Physical Removal and Transfer of Murine Norovirus and Hepatitis A Virus from Contaminated Produce by Scrubbing and Peeling

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ABSTRACT

Human noroviruses and hepatitis A virus are responsible for numerous outbreaks associated with handling fresh produce. In this study, physical removal of hepatitis A virus and murine norovirus, a human norovirus surrogate, from contaminated produce items (honeydew melons, cantaloupes, carrots, and celery) by scrubbing under running water with a nylon brush or scouring pad and by peeling (carrots and celery) with a peeler was investigated. The degree and extent of utensil contamination with viruses during these operations in the presence and absence of food residue also was investigated. Scrubbing or peeling produce initially inoculated with ~5.5 log PFU of each virus resulted in significant levels of virus removal, ranging from 0.93 to 2.85 log PFU. However, utensil cross-contamination occurred, with >2 log PFU of virus transferred from a single produce item. After preparation of a contaminated produce item, utensil cross-contamination resulted in virus detection on seven successively prepared produce items. Produce residue accumulation on utensils variably impacted virus transfer to utensil surfaces. Results indicate that scrubbing and peeling produce can reduce levels of viruses on contaminated produce, but the importance of utensil sanitation to prevent cross-contamination is highlighted. Findings also provide important information for modeling virus cross-contamination during food preparation.

Human norovirus and hepatitis A virus (HAV) are important viral agents of foodborne disease. A large number of outbreaks are caused by norovirus (accounting for ~58% of all foodborne illnesses with a known etiology), and high hospitalization and death rates are associated with HAV infection (32 and 2%, respectively) (29). Human norovirus causes a self-limiting illness marked by vomiting and diarrhea. High levels of virus particles (up to $10^{11}$ virus particles per g of stool sample) can be shed by infected persons, and the infectious dose is very low (~18 virus particles) (1, 27, 32). Currently, human norovirus cannot be propagated in cell culture, so estimating its infectivity requires the use of surrogate viruses, such as murine norovirus (2, 6). HAV causes acute viral hepatitis and has a long incubation period of ~28 days (range, 15 to 50 days), after which some patients develop characteristic symptoms of jaundice (17). Virus shedding can begin 10 to 14 days before the onset of symptoms (9) and can continue up to 3 months after resolution of symptoms (28, 38).

Both human norovirus and HAV are among the most common pathogens associated with produce, accounting for 40 and 4% of all produce-associated outbreaks, respectively (11). Fresh fruit and vegetable contamination by foodborne viruses can occur during production, harvesting, processing, transport, or preparation close to the point of consumption. Contamination of produce can occur through contact with contaminated water during irrigation or processing or contact with infected food workers via the fecal-oral route or aerosolized vomit (8, 16, 17). According to data obtained from 1927 to 2006, viruses caused 60% (491 of 816) of outbreaks in which food workers were implicated (14). Of these outbreaks, norovirus was responsible for 33% (274 of 816), and HAV was responsible for 10% (84 of 816) (14). When food workers were implicated, 16% of the norovirus and 12% of the HAV infection outbreaks involved produce (14).

Many studies have been conducted to assess virus removal by water washing procedures (3–5, 7, 10, 15, 20). Soaking murine norovirus— or HAV-contaminated produce in water generally results in ~1-log PFU reduction of viruses detected on produce surfaces. Baert et al. (3) found a 1.01-log reduction in murine norovirus 1 (MNV-1) on inoculated spinach leaves after one wash and a 1.26-log reduction after three washes. Croci et al. (10) also obtained approximately a 1-log 50% tissue culture infective dose reduction in HAV from inoculated fennel and carrots after washing with water. Lukasik et al. (20) found that hand rubbing of produce in a 22°C water bath for 5 s could lead to a 60 to 86% reduction of bacteria (Escherichia coli O157:H7 and Salmonella Montevideo), bacteriophage (MS2, ФX174, and PRD1), and poliovirus 1 inoculated on the surface of strawberries. Feline calicivirus was reduced by 2 log PFU from strawberries and lettuce after these items were stirred in water at room temperature for 10 min (15). However, few studies have been performed to determine virus removal by kitchen utensils during preparation of fresh produce.

