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RESEARCH ARTICLE

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Evaluation of glove type on survival and transfer of *Escherichia coli* in model systems and during hand harvesting of lettuce

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Abstract

Background: Both reusable and single-use gloves can be employed during hand harvesting of lettuce and leafy greens. The impact of glove type on survival and transfer of *Escherichia coli* was evaluated using agar or lettuce in a laboratory setting and during simulated lettuce harvesting in the field.

Results: Textured and smooth reusable latex and smooth disposable latex gloves inoculated with *E. coli* were sequentially touched to 10 or 20 agar plates or 20 lettuce leaves ($n = 6$; laboratory) or used to sequentially harvest 20 heads of lettuce ($n = 6$; field). *E. coli* was recovered by enrichment from significantly fewer leaves (46%; 55 of 120) or heads (26%; 31 of 120) of lettuce when inoculated reusable textured gloves were used compared with disposable gloves (leaves: 98%; 118 of 120, or heads: 74%; 89 of 120). In contrast, when a single head of lettuce was the point source for glove contamination, there was no significant difference in the number of *E. coli*-positive lettuce heads harvested with reusable textured (71%; 85 of 120) or disposable gloves (75%; 90 of 120). In either field-contamination scenario, at the 20th head of lettuce harvested with a single glove (final sample point), *E. coli* was recovered from one to five of six lettuce heads across experimental trials.

Conclusion: Contamination of a glove from a single point source can lead to subsequent contamination of multiple heads of lettuce during hand harvesting, showing the importance of policies to manage hand hygiene and glove use for harvest crews.

KEYWORDS
cross contamination, gloves, harvest, leafy greens, lettuce, STEC

INTRODUCTION

Gloves are often used when handling foods, on the assumption that a physical barrier will prevent the food handler from contaminating food. Early data on glove effectiveness were published in the healthcare literature.[1,2] These studies had limited application in food handling because of their focus on high-quality surgical gloves, which are not used in food service or food processing sectors. Many of these healthcare studies used a "watertight test," which may not be indicative of how gloves fare while in use.[3] Some studies have examined gloves in a foodservice setting. Bardell[4] inoculated droplets of saliva containing herpes simplex virus on the outside of latex disposable gloves and evaluated transfer to lettuce or ham; although virus could be isolated from the food in all five trials for each group, transfer was

Irene Y. Zhao and Jiin Jung should be considered joint first authors.

not quantified. Fendler et al.[5] used volunteers to handle ground beef containing *Escherichia coli* and showed that the outside of the glove was highly contaminated at the end of a 3-h period regardless of whether gloves had been changed or hands washed. Montville et al.[6] evaluated bacterial transfer through foodservice quality gloves but did not specifically evaluate transfer by gloved hands.

Leafy green vegetables including lettuce have been broadly recognized as vehicles for foodborne pathogens such as *E. coli* O157:H7. Lettuce was implicated in over 31 outbreaks of Shiga toxin–producing *E. coli* (STEC) O157:H7 between 1998 and 2020.[7–11] Investigations of multistate outbreaks associated with lettuce have noted multiple potential contamination points from production to final consumption.[12–14] Evidence for contamination via agricultural water,[15–17] soil,[15–17] wild or domestic animals,[17] adjacent land use,[12] and human handling[18–21] have all been suggested.

Microbial cross contamination from gloved hands has been shown to occur during hand harvesting of fresh produce, including lettuce,[19,20] basil,[20] peppers,[15,22] and tomatoes.[15,23] Bacterial transfer between gloves and produce may be affected by factors such as concentration or type of organism,[20,23] glove material,[23,24] presence of organic matter,[23,24] and environmental conditions.[15,23]

The U.S. Food and Drug Administration (FDA) Produce Safety Rule[25] and Good Agricultural Practices focus on prevention of contamination of produce during pre- and postharvest handling. The California Leafy Green Products Handler Marketing Agreement program provides best practices guidelines for lettuce and leafy greens production and harvest.[26] These include training employees to wash hands with soap and running water before donning gloves and when hands may have become contaminated, ensuring gloves are intact and sanitary, and prohibiting the use of personal gloves or taking gloves home. Although the risk of pathogen transmission can be reduced by proper glove use, gloves may still become contaminated by various soils, which may result in subsequent cross contamination during harvest.[20,24,27] While the influence of specific factors on transfer of microorganisms has been studied under laboratory conditions, this may not reflect the agricultural production environment. This study was undertaken to evaluate the impact of glove type on survival and sequential transfer of *E. coli* under both laboratory conditions and during simulated lettuce harvest in the field.

MATERIALS AND METHODS

Gloves

Four different glove types were selected based on field observations during harvest of leafy greens in the Salinas Valley and on glove material and texture: (a) reusable textured latex (embossed fish-scale pattern, 20 mil, Canners and Handlers 394, Ansell Ltd., Iselin, NJ); (b) reusable smooth latex (pebble embossed, 20 mil, Canners and Handlers 392, Ansell Ltd.); (c) disposable smooth latex (5.5 mil, EV2050 Evolution One, Microflex, Reno, NV); and (d) disposable smooth nitrile (3.5 mil, KC300 Sterling Nitrile, Kimberly-Clark Professional, Roswell, GA).

