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Short communication

Evaluation of transfer rates of *Salmonella* from single-use gloves and sleeves to dehydrated pork jerky



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ABSTRACT

Meat jerky is a popular dried snack food that is typically considered shelf-stable and ready-to-eat. Many jerky processes incorporate post-lethality handling that represents opportunities for contamination through contact with worker hands and gloves. The objective was to identify transfer rates of Salmonella enterica from gloves to dried jerky after handling with three types of single-use gloves (Nitrile, PVC, and PE) and one type of single-use PE-coated sleeve cover. Six Salmonella enterica seroyars were mixed and diluted to 7–8 log₁₀ CFU/mL and 2–3 log₁₀ CFU/mL for quantitative and qualitative transfer rate analyses, respectively. For quantitative analysis, high dose inoculum was applied evenly to the palm of the glove and the gloved hands were used to touch three jerky slices successively, simulating a major glove-jerky contact. A total of six inoculations were performed per material (n = 18). For qualitative analysis, low dose inoculum was applied evenly to the palm of the glove and the gloved hands were used to touch the jerky (n = 40) using two contact scenarios (contact with fingers only or fingers and palm) simulating activities associated with hand sorting and packaging. Salmonella were enumerated by plating onto XLT4 following serial dilution or after 24 h enrichment, Salmonella transfer to jerky was significantly greater (P < 0.05) from PE gloves (5.52 \pm 0.24 \log_{10} CFU/sample) and sleeves (6.16 \pm 0.49 \log_{10} CFU/sample) compared to Nitrile (4.47 \pm 0.47 \log_{10} CFU/sample) and PVC gloves (4.66 \pm 0.58 \log_{10} CFU/sample). In qualitative analysis, finger-only contact resulted in Salmonella transfer to 10/40 jerky slices from PE gloves and 1/40 slices from Nitrile gloves. However, when the palm of the glove was involved in the contact, Salmonella was detected on all 80 jerky slices, regardless of material type. Selection of materials associated with reduced transfer may be an important strategy for reducing bacterial crosscontamination in jerky production facilities.

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1. Introduction

Dried meat snack foods are popular for their high nutrient density, convenience, and good flavor. Jerky processing does not require investment in expensive facilities and equipment costs are modest, thus this product is produced by both small and large scale meat processors. To address safety concerns regarding the manufacture of jerky, the US Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) has published compliance guidelines for small plants with detailed, step-by-step guidelines for jerky processing developed to achieve a 5-log₁₀ reduction of Salmonella (USDA, 2014). However, jerky products are still not

without risks — deviation from processing parameters and cross-contamination after lethality treatment are example scenarios that bring potential risks to jerky.

Outbreaks of salmonellosis have been documented where jerky contamination was attributed in part to post-lethality handling by food workers and environmental cross-contamination (Centers for Disease Control and Prevention, 1995; Laufer et al., 2015). It is possible that raw meat material in jerky processing plant contains Salmonella, which may contaminate handler's gloves, and then contaminate post-processing jerky products. Salmonella enterica serovars, including Tennessee, Senftenberg, and Montevideo, can survive in low-moisture food matrices for a substantial period of time and are more resistant to heat treatment (Podolak, Enache, Stone, Black, & Elliott, 2010). Salmonella may persist for more than 60 days on jerky and this survival may be enhanced in certain marinades (Calicioglu, Sofos, Samelis, Kendall, & Smith, 2003).

Single-use gloves and sleeves are among the most commonly

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used personal protection equipment (PPE) which have been widely employed by food manufacturing and service. Glove use for ready-to-eat (RTE) food handling is recommended by Food and Drug Administration (FDA), and required if consumers are susceptible to foodborne diseases, such as elderly, children and immunocompromised group (FDA, 2009; annex 3). Single-use gloves are available in multiple types of materials such as latex, nitrile rubber ("nitrile"), polyvinyl chloride ("vinyl"), polyethylene ("poly"), and so forth. Proper use of PPE such as gloves can successfully reduce the transfer rate of pathogens; that said, it is difficult to totally eliminate the risk (Montville, Chen, & Schaffner, 2001; Robinson et al., 2016; Todd, Michaels, Greig, Smith, & Bartleson, 2010).