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In the present study, kitchen utensils (nylon brushes, scouring pads, and peelers) were applied to artificially contaminated produce items to determine the degree of virus decontamination that could be achieved during typical produce preparation. Produce (honeydew melons, cantaloupes, carrots, and celery) were chosen based on their variation in surface properties, softness, and compatibility with the various kitchen utensils chosen for the study. The extent of virus transfer to these utensils and cross-contamination in the kitchen also was investigated, as was the impact of produce residues accumulated on utensils on virus transfer. These investigations are important for determining the effectiveness of virus removal methods used in the kitchen environment and for better assessing the potential risk of virus cross-contamination via utensils during preparation.

**MATERIALS AND METHODS**

**Virus cultivation and plaque assay.** MNV-1 (a gift from Dr. Herbert W. Virgin, School of Medicine, Washington University, St. Louis, MO) was cultured in RAW 264.7 cells (ATCC TIB-71; American Type Culture Collection, Manassas, VA) in Dulbecco’s modified Eagles medium (DMEM) supplemented with 10% low-endotoxin fetal bovine serum (FBS: HyClone, Logan, UT), 100 U/ml penicillin (Invitrogen, Carlsbad, CA), 100 U/ml streptomycin (Invitrogen), 10 mM 4-(2-hydroxy-ethyl)-1-piperazineethanesulfonic acid (HEPES; Invitrogen) and 1 mM sodium pyruvate (Invitrogen). MNV-1 was cultured by infecting 80 to 90% confluent monolayers of RAW 264.7 cells until complete cytopathic effect (CPE) was observed (typically 48 h for MNV-1). HAV strain HM175 (ATCC VR-1402) was propagated in fetal rhesus monkey kidney cells (FRhK-4, ATCC CRL-1688) in DMEM containing 8% FBS, 100 U/ml penicillin, 100 U/ml streptomycin, 1% l-glutamine, and 1% nonessential amino acids. HAV was then infected into 80 to 90% confluent monolayers of FRhK-4 cells until complete CPE was observed (typically 7 days). Viruses were released from cells by three cycles of freezing-thawing. Cellular debris was precipitated by centrifugation for 15 min at 2,000 × g. The supernatant fluid was filtered through a 0.2-μm-pore-size membrane filter (Millipore, Billerica, MA) and then stored in 2-ml aliquots at −80°C until used.

Plaque assay of MNV-1 and HAV was performed similarly to previous reports with some modifications (19, 37). RAW 264.7 and FRhK-4 cells maintained in complete DMEM were grown until 80 to 90% confluence was reached. Tenfold serial dilutions of each virus-containing sample or stock prepared in phosphate-buffered saline (PBS; pH 7.4) were dispensed in (duplicate) over monolayers grown in 60-mm-diameter cell culture plates. The plates were incubated at 37°C with 5% CO₂ for 1 h with gentle rocking every 15 min to evenly distribute the inoculum. MNV-1 overlays consisted of 3 ml of 0.5% agarose (Lonza SeaKem LE, Rockland, ME) with complete Eagle’s medium (Cellgro, Mediatech, Manassas, VA) supplemented with 5% low-endotoxin FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, 10 mM HEPES, and 1 mM sodium pyruvate. HAV overlays consisted of 5 ml of 0.5% agarose with DMEM (HyClone, Logan, UT) supplemented with 8% FBS (Atlanta Biological, Atlanta, GA), 100 U/ml penicillin, 100 μg/ml streptomycin, 1% l-glutamine, and 1% nonessential amino acids. At the end of the virus incubation period (48 h for MNV and 7 days for HAV), 3 ml of second overlay medium containing 0.5% agarose and 1.0% 3.3 g/liter neutral red solution (Sigma) for MNV-1 and 0.5% agarose and 2.5% neutral red solution for HAV was applied for at least 5 h to allow neutral red uptake. Plaques were then counted, and virus titers were expressed in PFU. Titers of stocks cultures were 5.63 to 7.40 log PFU/ml for MNV-1 and 5.76 to 7.56 log PFU/ml for HAV.