Lettuce

Romaine lettuce cv. Paris Island (*Lactuca sativa*) seeds were planted in pots containing potting soil (Sun Gro Horticulture, Agawam, MA) and grown for 12 weeks in an environmental chamber (PGR15, Conviron, Pembina, ND) with a light intensity of 230 μmol m^{-2} s^{-2} set to a photoperiod of 12 h at 22°C and a 12-h dark cycle at 18°C, and at 60 ± 3% relative humidity throughout. The plants were fertilized weekly with Hoagland nutrient water, starting 2 weeks after emergence.

Bacterial strains and inoculum preparation

Three *E. coli* strains were used: (a) "generic" *E. coli* TVS 354 isolated from romaine lettuce on the Central Coast near Salinas Valley, CA (provided by Dr. Trevor Suslow, University of California, Davis); (b) stx-negative *E. coli* O157:H7 ATCC 700728 (stx_1^- and stx_2^-) (*stx*− *E. coli* O157) classified as BSL1 by ATCC and previously used for field trials;[28,29] and (c) stx-positive *E. coli* O157:H7 EC 4045 (stx_1^-, stx_2^+, and stx_2c^+) (*stx*+ *E. coli* O157), isolated from spinach plants during the 2006 spinach-associated outbreak (provided by Dr. Thomas A. Cebula, U.S. FDA).[30] Variants of all three strains resistant to 120 μg ml^{-1} rifampin (Gold Biotechnology, St. Louis, MO) were isolated through stepwise exposure;[31] 50 μg ml^{-1} in media was sufficient, when needed, to suppress background microbiota. A frozen culture of each strain was streaked onto tryptic soy agar (TSA; tryptic soy broth and 1.5% granulated agar; Difco, BD, Franklin Lakes, NJ) supplemented with 50 μg ml^{-1} of rifampin (TSAR) and incubated overnight at 37°C. A single isolated colony was transferred into tryptic soy broth supplemented with 50 μg ml^{-1} of rifampin (TSBR). The subsequent overnight culture was spread over TSAR using an automated spiral plater (Autoplate 4000, Spiral Biotech Inc., Norwood, MA) and incubated for 24 h to produce a bacterial lawn. The bacterial lawn was loosened by adding 5 ml of 0.1% peptone (Difco, BD) to each plate and collected with a sterile spreader (Lazy-L Spreader, Andwin Scientific, Tryon, NC). Appropriate dilutions of the cell suspensions were made in 0.1% peptone to achieve target inoculation levels.

Inoculation of gloves in laboratory-based studies

For all the laboratory-based studies, a 10 × 10 cm piece was aseptically cut from the palm area of each glove and then wrapped (with the outer glove surface exposed) around a foam sheet (5 × 5 cm, Elmer's Product Inc., Columbus, OH) that was on top of an acrylic block (5 × 5 cm, Fiskars Brands Inc., Madison, WI). The acrylic blocks were sprayed with 70% ethanol and allowed to dry between each use. The surface of the glove was spot inoculated using a repeater pipette dispenser (Eppendorf Repeater Plus, Eppendorf Inc., Hauppauge, NY) to deliver 20 1-μl spots across the 5 × 5 cm surface. The inoculated

gloves were then held at ambient conditions (~23°C, ~40% RH) for 30 min. The inoculum was visibly dry at this time.

Survival of different *E. coli* strains on gloves

Individual strains of *E. coli* were inoculated onto reusable textured latex gloves at 2.5, 4.5, and 7.0 log CFU glove^{-1}. Each glove piece was removed from the block and transferred to an individual 710-ml Whirl-Pak bag (Nasco, Fort Atkinson, WI) for enumeration or enrichment, as described below.

Transfer of *E. coli* TVS 354 from inoculated gloves to agar

Reusable textured latex, reusable smooth latex, disposable smooth latex, or disposable nitrile gloves were inoculated with *E. coli* TVS 354 at 3.5 or 4.5 log CFU glove^{-1}. The inoculated glove was pressed onto the surface of a TSAR plate and then a 200-g weight was placed on the top of the glove-wrapped block for 5 s, consistent with the approximate time needed for a harvester to cut one head of lettuce. The same glove was then pressed sequentially onto another 9 or 19 TSAR plates (depending on inoculum levels of 3.5 or 4.5 log CFU glove^{-1}, respectively) using the same procedure. Bacterial colonies were counted after incubation at 37°C for 24 h, and *E. coli* populations were expressed as log CFU plate^{-1}.

