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Cross contamination of *Escherichia coli* O157:H7 between lettuce and wash water during home-scale washing

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ABSTRACT

Lettuce and leafy greens have been implicated in multiple foodborne disease outbreaks. This study quantifies cross contamination between lettuce pieces in a small-scale home environment. A five-strain cocktail of relevant *Escherichia coli* O157:H7 strains was used. Bacterial transfer between single inoculated lettuce leaf pieces to 10 non-inoculated lettuce leaf pieces that were washed in a stainless steel bowl of water for 30 s, 1 min, 2 min, and 5 min was quantified. Regardless of washing time, the wash water became contaminated with 90–99% of bacteria originally present on the inoculated lettuce leaf piece. The *E. coli* O157:H7 concentration on initially inoculated leaf pieces was reduced ~2 log CFU. Each initially uncontaminated lettuce leaf piece had ~1% of the *E. coli* O157:H7 from the inoculated lettuce piece transferred to it after washing, with more transfer occurring during the shortest (30 s) and longest (5 min) wash times. In all cases the log percent transfer rates were essentially normally distributed. In all scenarios, most of the *E. coli* O157:H7 (90–99%) transferred from the inoculated lettuce pieces to the wash water. Washing with plain tap water reduces levels of *E. coli* O157:H7 on the inoculated lettuce leaf pieces, but also spreads contamination to previously uncontaminated leaf pieces.

Lettuce and leafy greens have been implicated in multiple foodborne disease outbreaks (Doyle and Erickson, 2008; Froder et al., 2007; Gorny et al., 2006; Lund and O'Brien, 2011; Smith et al., 2003). Pathogens, such as *Campylobacter*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella*, *Staphylococcus aureus*, *Shigella*, and *Yersinia enterocolitica* have all been linked to lettuce, salad, and leafy green outbreaks (Beuchat, 1996; Froder et al., 2007; Gorny et al., 2006). Head lettuce can harbor bacteria even after washing due in part to its large surface area and layering of the leaves (Solomon et al., 2002), and lettuce leaves can often have high populations of non-pathogenic bacteria (Smith et al., 2003; Soriano et al., 2000; Vijayakumar and Wolf-Hall, 2002).

Contamination of lettuce can occur at numerous points from food production through distribution including irrigation water, soil, harvesting, washing, packaging, storage, and in the kitchen (Beuchat, 1996; Froder et al., 2007; Gorny et al., 2006). Many pathogens, including *E. coli* O157:H7, survive and depending on temperature, grow on cut lettuce (Boyer et al., 2007; Gleeson and O'Beirne, 2005; Takeuchi et al., 2000; Wachtel et al., 2002). This is likely due to the cut leaf edges, which encourage attachment and contain nutrients that promote bacterial growth (Froder et al., 2007; Wachtel et al., 2002).

A consumer or restaurant employee may wash lettuce with the intention of removing dirt, debris, bacteria, or pesticides that may have accumulated during cultivation or processing. Prior research has demonstrated that multiple washes with plain water may not significantly reduce bacterial concentration on contaminated lettuce leaves or leaf pieces (Fink et al., 2012; Froder et al., 2007; Gil et al., 2009; Goularte et al., 2004; Nou and Luo, 2010; Wachtel et al., 2002; Zhang et al., 2009). A recent expert review of the literature concluded that washing ready-to-eat fresh cut leafy greens in the home was not likely to enhance safety but did significantly increase the risk for cross contamination (Palumbo et al., 2007). Microbial concentrations on some lettuce leaves or

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leaf pieces may increase in wash water that does not contain a sanitizer or which is improperly sanitized (Gil et al., 2009). Prior research has suggested that the primary benefit of sanitizing agents is to reduce cross contamination rates between produce by maintaining the quality of the wash water (Gil et al., 2009; Zhang et al., 2009). However, high organic loads, from dirt or produce debris, can rapidly reduce the effectiveness of wash water and sanitizers (Allende et al., 2008; Gil et al., 2009; Zhang et al., 2009).

