



The Spread of a Norovirus Surrogate via Reusable Grocery Bags in a Grocery Supermarket

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Abstract The conventional supermarket represents an important public access to a wide variety of food that is vital for healthy families. The supermarket is also a location where food, the public, and pathogens can meet. The purpose of this study was to develop and test a hypothesized norovirus transmission pathway via reusable grocery bags (RGBs) within a conventional grocery supermarket. An RGB was inoculated with a surrogate virus to assess potential transport of pathogens within a grocery store. Volunteer shoppers were given an RGB sprayed with a surrogate (bacteriophage MS2) upon entry to a grocery store. A surrogate is defined in this study as an organism, particle, or substance that is used to study the fate and transport of a pathogen in a specific environment (Sinclair, Rose, Hashsham, Gerba, & Haas, 2012). The study personnel swabbed all surfaces touched by the volunteer shopper to recover the MS2 surrogate. The data show that MS2 spread to all surfaces touched by the shopper; the highest concentration occurred on the shopper's hands, the checkout stand, and the clerk's hands. The high concentration of MS2 on hands justify a recommendation for in-store hand hygiene as a primary preventive measure against transmission of infectious pathogens. The high concentrations on the checkout stand justify a secondary recommendation for surface disinfection and public education about washing RGBs.

Introduction

Norovirus is a pathogenic RNA virus that is the leading cause of acute gastroenteritis from contaminated food in the U.S. Outbreaks occur in restaurants, schools, hotels, home care facilities, cruise ships, and in the wilderness tourism industry. Norovirus outbreaks occur often during times of low humidity such as the winter season in temperate zones (Colas de la Noue et al., 2014; Jones, Gaither, Kramer, & Gerba, 2009; Seitz

et al., 2011). These viruses are a major concern for surfaces and fomites in the food production, service, and grocery retail industries (U.S. Department of Health and Human Services, 2018).

The objective of this study was to test a hypothesized norovirus transmission pathway via reusable grocery bags (RGBs) within a conventional grocery supermarket. A true norovirus transmission pathway is not possible to evaluate in a public setting, so a bacte-

riophage is used as a safe surrogate to assess the presence and concentration of the virus. A surrogate is defined here as an organism, particle, or substance that is used to study the fate and transport of a pathogen in a specific environment (Sinclair et al., 2012).

The MS2 bacteriophage is a suitable surrogate for norovirus because it is a single-stranded RNA virus with a similar structure and size to most noroviruses (Beamer et al., 2014). The MS2 bacteriophage surrogate can be used to model the survival, morphology, and transport characteristics of norovirus without the infection risk or the necessity of mammalian cell culture facilities (Dawson, Paish, Staffell, Seymour, & Appleton, 2005). This experiment models norovirus transmission with the surrogate MS2 introduced into a grocery store through an experimentally contaminated RGB.

This study builds on a previous work that investigated the potential for contamination in RGBs. The findings were that over 10% of all bags obtained from shoppers contained fecal indicator bacteria and that only 3% of all shoppers had reported ever washing their bags (Williams, Gerba, Maxwell, & Sinclair, 2011). Other studies have linked reusable bags with a norovirus outbreak in the U.S. Northwest (Repp & Keene, 2012) where an RGB was contaminated with aerosolized norovirus from an infected individual. This study investigates the potential for contaminated RGBs to distribute viruses within a public grocery store.

This study's hypothesis is that norovirus could be spread from a contaminated RGB to various public surfaces in the grocery store (Figure 1). The study purpose is to provide data that can help identify critical control

TABLE 1

The Log Concentration (LC), Category (Cat), Arithmetic Mean (μ), Geometric Mean (GM), Standard Deviation (SD), Number of Items Swabbed in the Grocery Stores (n), and Percent of Initial Inoculum (%I)