Transfer of *E. coli* TVS 354 from inoculated gloves to lettuce

Reusable textured latex and disposable smooth latex gloves were inoculated with *E. coli* TVS 354 at ~4.5 log CFU glove^{-1} or at 7.0 log CFU glove^{-1} (disposable smooth latex only). Intact lettuce leaves (5–10 g each) were cut from 12-week-old lettuce plants and were laid flat, upper (adaxial) surface facing up, in a single layer, on sterile paper towels. Within 5 min, the inoculated glove-wrapped block was placed face down on the leaf surface and a 200-g weight was placed on top of the block for 5 s. The same glove was then placed sequentially onto another 5 (~7.0 log CFU glove^{-1}) or 19 (~4.5 log CFU glove^{-1}) leaves using the same procedure. The leaves were placed in individual 710-ml Whirl-Pak bags for enrichment, as described below. Populations of *E. coli* on the glove were determined, as described below, for control gloves prior to transfer and for gloves after 20 sequential transfers.

Transfer of *E. coli* TVS 354 from inoculated lettuce to gloves

The adaxial side of outer leaves of 12-week-old lettuce plants was inoculated with a calibrated spray bottle (60-µl spray^{-1}; target 5.5 or 6.5 log CFU leaf^{-1}) from approximately 2 cm to achieve a uniform population of ~5.0 log CFU leaf^{-1} of *E. coli* TVS 354 after drying at ambient conditions for 6 or 24 h, respectively. Populations of *E. coli* on control leaves were determined after 0, 6, and 24 h of drying. Dry inoculated leaves were placed adaxial side up on sterile paper towels. *E. coli* transfer from the inoculated lettuce leaves to reusable textured, reusable smooth, and disposable smooth latex gloves was evaluated by placing a latex glove-wrapped block face down on the surface of an inoculated lettuce leaf with a 200-g weight on top for 5 s. Both the lettuce leaf and glove were placed into individual 710-ml Whirl-Pak bags for enumeration, as described below.

Cross contamination during field harvesting of lettuce

Field trials were conducted in the Salinas Valley region of California during spring 2011 and summer 2012, as described in Moyne et al.[28] Permits and approvals for use of United States–owned land were granted by the U.S. Department of Agriculture. Romaine lettuce (*L. sativa*) cv. Green Towers (2011) or Braveheart (2012) seeds were planted in two rows per bed according to standard commercial practice.

Transfer of *E. coli* TVS 354 and *stx*– *E. coli* O157 from inoculated gloves to lettuce in the field under simulated conditions of lettuce harvest was determined using intact reusable textured, reusable smooth, or disposable smooth latex gloves. *E. coli* was spot inoculated (20 1-µl spots) onto the four fingers and thumb on both gloved hands at a target of 4.5 or 7.0 log CFU glove^{-1} and spread by rubbing the fingertips together. The gloves were visibly dry in ~2 min under field conditions. The right-hand glove was removed and placed into a 230-g specimen container (Thermo Fisher Scientific, Waltham, MA) containing 50 ml of 0.1% peptone to determine the initial level of *E. coli*. The harvesting was conducted by two harvesters who alternated after harvesting 20 lettuce heads; the order of the glove type (reusable or disposable) used for each replicate was randomized. Each harvester used the gloved left hand to harvest 20 lettuce heads sequentially, holding an uninoculated head of lettuce from the top and removing the head from the base with a sterile knife in the right hand. For each glove type, the procedure was repeated three times by each of the two harvesters (total 120 heads of lettuce for each glove type) using a new pair of gloves for each replicate. The 4–8 outer leaves that contacted the contaminated glove were placed into a 1600-ml Whirl-Pak bag for subsequent testing. All bagged glove and lettuce samples were placed on ice, transported to the laboratory, and analyzed for *E. coli* within 24 h, as described below.

Mature unharvested heads of lettuce were spray inoculated one time at approximately 10 cm from the top of the head with 7.0 log CFU ml^{-1} spray^{-1} as described above and then allowed to dry for 2 h. To evaluate the transfer of *E. coli* TVS 354 from inoculated lettuce to other heads of lettuce via gloved hands, a pair of reusable textured or disposable smooth latex gloves was used to harvest one head of inoculated lettuce by the procedure described above. The same glove was used to sequentially harvest 20 heads of uninoculated lettuce. The procedure was repeated six times (total 120 heads of lettuce for each glove type) using a new pair of gloves for each replicate.

Enumeration and enrichment of glove and lettuce samples

Sterile 0.1% peptone (30 or 50 ml) was added to glove samples, and 50 ml of 0.1% peptone was added to lettuce samples prior to stomaching (Stomacher 400, Seward, Westbury, NY) for 2 min at high speed. Samples were serially diluted in 0.1% peptone and plated on TSAR using an automated spiral plater. Gloves were placed into 100 ml of Dey-Engley neutralizing buffer (Thermo Fisher Scientific), and 50 ml of the diluent was filtered using disposable analytical filter units (0.45 μl; Nalgene, Thermo Fisher Scientific); filter membranes were removed and placed onto CHROMagar O157 (CHROMagar, Paris, France) when necessary to improve the limit of detection (LOD). Samples were incubated at 37°C for 24 h, colonies were counted, and *E. coli* populations were expressed as log CFU glove^{-1} or lettuce leaf^{-1}.