The goal of this study was to quantify cross contamination rates of *E. coli* O157:H7 from inoculated to non-inoculated lettuce leaf pieces via un-chlorinated tap water to mimic what might commonly occur in a consumer or small restaurant kitchen, using commonly available tools (i.e. stainless steel bowl and municipal tap water).

1. Materials and methods

1.1. Preparation of stainless steel bowls

Stainless steel bowls, with a 15 cm circumference and 4 cm depth, were purchased from a kitchen supply store (Winter Haven, FL). Stainless steel bowl surfaces were disinfected by soaking in 30% sodium hypochlorite (Clorox, Oakland, California) overnight before use. Bowl surfaces were then scrubbed in hot water with an anionic active detergent, and rinsed with hot water. Bowl surfaces were then soaked in 70% ethanol for 1 h, removed, and air-dried prior to each experiment, modeled after a previously published protocol (Kusumaningrum et al., 2003). Several methods of disinfection as well as mechanical scrubbing/heat were used in combination, since disinfectants vary in their spectrum and modes of action. Multiple disinfectant methods provide a broader base of cleaning, to ensure minimal carry-over of bacteria or soil.

1.2. Produce

One bag of fresh cut romaine lettuce and one bag of fresh cut romaine spring mix, in re-sealable bags or storage containers (clam shells), were purchased from a local supermarket (Winter Haven, FL) for each experiment. Produce was stored at 4 °C and brought to ambient temperature prior to starting the experiment. To standardize the size of the lettuce pieces, the lettuce was cut, with an autoclaved, non-serrated knife, into approximately 3 × 3 cm leaf pieces. A total of 11 lettuce pieces (~15 g) were used per experiment.

1.3. Selection of strains

A five-strain cocktail of *E. coli* O157:H7 isolates from produce or produce-related commodities were used. Strain designation and sources are: Odwalla outbreak (223), human isolate from a cantaloupe outbreak (F658), human isolate from a lettuce outbreak (H1730), human isolate from an alfalfa sprouts outbreak (F4546), and human isolate from a spinach outbreak (EC4042). All strains were adapted to grow in the presence of 80 μg/ml rifampicin (Thermo Fisher Scientific, Waltham, MA), through stepwise exposure (Parnell et al., 2005).

1.4. Inoculum preparation

Prior to each experiment, frozen cultures of each strain were streaked onto tryptic soy agar (TSA; Difco, BD, Sparks, MD) with 80 μg/ml rifampin (TSAR), and incubated at 37 °C for 24 h. One isolated colony from each strain was transferred to 10 ml of tryptic soy broth (TSB; Difco, BD) with 80 μg/ml rifampin (TSBR), and incubated at 37 °C for 24 h. Cultures were then subcultured twice by transferring 0.1 ml of an overnight culture to 10 ml of fresh TSBR and incubated at 37 °C for 24 h. Each strain was subjected to centrifugation at 0.6 × *g* for 10 min (Allegra X-12, Beckman Coulter, Fullerton, CA). Cells were washed twice by removing the supernatant and suspending the cell pellet in 10 ml of 0.1% peptone (Difco, BD). Washed cells were suspended in 0.1% peptone at half the original culture volume. Strains were diluted in 0.1% peptone and combined in equal volumes to achieve a concentration of 9 log CFU/ml. This concentration of peptone is generally below the protein concentration needed to promote attachment (Barnes et al., 1999). Final concentrations were verified for each strain by enumeration on TSAR.

1.5. Transfer between lettuce pieces

Ten leaf pieces of green romaine lettuce were used as the non-inoculated lettuce. One leaf piece of red lettuce was used as the inoculated lettuce. Ten microliters of *E. coli* O157:H7 cocktail were spotted onto the abaxial side of the red lettuce piece and dried for ~10 min to simulate the type of cross-contamination that might happen by a splash event in a home kitchen during meal preparation. The spots were visibly dry before proceeding. *E. coli* O157:H7 concentration on inoculated lettuce was confirmed as ~6 log CFU/leaf piece by enumeration on TSAR in triplicate. One hundred milliliters of room temperature sterile potable city water was poured into a sterile stainless steel bowl. All the lettuce pieces (both inoculated and non-inoculated) were added to the bowl and mixed with constant agitation using a sterile spoon for 30 s, 1, 2 or 5 min. Each lettuce piece was pulled out of the mixing bowl using sterile forceps, and 1 ml of the wash water was sampled. Each experiment was performed in triplicate.