**Inoculation of fresh produce with viruses.** Produce items with visually consistent sizes and weights were purchased from local grocery stores. Before inoculation, each produce item was surface disinfected by exposure to UV light (254 nm, 100 μW/cm²) for 30 min (15 min on each side). Western cantaloupes (Cucumis melo L. var. reticulatus) and honeydew melons (C. melo) were cut into halves, and six equally distributed dots were marked on the rind of each half, 6 cm away from the apex. A 25-μl inoculum of MNV-1 or HAV was applied in a straight line between each set of dots for a total of 150 μl. For carrots (Daucus carota) and celery (Apium graveolens), a mark was made 8 cm from the stem or base and another was made 6 cm away. A 50-μl inoculum was dispensed in 12.5-μl portions by drawing four parallel lines around the circumferences of the produce item, between the marks. After inoculation, all the produce items were kept under the laminar flow hood at room temperature until the liquid of each inoculum was visibly dry (−1 h). To prevent moisture condensation on the surface after drying, the produce items were sealed in a container containing anhydrous calcium sulfate with a cobaltous chloride indicator (Drierite, Xenia, OH) for overnight storage at 4°C until they were scrubbed or peeled the next day.

**Inoculation of utensils with viruses.** Utensils were inoculated with 50 μl of virus stock and dried under the laminar flow hood at room temperature until the liquid of the inoculum was visibly dry (−1 h). Nylon brushes (10 cm long, 5 cm wide; OXO, Chambersburg, PA) and scouring pads (6 cm long, 3 cm wide; Scotch-brite, St. Paul, MN) were dotted with 35 to 40 spots equally distributed only on the surface that was to come into contact with the produce. Peelers (blade size 4 cm long, 0.2 cm in wide; Hamilton Beach, Southern Pines, NC) were inoculated with 25 μl of virus stock on both sides of the direct contact blade. Utensils were stored overnight at 4°C in a container containing anhydrous calcium sulfate with a cobaltous chloride indicator to maintain a low relative humidity and prevent moisture condensation on the utensil.

**Protocol for virus removal and transfer during washing and peeling.** Melons were placed on a circular rotating pad and stabbed with a pair of forceps at the apex so the melons could be rotated and gripped. During the scrubbing procedures with nylon brushes and scouring pads, melons were rotated two complete turns in 20 s under running sterile deionized water flowing at 10 ml/s. During each turn, the inoculated area of each melon was scrubbed with a nylon brush or scouring pad. For carrots, the uncontaminated tip of each carrot was removed and the top (stem side) of the carrot was held. Carrots were rotated in two turns in 10 s under running water, flowing at 10 ml/s, while scrubbing the inoculated area. After the noninoculated tips were removed, carrots and celery were peeled from a location approximately 1 cm above the mark next to the stem to the end until all the inoculated area was removed. After completing the washing (scrubbing) and peeling procedures, viruses were recovered and quantified from the surfaces of produce items and/or utensils.

**Protocol for virus transfer from contaminated utensils to multiple uncontaminated produce items.** Nylon brushes and peelers were first used on carrots or celery, respectively, to obtain utensils contaminated with MNV-1 or HAV. Immediately after contamination, the utensil was then applied successively to seven
TABLE 1. MNV-1 and HAV reduction on inoculated produce items after overnight storage at 4°C

<table>
<thead>
<tr>
<th>Produce item</th>
<th>Virus reduction (log PFU) per produce item$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MNV-1</td>
</tr>
<tr>
<td>Carrot</td>
<td>1.05 ± 0.15 C</td>
</tr>
<tr>
<td>Celery</td>
<td>1.49 ± 0.00 A</td>
</tr>
<tr>
<td>Cantaloupe</td>
<td>1.26 ± 0.65 B</td>
</tr>
<tr>
<td>Honeydew melon</td>
<td>1.07 ± 0.29 C</td>
</tr>
</tbody>
</table>

$^a$ Values are means ± standard deviations of three replicates with five samples each. Within a column, means with the same letter are not significantly different (P > 0.05).

noncontaminated produce items, and the extent of virus transfer to these produce items was determined. Results were reported as positive or negative for detection of viruses on each produce item (unit).

**Artificial residue accumulation on utensils.** All the utensils were dried in a 37°C oven overnight to remove excess moisture and were weighed immediately after drying. Organic carrot juice (Bolthouse, Bakersfield, CA) was purchased from a local grocery store, and 10 ml was transferred to a petri dish. Two-thirds of the bristles from each nylon brush were placed inside the dish to absorb carrot juice for 5 min at room temperature. Brushes were then placed into a 37°C oven for 80 to 90 min. The nylon brushes were then flipped over until completely dried in the oven. For peelers, a more concentrated residue accumulation level was desired: 45 ml of juice was pipetted into 50-ml tubes and centrifuged (4,000 × g, 15 min, 21°C). The pellet was resuspended in supernatant to a final volume of 2 ml. Peellers were then spiked with 100 µl of concentrated juice (50 µl on each blade) and dried for 20 min in a 37°C oven. A second layer of residues was added to the blades using the same procedure. Utensils were weighed immediately after the residues on the utensils were completely dried. The weight of residues on each utensil was recorded by calculating the difference in weights before and after the residue accumulation procedure.