Item	Identification Letters	LC	Cat	μ	GM	SD	n	%I
Seeded reusable grocery bag	a	9	–	4.81×10^9	8.58×10^8	7.27×10^9	25	100
Hands								
Customer	b	7	C1	7.02×10^7	4.10×10^7	7.52×10^7	9	1.4600
Clerk	c	7	C2	9.30×10^7	9.30×10^7	9.30×10^7	4	1.9300
Cart								
Handle	d	5	C1	3.46×10^5	2.91×10^5	1.59×10^5	9	0.0072
Surface	e	5	C1	6.47×10^5	2.38×10^5	8.13×10^5	6	0.0130
Checkout								
Scale	f	5	C1	1.43×10^5	9.05×10^4	1.52×10^5	4	0.0030
Conveyor	g	4	C1	6.95×10^4	2.60×10^4	5.73×10^4	13	0.0014
Customer bumper	h	4	C1	6.07×10^4	1.23×10^4	7.97×10^4	6	0.0013
Clerk keyboard	i	4	C2	5.58×10^4	2.54×10^4	4.51×10^4	4	0.0012
Clerk table	j	4	C2	5.25×10^4	1.56×10^4	5.00×10^4	4	0.0011
Customer keyboard and table	k	5	C2	1.54×10^5	1.50×10^5	3.71×10^4	4	0.0032
Food								
Packaged	l	7	C2	2.90×10^7	3.75×10^5	5.91×10^7	11	0.6020
Unpackaged produce	m	6	C2	2.36×10^6	3.04×10^5	4.22×10^6	9	0.0490
Other								
Freezer handle	n	4	C2	2.45×10^4	1.94×10^4	1.17×10^4	6	0.0005
Other handle	o	4	C2	2.42×10^4	1.39×10^4	2.86×10^4	5	0.0005
Incentive card	p	5	C2	3.91×10^5	3.34×10^5	1.90×10^5	6	0.0081

Note. Darker shades of grey indicate a higher LC. The identification letters are used in reference to Figure 3.

points that could be the focus of improved norovirus management strategies in grocery stores.

Methods

Our hypothesized virus transmission pathway was developed in this field study using RGBs and a nonpathogenic microbial surrogate for norovirus. Volunteer shoppers were recruited in front of three grocery stores in California and instructed to complete their planned shopping trip using an RGB that the study team provided. The volunteer shopped using a store-provided grocery cart and was followed by a study team member who swabbed surfaces and items contacted by the volunteer shopper. The three site visit trips involved traveling to Atascadero, Ceres, and Madera in the Central Valley of California. The temperature and humidity were recorded

after the study using historical data from the closest National Climatic Data Center (NCDC)-affiliated weather stations to each grocery store site (NCDC, 2012).

A microbial surrogate was used to safely trace the norovirus transmission pathway in the presence of customers. The norovirus surrogate chosen was the MS2 single-stranded RNA bacteriophage obtained from American Type Culture Collection (ATCC #15597-B1). The MS2 surrogate was used because it can be produced in large numbers at low cost, can be easily detected, and is nonpathogenic (Sinclair et al., 2012). Also, MS2 is a safe, noninfectious laboratory strain not found in the natural environment or on fomites. For this reason, it was not necessary to decontaminate the bags and surfaces in the store before the study initiation.

Grocery stores were selected by contacting the California Grocers Association and the Environmental Safety Alliance. These two stakeholders were able to obtain access permissions and staff participation from three stores owned by PAQ, Inc. The three supermarkets were a Food4Less store in Atascadero, California, measuring 3,530 m²; a Food4Less store in Ceres, California, measuring 2,880 m²; and the Rancho San Miguel Market in Madera, California, measuring 5,561 m². The store area estimates were calculated using the polygon tool in Google Earth version 7.0.3.8542 (2013).

All three stores were of a similar layout with an identical checkout stand, allowing the customer to self-bag the groceries. The Rancho San Miguel Market is a larger store that closely resembles a Food4Less with added Latino food

