Presence of SARS-CoV-2 on surfaces and materials in supermarket social areas in Türkiye

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ABSTRACT

The aim of this study was to monitor the presence of SARS-CoV-2, the virus that cause the coronavirus disease 2019 (COVID-19), particularly on certain foods and surfaces that come in contact with food in district supermarkets in Ankara, Türkiye, where the highest number of COVID-19 cases was reported based on data from the Ministry of Health. For this purpose, a total of 172 samples were taken from 5 supermarkets in 4 districts in Ankara. RNA was extracted from the samples and RdRp gene-targeting reverse transcription quantitative polymerase chain reaction (RT-qPCR) assays were used to determine the presence of SARS-CoV-2. The results showed that all the supermarket samples collected during the period when there was a high number of COVID-19 cases in the district did not have SARS-CoV-2 except for one sample that was taken from a supermarket where COVID-19 had been detected among the staff. In this supermarket, COVID-19 RNA was detected with a high number of copies of 5 000, using Real-Time RT-PCR assay in pooled swab samples taken from salt shakers, pepper shakers, red pepper shakers, and vinegar and oil bottles in the social area that the staff used for lunchbreaks and other breaks. This finding shows that it is of great importance for public health agencies to monitor COVID-19 cases in food businesses in regions with a high number of cases and to take samples from these businesses at certain intervals, as a form of "early warning system."

Introduction

Coronoviruses (CoVs) were first known as viral agents related to the *Coronaviridae* family, which caused significant health problems mainly in mammals, birds, and fish. Later, human CoVs were discovered, associated with mild respiratory disorders (9, 17). In 2003, the severe acute respiratory syndrome-associated coronavirus (SARS-CoV) broke out globally; followed 10 years later by the Middle East respiratory syndrome-related coronavirus (MERS-CoV), which affected a more limited geographic area (26). In December 2019, a new coronavirus emerged in Wuhan, China. The novel coronavirus (2019-nCoV) was identified from a patient's broncho-alveolar lavage fluid (28). Full genome sequencing and phylogenetic analysis revealed that it belongs to a distinct clade from the betacoronaviruses associated with the previous SARS-CoV and MERS-CoV (28). The International Virus Taxonomy Committee named the virus SARS-CoV-2 and classified it under the genus *Betacoronavirus*. Although the main source of the transmission of SARS-CoV-2 has not yet been clearly determined, it is presumed to have been transmitted to humans from wild animals sold in the Huanan Seafood Wholesale Market in Wuhan (5). The disease caused by

this virus was named coronavirus disease 2019 (COVID-19) by the World Health Organization (WHO).

A total of 124,218,483 cases of COVID-19, 2,734,668 of which resulted in death, have been reported worldwide from the start of the epidemic to March 28, 2021 (1). Official authorities, especially WHO, emphasize that the main route of transmission of SARS-CoV-2 is person to person through respiratory droplets released during coughing, sneezing, and/or speaking (25). Studies have reported that other potential routes of transmission are droplet-contaminated surfaces or aerosols (21). Therefore, people can indirectly come in contact with COVID-19 by touching a contaminated surface and then touching their mouth, nose, or eyes (27). Although no direct foodborne transmission has been reported, examination of clinical records has revealed high incidence rates of gastrointestinal symptoms, particularly diarrhea, nausea, vomiting, and abdominal discomfort (2, 11, 14, 29, 12).

Considering this information, this study was carried out to investigate if SARS-CoV-2 can be detected in foods offered for sale in supermarkets and on surfaces that come in contact with food in such supermarkets in the districts of Ankara province in Türkiye, where the number of COVID-19 cases was reported by the country's Ministry of Health as the highest in the country. In addition, samples taken from the materials and surfaces used by the supermarket staff while eating in the social areas of the supermarket were collected and included in the study.

Materials and Methods

A total of 172 samples were taken from 5 supermarkets in 4 districts in Ankara. First, samples were collected from a supermarket in district that was closed officially because COVID-19 had been detected in some staff. The samples were taken both from the staff's social area and from the sales areas. Next, after examining the data from the Ministry of Health on districts with high numbers of COVID-19 cases, the districts of were chosen for further sampling. All samples were collected from popular supermarkets in these districts.

Collection of samples: All staff who participated in the collection of the samples were trained on sample collection, labeling, documentation, and biosecurity measures, and were transported from the laboratory to the collection sites and back to the laboratory. During the sampling, the sample collectors wore high-level biosecurity clothing. All the samples were collected after the closing times of the supermarkets, when all customers and staff have left and before the routine cleaning procedures. The surface samples were collected using the swab method, but fresh vegetables and fruits that could be consumed unpeeled were collected as whole foods. The surfaces from which samples were taken were "high-touch surfaces" by customers and supermarket personnel, as identified in literature (24).