**Protocol for virus transfer from contaminated produce to utensils with or without residue.** Nylon brushes and peelers with and without residue were used to peel inoculated carrots. The degree of virus transfer to utensils was then determined.

**Virus elution from produce items and utensils.** Cantaloupe and honeydew melon rinds were cut along the inoculation line into six squares (2 by 2 cm). Inoculated rind squares from each melon and inoculated portions of carrots or celery were placed in a 50-ml centrifuge tube containing 20 ml of elution buffer (PBS containing 1 M NaCl and 0.05% Tween 20) and vortexed for 30 s to elute the viruses. The pieces of produce were removed, and the virus-containing elution buffer was retained for further analysis.

Nylon brushes, scouring pads, and peelers used for processing contaminated produce items were placed into stomacher bags containing 20 to 30 ml of elution buffer and massaged by hand for 30 s for virus elution. The utensils were removed, and the virus-containing elution buffer was retained for further analysis.

**Concentration of eluted viruses.** Polyethylene glycol (PEG; molecular weight 10,000 g/mol) was added to each virus-containing eluent to give a final concentration of 8%. Samples were shaken for at least 4 h (200 rpm on a shaking platform) to dissolve PEG before centrifugation at 4°C. Pellets were obtained after centrifugation (9,000 × g, 30 min, 4°C) and resuspended in 1 ml of PBS. Samples were then either processed immediately or frozen at −70°C for less than 2 weeks before plaque assay. When the samples contained visible produce residue, chloroform extraction was conducted before the plaque assay at a of 1:1 ratio (vol/vol). After phase separation by centrifugation (17,000 × g, 10 min, room temperature), the aqueous phase was retained for plaque assay.

**Calculations and statistical analysis.** Log reductions in viral PFU were obtained by subtracting the amount of virus recovered (log PFU) after scrubbing or peeling from the amount of virus recovered (log PFU) from produce that did not undergo treatment. Percent recoveries of viruses from nylon brushes, scouring pads, and peelers (with or without residues) were calculated to determine the effectiveness of virus recovery protocols. Because of the differences in recovery efficiencies between utensil types and produce types, all virus concentration values obtained after transfer experiments were adjusted for the percent recoveries for each utensil type, so that direct comparisons could be made between transfer studies with different utensil plus produce combinations. Percent recovery and degree of virus transfer was calculated as follows:

\[
\% \text{ recovery} = \frac{\text{titer of viruses recovered after overnight storage}}{\text{titer of viruses initially inoculated}} \times 100
\]

\[
\% \text{ recovery} = \frac{\text{titer of virus transferred to utensil}}{\text{titer of viruses recovered from utensil}} \times 100
\]

Experiments were conducted with three or five samples in each of three replicates. Data were analyzed using SAS software (version 9.2, SAS Institute, Cary, NC). Values obtained from different combinations of factors were analyzed by analysis of variance (ANOVA) using a generalized linear model. Significant differences between the least square means of treatment groups were indicated at P < 0.05. In the studies of sequential virus transfer, data were analyzed by ANOVA using a mixed model ANOVA for the binary response. Least-square means were separated using pairwise t tests, and significant difference was indicated at P < 0.05.

**RESULTS**

**Recovery and survival of viruses on the surface of produce after overnight storage at 4°C.** A study was conducted to determine the survival rates of infectious virus particles (both MNV-1 and HAV) after overnight storage on produce at 4°C before the storage procedure was adopted in subsequent protocols. The log PFU reductions of both viruses on different produce items after overnight storage are listed in Table 1. Initial titers of viruses inoculated on produce items (cantaloupes, honeydew melons, carrots, and celery) were 5.91 ± 0.47, 5.80 ± 0.41, 4.69 ± 0.25, and 4.74 ± 0.07 log PFU for MNV-1 and 6.42 ± 0.26, 6.13 ± 0.24, 5.73 ± 0.59, and 4.91 ± 0.03 log PFU for HAV. The percent recoveries from produce after overnight storage were 0.5 to 13% of the initial inoculum titers. After overnight storage, the infectivity of viruses on produce decreased by at least 1 log PFU regardless of differences in inoculation virus titers for melons, carrot, and celery. Reductions in virus titers were 1 to 2 log PFU for most
produce types, with the exception of HAV on carrots, which was reduced by 2.75 log PFU.

