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Tracking and modeling norovirus transmission during mechanical slicing of globe tomatoes

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ABSTRACT

Recent epidemiological evidence indicates that preparation of fresh produce for use as ingredients in ready-to-eat food in commercial settings has been a significant source of the norovirus (NoV) infections in the U.S. This research investigated the dissemination of NoV from a single tomato to many others via the use of an 11-horizontal blade slicer commonly found in restaurants or sandwich shops. A total of eight trials were conducted. The source of contamination in each trial was a soak-inoculated, air-dried globe tomato containing ~8 log10 murine norovirus (MNV). Each trial began by slicing a single un-inoculated tomato in the slicer, followed by slicing an inoculated tomato. This was then followed by slicing 9 to 27 un-inoculated tomatoes. A similar and constant hand pressure on the slicer was used in every trial. Three slices from each tomato were collected for virus elution, concentration, and extraction before RT-PCR detection of MNV. The change in MNV per sliced tomato was averaged over all eight trials, and two mathematical models were fit to the average data using a logarithmic model or a power model. Regression analysis determined that the equation that best fit the data was $y = -0.903 * ln(x) + 7.945$, where $y =$ log10 MNV per slicing and $x =$ tomato slicing number. An acceptable fit ($R^2 = 0.913$) was indicated. The MNV levels transferred (y) generally decreased as the number of tomatoes sliced (x) increased, with some exceptions. Infrequent but erratic transfers, where the MNV level of a subsequent tomato was higher than that of a preceding tomato, occurred in later transfer of some trials. In contrast, the first and second transfers of each trial were always shown to have sharply decreased levels of MNV from the inoculum. The MNV log10 reduction per slicing event changes throughout the process: with a predicted 0.63 log10 reduction from tomato 1 to tomato 2 (76% reduction); a 0.07 log10 reduction predicted from tomato 13 to tomato 14 (a 14% reduction); and 0.03 log10 reduction predicted from tomato 27 to tomato 28 (a 7% reduction). Virus transfer is clearly variable even given the consistent slicing procedure used throughout each trial. This study illustrates the complex nature of risk prediction associated with NoV cross-contamination during food preparation in commercial establishments.

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1. Introduction

Noroviruses (NoVs) far exceed the other known agents of gastroenteritis as a cause of illness in the U.S. (Scallan et al., 2011). For the years 2000–2008, over 5 million cases of foodborne illness were caused each year by NoV alone, representing nearly 60% of the cases attributed to known agents (Scallan et al., 2011). Fresh produce and fresh produce-containing ready-to-eat foods (e.g., salads or sandwiches) have been linked to NoV infection outbreaks, and food preparation or food service procedures, rather than production or processing operations, often have been implicated as points of contamination (Hall et al., 2012). Commercial settings, especially restaurants and delicatessens, accounted for 83% of the food preparation settings linked to the outbreaks, and food handler contact during preparation of uncooked or ready-to-eat foods most commonly contributed to foodborne NoV outbreaks (Hall et al., 2012). Fresh produce in the commercial food preparation setting is clearly a significant source of the NoV disease burden.

NoVs are highly contagious with only 10 to 18 virus particles needed to cause infection in some cases (Teunis et al., 2008; CDC, 2013b). Virus particles are easily transmitted by person-to-person contact as well as through contact with contaminated surfaces (CDC, 2013a). Environmental vehicles including utensils, equipment, and gloves have been contributing factors in NoV outbreaks (Dreyfuss, 2009). Although studies of virus transfer from food handlers and environmental vehicles to food matrices have been published (Bidawid et al., 2000, 2004; D'Souza et al., 2006; Wei et al., 2010), there are many knowledge gaps, and the mechanisms by which NoVs are transmitted are not well understood.