The samples were delivered to the Etlik Veterinary Control Central Research Institute Virology Laboratory under a cold chain within two hours, where they were subjected to PCR analyses.

Sampling model: In each supermarket, 3 swab samples from each of the surfaces in the list below (in nos. 1 and 2) and 3 whole food samples of the fruits and vegetables listed below (in no. 3) were taken.

1. Surfaces
   a. Handles of supermarket trolleys
   b. POS device
   c. Cupboard and refrigerator covers and holders
   d. Cashier counters (movable bands)
   e. Butcher counter and cutting boards
   f. Staff gloves from different sections
2. Food packaging surfaces
   a. PET bottles (water bottles, other drink bottles, etc.)
   b. Glass vials
   c. Paper packaging (of flour, chocolate, etc.)
   d. Plastic packaging (of chips, pasta, yoghurt/cheese, etc.)
   e. Cardboard packaging products
3. Vegetables and fruits
   a. Green leafy vegetables (lettuce, roka, parsley, etc.)
   b. Fruits (plums, strawberries, etc.)

Apart from the above samples, a total of 33 samples were taken from plastic surfaces such as salt shakers, pepper shakers, red pepper shakers, and vinegar and oil bottles used only by the personnel in the staff social areas of the supermarket with confirmed COVID-19 cases among its personnel. The samples were taken 36 hours after the supermarket was closed down due to the COVID-19 cases. No one had entered the officially closed plant during this period, and the samples were collected before the supermarket was cleaned and disinfected.

The surface swab samples were taken using a swab with a synthetic tip and a plastic shaft. The swab specimen collection vials contained 1.5-2.0ml sterile water. The sampling was done from 3 to 4 different material surfaces. The swab surface area was planned to be approximately 25 cm^2 (24). To increase the positive predictive value of the sampling process, each sampling area was swabbed several times. All the swabs were transported to the laboratory under a cold chain within two hours.

Extraction and real-time RT-PCR protocol: The swab samples were vortexed and 200µl samples were separated for RNA extraction. The RNA was extracted with the

cador Pathogen 96 QIAcube HT Kit using the QIAcube automated extraction robot (Qiagen, Hilden, Germany).

Primers and probe sets that targeted the SARS-CoV-2 RdRp gene were used (7). A 20μl reaction was set up that contained 5 μl of RNA and 12.5 μl of the 5x reaction buffer provided in the QuantiNova Pathogen+IC Kit (Qiagen, Hilden, Germany) that contained 0.8 nM each of a forward primer and a reverse primer and a 0.25nM probe. Thermal cycling was performed at 50°C for 10 minutes for the reverse transcription, followed by 95°C for 3 minutes and then 45 cycles each of 95°C for 5 s and of 58°C for 30 s. The RT-qPCR assays were performed using a Bio-Rad CFX96 thermal cycler (Bio-Rad Laboratories, Montreal).

Positive and negative controls were used for accuracy. The ETLVET3 (MW306668) isolate was used as the positive control. The DNA amount of the SARS-CoV-2 RdRp gene region was also measured. The SARS-CoV-2 RdRp gene region base was serially diluted according to log10 and was used as a standard in the evaluation of the results.

Results

All the samples from the supermarkets that were selected based on the high number of COVID-19 cases in their district were SARS-CoV-2-negative. However, the COVID-19 RNA was detected by real-time RT-PCR in the samples from the supermarket that had positive cases among its personnel.

In that supermarket, the pooled swab samples taken from salt shakers, pepper shakers, red pepper shakers, and vinegar and oil bottles that the staff used at meal times in their social area were positive.

The raw data, standard curve results, and copy number quantification summary were exported from Biorad CFX Manager Version 3.1. The slope of the standards was determined as -3.25 with an R^2 value of 0.99 and a reaction efficiency of 103%.

As a result of the quantitation, the virus copy numbers in these samples were determined as 5 000 with a Ct value of 32. On the other hand, the other samples from this supermarket were determined as negative. The real-time RT-PCR result diagram is shown in Figure1.