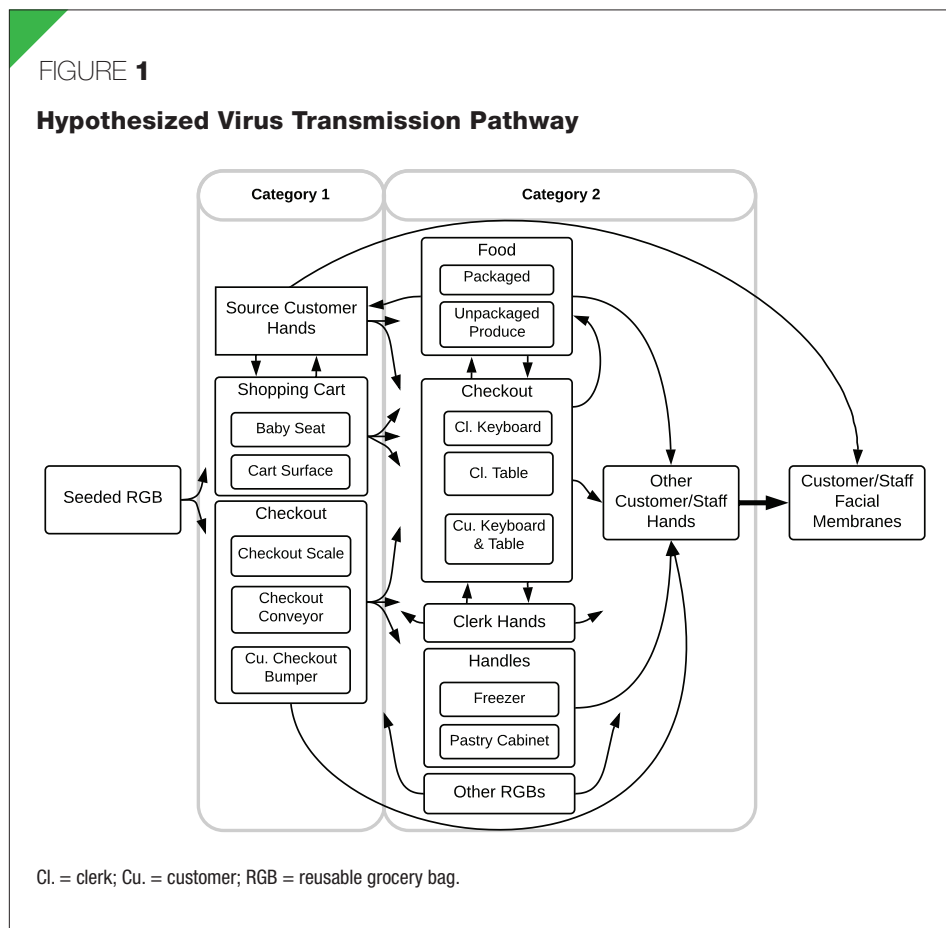
products. The majority Latino population in Madera (76.7%) and Ceres (56.0%) represent young Latino families, with the Atascadero market catering towards a White community (76.7%) (U.S. Census Bureau, 2010). The Atascadero location is a cooler coastal climate, while the other two stores are in the Central Valley of California.

The new RGBs used in the study were purchased for 98 cents each from the three stores on the date of the study. The bag material was unwoven polypropylene, a common cloth-like synthetic typically used for inexpensive RGBs. The same bag was available at each of the three stores and was sized at 36 x 34 x 18 cm and printed with a logo of Food4Less or Rancho San Miguel markets.

The bag and its handles were thoroughly sprayed with 5 mL of a 10^9 PFU/mL-concentration MS2 solution suspended in sterile Ringer's solution (Fisher Scientific). The spray bottle was a sterile 250 mL low-density polyethylene plunger-style bottle (Bel-Art, Fisher Scientific) that was prepped with a fresh MS2 surrogate stock for each of the study days. The MS2 bacteriophage #15597 was obtained from ATCC and propagated using the U.S. Environmental Protection Agency single-agar layer method (Ohio Environmental Protection Agency [EPA], 2005). A lawn of the host *E. coli* strain (ATCC #15597) allowed heavy plaque formation on trypticase soy agar (Difco) after 24 hr of incubation at 37 °C. The plaques were scraped from the surface of the agar and placed in 50 mL sterile centrifuge tubes along with 30 mL of sterile Ringer's solution buffer.

The MS2 surrogate concentration used in this study is a similar high concentration to norovirus that can be shed by an infected individual's vomit or feces (Sinclair, Jones, & Gerba, 2009). The RGBs were hung to dry in ambient outdoor air for 15 min, then folded and placed in individual, sealed Ziplock bags.