The transfer of microorganisms during slicing of foods has been a topic of research in the past with the focus on bacterial transfer in meats. Earlier research (Farrell et al., 1998; Flores and Tamplin, 2002) showed that *Escherichia coli* O157:H7 in beef could be disseminated to

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specific areas of the meat grinder and to portions of ground beef, with more *E. coli* O157:H7 distributed in portions collected immediately after contamination was introduced. When deli meats were inoculated with *Listeria monocytogenes* and sliced with a commercial deli slicer, transfer of the pathogen to over at least 30 slices was observed (Vorst et al., 2006). In addition, *L. monocytogenes* could be transferred from an inoculated slicer onto meats (Lin et al., 2006).

More recently, mathematical models for bacterial transfer have been developed. Aarnisalo et al. (2007) showed that lower numbers of *L. monocytogenes* were transferred to salmon slices when the inoculum level was lower, when the temperature was colder or when the attachment time was longer. These researchers also showed a progressive exponential reduction in *L. monocytogenes* transfer during slicing. Sheen (2008) developed two empirical models for *L. monocytogenes* transfer from contaminated slicers to salami loaf. These models were reasonably accurate with high starting concentrations (>5 log CFU) and less accurate with lower starting concentrations of *Listeria*. Møller et al. (2012) developed a five parameter semi-empirical model for *Salmonella* transfer from a contaminated grinder to pork meat. This model hypothesized that transfer occurred from two environmental matrices inside the grinder, and predicted the observed concentrations of *Salmonella* during grinding of up to 110 pork slices. Hoelzer et al. (2012) summarized the probability distributions and mathematical models of *L. monocytogenes* transfer as an aid to quantitative microbial risk assessment.

This study used a mechanical, commercial-grade slicer, and investigated its capability for disseminating, transferring or cross-contaminating viruses from a single produce item to many others. Mechanical slicers are frequently used in food service, especially for high moisture content produce that is not precut prior to arrival in the restaurant (e.g., globe tomatoes or cucumbers). The tomato matrix was chosen in the current study because it is a common ready-to-eat ingredient in food service operations and has been implicated as the food vehicle in multiple outbreaks of NoV-associated gastroenteritis (CDC, 2013c). The current report is the first to characterize virus transfer to fresh produce during mechanical slicing with the intent to develop data and models for use in future risk assessment.

2. Materials and methods

2.1. Murine norovirus (MNV)-soaked tomato sliced by a mechanical slicer

A single large globe tomato freshly purchased from a local market was inoculated and used to contaminate the mechanical slicer. Soak-inoculation was chosen due to the impracticality of using (1) spot-inoculation over the large surface area of a ~180 g globe tomato, or (2) spray-inoculation on glassy surface of the tomato. The tomato was soaked in a Fisher sampling bag in 15 ml of ~10^7 pfu/ml of MNV-1 stock solution (originally given by Dr. Virgin at Washington University), and prepared by propagation in Raw cells 264.7 (Wobus et al., 2004). A vacuum sealer (ULINE, Chicago, IL) was used to seal the sampling bag-top, to maximize contact between the MNV and tomato surface. The sealed bag containing the tomato and MNV solution was gently shaken in an ice bath (approximately 1 to 2 °C to maintain MNV viability) at 35 rpm for 20 min, with a change to the bag-position after 10 min. The inoculated tomato was transferred to a sterile Petri dish and placed in a bio-safety hood level II for approximately 40 min until it was visibly dry. A commonly used restaurant-type mechanical slicer with 11 horizontal stainless steel blades 1/4" apart (Easy Tomato Slicer II, Nemco Food Equipment, Hicksville, OH) was used to slice 10 to 28 globe tomatoes per trial, with a total of eight trials used in the study. This slicer produced 11 to 12 tomato slices with a single hand-push. An un-inoculated tomato was sliced prior to beginning the trial. An inoculated tomato was then sliced, followed by up to 27 individual un-inoculated tomatoes sliced one by one.