Discussion and Conclusion

This study was performed to investigate the presence of SARS-CoV-2 in some foods, different contact surfaces, and different food packages in supermarkets in regions where the number of COVID-19 cases is high. According to our results, all the samples were negative except for one supermarket that had positive cases among its staff, where SARS-CoV-2 RNA was detected with a very high copy number, due to which the supermarket was officially closed. The detection of SARS-CoV-2 in the swab samples taken from the salt shakers, pepper shakers, and vinegar and oil bottles that were used by the personnel and were pooled 36 hours after the official closure of the supermarket is of great importance.
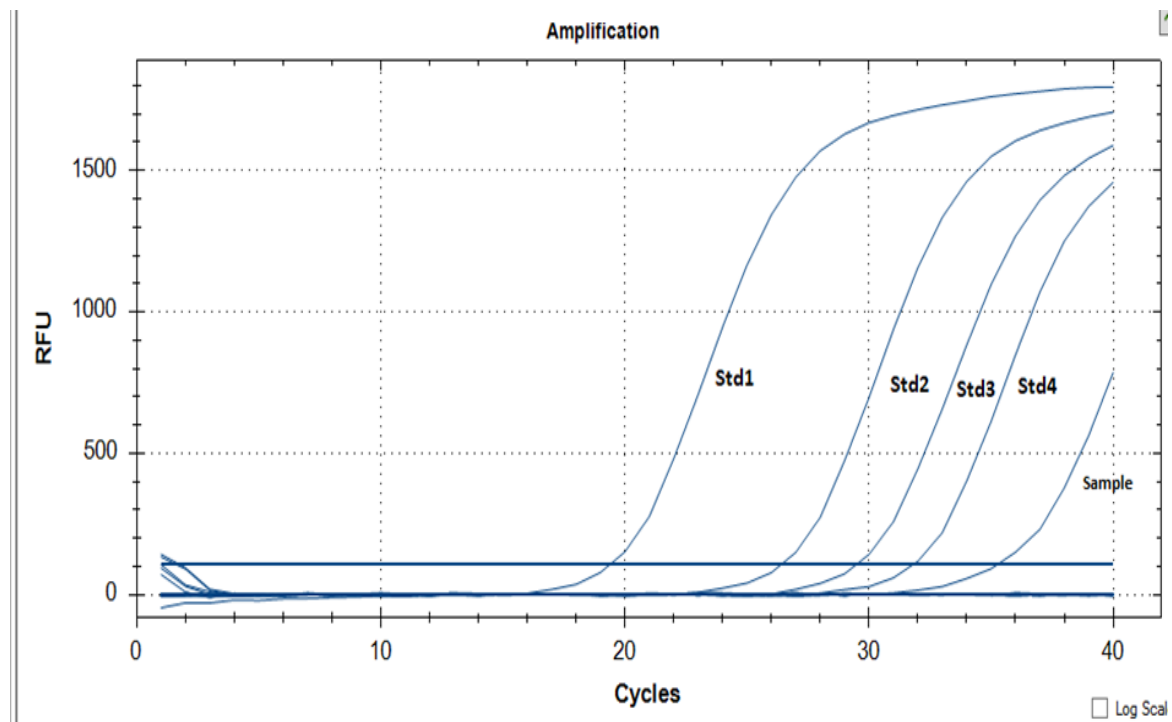

Figure 1. Real time RT-PCR result of a positive sample.

These results show that if personnel in food businesses are infected with SARS-CoV-2, they may infect the surfaces that food and customers come in contact with. Considering that the sampling was done 36 hours after the supermarket was closed, it showed that the virus RNA could be found on surfaces for more than 24 hours. Furthermore, the surfaces where the samples were found to be positive were used during eating and socializing, at which times people have a lot of contact with their mouths and noses. Although the customers were unlikely to have come in contact with infected droplets here, the results emphasize the negative impact of hygiene and attention deficit, considering the mouth-nose-eyes infection route. Cai et al. (2) reported that people may indirectly contract SARS-CoV-2 by touching their mouth, nose, or eyes after touching a contaminated surface. Considering our findings, it is thought that personnel working in food businesses, with or without symptoms, may pose significant public health hazards in cases where the necessary precautions are not taken, considering the potential droplet contamination.

It is general knowledge that viruses are obligate intracellular microorganisms. Thus, they cannot reproduce in environments other than living bodies, and they lose their vitality and infectivity doses over time. On the other hand, there have been studies on the viability of SARS-CoV-2 on different surfaces under approximate room temperature conditions. In one such study, cardboard, stainless steel, and plastic surfaces that were experimentally contaminated with 10^5 viruses were kept under room temperature conditions with 40% humidity. It was found that SARS- CoV-2 can survive on cardboard, stainless steel, and plastic surfaces for 24 hours, 48 hours, and 72 hours, respectively (21). Another experimental study with a similar number of viruses showed that the virus could survive for up to 21 days in biosafety equipment, especially in masks and gloves (13). Although this result was extreme, the findings from these two studies coincide with our findings from this study. We also detected SARS-CoV-2 RNA in the samples that we took 36 hours after the closure of the infected supermarket.

