune (6)	13	0	13
Grape	Maharashtra: Shewalwadi, Pune (7); Ahmednagar (14); Nasik (12); Sangli (10); Tasgaon, Sangli (12) Tamil Nadu: Chennai (3) Karnataka: Vijayapura (10); Bengaluru (4) Telangana: Hyderabad (8)	80	0	80
Guava	Maharashtra: Uruli kanchan, Pune (2); Theur, Pune (8) Telangana: Hyderabad (3)	13	0	13
Lemon	Maharashtra: Manjri, Pune (3); Hadapsar, Pune (3); Magarpatta, Pune (5); Saswad, Pune (3) Telangana: Hyderabad (3)	17	0	17
Muskmelon	Maharashtra: Satara (2); Shewalwadi, Pune (2); Solapur (4) Karnataka: Vijayapura (4)	12	0	12
Orange	Maharashtra: Nagpur (7); Nasik (2) Karnataka: Hosur, Bengaluru (5)	14	0	14
Papaya	Maharashtra: Hadapsar, Pune (3); Shewalwadi, Pune (3); Tamgaon, Kolhapur (6); Sinhagad road, Pune (2)	14	0	14
Pineapple	Maharashtra: Pune (2); Solapur (3); Sangli (5)	10	0	10
Pomegranate	Maharashtra: Solapur (4), Pandharpur (3); Satara (5); Osmanabad (4) Karnataka: Vijayapura (4)	20	0	20
Sapodilla	Maharashtra: Hinjewadi, Pune (2); Sangli (5) Telangana: Hyderabad (7)	14	0	14
Watermelon	Maharashtra: Wagholi, Pune (3); Hadapsar, Pune (2); Solapur (6) Karnataka: Vijayapura (5)	16	0	16
Sugarcane	Maharashtra: Chikodi, Kolhapur (2); Solapur (6); Nasik (4)	12	0	12
Vegetables				
Beans	Maharashtra: Katraj, Pune (4); Hadapsar, Pune (3); Osmanabad (4)	11	0	11
Betel leaf	Maharashtra: Chikodi, Kolhapur (3) Rajasthan: Udaipur (7)	10	0	10
Bitter gourd	Maharashtra: Bibwewadi, Pune (3); Hadapsar, Pune (2); Panvel, Navi Mumbai (6)	11	0	11
Bottle gourd	Maharashtra: Shewalwadi, Pune (3); Wagholi, Pune (2); Bengaluru (4); Anand, Gujarat (4)	13	0	13
Brinjal	Maharashtra: Kolhapur (2); Sangli (2); Karad (11)	15	0	15
Cabbage	Maharashtra: Shewalwadi, Pune (5); Wagholi, Pune (4); Solapur (2)	11	0	11
Capsicum (green)	Maharashtra: Pimpri Chinchwad, Pune (3); Chikodi, Kolhapur (4); Karnataka: Vijayapura (3)	10	0	10
Capsicum (red and yellow)	Maharashtra: Loni Kalbhor, Pune (4); Magarpatta, Pune (2); Uruli Kanchan, Pune (4)	10	0	10
Carrot	Maharashtra: Warje, Pune (2); Solapur (3) Karnataka: Bengaluru (3); Gujarat: Ahmadabad (4)	12	0	12
Cauliflower	Maharashtra: Pashan, Pune (2); Hadapsar, Pune (4) Karnataka: Bengaluru (3) Telangana: Hyderabad (2)	11	0	11
Chilli	Maharashtra: Shewalwadi, Pune (2); Hadapsar, Pune (4); Wagholi (4); Telangana: Hyderabad (4) Karnataka: Bengaluru (3) Andhra Pradesh: Guntur (4)	21	0	21

(*Contd*)

Table 1. (*Contd*)