Transfer of pathogens has been documented from a variety of surfaces and food products (Jensen, Danyluk, Harris, & Schaffner, 2017; Jensen, Friedrich, Harris, Danyluk, & Schaffner, 2013; Knobben, van der Mei, van Horn, & Busscher, 2007; Rönngvist et al., 2014). However, only a few studies have examined transfer from gloves (Brar & Danyluk, 2013; Moore, Dunnill, & Wilson, 2013). Specifically for jerky production, transfer rate associated with different types of single-use gloves remains undiscussed. In this study, several cross-contamination scenarios during jerky production were simulated, including touching jerky slices when moving racks, touching slices with sleeve covers while reaching out further located slices, handling slices with palm and fingers, and picking up slices with fingers. A cocktail of Salmonella strains that are tolerant to low moisture were used to simulate the source of contamination. Either high $(7-8 log_{10} CFU/glove)$ or low (2log₁₀ CFU/glove) dose inoculum was used, and the different transfer rates of Salmonella associated with different types of single-use gloves and sleeves were compared.

2. Materials and methods

2.1. Meat preparation

Raw, marinated pork slices (pH = 6.18 ± 0.10 , no cure, 55-75 g/slice) were obtained from a meat producer. Single layer of pork slices were spread on one-inch mesh stainless steel racks and dehydrated in a smokehouse (Alkar 1000, PN 045122, Alkar-Rapidpak, Lodi, WI) until slices acquired the target water activity (a_w) range (0.80–0.85), as recommended by FSIS jerky guidelines (USDA 1999; 2014).

2.2. Bacterial strains and preparation of inoculum

Bacterial culture preparation methods were adopted from Bowman, Waterman, Williams, and Ponder (2015), with minor modifications. Briefly, six isolates of Salmonella enterica strains previously associated with low aw outbreaks or used in thermal processing of jerky validations (Porto-Fett, Call, & Luchansky, 2008; Senftenberg 775W ATCC 43835, Montevideo 1449, Tennessee, Typhimurium JBL 3269, Typhimurium JBL 3270, and Typhimurium JBL 3271) were resuscitated from -80 °C freezer stocks and streaked onto Xylose Lysine Tergitol-4 (XLT4, Becton Dickinson, Sparks, MD) agar plates and incubated for 24 h at 37 °C to obtain isolated colonies. One isolated colony of each culture was transferred to Tryptic Soy Broth (TSB, Becton Dickinson, Sparks, MD) and incubated with shaking (180 rpm) for 24 h at 37 °C. After incubation, 500 μ L of each culture was plated onto a 100 \times 15 mm Petri plate (Fisher Scientific, Pittsburgh, PA) of Tryptic Soy Agar (TSA, Becton Dickinson, Sparks, MD) and incubated for 24 h at 37 °C to cultivate a lawn of bacteria. Cells from each plate were scraped from the agar surface using a sterile swab and suspended in 9 mL of peptone (Becton Dickinson, Sparks, MD) buffer (0.1% w/v peptone in water). The suspensions in peptone buffer of all six strains were combined and diluted to provide a bacterial cocktail of two different dilutions, approximately 10^7-10^8 CFU/mL for quantitative studies and 10^2-10^3 CFU/mL for qualitative studies respectively.