WHO and the United Nations Food and Agriculture Organization (FAO) stated in their guidelines published in April 2020 that the possibility of COVID-19 transmission through food or food packaging is low. This guide noted that COVID-19 is a respiratory disease, and the primary route of transmission is through person-to-person contact through direct contact with respiratory droplets from the infected person when the person coughs or sneezes. The guidelines also state that there is no evidence of SARS-CoV-2 transmission through food or food packaging (23). Since the publication of the guide, no other source of transmission of the disease has been reported besides the respiratory tract. However, this does not eliminate the possibility of fecal-oral contamination of food packaging or contact surfaces or their contamination with droplets from infected persons.

According to many studies conducted in different countries, sewage systems can be considered an important sources of transmission of coronavirus (14, 18). The similarity between the results obtained in the first SARS-CoV epidemic and the current findings is striking. In 2003, during the SARS-CoV outbreak, Lee (15) reported that the sewage system in Hong Kong served as a reservoir because the sewage system accommodated the feces of SARS-CoV patients. During the same period, SARS-CoV RNA was found in the sewage systems of two hospitals in Beijing (22). Chan et al. (4) also stated that the fecal excretion of SARS-CoV RNA may take more than 30 days.

The presence of SARS-CoV-2 in the feces of patients, in wastewater, on surfaces used in experimental studies, and finally, especially in frozen foods, is of great importance in terms of the characteristics of the agent, its spread, and its different potential transmission routes (3, 19, 20, 10, 12, 16).

Considering the homology between these three coronaviruses (SARS-CoV-2, SARS-CoV, and MERS-CoV), another confirmation of possible fecal-oral contamination emerges with a low-temperature tolerance of up to two weeks and a survival capacity of 20–30°C. The longer-term persistence of SARS-CoV-2 in the stool compared to respiratory swabs provides strong clinical evidence that fecal-oral transmission is likely (8, 11). Cheung et al. (6) reported that while 70.3% of the stool tests of the patients remained positive for SARS-CoV-2 RNA, their respiratory tract samples were negative. Additionally, Zhang et al. (27) confirmed the presence of live viruses in stool samples; and stool can contaminate food, water, hands, etc. and cause infection. Although these results strengthen the hypothesis that the virus may have a chance of spreading through the fecal-oral route, such hypothesis has not yet been proven.

To the best of our knowledge, this study is the first to detect SARS-CoV-2 from the salt shakers, pepper shakers, and vinegar and oil bottles used on tables during staff meals in the staff social area of a supermarket where some staff contracted COVID-19. The results show how important it is for public health agencies to monitor food businesses for COVID-19 cases and to take and test samples from these businesses. In our results, in addition to the detection of the SARS-CoV-2 RNA in the supermarket with active staff cases, the copy number there was found to be high. It is likewise important that the RNA of the agent was not detected in the supermarkets whose staff were not known to be positive for COVID-19. For these reasons, collection and testing of samples from supermarkets, restaurants, and other food-related

establishments in regions with high numbers of COVID-19 cases at certain intervals should continue as a form of "early warning system," as it has been determined that there is a possibility of transmission of the virus to surfaces that food and customers come in contact with, especially in food businesses, if the personnel carry the agent, whether symptomatically or asymptomatically. Considering that the sampling was done 36 hours after the establishment was closed, it was determined that the virus could be found on the surfaces of the materials used during eating for more than 36 hours. Thus, it is thought that the people who came in contact with the droplets there also had hygiene and attention deficit, since they acquired the agent through their mouth, nose, and eyes.

Conflict of Interest
The authors declared that there is no conflict of interest.

Author Contributions
MG, NDA and HS conceived and planned the study. BP and HS approved the plan and publication of the study. MG, NDA, SH, SÖY, ÖY and CY contributed the sample collection procedures. SH carried out the experiments. MG, NDA, SH and CY contributed the interpretation of the results. MG took the lead in writing the manuscript. All authors provided critical feedback and helped the research, analysis and manuscript.

Data Availability Statement
The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement
This study does not present any ethical concerns.

Animal Welfare
Not applicable.

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