Sample	Location (no. of samples)	Total no. of samples	Positive samples	Negative samples
Coriander	Maharashtra: Magarpatta, Pune (3); Solapur (3); Hadapsar, Pune (4)	10	0	10
Cucumber	Maharashtra: Hadapsar, Pune (3); Aurangabad, Pune (4) Karnataka: Bengaluru (3)	10	0	10
Drumstick	Maharashtra: Wagholi, Pune (4); Sangli (4) Telangana: Hyderabad (3)	11	0	11
Fenugreek	Maharashtra: Hadapsar, Pune (2); Shivaji Nagar, Pune (4); Gujarat: Ahmadabad (4)	10	0	10
Garlic	Maharashtra: Rajgurunagar, Pune (3); Navi Mumbai (4) Rajasthan: Udaipur (3)	10	0	10
Ginger	Maharashtra: Aurangabad (6); Hadapsar, Pune (2) Telangana: Hyderabad (3)	11	0	11
Okra	Maharashtra: Kolhapur (4) Karnataka: Bengaluru (3) Telangana: Hyderabad (4)	11	0	11
Onion	Maharashtra: Hadapsar, Pune (3); Shewalwadi, Pune (3); Navi Mumbai (4)	10	0	10
Peas	Maharashtra: Hadapsar, Pune (2); Magarpatta, Pune (2) Telangana: Hyderabad (4) Gujarat: Ahmadabad (4)	12	0	12
Potato	Maharashtra: Hadapsar, Pune (3); Shewalwadi, Pune (5); Ahmednagar (5)	13	0	13
Ridge gourd	Maharashtra: Shivaji Nagar, Pune (2); Bhopal (4); Solapur (4); Akola (2)	12	0	12
Spinach	Maharashtra: Magarpatta, Pune (2); Hadapsar, Pune (4) Karnataka: Bengaluru (3) Gujarat: Ahmadabad (3)	12	0	12
Sprouts	Maharashtra: Hadapsar, Pune (2); Navi Mumbai (4); Solapur (4)	10	0	10
Sweet lemon	Maharashtra: Shewalwadi, Pune (2); Nagpur (6); Bhandara (2)	10	0	10
Sweet potato	Maharashtra: Shewalwadi, Pune (4); Nagpur, Maharashtra (4); Solapur, Maharashtra (3)	11	0	11
Tomato	Maharashtra: Karad (4); Hadapsar, Pune (5) Karnataka: Bengaluru (5) Telangana: Hyderabad (3)	17	0	17
Zucchini	Maharashtra: Shewalwadi, Pune (2); Magarpatta, Pune (2) Karnataka: Bengaluru (4) Tamil Nadu: Chennai (2)	10	0	10
Packaging material				
Cardboard box	Maharashtra: Tasgaon, Sangli (7); Narayangaon, Pune (5)	12	0	12
Hand gloves	Maharashtra: Narayangaon, Pune (4); Ahmednagar (4); Nasik (3); Sangli (5); Tasgaon, Sangli (5); Nagpur (3); Navi Mumbai (3) Tamil Nadu: Chennai (3) Karnataka: Vijayapura (5); Bengaluru (4) Telangana: Hyderabad (4) Gujarat: Anand (3)	46	0	46
Newspaper	Maharashtra: Sangli (8); Nashik (5); Baramati (4)	17	0	17
Plastic bag	Maharashtra: Baramati (5); Sangli (3); Nasik (2) Tamil Nadu: Chennai (2); Gujarat: Anand (3)	15	0	15
Punnet box	Maharashtra: Narayangaon, Pune (12); Nasik (8)	20	0	20
Paper wrapper	Maharashtra: Nasik (10) Telangana: Hyderabad (4)	14	0	14
Total number of samples				748

ranged from 6,553,961 to 7,868,452 over time period[35]. The samples were not prepackaged but readily available in the open markets, where sellers and buyers frequently handled them with their bare hands, and thus the risks were more (Figure 1). Swabs were obtained at random from the surface of these products. Additionally, swabs were taken from used hand gloves and packaging materials such as cardboard boxes, newspapers, plastic bags, wrappers and punnet boxes just after use by farm and pack-house workers. Swabs were also randomly collected from the glove surfaces of field labourers, shopkeepers and roadside fruits and dipped in a viral transport medium (BioEra CAT. No.: BTK/VLTM/01).

They were transported to the laboratory under refrigerated conditions (4°C) and stored at –80°C until RNA extraction was performed.