To avoid experimental error or laboratory cross-contamination during slicing, 3 researchers with gloves each handled one of the following 3 steps: (1) placing clean tomatoes on the slicer, (2) manipulating the slicer and tomato sampling, and (3) sealing sample bags each with 120 ml-eluent and tomato slices. For each tomato sliced, 3 slices were collected for MNV-quantification. The MNV level in each tomato was calculated by multiplying the virus level of the 3 slices by a factor of 3.3. The selection of the 3 representative slices (nos. 3, 5, and 7) was determined by typical hand contamination patterns, showing the most probable contamination areas encountered during handling tomatoes (described in Section 3.3). Briefly, the hand contamination experiments were conducted as follows. Four volunteers were asked to pick up stem-down globe tomatoes, ranging from 1 to 4 trials for each volunteer. The areas where volunteers' fingertips contacted the surface of tomatoes were marked with a permanent marker and then the tomatoes were sliced through the 11 blade mechanical slicer. The slices with the marker-mark were tallied for each tomato, with the stem end counted as slice no. 1. Tomato slice nos. 3, 5, and 7 with tallies of at least 7 out of 11 (trials) by 4 volunteers were the most probable hand-contamination slices of tomatoes during picking (data in Table 2).

2.2. MNV eluted from tomato slices

Three slices of each tomato were aseptically transferred to a sampling filter bag containing 120 ml of ice-cooled eluent previously developed in our laboratory: 0.3% beef extract (BE powder, Becton Dickinson, Sparks, MD), pH adjusted to 8.5, prepared by tissue culture grade-phosphate buffer saline 10-fold dilution of 3% BE (originally made in water). Sealed filter bags (BagPage F, 63 micron porosity, Interscience, France) from each trial were shaken together at 125 rpm for 10 min at 10 °C. The eluates were collected for (1) direct quantitative reverse transcription-polymerase chain reaction (qRT-PCR) examination of the samples containing high levels of viruses, or (2) RNA extraction before qRT-PCR examination of the samples containing probable intermediate levels of viruses. Direct qRT-PCR was conducted simply by diluting the eluates 25-fold with water and heat-releasing virion RNAs at 100 °C for 10 min (Sun et al., 2012). In 6 of 8 trials, the first 5 tomato eluates were examined by direct qRT-PCR. From the 6th to the 28th tomato, the eluates were RNA-extracted (Mullendore et al., 2001) prior to qRT-PCR quantification with dual purposes of removing inhibitors and concentrating viruses. For the remaining 2 trials, tomato eluates were all RNA-extracted with some later samples concentrated first (with Amicon Ultra columns approximately 18-fold concentrated, 9 ml concentrated to 0.5 ml) before RNA extraction. When different detection or concentration procedures were used in a slicing trial, at least two samples were randomly selected and run by different procedures in order to line up or normalize the data using the adjustment factor. An example of an adjustment factor used is shown in Table 1.

2.3. MNV in eluates detected by qRT-PCR, with and without RNA extraction

Tomato eluate samples or eluate concentrates, 300 μl per sample, were RNA-extracted using RNeasy mini kit (Qiagen, Hilden, Germany), with the eluate concentrates first prepared by Amicon Ultra (100K) centrifugal filter units (Millipore, Billerica, MA). The final 60 μl RNA per sample was immediately frozen at −80 °C. Three types of templates were incorporated into qRT-PCR reactions of UltraSense qRT-PCR kit (Invitrogen, Carlsbad, CA): (1) RNAs of eluates, (2) RNAs of eluate concentrates, and (3) directly diluted eluate samples (for high MNV titer samples only). In each reaction of reverse transcription-polymerase chain reaction (RT-PCR), 6 μl of each RNA sample or a sample diluted 25-fold in water was added to a microtube containing a forward primer, a reverse primer, 0.5 μl RNAse inhibitor (Promega, Madison, WI), and water. All reaction mixtures were placed in a closed microtube, heated for 10 min at 100 °C in PTC 200 Thermal Cycler (Bio-RAD, Hercules, CA), and cooled down to 4 °C. Each of the heated and cooled mixtures was spun first, adding 4 μl of 5X reaction mix, fluorogenic probe (final 500 nM), RT-PCR enzymes (reverse transcriptase and polymerase),