For enrichment, TSBR (200 ml) was added to the glove or lettuce sample followed by stomaching for 1 min at high speed. Samples were incubated at 42°C for 18 ± 4 h, plated onto CHROMagar ECC or CHROMagar O157, and incubated at 37°C for 24 h. Colonies were evaluated for those typical of *E. coli* (blue colonies) on CHROMagar ECC and of *E. coli* O157:H7 (mauve colonies) on CHROMagar O157.

Levels of *E. coli* were determined in one study by a modification of the Quanti-Tray (Idexx, Westbrook, ME) most-probable-number (MPN) method as previously described[28] to lower the LOD to 1 MPN per sample. Subsamples (200 μl) were distributed into 48 350-μl wells of a 96-well plate. The remaining cell suspension was distributed in 2-ml aliquots per 2.2-ml wells of a 96-well plate. All plates were sealed with microplate adhesive film and incubated at 42°C for 24 h. Adhesive film was removed and the enrichment broth was transferred with a 96-pin sterile replicator (Phenix Research Products, Candler, NC) to a 96-well plate containing 100 μl per well of CHROMagar O157 supplemented with rifampin at 50 mg/L; plates were sealed and incubated at 37°C for 24 h. Positive wells (mauve color) were scored and MPN estimated as described in Moyne et al.[28]

Data analysis

In the laboratory-based survival and transfer studies, the mean populations of *E. coli* enumerated from three glove, plate, and lettuce samples from each of two replicate experiments ($n = 6$) were analyzed using Tukey's HSD multiple comparison tests. Statistical tests used a level of significance of 0.05 or 0.0001 and were performed with JMP Pro 15 software (SAS Institute, Cary, NC) to determine significant differences between glove types and *E. coli* transfer to plates, lettuce, and gloves. *E. coli* recovery by enrichment results for laboratory and field trials were analyzed for statistically significant differences. Mann–Whitney *U*, a nonparametric statistical hypothesis test for assessing whether one of two samples of independent observations tends to have larger values than the other, was performed using an online calculator (https://www.socscistatistics.com/tests/mannwhitney/default2.aspx). Results were also analyzed by two-sample *t* test, assuming unequal variances using Excel (Microsoft).

RESULTS

Survival of different *E. coli* strains on gloves

Preliminary experiments showed that survival of *E. coli* TVS 354 and *stx*+ *E. coli* O157 (EC 4045) was similar and significantly better than *stx*− *E. coli* O157 (ATCC 700728) on reusable textured latex gloves after 30 min of drying at ambient temperature. Populations of *E. coli* TVS 354 and *stx*+ *E. coli* O157 inoculated at 4.5 or 7.0 log CFU glove^{-1} decreased by approximately 2.0 log after drying, while populations of *stx*− *E. coli* O157 decreased by approximately 5.0 log when inoculated at 7.0 log CFU glove^{-1} and by >4.5 log to below the LOD by plating (1.70 log CFU glove^{-1}) or by enrichment when inoculated at 4.5 log CFU glove^{-1}. When either *stx*+ *E. coli* O157 or *stx*− *E. coli* O157 was inoculated at 2.5 log CFU glove^{-1}, none of the glove samples were positive by plating or enrichment, but two of three samples were positive by enrichment for *E. coli* TVS 354. *E. coli* TVS 354 was used for all subsequent laboratory transfer studies because survival of *E. coli* TVS 354 and *stx*+ *E. coli* O157 was similar.

Transfer of *E. coli* TVS 354 from inoculated gloves to agar

When inoculated at 4.5 log CFU glove^{-1}, *E. coli* TVS 354 populations recovered from the disposable gloves were significantly higher than those recovered from the reusable gloves after 30 min of drying, regardless of glove texture ($p < 0.0001$). Populations of *E. coli* were 2.94 ± 0.20 log CFU glove^{-1} on reusable textured latex, 3.11 ± 0.32 log CFU glove^{-1} on reusable smooth latex, 4.23 ± 0.06 log CFU glove^{-1} on disposable smooth latex, and 4.38 ± 0.18 log CFU glove^{-1} on disposable nitrile gloves after drying.

Counts on the agar plates were above the upper limit of quantification (300 CFU or 2.48 log CFU plate^{-1}) for the first five (for reusable) or six (for disposable) sequential transfers from gloves (Figure 1). The number of CFU transferred to each sequential plate declined from a mean of 148 and 279 colonies at plate 6, to 51 and 58 colonies at plate 20 for reusable textured and reusable smooth latex gloves, respectively. The number of CFU of *E. coli* transferred to each sequential plate decreased more rapidly for the disposable gloves; mean colony counts ranged from ~6 (disposable nitrile) to ~15 (disposable smooth latex) after 20 transfers.