RNA extraction

RNA was extracted using HiPurA™ Viral RNA Purification Kit (Cat No: MB615), HiMedia, Bengaluru, India. In brief, 140 µl of the RNA sample was taken in a 2 ml Eppendorf tube. To this, 560 µl of carrier RNA-lysis solution was added, and incubated for 10 min at room temperature (24–25°C). Thereafter, absolute ethanol (560 µl) was added and the mixture was transferred to the HiElute miniprep spin column, followed by centrifugation at 8000 rpm for 1 min. Then, the liquid portion was discarded, and the column with the bounded RNA was washed with 500 µl of wash solution (WS). The liquid portion was removed, and the column containing the bound RNA was washed with 500 litres of WS. The washing and centrifugation processes were performed twice. The miniprep spin column containing the bound RNA was dried at 14,000 rpm for 1 min. This column was transferred to a 1.5 ml Eppendorf tube, and RNase-free water was added. The sample was subsequently exposed to RT-LAMP testing.

Figure 1. Representative samples used in the reverse-transcription loop-mediated isothermal amplification (RT-LAMP) assay for SARS-CoV-2.

Table 2. RT-LAMP reaction set up for the detection of SARS-CoV-2

Component	Each 10 µl of RT-LAMP reaction contained	
	N-gene (µl)	*E*-gene (µl)
Covi Qwik master mix	5	5
N-gene primer mix	1.4	–
E-gene primer mix	–	1.4
β-actin primer mix	–	–
Enhancer	0.4	0.4
Sample RNA	3.2	3.2
Total volume	10	10

RT-LAMP

The RT-LAMP reaction was set-up according to the manufacturer's protocol (Table 2). In brief, a reagent mix was prepared freshly by adding the Covi Qwik master mix (5 µl), N gene or E gene (1.4 µl), and enhancer (0.4 µl) and distributed in PCR tubes (6.8 µl), followed by the addition of 3.2 µl of test RNA (RNA sample). Then, the tubes were incubated at 65°C for 30 min in a thermal cycler (GeneAmp PCR system 9700, Applied Biosystems, USA).

Interpretation of RT-LAMP

The RT-LAMP end-point was visually recorded when the colour turned pink to yellow (Figure 2). This change in colour indicated a positive reaction. For a valid test, the positive test control (PTC) tubes should turn yellow, and the negative test control (NTC) tubes should stay pink. The test sample results were recorded separately for the E gene and N gene.

Spiking of SARS-CoV-2 RNA on the surface of samples

The spiking of RNA of SARS-CoV-2 was performed on the surface of a range of selected fruits (apple, muskmelon, grape, sapota, guava and pomegranate) and vegetables (okra, tomato, chilli, potato, onion and curry leaves). The samples were collected from a local market in Hadapsar, Maharastra, Pune. All the samples were thoroughly washed with water to remove debris from their exteriors. Moreover, each sample was spiked with 10 litres of SARS-CoV-2 RNA, which was included in the RT-LAMP kit. After allowing the samples to stand for 5 min, swabs were taken from their spiked surfaces. The RNA extraction (mentioned above) was followed by the RT-LAMP reaction. For confirmation, it was also tested by RT-PCR (Lab Genomics Co Ltd, R, Cat. No. CV9017, Republic of Korea). Each sample analysis was repeated twice. Finally, the outcomes were compiled and compared.

Results and discussion

A total of 748 samples (including fruits, vegetables and packaging materials) from various Indian cities were tested using RT-LAMP. All these samples were found to be free from SARS-CoV-2 RNA. These findings suggest that SARS-CoV-2 may not survive on fruits, vegetables or packaging materials.