1.6. Enumeration of cells

Lettuce pieces were placed in a sterile 207 ml Whirl-Pak filter bag (Nasco, Fort Atkinson, WI, USA), 40 ml of DE neutralizing buffer (Dey/Engley, Thermo Fisher Scientific, Waltham, MA) was added, and the samples were macerated in a smasher (AES Laboratories, Chemunex, France) for 1 min. Homogenized lettuce samples and wash water were serially diluted in 0.1% peptone and surface plated (0.1 ml) onto TSAR and sorbitol MacConkey agar (SMAC; Difco, BD, Sparks, MD) with 80 μg/ml rifampicin (SMACR). TSAR and SMACR plates were incubated at 37 °C for 24 h. Colonies were counted by hand following incubation, and *E. coli* O157:H7 population levels were expressed in log CFU/g for produce and log CFU/ml for wash water. The total number of cells added to the system on the inoculated leaf piece at the beginning of the experiment was compared to the total number of cells at the end of the experiment (inoculated leaf piece post wash, plus amount transferred to the non-inoculated leaf pieces, plus the amount transferred to the water). The number of cells recovered pre- and post-experiment showed statistically significantly ($p = 0.003$) lower overall recoveries on SMAC (-0.06 ± 0.13 log CFU) vs. TSAR (0.15 ± 0.11 log CFU), as expected, since SMAC is a more selective medium.

1.7. Data analysis

Data were compiled, log transformed, and used to create histograms, using Microsoft Excel (Microsoft, Redmond, Washington). The number of times a particular transfer rate occurred within a target data set (i.e. it's frequency) was plotted on the *y*-axis to visualize variability in log percent transfer rates during the different transfer events. The *x*-axis in these histograms is log percent transfer, as previous research in our laboratory has

indicated that this transformation generally produces normally distributed data (Schaffner, 2003). The *x*-axis bin width used to create these histograms was 0.5 log percent transfer, as past experience in our lab indicates that this is generally satisfactory. Optimal bin size is determined by multiple factors, but generally the fewer observations available, the larger the bin needs to be to visualize meaningful trends.

Because of the complexities in cross-contamination research, such that any measurement of a contaminated surface will change the concentration on that surface, the following equations were used for log reduction and percent transfer calculations.

Log reduction on each initially inoculated piece was calculated as the difference between the initial level on individual inoculated leaf pieces and the level on the leaf piece after washing. The initial level is calculated indirectly as the sum of the amount transferred to previously a non-inoculated leaf piece and the corresponding wash water plus the amount on the leaf piece after washing:

$$\begin{aligned}&\text{Log reduction on the initially inoculated piece}\\&= \text{Log}(\text{CFU previously non} - \text{inoculated lettuce pieces}\\&\quad + \text{CFU wash water} + \text{CFU initially inoculated piece post}\\&\quad - \text{wash}) - \text{Log}(\text{CFU initially inoculated piece post}\\&\quad - \text{wash})\end{aligned}$$

Since calculations are done on a per leaf piece basis, the weight of an individual leaf piece was not measured.

Percent transfer to non-inoculated pieces is calculated as the ratio between the level on previously non-inoculated pieces after washing and level on the inoculated pieces, where the level on the inoculated pieces is calculated indirectly as the sum of the amount transferred to previously non-inoculated pieces and the wash water:

$$\text{Transfer Rate to non} - \text{inoculated pieces}(\%) = \frac{\text{CFU on previously non} - \text{inoculated lettuce piece}}{\text{CFU previously non} - \text{inoculated lettuce pieces} + \text{CFU wash water}} * 100$$