When inoculated at 3.5 log CFU glove^{-1}, populations of *E. coli* recovered after 30 min of drying from disposable smooth latex gloves (3.26 ± 0.30 log CFU glove^{-1}) were not significantly different from those recovered from reusable textured (2.74 ± 0.40 log CFU glove^{-1}) or reusable smooth (2.79 ± 0.30 log CFU glove^{-1}) gloves. Significantly greater ($p = 0.0003$) numbers of *E. coli* were transferred from the disposable smooth gloves (258 ± 27 CFU plate^{-1}; 2.40 ± 0.09 log CFU plate^{-1}) to the agar plates during the initial transfer compared with reusable smooth (103 ± 30 CFU plate^{-1}; 1.92 ± 0.30 log CFU plate^{-1}) or reusable textured (47 ± 7.5 CFU plate^{-1}; 1.65 ± 0.14 log CFU plate^{-1}) gloves (Figure 2). There was no significant difference ($p = 0.9615$) in the

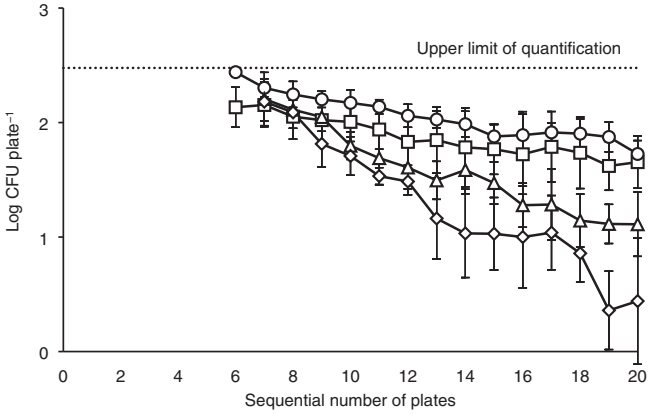

FIGURE 1 Mean populations of *E. coli* TVS 354 sequentially transferred to agar plates from the outer palm surface of reusable textured latex (□), reusable smooth latex (○), disposable smooth latex (△), or disposable nitrile (◇) gloves inoculated at 4.5 log CFU glove^{-1}. Counts were above the upper limit of quantification (2.48 log CFU plate^{-1}) in all cases for plates 1 through 5. Error bars indicate standard deviation ($n = 6$)

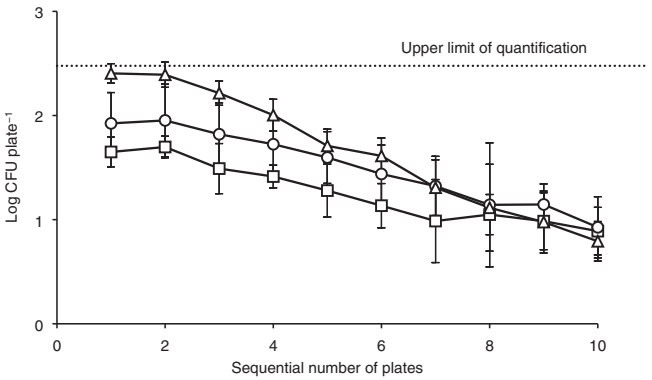

FIGURE 2 Mean populations of *E. coli* TVS 354 sequentially transferred to agar plates from the outer palm surface of reusable textured latex (□), reusable smooth latex (○), or disposable smooth latex (△) gloves inoculated at 3.5 log CFU glove^{-1}. Error bars indicate standard deviation ($n = 6$)

mean numbers of *E. coli* TVS 354 transferred to agar for each type of glove between the 8th and 10th transfer. The level of *E. coli* remaining on the disposable smooth gloves (1.72 ± 0.60 log CFU glove^{-1}) was significantly lower than that on the reusable gloves (2.53 ± 0.19 and 2.37 ± 0.29 log CFU glove^{-1} for textured and smooth, respectively) after sequential transfer to 10 agar plates ($p = 0.0077$).

Transfer of *E. coli* TVS 354 from inoculated gloves to lettuce under laboratory conditions

Initial populations of *E. coli* TVS 354 were significantly ($p < 0.0001$) higher on the disposable smooth latex (4.30 ± 0.08 log CFU glove^{-1}) than on the reusable textured latex (2.42 ± 0.43 log CFU glove^{-1}) gloves after 30 min of drying at ambient temperature. The numbers of

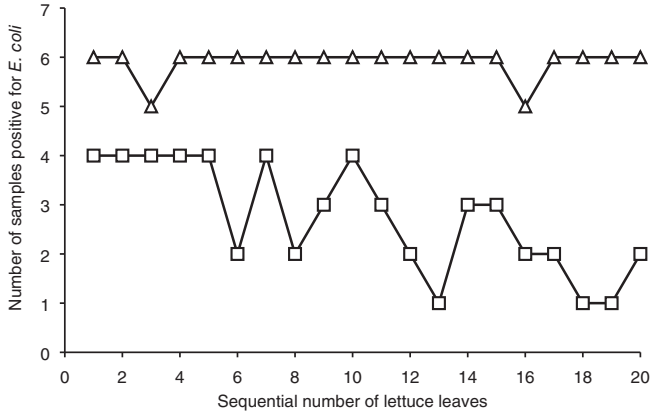

FIGURE 3 The number of lettuce leaves positive by enrichment for *E. coli* TVS 354 out of six independent replicates after sequential transfer from reusable textured latex (□) or disposable smooth latex (△) gloves inoculated with *E. coli* TVS 354 at 4.5 log CFU glove^{-1} under laboratory conditions