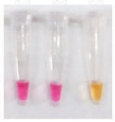
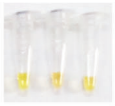
RT-LAMP end-point colour	Visual observation	Interpretation
E N B 	Both E and N gene reaction tubes are pink B-actin gene reaction tube is yellow	Negative for both E and N genes Negative for SARS-CoV-2 Internal control is positive
E N B 	E gene reaction tube is pink and N gene reaction tube is yellow B-actin gene reaction tube is yellow	Negative for E gene and positive for N gene Positive for SARS-CoV-2 Internal control is positive
E N B 	E gene reaction tube is yellow and N gene reaction tube is pink B-actin gene reaction tube is yellow	Positive for E gene and negative for N gene Positive for SARS-CoV-2 Internal control is positive
E N B 	Both E and N gene reaction tubes are pink B-actin gene reaction tube is yellow	Positive for both E and N gene. Positive for SARS-CoV-2 Internal control is positive

Figure 2. Visual observations and interpretation of results for the samples tested using RT-LAMP assay.

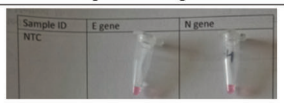
Sample ID	Fruit/ vegetable	RT-LAMP results E gene N gene	Interpretation
NTC	NTC		The test is valid
PTC	PTC		The test is valid
1	Fruit (grape)		Negative for SARS-CoV-2
2	Vegetable (potato)		Negative for SARS-CoV-2
3	Spiked sample (apple)		Positive for SARS-CoV-2

Figure 3. Results for samples tested with the RT-LAMP assay.

Spiking of SARS-CoV-2 RNA on the sample surface

Six fruit samples (apple, grape, guava, muskmelon, pomegranate and sapota) and six vegetable samples (chilli, curry leaves, okra, onion, potato and tomato) were spiked and tested for SARS-CoV-2 RNA to validate the method. The RT-LAMP kit demonstrated a positive test for all spiked samples by turning them yellow after 30 min of incubation at 65°C (Figure 3). When the samples were analysed using RT-PCR, they all yielded similar results. These findings suggest that the methodology used in this study for sample collection and processing is appropriate. Furthermore, to confirm the validity of this method, a few samples were spiked with SARS-CoV-2 RNA. All the spiked samples were reported positive by RT-LAMP and RT-PCR kits, ensuring satisfactory performance (Table 3). In our study, however, no trace of SARS-CoV-2 was detected on the hand gloves of fruit and vegetable handlers and farmers (Table 1).

Previously, Shah *et al.*[11] had used RT-PCR to detect SARS-CoV-2 on the surfaces of fruits and vegetables and showed a 1 in 140 chance of detecting the virus on supermarket produce. Elsewhere, Telang *et al.*[15] suggested that

Table 3. Performance check by spiking of SARS-CoV-2 RNA on the surface of the samples

Sample	RT-LAMP			Real-time RT-PCR
	E gene	*N* gene	Result	
Apple	Yellow	Yellow	Positive	Positive
Muskmelon	Yellow	Yellow	Positive	Positive
Grape	Yellow	Yellow	Positive	Positive
Sapota	Yellow	Yellow	Positive	Positive
Guava	Yellow	Yellow	Positive	Positive
Pomegranate	Yellow	Yellow	Positive	Positive
Okra	Yellow	Yellow	Positive	Positive
Tomato	Yellow	Yellow	Positive	Positive
Chilli	Yellow	Yellow	Positive	Positive
Potato	Yellow	Yellow	Positive	Positive
Onion	Yellow	Yellow	Positive	Positive
Curry leaves	Yellow	Yellow	Positive	Positive

fruits and vegetables most probably do not act as a source of fomite and do not play a significant role in the spread of SARS-CoV-2. They reported that even after fruits and vegetables were handled by COVID-19 patients, no SARS-CoV-2 was detected on the produce within 1 h of storage in areas with a free flow of natural air. We opine that regulatory agency guidelines are adequate and that fruits and vegetables sold in open markets and supermarkets are not a major source of virus transmission, despite the fact that they are not risk-free.