Physical removal of viruses from contaminated produce items by scrubbing or peeling. Before scrubbing or peeling but after overnight storage at 4°C, titers recovered from cantaloupe, honeydew melon, carrot, and celery surfaces were 4.66 ± 0.44, 4.77 ± 0.14, 3.65 ± 0.30, and 3.25 ± 0.09 log PFU for MNV-1 and 5.00 ± 0.39, 4.52 ± 0.18, 3.05 ± 0.72, and 3.93 ± 0.21 log PFU for HAV, respectively. After scrubbing or peeling, log titers recovered from produce were again determined and subtracted from initial recovery values to calculate log reductions for MNV-1 and HAV.

Significant reductions of both viruses were observed after scrubbing under running water or removing the peel (P < 0.05), with reductions of 0.93 to 2.85 log PFU (Table 2). For a few pieces of produce, virus was not detected (lower than the limit of detection: 1 log PFU per sample), but complete removal was not obtained for most of the produce items tested. When each virus type was examined separately, the removal of MNV-1 was significantly greater than that of HAV for carrots that were scrubbed with scouring pads (P < 0.05). Compared with nylon brushes and peelers, scouring pads achieved a significantly greater removal of MNV-1 from carrots (P < 0.05), but MNV-1 and HAV removal was not significantly different when comparing the use of nylon brushes and scouring pads on both types of melons (P > 0.05). Comparing virus removal from different produce items when using the same utensil, more virus particles (both MNV-1 and HAV) were removed with a nylon brush (P > 0.05). The scouring pad also removed significantly higher numbers of HAV particles from honeydew melons than from cantaloupes (P > 0.05). However, similar levels of virus reduction were achieved for carrots and celery with a peeler, regardless of virus type (P > 0.05).

Virus transferred to utensils during processing. The ability of viruses to transfer to utensils during scrubbing or peeling of contaminated produce also was examined (Table 3). Qualitative analysis (the ratio of positive samples to the total number of samples tested) indicated that viruses frequently were transferred from contaminated produce to common kitchen utensils. The extent of virus transfer to utensils also was determined quantitatively with an adjustment for loss occurring during the recovery procedure (adjusted by the percent recovery).

Comparing the transfer of different virus types across produce items with the same utensil, significantly more HAV than MNV-1 particles transferred to nylon brushes and scouring pads from cantaloupes; however, more MNV-1 than HAV particles were transferred from honeydew melons to nylon brushes and from carrots to scouring pads (P < 0.05). MNV-1 was more easily transferred from honeydew melons to nylon brushes than to scouring pads, and HAV was more easily transferred from carrots to nylon brushes and peelers than to scouring pads (P < 0.05). However, no significant difference was found for MNV-1 transfer from cantaloupes and carrots or for HAV transfer from honeydew melons and cantaloupes to any type of utensil tested (P > 0.05). More MNV-1 particles were transferred to nylon brushes from honeydew melons than from cantaloupes and carrots, whereas more HAV particles were transferred to peelers from celery than from carrots (P < 0.05). No significant difference was found in the amount of virus transfer when cantaloupes or honeydew melons were compared after scrubbing with scouring pads regardless of the virus type (P > 0.05).

Extent of virus cross-contamination from contaminated utensils to uncontaminated produce during utensil application. After the first use of each utensil on an inoculated produce item, the newly contaminated utensils were used successively to process seven uncontaminated produce units of the same type (units 1 through 7). The frequency of virus transfer from contaminated nylon brushes and peelers to uncontaminated carrots and celery is presented in Tables 4 and 5, respectively. In both scenarios, contamination occurred in at least one sample in every unit examined. For unit 1 samples, viruses could be detected on
and HAV inoculated on carrots were $5.30 \pm 0.50$ and $5.41 \pm 0.54 \log \text{PFU}$, respectively, which were higher than those previously used in virus removal investigations. No difference in MNV-1 and HAV transfer was found for nylon brushes with or without residues; however, significantly more MNV-1 was transferred to peelers with residues than to peelers without residues. In contrast, HAV transferred more easily to peelers without residues than to peelers with residues.