Calculations with wash water are based on the total amount of bacteria in the entire volume of wash water (100 ml). Percent transfer to the wash water is calculated as the ratio between the level in the wash water and level on the inoculated pieces, where the level on the inoculated pieces is calculated indirectly as the sum of the amount transferred to previously non-inoculated pieces and the wash water:

$$\text{Transfer Rate to wash water}\left(\%\right) = \frac{\text{CFU wash water}}{\text{CFU on previously non} - \text{inoculated lettuce piece} + \text{FU wash water}} * 100$$

A “mass-balance” style validation of the suitability of the calculations shown above was performed. Comparisons between *E. coli* O157:H7 CFU recovered from control inoculated lettuce pieces not used for experiments and total *E. coli* O157:H7 CFU recovered from inoculated pieces post-wash plus CFU in wash water plus CFU recovered from previously non-inoculated pieces showed good agreement. The mean difference between the total CFU introduced into the system and the total CFU recovered across all experiments was 0.02 ± 0.16 log CFU.

2. Results

The reduction of *E. coli* O157:H7 on the initially inoculated piece of lettuce after being washed with the non-inoculated pieces, when samples were plated onto TSAR is shown in Fig. 1. At the shortest wash time (30 s), two observations had a ~2-log reduction, and one observation had a slightly higher log reduction (~2.5 log). This trend reversed for the 1 min wash time; with one observation of a 2-log reduction, and two with a slightly higher log reduction (~2.5 log). When wash time was increased to 2 or 5 min, all three observations were ~2.5 log CFU/g. Fig. 2 shows the equivalent data where the *E. coli* O157:H7 cells were recovered on SMACR. The shortest and longest wash times (30 s and 5 min) both had two instances of a ~2 log CFU reduction and one of ~2.5 log reduction, while the intermediate wash times of 1 min 2 min wash showed the reverse (two instances of ~2.5 log CFU reduction and one of ~2 log reduction). No differences in log reduction at different wash times were significant ($p = 0.05$). The choice of a nonselective (TSAR) vs. selective (SMACR) medium seems to have little effect on recovery from initially inoculated leaf pieces. When all data were pooled the overall reduction was 2.05 ± 0.18 log CFU. In all scenarios, most of the *E. coli* O157:H7 (90–99%) transferred from the inoculated lettuce pieces to the wash water (data not shown).

The distribution of log percent transfer of *E. coli* O157:H7 from the piece of inoculated lettuce to each of the non-inoculated pieces of lettuce during washing, when samples were plated onto TSAR are shown in Fig. 3. The shortest and longest wash times (30 s and 5 min), showed the greatest transfer rates, most commonly transferring 0 log % (~1%) of the *E. coli* O157:H7 originally present on the inoculated lettuce leaf piece to the non-inoculated leaf pieces. In some instances, slightly higher (0.25 log; 1.8%) or slightly lower (−0.25 log; 0.6%) transfer rates were observed. In one instance with the 30 s wash time ~5% transfer (0.75 log) was observed, but the transfer rate never exceeded 10% (1 log). The intermediate wash times showed slightly lower (~0.25 log; 0.6%) transfer rates, most commonly 0.25 log (~0.6%) of the *E. coli* O157:H7 originally present on the inoculated lettuce piece transferred to the non-inoculated leaf pieces. In some instances, slightly higher (0.0-log; 1%) or slightly lower (−0.5-log; 0.3%) transfer rates were observed. In one instance with the 2 min wash time ~1.8% transfer (0.25-log) was observed, but the transfer rate never exceeded 3.2% (0.5 log percent transfer).

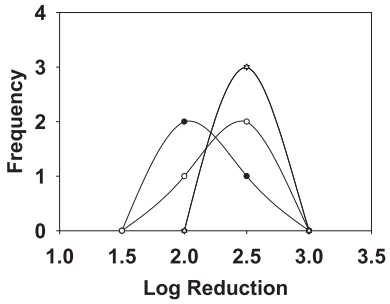

Fig. 1. Log reduction of *E. coli* O157:H7 on initially inoculated lettuce pieces after being washed with the non-inoculated pieces and plated on tryptic soy agar with 80 μg/ml rifampicin. Wash times are 30 s (●), 1 min (○), 2 min (▼), and 5 min (△).