Conclusion

The present study provides a comprehensive understanding of the SARS-CoV-2 transmission through fresh fruits and vegetables, hand gloves and packaging materials collected from different locations in India using RT-LAMP assay. This approach offers a holistic analysis of the risk of transmissibility of SARS-CoV-2 from the surfaces of fresh food. We anticipate that the study would facilitate the domestic and international trade of fruits and vegetables by nullifying consumer apprehensions. Nevertheless, this study is limited to specific localities and matrices, and hence foodborne transmission of SARS-CoV-2 should be further examined, with more food types and sampling from different regions in the future. Despite current evidence suggesting that SARS-CoV-2 does not cause foodborne illness through fresh foods, we recommend that they must be regularly monitored to prevent the virus from spreading in the food chain. We also recommend that our national food agencies should update their buy-local regulations and take initiatives to educate vendors in neighbourhoods to reduce infection size and prevent future outbreaks. The implications of the findings will help government agencies develop strategies for long-term food supply systems. Prior to making important decisions, trade-offs between the health and economic crises must be thoroughly analysed in light of the local context. This can turn the current food safety crisis into an opportunity to create more resilient and sustainable food safety systems.

Ethical approval: The Ethics Committee of each organization involved in this study has approved the manuscript.

1. Yang, P. and Wang, X., COVID-19: a new challenge for human beings. *Cell. Mol. Immunol*., 2020, **17**(5), 555–557.
2. Yu, P., Zhu, J., Zhang, Z. and Han, Y., A familial cluster of infection associated with the 2019 novel coronavirus indicating possible person-to-person transmission during the incubation period. *J. Infect.*, 2020, **221**(11), 1757–1761.
3. Coronavirus: no evidence that food is a source or transmission route, 2020; https://www.efsa.europa.eu/en/news/coronavirus-no-evidence-food-source-or-transmission-route (accessed on 4 May 2022).
4. CDC, Food and coronavirus disease 2019 (COVID-19). 2020; https://www.cdc.gov/coronavirus/2019-ncov/daily-life-coping/food-and-COVID-19.html (accessed on 4 May 2022).
5. Zhang, H., Kang, Z., Gong, H., Xu, D., Wang, J., Li, Z. and Xu, H., The digestive system is a potential route of 2019-nCov infection: a bioinformatics analysis based on single-cell transcriptomes. *BioRxiv*, 2020.
6. Chen, N. *et al.*, Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *The Lancet*, 2020, **395**(10223), 507–513.
7. Sharma, V. K., Jinadatha, C. and Lichtfouse, E., Environmental chemistry is most relevant to study coronavirus pandemics. *Environ. Chem. Lett*., 2020, **18**, 993–996.
8. Eslami, H. and Jalili, M., The role of environmental factors to transmission of SARS-CoV-2 (COVID-19). *AMB Express*., 2020, **10**(1), 92; doi:10.1186/s13568-020-01028-0.
9. Shenzhen Municipal Health Commission (SMHC) detection of SARS-CoV-2 on an imported chicken wing sample. 2020; https://wjw.sz.gov.cn/yqxx/content/post_7998108.html (accessed on 4 May 2022).
10. Ceylan, Z., Meral, R. and Cetinkaya, T., Relevance of SARS-CoV-2 in food safety and food hygiene: potential preventive measures, suggestions and nanotechnological approaches. *Virus Dis.*, 2020, **31**(2), 154–160.
11. Shah, A., Sobek, E. and Shah, V., Presence of SARS-CoV-2 on the surface of fruits and vegetables. *ACS Food Sci. Technol.*, 2021, **1**(3), 316–317.
12. WHO, Critical preparedness, readiness and response actions for COVID-19: interim guidance, 2020; https://www.who.int/publications/i/item/critical-preparedness-readiness-and-response-actions-for-covid-19 (accessed on 4 May 2022).