### DISCUSSION

In this study, both MNV-1 and HAV were capable of persisting on the surface of produce items during overnight storage at 4°C. This finding is not surprising; MNV-1 and HAV have been previously reported as relatively resistant to dry conditions and low temperatures (6, 22, 23). Virus survival rates differed by virus type and produce type, with the greatest reduction in survival observed for HAV on carrots. Croci et al. (10) reported that inactivation of HAV during produce storage (lettuce, fennel, and carrots) at 4°C was greatest for HAV-contaminated carrots and postulated

#### TABLE 4. Ratio of positive samples to total number tested after contaminated nylon brushes were sequentially used on multiple units of carrots

<table>
<thead>
<tr>
<th>Virus</th>
<th>Trial no.</th>
<th>Unit 1</th>
<th>Unit 2</th>
<th>Unit 3</th>
<th>Unit 4</th>
<th>Unit 5</th>
<th>Unit 6</th>
<th>Unit 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>MNV-1</td>
<td>1</td>
<td>3/5</td>
<td>2/5</td>
<td>0/5</td>
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<td>0/5</td>
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<td>1/5</td>
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</tr>
<tr>
<td>HAV</td>
<td>1</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
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</table>

*a Transfer ratio is the number of positive samples/number of samples tested in three replicate trials with five samples per trial. Within a row, ratios with the same letter are not significantly different ($P > 0.05$).

87 to 93% of the celery samples that were peeled and 27 to 47% of the carrots that were scrubbed. For each subsequent use of the contaminated utensils on uncontaminated carrots (units 2 through 7), there was a general trend of decreasing number of virus-positive samples. Virus transfer from nylon brushes to carrots was generally lower than from contaminated peelers to celery. Within each experimental trial, virus transfer was not always continuous; contamination sometimes occurred on a subsequent unit within the same experimental trial after skipping one or two units.

#### Degree of virus transfer to utensils with or without residues from contaminated carrots

The common practice of reusing utensils in the kitchen without washing between uses can result in accumulation of produce residues on utensil surfaces. Transfer of viruses to nylon brushes and peelers after scrubbing or peeling of contaminated carrots and the impact of carrot residues on each utensil are shown in Table 6. Carrot residues coated on nylon brushes and peelers weighed ~0.8 and ~0.03 g, respectively, before transfer experiments were conducted. The titers of MNV-1...
that the natural antimicrobial properties of carrots may be responsible.

Rates of virus survival and recovery from the surface of produce can be affected by the unique surfaces properties of each type of produce or by virus-specific properties. For instance, carrots have relatively rough skins that are prone to drying out after overnight storage. Similarly, the virus inoculum was more easily absorbed by the netted cantaloupe rinds, perhaps making it more difficult to elute viruses from these surfaces than from smooth surfaces such as those of honeydew melons. To make direct comparisons of virus transfer between different utensils or produce items with variable percent recoveries of virus, the percent recovery for each utensil and produce type was included in the calculations to determine the titers of viruses recovered from utensils and produce. However, in studies of changes in virus populations due to treatment (i.e., virus survival during storage and virus removal after processing with a utensil), log PFU reductions of viable virus were determined by comparison with an untreated control of the same produce type. Therefore, no adjustment for percent recovery was needed to determine log PFU reductions before and after treatment (i.e., storage, washing, or peeling). Both the ratio of positive sample to the number of samples tested and the average log PFU reduction per milliliter were included in the results because only samples that were positive for viruses were considered in the statistical analysis.

The number of virus particles detected on produce was significantly reduced after scrubbing or peeling of produce items under running water (0.93 to 2.85 log PFU), but the produce items were not completely decontaminated after preparation. Similar conclusions have been reached in previous studies of produce washed with water (4, 5, 12).

Significantly more virus particles were removed with nylon brushes from honeydew melons than from cantaloupes, possibly because of the weak attachment of viruses to the smooth surfaces of the honeydew melons or because the physical features of the cantaloupe (roughness and absorptive nature) made it difficult to elute viruses. Other researchers have found similar results; a 5-min water-wash treatment of produce surfaces led to reduced removal of E. coli O157:H7 from cantaloupe than from honeydew melons (26).