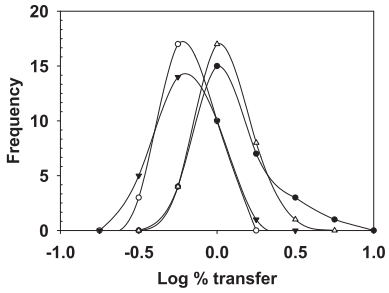

Fig. 3. Frequency distributions of log % transfer of *E. coli* O157:H7 from the inoculated lettuce pieces to non-inoculated lettuce pieces during washing when plated on tryptic soy agar with 80 μg/ml rifampicin. Wash times are 30 s (●), 1 min (○), 2 min (▼), and 5 min (△).

The log percent transfer of *E. coli* O157:H7 from the piece of inoculated lettuce to the non-inoculated pieces of lettuce when samples were plated onto the more selective SMACR are shown in Fig. 4. All wash times showed ~1% (0-log percent transfer) of the *E. coli* O157:H7 most frequently transferring from the inoculated leaf pieces to the non-inoculated leaf pieces. In some instances the log percent transfer rates were 0.25 log percent higher or lower, or rarely 0.5 log percent higher or lower. As with the inoculated leaf pieces, the choice of a nonselective (TSAR) vs. selective (SMACR) medium had little effect on recovery. In all cases the distribution of log percent transfer rates were essentially normally distributed.

3. Discussion

Studies that quantify the effect of washing and/or sanitizer use on the concentration of pathogens on leafy green produce are numerous (Akbas and Olmez, 2007; Goularte et al., 2004; Inatsu et al., 2005; Lang et al., 2004; Smith et al., 2003; Wachtel et al., 2002), while published data on the occurrence of cross-contamination between inoculated and non-inoculated produce are more limited (Wachtel and Charkowski, 2002; Zhang et al., 2009). We are not aware of any published research that quantifies the spread of contamination between lettuce leaf pieces during washing after some of those leaf pieces have been cross-contaminated in scenario mimicking a home kitchen environment. Understanding and quantifying all of these aspects of the issue are important in managing risk.

Under the contamination scenario mimicked in this study (e.g., cross contamination in the kitchen during preparation) our results show the concentration of *E. coli* O157:H7 on the inoculated leaf pieces is reduced by 2–2.5 log CFU/g during washing, with most (>90%) of the removed cells ending up in the wash water. Previous studies suggest that washing with tap water can achieve a 0.5–2 log CFU/g reduction on leafy green pieces when the initial inoculation level was 5–6 log CFU/g (Akbas and Olmez, 2007; Goularte et al., 2004; Inatsu et al., 2005; Lang et al., 2004; Smith et al., 2003; Wachtel et al., 2002). While our results indicate a slightly greater reduction than that observed in the literature, it should be noted that our experimental design was also different. In most previous studies, all the leaf pieces to be studied were inoculated, and no non-inoculated leaf pieces were used (Akbas and Olmez, 2007; Goularte et al., 2004; Inatsu et al., 2005; Smith et al., 2003). Our experimental design included a substantial number of non-inoculated leaf pieces, which could also become contaminated in the course of the experiment. Many other methodological differences could also have influenced the results including strain selection, inoculum preparation, inoculation method, drying time, etc.

A number of articles related to cross-contamination between inoculated and non-inoculated produce have been published (Allende et al., 2008; Holvoet et al., 2014; Luo et al., 2012; Pérez Rodríguez et al., 2011; Wachtel and Charkowski, 2002; Zhang et al., 2009). Wachtel and Charkowski (2002) studied leaf piece to piece cross-contamination during dry mixing that mimicked restaurants procedures. Zhang et al. (2009) quantified cross-contamination during lab-scale washing. Allende et al. (2008) studied the degree of generic *E. coli* cross-contamination between inoculated and uninoculated escarole during washing. Pérez Rodríguez et al. (2011) used stochastic simulation modeling based on a physical indicator (Glo Germ) to represent *E. coli*. Luo et al. (2012) studied non-pathogenic *E. coli* O157:H7 on baby spinach leaves washed with large amounts of uninoculated iceberg lettuce shreds in a pilot plant scale washing system. Holvoet et al. (2014) quantified two cross-contamination processes (from lettuce to