Volunteer shoppers were recruited from the entryway of the store after verbal consent, a brief introduction to the study purpose, and instructions. The shoppers agreed to shop for their normal items and to shop for the included list of items needed for the study. The volunteers were provided a list of items designed to control the travel and contact that the customers had throughout the store. Shoppers were motivated to follow through with the study by being informed



that they would receive an incentive at the end of the study.

The incentive was not disclosed upon recruitment; however, at the end of the study volunteers were given \$10–\$15 store credit on a gift card. The volunteers were then given the RGB to use when they checked out at the designated checkout stand after the completion of their shopping errand. Volunteers were given the RGB and intentionally not told what to do with the bag as they carried or carted it throughout the store. The study team ensured that the volunteer shoppers did not use a grocery cart used by a previous study participant. The Loma Linda University Institutional Review Board granted this study a waiver, because there were no health risks, no individual identifiers, and no health data were recorded.

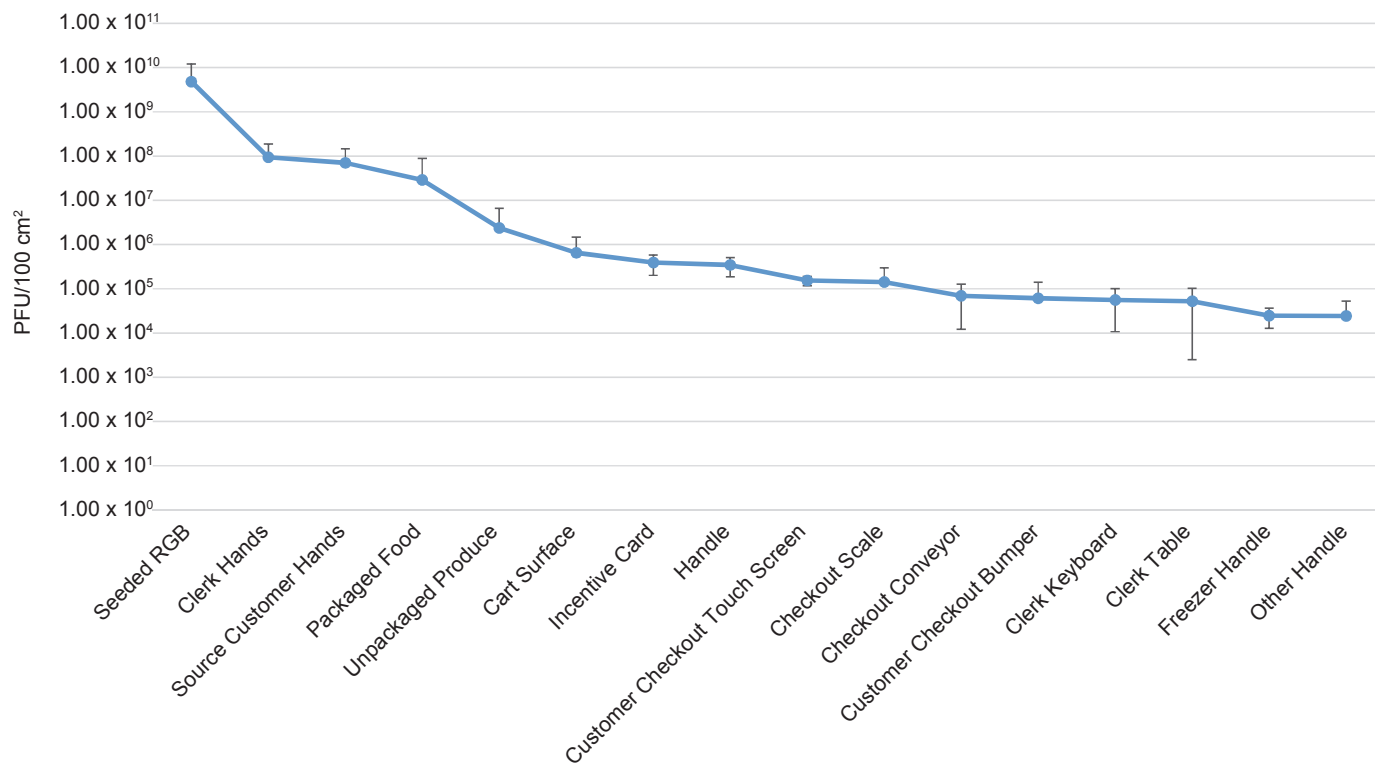
As the volunteer shopper moved throughout the store, a study team member swabbed a 100 mm² area of various surfaces that the shopper touched. For handles and other items that were not flat, the study team