Similar to the effectiveness of brushes, peelers only partially removed virus particles from the surface of carrots and celery, but cross-contamination of the peelers and transfer of virus particles to underlying tissues of the produce likely occurred. Thus, utensils had a significant effect on virus removal but complete elimination of virus did not occur.

### Table 5. Ratio of positive samples to total number tested after contaminated peelers were sequentially used on multiple units of celery

<table>
<thead>
<tr>
<th>Virus</th>
<th>Trial no.</th>
<th>Unit 1</th>
<th>Unit 2</th>
<th>Unit 3</th>
<th>Unit 4</th>
<th>Unit 5</th>
<th>Unit 6</th>
<th>Unit 7</th>
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<tr>
<td>MNV-1</td>
<td>1</td>
<td>3/5</td>
<td>3/5</td>
<td>4/5</td>
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<td>1/5</td>
<td>2/5</td>
<td>2/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4/5</td>
<td>5/5</td>
<td>3/5</td>
<td>3/5</td>
<td>3/5</td>
<td>4/5</td>
<td>3/5</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>14/15</td>
<td>A</td>
<td>15/15</td>
<td>A</td>
<td>9/15</td>
<td>A</td>
<td>10/15</td>
</tr>
</tbody>
</table>

*Transfer ratio is the number of positive samples/number of samples tested after contaminated peelers were sequentially used on multiple units of celery. Within a row, ratios with the same letter are not significantly different (*P* > 0.05).

### Table 6. Transfer of MNV-1 and HAV to nylon brushes and peelers following scrubbing or peeling contaminated carrots and the impact of carrot residues on each utensil surface

<table>
<thead>
<tr>
<th>Utensil</th>
<th>Virus</th>
<th>Carrot residue</th>
<th>Residue wt (g)*</th>
<th>Virus transfer/utensil (log PFU)*</th>
<th>Transfer ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nylon brush</td>
<td>MNV-1</td>
<td>With</td>
<td>0.84 ± 0.10</td>
<td>2.95 ± 1.77 A</td>
<td>7/9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Without</td>
<td></td>
<td>3.57 ± 0.96 A</td>
<td>9/9</td>
</tr>
<tr>
<td></td>
<td>HAV</td>
<td>With</td>
<td>0.78 ± 0.10</td>
<td>2.74 ± 0.96 A</td>
<td>9/9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Without</td>
<td></td>
<td>2.80 ± 1.01 A</td>
<td>7/7</td>
</tr>
<tr>
<td>Peeler</td>
<td>MNV-1</td>
<td>With</td>
<td>0.03 ± 0.01</td>
<td>3.63 ± 0.85 A</td>
<td>9/9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Without</td>
<td></td>
<td>3.20 ± 1.00 A</td>
<td>9/9</td>
</tr>
<tr>
<td></td>
<td>HAV</td>
<td>With</td>
<td>0.03 ± 0.01</td>
<td>1.11 ± 1.22 A</td>
<td>7/9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Without</td>
<td></td>
<td>1.96 ± 1.68 A</td>
<td>6/9</td>
</tr>
</tbody>
</table>

*Values are means ± standard deviations of three replicates with three samples each.

*Values are means ± standard deviations of three replicates with three samples each with adjustment for percent recovery. Within this column, means with the same letter are not significantly different (*P* > 0.05) when comparing transfer of MNV-1 and HAV between utensils with or without residue.

*Transfer ratio is the number of positive samples/number of samples tested.*
The unique surface properties of viruses also could affect the degree of MNV-1 and HAV attachment to a produce item and in turn could explain some of the differences in virus removal observed for a given utensil. Virus attachment to produce is affected by various interactions (18, 31, 34–36). For example, both the electrostatic and hydrophobic interactions between viruses (e.g., feline calicivirus, echovirus 11, bacteriophage MS2, and ΦX174) and surfaces (e.g., membrane and lettuce) have been observed (31, 34, 35). More specifically, the ability of HAV to attach to stainless steel is dependent on surface energy parameters (e.g., total surface energy, nonpolar Lifshitz–Van der Waals reactions, and polar short-range hydrogen bonding components) and the total free energy of the environment (18). Future studies should be conducted to measure virus-produce interactions and compare the strength of MNV-1 and HAV attachment to produce surfaces.