2.3. Qualitative glove transfer rate

Two types of single-use gloves, nitrile rubber glove ("nitrile", Fisher Scientific, Pittsburgh, PA) and polyethylene food service glove ("poly", US Foods Inc., Rosemont, IL), were tested for the rate of a successful Salmonella transfer from glove to meat. Each tested glove was put on investigator's left hand. An aliquot of 250 µL from the Salmonella cocktail $(10^2-10^3 \text{ CFU/mL})$ was pipetted in the middle of left palm. The investigator then rubbed the inoculated, gloved hand itself carefully for 90 s to spread inoculum across palm and fingers, then the transfer was conducted immediately. A majorcontact and a minor-contact test were performed. In the majorcontact test, twenty jerky slices were placed on wire cooling racks (Wilton Industries Inc., Woodridge, IL). Using the inoculated glove, each individual slice was mildly pressed with palm and fingers (approx. 0.3 PSI), then picked up by three fingers (thumb, index finger and middle finger) to place in a sample bag. A 50-mL volume of lactose broth (Remel, Lenexa, KS) was added to the bag, and hand massaged for one min followed by incubation for 24 h at 37 °C. In the minor-contact scenario, the same inoculation method (250 µL, 10^2-10^3 CFU/mL) was used. Twenty jerky slices were picked up individually using three fingers of the inoculated glove, then enriched as described above.

2.4. Quantitative glove transfer rate

Transfer from three types of single-use gloves was examined: nitrile glove and poly glove as described previously, and polyvinyl chloride glove ("vinyl", Ambitex, Cleveland, OH). Tested gloves were inoculated as described above, except for a different inoculum dose (250 $\mu L, 10^7 - 10^8$ CFU/mL). Three dehydrated jerky slices were placed on a wire rack and the palm and fingers of the inoculated gloves were faced down and mildly pressed against one jerky slice. The contact time was 10 s for each slice, and three slices were consecutively pressed by each inoculated glove. Pre-contact and post-contact gloves, as well as post-contact jerky slices were collected for microbiological analysis.

2.5. Quantitative sleeve cover transfer rate

One single-use polyethylene-coated polypropylene woven sleeve cover ("sleeve", Sunsoft, Sunrise Industries Inc., Guntersville, AL) was laid flat in a biosafety cabinet. An aliquot of 250 μL of Salmonella cocktail (10^7-10^8 CFU/mL) was pipetted in a rectangular area (7.9 \times 15.8 cm, 125 cm²) that was marked in the middle portion of the sleeve, and spread across the area for one min, then carefully placed on researcher's left front arm. The marked, inoculated area of the sleeve was placed face down onto three jerky slices and gently pressed for 10s against each jerky slice. Precontact and post-contact sleeves, as well as post-contact jerky slices were collected for microbiological analysis.

2.6. Microbiological analysis

Qualitative samples were enriched as described above, and enrichment broth streaked for isolation on XLT4 agar plates incubated 24 h at 37 °C for *Salmonella* presence/absence. Transfer rate was defined as number of positive samples/40. Quantitative test samples were added with appropriate amount (245 mL for glove, 240 mL for sleeve, and 215 mL for pork slice) of sterile lactose broth and the mixture was stomached in a lab blender (Interscience

BagMixer[®], Guelph, Ontario, Canada) for 120 s. The rinsate was diluted and surface plated onto TSA plates using a spiral plater (Autoplate 4000, Spiral Biotech, Norwood, MA). After 3 h incubation at 37 °C the TSA plates were overlayed with 7 mL of XLT4 (BD, Franklin Lakes, NJ) for better recovery (Caver, 2016) and incubated for 24 h at 37 °C. Colonies were counted using a colony counter (ProtoCOL SR, Microbiology International). Samples without *Salmonella* recovery were enriched and streaked on XLT4 for qualitative determination of *Salmonella* presence. Percentage quantitative transfer rates are defined as follows:

Percentage recovery rate of inoculum from glove T_{i/g}:

 $T_{i/g} = \text{total CFU on glove} \ / \ \text{total CFU in inoculum} \times 100;$

Percentage transfer rate from inoculated glove to jerky slice T_{g/j}:

 $T_{g/j}$ = total CFU on jerky slice / total CFU on glove × 100;

Percentage overall transfer rate of inoculum to jerky slice T_{i/i}:

 $T_{i/j} = \text{total CFU}$ on jerky slice / total CFU in inoculum $= T_{i/g} \times T_{g/j} / 100$

2.7. Statistical analysis

Statistical analysis was conducted on quantitative test data. Bacterial counts and transfer rates were logarithmically transformed to approximate normal distribution. For each material type, duplicated samples of glove or sleeve were tested, and triplicated jerky slices were touched by each glove or sleeve. At least 18 jerky samples were performed per glove or sleeve type. Statistical analyses were performed using JMP statistical software (version 10, SAS, Cary, NC). The effect of glove and sleeve type on *Salmonella* transfer rate (in log₁₀ CFU/sample) were analyzed using one-way ANOVA and compared using Tukey's HSD method. P-values < 0.05 was considered significant.