member swabbed the area touched by the volunteer shopper and then labeled the tube with the surface area of that particular surface. The rayon-tipped swabs (Fisher Scientific) were stored in 5 mL of Ringer's solution (Fisher Scientific) as a transport buffer, placed in an ice cooler, and transported back to the Loma Linda Environmental Microbiology Research Laboratory.

The study team also swabbed all surfaces at the checkout area, the grocery cart, the RGB, the fingers of the shoppers, and the fingers of the checkout counter clerk. The study also collected a series of negative controls such as the fingers of the volunteer–customers before they entered the store, several surfaces before study initiation, and a noncontacted shopping cart. The samples were processed using the U.S. EPA single-agar layer method (Ohio EPA, 2005) with antibiotics to remove background bacteria. The *E. coli* strain (ATCC #15597) was used to host the MS2 plaque formation. The plates were incubated at 37 °C for 24 hr and then counted for plaques.

FIGURE 2

Average Concentrations of MS2 Surrogate on Surfaces Contacted by the Reusable Grocery Bag or Shopper's Hand in the Grocery Store



PFU = plaque-forming unit; RGB = reusable grocery bag.

Results

This study presents concentrations of the MS2 bacteriophage on varying grocery store surfaces (Table 1). The data are presented with standard measures of central tendency such as the geometric means (GM) to show differences in magnitude, the percentage of the initial inoculum (%I), the number of surfaces sampled (*n*), and the standard deviation (*SD*). Table 1 also presents the log concentration of the MS2 bacteriophage (LC) and a category for the hypothesized exposure route.

Data are summarized for each surface and category per the hypothesized model presented in Figure 1. The far-left box is the initial contaminated RGB, while the far-right box indicates hand-to-face exposure that signifies the end of the exposure route and potential infection. Surfaces are categorized by the order of contact with a contaminated RGB. The category 1 (C1) surfaces include items that come

in direct contact with the RGB and then a shopper's hand. The category 2 (C2) surfaces come in contact with a C1 surface or hand before becoming contaminated. An example of C2 is the clerk's keyboard at the checkout counter, which would not become contaminated until the clerk first touched the contaminated RGB or contaminated food item.

For simplicity, only two contact categories are presented here, as there are hundreds of possible routes of exposure. Some surfaces in Figure 1 were included for continuity (e.g., facial membranes and other RGBs) and not measured during the experiment. The C1 surfaces with direct contact to the volunteer shopper's hand or RGB had higher overall concentrations of MS2 than the C2 surfaces. The hypothesized virus transmission model was largely validated and the only exception was the high concentration on the checkout clerk's hands (Figures 1 and 2).

The highest concentration of 10⁹ PFU/cm² was the RGB seeded in this study. The source customer hands had the second highest concentration at 9.3 x 10⁷ PFU/cm² (or 1.93% of initial inoculum), with the packaged food as the third most contaminated (Figure 2). The initial concentration of 10⁹ PFU/100 cm² is shown in Table 1 with the log concentration of other items in lighter shades of grey. Due to the high concentration in the initial RGB, the lightest shade of grey still represents a relatively high concentration of virus at 10⁴ PFU/100 cm². The initial concentration of 10⁹ PFU/100 cm² represents environmental concentrations of infectious norovirus particles that have been reported as higher than 10⁹ genome copies/g feces (Lee et al., 2007).

The packaged food was sampled on the handle portion of the plastic bag and had a higher concentration of MS2 than unpackaged food. This finding was not statistically

significant ($t = 0.35$, $p = .72$, $df = 17$) but might indicate a higher percent transferability to the plastic from hands and a higher percent recovery from plastic material to the sample swabs. Most packaged food sampled in this study was in typical grocery store packaging: polypropylene fibrous sacks for produce, clear bags made from polypropylene resins or polyethylene terephthalate (PET) for breads and potatoes. All other C2 handles in the three stores were found to have a lower concentration of the bacteriophage surrogate.