Contamination of kitchen utensils used to prepare artificially inoculated produce readily occurred, although not every time the utensil was used. The negative results may be due to the absorptive and/or adsorptive properties of the produce and the occasional sequestration of viruses within tissues or on the surface of the produce item. Nylon brushes and scouring pads were used under running water, which could have removed some virus particles. This hypothesis was supported in preliminary studies when collected wash water was confirmed to be contaminated with infectious viruses (data not shown). The absence of contamination of the peelers, however, may be due to limited opportunities for the peeler blade to contact the contaminated surface because most of the blade is cutting under the surface.

More virus particles (MNV-1 and HAV) were removed from honeydew melons than from cantaloupes with nylon brushes, and more MNV-1 particles were transferred from honeydew melons to nylon brushes than to scouring pads. MNV-1 may more readily attach to nylon brushes than to scouring pads. HAV did not appear to adhere to nylon brushes to the same extent as did MNV-1; little difference in HAV transfer to nylon brushes from both types of melons was noted.

Cross-contamination occurred when nylon brushes and peelers were applied to seven un inoculated carrots sequentially after being used on an inoculated carrot. These observations are in agreement with those from studies of bacterial cross-contamination during food preparation. Salmonella, E. coli, and Listeria inoculated on the surfaces of oranges contaminated cutting board, knives, and juice makers, and bacteria were found subsequently in the juice from the un inoculated oranges that came into contact with the utensils used on contaminated oranges (21). Zhao et al. (39) and Montville et al. (25) found Enterobacter aerogenes in lettuce after the lettuce contacted cutting boards, gloves, or hands used to prepare chicken contaminated with this bacterium. In the present study, contamination gradually decreased from unit 1 to unit 7, which can be explained by the decreasing number of virus particles on the utensils due to sequential transfers (dilution effect). Gill and Jones (12) found a similar trend when E. coli was transferred by handling inoculated meat and then handling multiple items of uncontaminated meat. The fact that viruses could be detected on the seventh uncontaminated produce item in the present study was surprising, even when they were not always found on all intervening items. A reason for this variation may be that the virus particles were unevenly distributed on the produce or the utensils or the viruses may have clustered at some sites on the utensils. Therefore, the contaminated sites might not have come into contact with some of the sequentially prepared items, so virus transfer could have skipped one or two units before being deposited on a unit that was processed later.

The hypothesis was that residue accumulation provides surface area that can facilitate virus transfer and lead to increased transferability. In the present study, more MNV-1 particles were transferred from contaminated carrots to peelers with residues than to peelers without residues. However, more HAV particles were transferred to peelers without residues than to peelers with residues. No difference in virus transfer was observed between nylon brushes regardless of residue presence. The majority of residue applied to nylon brushes was absorbed into the bristles, and although the viruses may have been detached to surface residue, the residues and any attached viruses may have been washed away, negating a residue effect. Differences between MNV-1 and HAV in their adherence to peelers also may be due to unique viral characteristics. For example, the isoelectric point of viral capsid proteins affects the attachment of viruses to surfaces (36), and the isoelectric points of noroviruses and HAV are different (5.9 to 6.0, and 2.8, respectively) (13, 24). Transfer of human norovirus (GI and GII strains) and MNV-1 has occurred from contaminated fingertips (30).

In summary, scrubbing and peeling can remove significant numbers of virus particles from the surfaces of produce items. Because the infectious dose of human norovirus and HAV is ~10 to 100 viral particles (27, 32, 33), scrubbing and peeling could prevent illness when the level of contamination is less than 2 to 3 log PFU. However, these methods would not be sufficient to decontaminating produce contaminated with the higher titers of viruses, as in this study.

This study provides a model for investigating the extent of virus removal and transfer during food preparation with kitchen utensils. The findings suggest that scrubbing and peeling of produce are important ways to improve the safety of these items. Combined with other good hygienic practices and/or the use of disinfectants and sanitizers on utensils or the exterior surfaces of produce, these physical removal methods may benefit the public health. However, utensils play an important role in virus cross-contamination in the kitchen environment. Thus, food workers and consumers must be equipped with knowledge of those procedures that remove viruses from produce and those that limit contamination and cross-contamination.

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