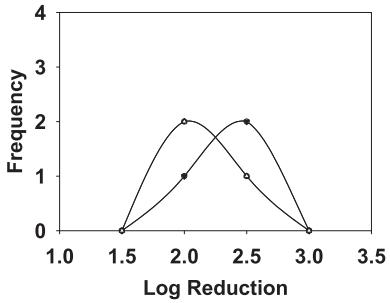

Fig. 2. Log reduction of *E. coli* O157:H7 on initially inoculated lettuce pieces after being washed with the non-inoculated pieces and plated on sorbitol MacConkey agar with 80 μg/ml rifampicin. Wash times are 30 s (●), 1 min (○), 2 min (▼), and 5 min (△).

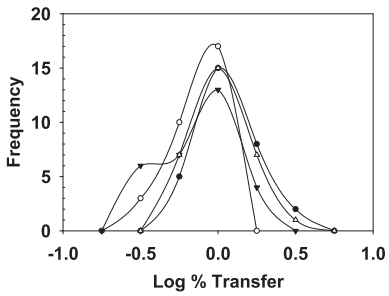

Fig. 4. Frequency distributions of the log % transfer of *E. coli* O157:H7 from the inoculated lettuce piece to each of the non-inoculated pieces of lettuce during washing is as plated on sorbitol MacConkey agar with 80 μg/ml rifampicin. Wash times are 30 s (●), 1 min (○), 2 min (▼), and 5 min (△).

water and from water to lettuce) using generic *E. coli* and *E. coli* O157.

Wachtel and Charkowski mixed dry uncontaminated lettuce leaf pieces with a single *E. coli* O157:H7 inoculated lettuce leaf piece. These researchers tested for presence of *E. coli* O157:H7 on the cross-contaminated leaf pieces, but did not quantify the cross contamination. Their results showed that when an initially inoculated lettuce leaf piece contained 3 log CFU *E. coli* O157:H7, the cross contamination between lettuce leaf pieces was not detectable, at a detection limit of 10 CFU/lettuce piece, implying less than 1% transfer (0-log percent transfer). When they used a lettuce leaf piece inoculated with 4 log CF *E. coli* O157:H7, 7% of the cross-contaminated lettuce leaf pieces contained detectable *E. coli* O157:H7. When the inoculated leaf piece contained 5-log CFU *E. coli* O157:H7 then 96% cross-contaminated lettuce leaf pieces contained detectible contamination, indicating the transfer rate was usually greater than 0.01% (−2 log percent transfer).

Zhang et al. performed a similar study to ours (Zhang et al., 2009). In their study the iceberg lettuce leaf pieces were spot inoculated with an *E. coli* O157:H7 cocktail to achieve ~5.6 log CFU per piece. The conditions used by Zhang et al. that was most similar to our conditions was a 1.5 min wash with tap water, with agitation, and no organic load. Zhang et al. observed a ~2 log CFU/piece reduction on the inoculated leaf pieces after washing, which was similar to what we observed (2–2.5 log reduction per leaf piece). Their post wash water contained 1.8 log CFU/ml after wash, while our wash water contained 3.7 log CFU/ml. Their non-inoculated lettuce pieces had 2.5 log CFU/piece after washing, while ours had ~4 log CFU/piece post wash. Their washing regime differed from ours in several ways. We used a smaller water volume (100 vs. 500 ml) and we used more non-inoculated leaf pieces per experiment (10 vs. 5 leaf pieces). Both differences would favor both an increased concentration in the wash water and a higher transfer to non-inoculated leaf pieces. These differences point out the importance of total water volume and produce concentration in that water as key variables in these types of experiments. Other experimental differences including holding conditions for inoculated leaf pieces (4 °C for 2 h for Zhang et al. vs. room temperature for 10 min in our study) as well as differences in washing temperature (4 °C for Zhang et al. vs. room temperature) may also have played a role in the differences observed. As others have noted (Lang et al., 2004), storage of inoculated lettuce at 4 °C reduces recovery relative to storage at 22 °C.