E. coli transferred from both glove types to lettuce leaves were not quantifiable by direct plating (LOD = 2.30 log CFU leaf^{-1}), so data were expressed as the number of enrichment-positive leaves out of six replicate transfers from gloves (Figure 3). Six of six lettuce leaves were positive for *E. coli* for all but two of the 20 sequential transfers with the disposable smooth latex gloves (98%; 118 of 120). Significantly fewer lettuce leaves were positive when reusable textured gloves were used. Four of six lettuce leaves were positive for *E. coli* for the first five transfers from the reusable textured gloves and between one and four leaves were positive between transfer six and 20 (46%; 55 of 120). Populations of *E. coli* recovered from the disposable smooth gloves (4.24 ± 0.04 log CFU glove^{-1}) and reusable textured latex gloves (2.31 ± 0.39 log CFU glove^{-1}) after sequential transfer to 20 lettuce leaves were not significantly different from the initial counts.

When lettuce sample diluent was filtered to improve the LOD (0.30 log CFU leaf^{-1}) mean *E. coli* populations on gloves were 6.77 ± 0.08 log CFU glove^{-1} after 30 min of drying. Mean *E. coli* populations recovered from leaf one through six after sequential transfer were 2.08 ± 0.14, 1.92 ± 0.53, 1.86 ± 0.50, 2.04 ± 0.37, 2.43 ± 0.43, and 1.98 ± 0.46 log CFU leaf^{-1}.

Transfer of *E. coli* TVS 354 from inoculated lettuce to gloves under laboratory conditions

Populations of *E. coli* TVS 354 recovered from the lettuce leaves were 5.59 ± 0.60 before drying and 4.81 ± 0.26 log CFU leaf^{-1} after 6 h of drying, or 6.51 ± 0.36 log CFU leaf^{-1} before drying and 5.46 ± 0.47 log CFU leaf^{-1} after 24 h of drying. The transfer of *E. coli* from the inoculated lettuce to the three types of latex gloves was variable, irrespective of glove type or drying time (Figure 4). There were no significant differences ($p = 0.2671$) in mean *E. coli* populations transferred to the different types of latex gloves and no significant differences

($p = 0.1152$) between drying times. The mean populations of *E. coli* transferred from inoculated lettuce to the different latex gloves after 6 h of drying ranged from 1.35 ± 1.04 log CFU glove^{-1} (disposable smooth) to 1.88 ± 0.72 log CFU glove^{-1} (reusable textured), and after 24 h of drying ranged from 0.57 ± 0.53 log CFU glove^{-1} (reusable smooth) to 1.64 ± 0.78 log CFU glove^{-1} (disposable smooth).

Transfer of *E. coli* between gloves and lettuce during in-field harvest

After 2 min of drying, *stx*− *E. coli* O157 declined from 7.0 log CFU glove^{-1} to 4.97, 4.16, or 5.04 log CFU glove^{-1} for reusable textured, reusable smooth, or disposable smooth gloves, respectively. *E. coli* was recovered from a significantly greater number of heads of lettuce harvested with disposable smooth latex gloves (82%; 98 of 120) than with either reusable textured (58%; 70 of 120) or reusable smooth latex gloves (54%; 65 of 120) as shown in Figure S1 (see Supporting Information). Transfer of *E. coli* from inoculated gloves to heads of lettuce ranged from undetectable on enrichment of the whole sample to 4.37 log MPN or CFU lettuce^{-1}. Approximately 3.0 log CFU lettuce^{-1} of *E. coli* was transferred from inoculated gloves to lettuce during sequential harvest of three lettuce heads (Figure S2); with no significant difference among the three latex glove types.

Populations of *E. coli* TVS 354 were 3.54 log CFU glove^{-1} on disposable smooth gloves and 3.05 log CFU glove^{-1} on reusable textured gloves after 2 min of drying when inoculated at 4.5 log CFU glove^{-1}. *E. coli* was recovered from a significantly greater number of heads of lettuce when disposable smooth latex gloves were used for sequential harvesting (74%; 89 of 120) than when reusable textured latex gloves were used (26%; 31 of 120) (Figure 5).

Populations of *E. coli* TVS 354 were 6.91 ± 0.39 CFU ml^{-1} ($n = 8$) in the inoculum and 7.04 ± 0.12 log CFU head^{-1} of lettuce ($n = 6$) after 2 h of drying under field conditions. There was no significant difference in total numbers of *E. coli*–positive lettuce heads recovered when a single inoculated head of lettuce was first harvested using disposable smooth latex gloves (75%; 90 of 120) or reusable textured gloves (71%; 85 of 120) (Figure 6).