13. CDC, Water transmission and COVID-19: questions and answers. 2020; https://www.cdc.gov/coronavirus/2019-ncov/php/water.html (accessed on 4 May 2022).
14. BfR, Can the new type of coronavirus be transmitted via food and objects? 2020; https://www.bfr.bund.de/en/can_the_new_type_of_coronavirus_be_transmitted_via_food_and_objects_244090.html (accessed on 4 May 2022).
15. Telang, K., Jain, R., Sodani, A., Shaw, P. and Kosta, S., Do vegetables/fruits act as a vehicle in the spread of COVID-19. *Int. J. Community Med. Public Health*, 2020, **7**, 4150.
16. WHO, COVID-19 and food safety: guidance for food businesses, 2020; https://www.who.int/publications-detail/covid-19-and-food-safety-guidance-for-food-businesses (accessed on 4 May 2022).
17. Chin, A. W. H. *et al.*, Stability of SARS-CoV-2 in different environmental conditions. *Lancet Microbe*, 2020, **1**, 10.
18. *Global Times*, Beijing supermarkets stop selling salmon after wholesalers test positive for coronavirus. 2020; https://www.globaltimes.cn/content/1191462.shtml (accessed on 4 May 2022).
19. Han, J., Zhang, X., He, S. and Jia, P., Can the coronavirus disease be transmitted from food? A review of evidence, risks, policies and knowledge gaps. *Environ. Chem. Lett.*, 2021, **19**(1), 5–16; doi:10.1007/s10311-020-01101-x.
20. Wang, D., Frozen South American white shrimp imported into Chongqing were found positive for nucleic acid of the new coronavirus in some of the outer packaging samples. 2020; http://www.xinhuanet.com/politics/2020-07/15/c_1126242315.htm (accessed on 4 May 2022).
21. Kampf, G., Todt, D., Pfaender, S. and Steinmann, E., Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. *J. Hospital Infect.*, 2020, **104**(3), 246–251.
22. Van Doremalen, N. *et al.*, Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. *N. Engl. J. Med*., 2020, **382**(16), 1564–1567.
23. Wei, L., Lin, J., Duan, X., Huang, W., Lu, X., Zhou, J. and Zong, Z., Asymptomatic COVID-19 patients can contaminate their surroundings: an environment sampling study. *Msphere*, 2020, **5**(3), e00442-20; doi:10.1128/msphere.00442-20.
24. Rizou, M., Galanakis, I. M., Aldawoud, T. M. and Galanakis, C. M., Safety of foods, food supply chain and environment within the COVID-19 pandemic. *Trends Food Sci. Technol.*, 2020, **102**, 293–299.
25. Guidance Note on COVID-19. 2020; https://fssai.gov.in/upload/uploadfiles/files/Guidance_Note_COVID_15_04_2020.pdf (accessed on 4 May 2022).
26. Bosch, A. *et al*., Foodborne viruses: detection, risk assessment, and control options in food processing. *Int. J. Food Microbiol*., 2018, **285**, 110–128.
27. Wu, L. *et al.*, Highly sensitive enzyme-free immunosorbent assay for porcine circovirus type 2 antibody using Au-Pt/SiO_2 nanocomposites as labels. *Biosens. Bioelectron*., 2016, **82**, 177–184.
28. Wu, L., Li, G., Xu, X., Zhu, L., Huang, R. and Chen, X., Application of nano-ELISA in food analysis: recent advances and challenges. *TrAC – Trends Anal. Chem*., 2019, **113**, 140–156.
29. Guo, Z. D. *et al*., Aerosol and surface distribution of severe acute respiratory syndrome coronavirus 2 in hospital wards, Wuhan, China. *Emerg. Infect. Dis.*, 2020, **26**(7), 1583–1591; doi:10.3201/eid2607.200885.
30. Li, Q. *et al.*, Early transmission dynamics in Wuhan, China of novel coronavirus-infected pneumonia. *N. Engl. J. Med.*, 2020, **382**(13), 1199–1207.
31. Chai's Coronavirus environmental test kit. 2020; https://www.chaibio.com/coronavirus-environmental-test-kit (accessed on 4 May 2022).
32. Eurofins, Detection of SARS-CoV-2 in swabs of environmental surfaces. 2020; https://www.eurofins-technologies.com/products/viruses.html (accessed on 4 May 2022).
33. Hawks, L., Woolhandler, S. and McCormick, D., COVID-19 in prisons and jails in the United States. *JAMA Intern. Med.*, 2020, **180**(8), 1041–1042.
34. Tentamus, New sampling kit to test your surfaces for coronavirus. 2020; https://www.tentamus.com/new-test-kit-surfaces-coronavirus/?cn-reloaded=1&cn-reloaded=1 (accessed on 4 May 2022).
35. COVID-19 dashboard Maharashtra government; https://www.covid19maharashtragov.in/mh-covid/dashboard (accessed on 4 November 2021).

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