Allende et al. (2008) studied the degree of *E. coli* cross-contamination between inoculated and uninoculated Escarole during washing. These researchers used three generic *E. coli* strains of unreported origin, spot inoculated on to escarole in 0.1% peptone water and held a 4 °C for 60 min. One hundred gram samples of fresh-cut escarole inoculated with 5 and 3 log CFU/g were washed in 5 L of water. Subsequently, 900 g of uninoculated fresh-cut escarole was washed in the same contaminated water. Counts on inoculated leaf pieces were reduced, but not significantly. Uninoculated pieces had significantly fewer *E. coli* (1 log) than inoculated in some water types tested but not others. Levels in water ranged from undetectable (no detection limit reported) to 3.6 log CFU/ml and 5.5 log CFU/ml. The highest *E. coli* levels in water reported are 2 orders of magnitude higher than the total amount of *E. coli* introduced into the system, highlighting the importance of a mass-balance style calculation as we indicate at the end of the methods above.

Pérez Rodríguez et al. (2011) used a stochastic simulation modeling approach to predict the extent of *E. coli* O157:H7 contamination in fresh-cut bagged lettuce leaving the processing plant. Transfer data for the simulation were taken from a poster presentation entitled “Glo Germ as a cross contamination indicator during processing of leafy greens” and do not appear to be based on data collected using microorganisms, and are not publicly available. The manuscript did not explicitly model transfer from water to non-contaminated lettuce.

Luo et al. (2012) used a non-pathogenic strain of *E. coli* O157:H7 inoculated onto baby spinach leaves washed with large amounts of freshly cut uninoculated iceberg lettuce shreds in wash water in a pilot plant scale washing system. The system had fluctuating free chlorine levels, hence a direct comparison to our experimental system is not possible. The washing of inoculated spinach leaves reduced pathogen counts on spinach by 0.8–0.9 log CFU/g, and *E. coli* O157:H7 was recovered in the control wash water solution in concentrations ranging from 0.2 to 8.1 MPN/ml, when free chlorine was 0.5 ppm or less. Levels of *E. coli* in un-inoculated lettuce ranged from 0.1 MPN/g to 2 MPN/g.

Holvoet et al. (2014) quantified two cross-contamination processes (from lettuce to water and from water to lettuce) using generic *E. coli* from lettuce, and two strains of *E. coli* O157 with unspecified origin. These researchers did not study simultaneous cross-contamination from lettuce to water and to previously uninoculated leaves as we did, but rather broke the process into two steps. Slices of lettuce 3 cm long were dip inoculated, spun dry and stored refrigerated overnight prior. Water was directly inoculated to study cross-contamination to lettuce. The authors did not report a mass-balance style calculation, but appear to consider full lettuce and water masses in their calculations. We calculate from the data they report that 20–25% of generic *E. coli* transferred to water and they report a 0.33 log reduction on lettuce with washing. They report a mean transfer ratio of *E. coli* O157 from water to the lettuce of 1.0% ± 0.3% overall inoculation experiments.

Our study builds on this prior literature by providing quantitative data on magnitude and variability of the cross-contamination that occurs when leafy green produce contaminated during preparation is subsequently washed. It confirms prior research that plain water washing may, under some circumstances, produce a 2-log reduction in the bacterial concentration on leafy green produce (Smith et al., 2003) although this observation is by no means universal (Lang et al., 2004). It shows that increasing plain water wash times from 30 s to 5 min has little effect on wash effectiveness or cross-contamination rates. More importantly it demonstrates that plain water washing moves bacterial contamination to wash water (Danyluk and Schaffner, 2011), and that wash water can in turn serve to contaminate previously uncontaminated leaves and leaf pieces (Palumbo et al., 2007). This research highlights the importance of total water volume and produce concentration in that water as key variables in these types of experiments. Finally, this research further underscores the need for proper sanitation of the wash water (Zhang et al., 2009), and will aid in the development more accurate risk analysis for leafy green related outbreaks.

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