3. Results

Inoculation of gloves with a low dose inoculum (1.5 log₁₀ CFU/glove) resulted in transfer of *Salmonella* that could be detected by enrichment and subsequent plating. When the palm was placed in contact with the jerky, each sample was *Salmonella* positive regardless of glove type, indicating a high incidence of *Salmonella* transfer. With finger only contact, the incidence of *Salmonella* positive gloves was reduced compared to the palm contact. The incidence of transfer from poly glove (10 out of 40 samples) was

significantly greater than the incidence of transfer from nitrile gloves (1/40 samples).

The amount of *Salmonella* (log₁₀ CFU/sample) transferred to the sample was significantly affected by the glove or sleeve material (Table 1). Inoculum of *Salmonella* ranged from 6.98 to 7.47 log₁₀ CFU/sample across glove type and batches. The smallest inoculum-to-glove transfer occurred for nitrile gloves (lowest) followed by vinyl gloves, both of which were approximately 1 log lower than the poly gloves and sleeves.

Salmonella transfer rates during each transfer stage, inoculumglove $(T_{i/g})$, glove-jerky $(T_{g/i})$ and overall inoculum-glove-jerky $(T_{i/g})$ i) were logarithmically transformed and the distributions were plotted in Figs. 1–3 respectively. The interval of 0.25 was chosen to best demonstrate distributions of all four glove/sleeve types. $T_{i/g}$ indicates the ability of the glove to retain Salmonella cells after inoculation. Log₁₀ T_{i/g} of nitrile glove and vinyl glove distributed around 0-1 and 0.5-1.5 respectively, while log $T_{i/g}$ of two polyethylene-surfaced PPE distributed around 2 (Fig. 1). Wide distributions of Log₁₀ $T_{g/j}$ and Log₁₀ $T_{i/j}$ in Fig. 2 (ranged from -1.5 to 2) and 3 (ranged from -2.5 to 1.25) revealed the high degree of variability of Salmonella migration from glove to jerky slices. Overall, distributions of Log₁₀ T_{i/j} of nitrile glove and vinyl glove overlap substantially, while $Log_{10} T_{i/j}$ of poly glove and poly-coated sleeve overlap for the most part, indicating the nitrile and vinyl materials have similar Salmonella transfer rates. Both made with polyethylene surface, poly glove and poly-coated sleeve have similar Salmonella transfer potential, which is significantly different than nitrile and vinvl gloves.

4. Discussion

Raw meat may carry a variety of microorganisms including pathogens such as Salmonella, E. coli, and other human pathogens (Zhao et al., 2001). For small establishments with limited space or personnel, the complete segregation of raw and cooked products may be difficult to implement, which indicates an increased possibility of cross-contamination. Pathogens originating in raw meat may be transferred to a finished product indirectly, through contact between surfaces that may have dual contact such as stainless steel, wood, synthetics and food workers (Cliver, 2006; Kusumaningrum, Riboldi, Hazeleger, & Beumer, 2003). The scenario simulations in these experiments were designed based on observation of operational personnel during manufacture of jerky product as well as our experiences in pilot-scale jerky production. It would be considered standard practice to segregate physical spaces between raw and finished product if possible, however there are instances where personnel may move and work in both of these spaces creating the potential for cross-contamination. From the results of the qualitative transfer test, it can be seen that contact pattern affects transfer

Table 1Quantitative transfer rates of *Salmonella* inoculum to glove/sleeve and jerky. *Salmonella* population was logarithmically transformed and expressed as log₁₀ CFU/sample. During each transfer stage, different population drop of *Salmonella* per glove/sleeve type is annotated with different letters.