The data in Table 1 are presented for all three grocery stores because they were found to be statistically similar; analysis of variation (ANOVA) showed no significant difference in the mean concentrations of MS2 on the seeded RGB across three different stores ($df = 2$, $F = 2.50$, $p = .151$). The samples were collected in the late afternoon to early evening in the three stores to characterize the time with the most customer traffic. The relative humidity (RH) and temperature were typical for the temperate climate zone during the winter visits on December 15, 2012, and February 8, 2016. The RH and temperatures were 64% and 10.6 °C for Ceres, California; 51% and 22.2 °C for Madera, California; and, 74% and 7.8 °C for Atascadero, California (NCDC, 2012).

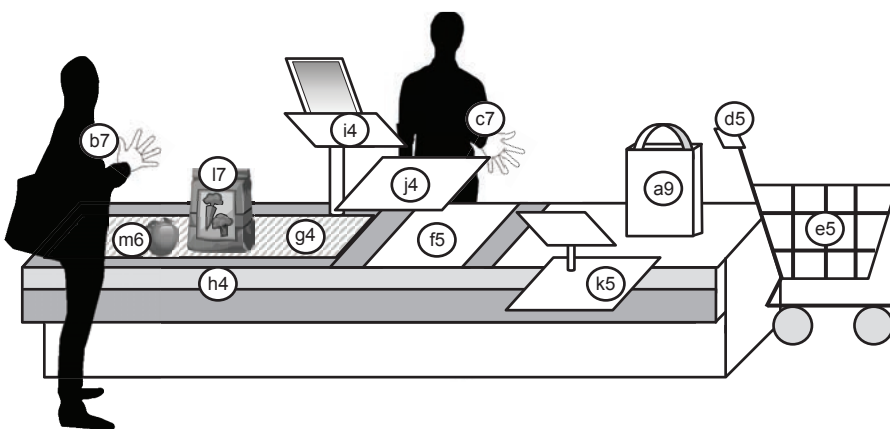
Discussion

The lowest mean concentration of virus detected on a surface was 10^4 PFU/cm². This concentration would represent a virus transmission risk for most individuals encountering any of the surfaces touched by the RGB directly or indirectly through at least one other contact. All C1 and C2 surfaces in the grocery store had detectable MS2, while all control surfaces were appropriately negative or positive. Further study is needed to characterize additional surfaces that were not contacted by the volunteer shoppers.

The high recovery of MS2 from packaged foods could be attributed to the increased adhesion of enteric viruses to hydrophobic PET plastic surfaces (Butot et al., 2007) or similar mechanisms for biofilm adherence within the high density polyethylene (HDPE) pipes (Rozej, Cydzik-Kwiatkowska, Kowalska, & Kowalski, 2015). The surfaces sampled in this study include the polypropylene (PP) fibrous sacks for potatoes and oranges, the clear bags made from PP resins used for table grape bags, unwoven PP sacks

FIGURE 3

Checkout Counter With Contamination Sites Represented by Circles With an Identification Letter and a Number Indicating the Log Concentration of MS2 Surrogate



Note. The letter indicates the surface specified in Table 1 (e.g., e = cart surface). The number abbreviates the log concentration of the MS2 surrogate (e.g., 1×10^5 PFU = 5).

PFU = plaque-forming unit.

From Table 1: Seeded reusable grocery bag = a; customer hands = b; clerk hands = c; cart handle = d; cart surface = e; checkout scale = f; checkout conveyor = g; customer bumper = h; clerk keyboard = i; clerk table = j; customer keyboard and table = k; packaged food = l; unpackaged produce = m.

used in this study for RGBs, and HDPE bags used for self-bagging unpackaged vegetables. Some of these adhesion mechanisms include the material's electrostatic surface charge, the material's hydrophobicity, the influences of temperature and humidity on the material, and other physiochemical parameters within the virus (Langlet, Gaboriaud, Gantzer, & Duval, 2008).