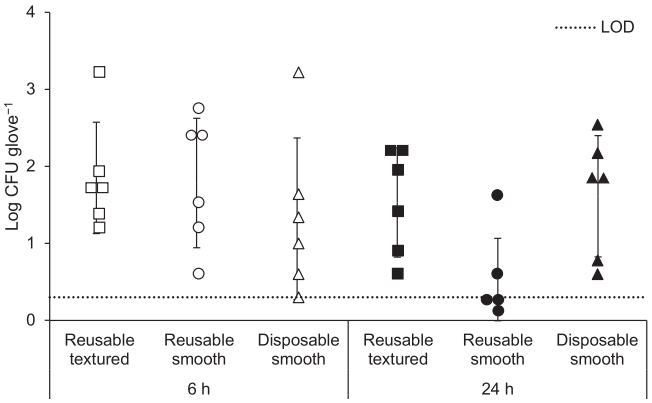

FIGURE 4 Mean populations of *E. coli* TVS 354 transferred from ~5.0 log CFU leaf^{-1} inoculated lettuce leaves to various latex gloves after contact with 200 g weight for 5 s, under laboratory conditions. Each data point represents a single glove applied once to a single lettuce leaf ($n = 6$). Limit of detection (LOD) = 0.30 log CFU glove^{-1}. Horizontal lines represent mean values, and vertical lines indicate standard deviation. Mean *E. coli* populations transferred to glove types or between drying times were not significantly different ($p > 0.05$)

DISCUSSION AND CONCLUSION

Survival of *E. coli* on glove surfaces during drying was significantly impacted by strain. Population declines for *stx*− *E. coli* O157 during post-inoculation drying were significantly greater than for either *E. coli* TVS 354 or the single *stx*+ *E. coli* O157 strain evaluated. In the field trials, *stx*− *E. coli* O157 (ATCC 700728) was included along with

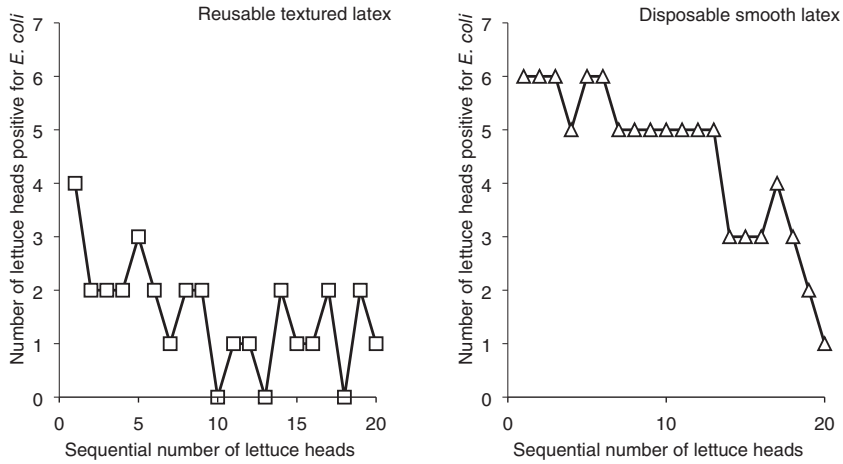

FIGURE 5 Number of lettuce heads positive for *E. coli* TVS 354 out of six independent replicates after sequential harvest in the field using reusable textured latex (□; 26% positive out of 120) or disposable smooth latex (△; 74% positive out of 120) gloves inoculated at 4.5 log CFU glove^{-1} and then dried for 2 min under ambient field conditions

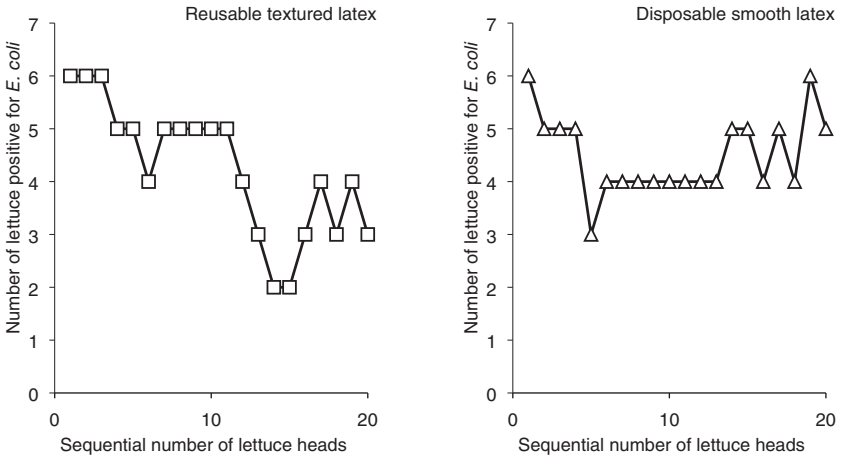

FIGURE 6 Number of lettuce heads positive for *E. coli* TVS 354 after harvest using the same reusable textured latex (□; 71% positive out of 120) or disposable smooth latex (△; 75% positive out of 120) glove ($n = 6$) used to first harvest a single inoculated head of lettuce (inoculated at 7.0 log CFU lettuce^{-1} and dried for 2 h under field conditions)

E. coli TVS 354 because it was previously and concurrently used in field trials at that location.[28] Glove-to-food transfer studies often have used single strains of surrogate organisms[6,32–34] or pathogens[19,22] and occasionally cocktails;[23] but our results show that strain choice may influence results and should be considered when designing and comparing studies.