A laboratory study found the attachment of MS2 and noroviruses to polypropylene surfaces to be between 0.1–3% adherence (Deboosere et al., 2012). The referenced MS2 adherence matches well with this current field study that achieved virus concentration recovery ranging from 0.6–1.93% among C1 surfaces (those surfaces that made direct contact with the initial contaminated RGB).

Our study findings are consistent with others who have found that hand washing can be the most important step for customers to reduce their risk of norovirus infection (Hall et al., 2011). The grocery store represents a public area where many individuals mix and touch many common surfaces. A contaminated grocery cart, basket, or RGB

could present the virus transmission pathway hypothesized in this study.

This study develops exposure assessment data that can be used for a quantitative microbial risk assessment (QMRA). More work is necessary to use the QMRA framework on our current dataset to characterize the uncertainty and describe the risk of virus infection from nondietary contact in a grocery store (Figure 1). A similar study used the QMRA framework to determine that the concentration of virus on fomites is the parameter most strongly linked to the estimated dose of the virus to cause a nondietary infection (Julian, Canales, Leckie, & Boehm, 2009).

Conclusion

This study presents various surfaces in the grocery store as potentially contaminated after contact with an RGB containing a surrogate for norovirus. An additional microbial risk assessment should take the data presented in this study and evaluate each surface from a hazard analysis and critical control point (HACCP) perspective. Each of the volunteer shoppers contacted a small percent-

age of available surfaces in each store with one area in common: The checkout stand is touched by every customer. The checkout stand surfaces and the grocery cart present ideal targets for new industry cleaning standards or new materials (Figure 3). This opportunity is also a consideration for small and large grocery stores alike during times of low customer volume because all customers filter through only one checkout stand.

The grocery cart is another surface to target in a HACCP plan, as the virus concentrations were also high. Grocery carts are not contacted as frequently as the checkout stand, and in many climates, are often in the parking lot where they are unintentionally disinfected from exposure to high temperatures, UVA,

and UVC from sunlight. Despite this potential natural process, one study found high concentrations of fecal indicator bacteria on grocery carts collected from stores in Southern California and other metropolitan areas around the U.S. (Gerba & Maxwell, 2012).

This study suggests that a virus-contaminated RGB presents a public health risk if it is brought into a contemporary grocery supermarket. The RGBs are contacted by people, contact many surfaces, and are used to carry a variety of household items in addition to groceries. The bags traverse the hygienic boundaries between private homes and public spaces such as grocery carts and checkout stands.

As the highest concentration of MS2 was found on hands (Figure 3), the health risk

first should be mitigated through promotion of an in-store hand hygiene campaign. The additional surface contamination findings of this report justify additional measures including more frequent surface disinfection with an emphasis on checkout stand surfaces, antimicrobial RGBs, and the use of antimicrobial surfaces to be built into checkout stands. 🐼

Acknowledgement: The authors would like to thank the Environmental Safety Alliance of Sacramento, California.

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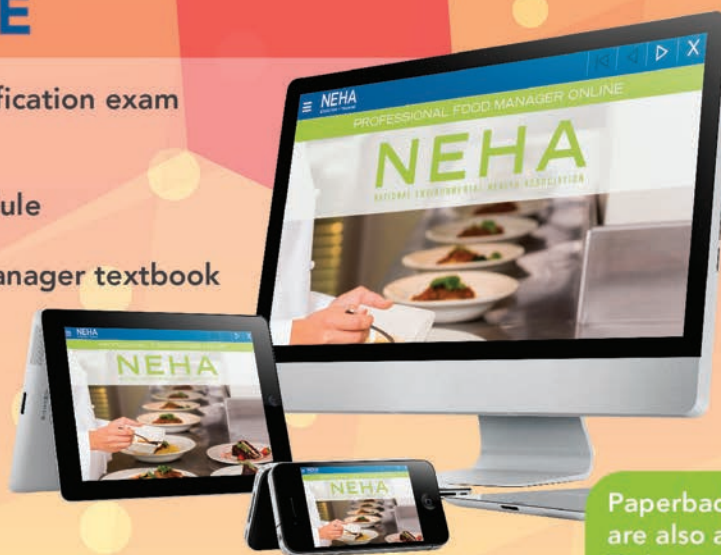
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