The levels of microorganisms found on gloves used to harvest lettuce are unknown and would depend on the source and timing of contamination. Because microbial counts are useful in quantitative microbial risk assessments, studies evaluating cross contamination between hands (bare or gloved) and foods typically quantify bacterial transfer rates (often expressed as percent log transfer). Experiments using one high (>6 log CFU) inoculum level are commonly used to facilitate enumeration after transfer.[19,22,23,32,34,35] Inoculum level is known to influence calculated transfer rates in cases of single transfer events,[6,33] with higher log percent transfer at lower inoculum levels. The inoculum levels used in the current study ranged from 3.5 to 7.0 log CFU and enumeration after transfer between gloves and lettuce was not always possible. Enrichment-positive samples were detected, so overall percent positive samples could be reported where transferred cells were below the LOD by enumeration. Such methods could be used in subsequent studies to evaluate lower and potentially more realistic cross-contamination scenarios for quantitative microbial risk assessment.

Inoculated populations recovered from disposable gloves after drying were significantly higher than from reusable latex gloves. This finding is consistent with studies using similar gloves.[24,36] The rough texture of reusable gloves may offer increased surface area and thus exposes bacteria to greater desiccation stress,[24] resulting in lower transfer from gloves to produce. Glove hydrophobicity also has been reported to play a role in bacterial transfer from and to gloves.[37]

Greater transfer to agar surfaces as measured by log CFU (Figures 1 and 2), to lettuce leaves in the lab (Figure 3), or to lettuce heads in the field (Figure 5) as measured by percent positive samples, occurred with disposable smooth latex gloves than with either of the reusable latex glove types. In contrast, Brar and Danyluk[23] reported no significant differences in the sequential transfer of *Salmonella* from single-use or reusable latex gloves to up to 25 tomatoes by plating or enrichment. These different results may be due to the organism (*E. coli* vs. *Salmonella*) or strains evaluated, the produce item (lettuce vs. tomato), or other differences in the experimental design. More research exploring these factors is needed, given the limited amount of published data on this topic.

When lettuce rather than gloves was inoculated and the transfer to gloves quantified in the laboratory (Figure 4) or by percent positive heads of lettuce harvested in the field (Figure 6), differences between disposable and reusable gloves were not significant. Likewise, reported *Salmonella* transfer from dried inoculated tomatoes to disposable and reusable gloves was not significantly different.[23]

Gloves used during harvesting will become soiled over time, which may affect bacterial survival and transfer. Previous studies[23,24] suggest that the presence of organic matter on gloves does not increase the risk of microbial transfer. Greater inactivation of *E. coli* O157:H7 and *Salmonella* was observed after inoculation and during drying on soiled reusable latex and disposable latex gloves.[24] Organic matter on gloves also reduced *Salmonella* transfer to subsequently touched tomatoes compared with clean gloves.[23] Those authors have suggested that the surface of gloves soiled with organic matter may be rougher and more porous, and may bind bacteria to the gloves, preventing transfer.[23,24]

When gloves are used to harvest or handle produce, the selection of glove type may be based on factors such as type of product harvested and harvest mechanism, comfort, ability to feel through the glove, cost, and/or disposal. Reusable gloves are intended to be used for longer times than disposable gloves but data on actual documented practice in the field are limited. The California Leafy Greens Marketing Agreement food safety guidelines outline best practices for personnel when gloves are used for handling or harvesting lettuce or

leafy greens.[26] These include prohibition of personal gloves or taking gloves home, washing hands with soap and running water before putting on gloves, and replacing gloves when they are no longer intact or in sanitary condition.

Washing disposable gloves is not recommended.[20] The effectiveness of washing and sanitizing reusable gloves depends on several factors including the concentration and strain of contaminating bacteria, disinfectant agent and concentration, presence of organic matter, and glove type.[20,27] Additional data are needed to evaluate the impact of soil buildup and cleaning and sanitation methods and frequency on glove contamination and cross contamination to fresh produce.

This study demonstrates that contamination of a glove from a single point source can lead to subsequent contamination of multiple heads of lettuce during hand harvesting under field conditions. This finding highlights the need for the implementation of company-based glove use and hand hygiene policies for harvest crews. Although the data presented here indicate a small potential advantage when using reusable gloves, this advantage may be offset by other factors (e.g., greater cost of reusable gloves and the need to implement cleaning and sanitizing programs for reusable gloves). Further analysis is needed to be able to determine whether reusable or disposable gloves are an appropriate choice. That choice may also depend upon the specific context.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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