| | Glove and sleeve type | Nitrile glove | Vinyl glove | Poly glove | Poly-coated Sleeve | P-value |
|---------------------------------|---|---|---|---|---|---------|
| Test Stage Glove Inoculation | Salmonella Inoculum ¹ Recovery from glove, before contact with jerky slices ¹ Population drop from inoculum to glove ¹ | 7.07 ± 0.21 5.57 ± 0.36 1.49 ± 0.43^{a} | 7.37 ± 0.47 6.55 ± 0.47 0.82 ± 0.39^{b} | 6.98 ± 0.07 6.82 ± 0.10 0.16 ± 0.05^{c} | 7.47 ± 0.51 7.25 ± 0.60 0.21 ± 0.18^{c} | <0.0001 |
| Jerky Contact | Recovery rate $T_{i/g}^2$ Recovery from jerky ¹ Population drop from glove to jerky ¹ | 5.10 4.47 ± 0.47 1.11 ± 0.47^{a} | 20.89 4.66 ± 0.58 1.89 ± 0.77 ^b | $69.325.52 \pm 0.241.30 \pm 0.26^{a}$ | 66.11 6.16 ± 0.49 1.09 ± 0.41^{a} | <0.0001 |
| Overall | Transfer rate $\Gamma^2_{g,j}$ Overall population drop from inoculum to jerky 1 Transfer rate Γ^2_{ij} | $13.76 \\ 2.60 \pm 0.40^{a} \\ 0.36$ | 3.44 2.70 ± 0.79^{a} 0.55 | 5.84 1.46 ± 0.27^{b} 4.02 | 11.64 1.30 ± 0.33 ^b 6.31 | <0.0001 |

 $^{^{1}}$ Values are expressed as mean \pm standard deviation. Calculation was based on log10 CFU/sample.

²Values are means of individual transfer rates/recovery rates.

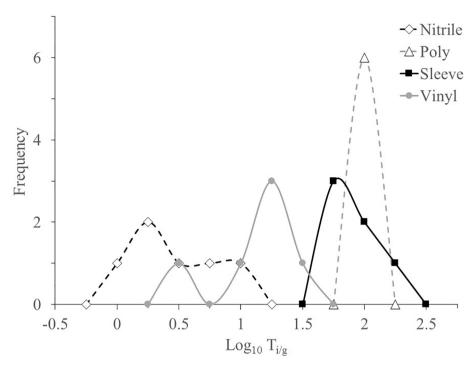


Fig. 1. Observed log percentage recovery of Salmonella from inoculation to glove/sleeve. For each glove/sleeve type, duplicate samples were enumerated per experiment, and triplicate experiments were conducted (n = 6). Frequency indicates the number of samples where Salmonella was detected.

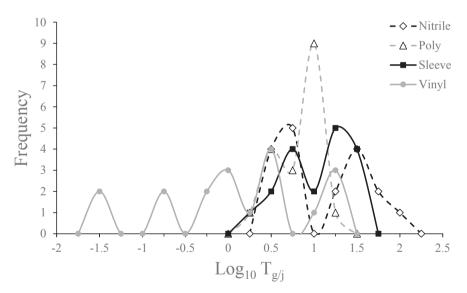


Fig. 2. Log percentage transfer of *Salmonella* from glove/sleeve to jerky slices. For each glove/sleeve type, 6 jerky slices were enumerated per trial, and triplicated trials were conducted (n = 18). Frequency indicates the number of samples where *Salmonella* was detected.

rate. At a very low level of *Salmonella* (~100 CFU per glove), *Salmonella* still succeeded in migration from glove to jerky when the palm was involved in the contact, indicating a potential risk to consumers emphasizing the need for frequent glove changes. It is worth mention that under low aw conditions, many *Salmonella* serotypes are able to survive and even show increased resistance to heat in low-moisture foods (Ma et al., 2009; Mattick et al., 2001; Podolak et al., 2010). *Salmonella* outbreaks attributed to low water activity foods have been associated with very low infectious doses, between 10 and 1000 CFU (Blaser & Newman, 1982) indicating that steps to reduce transfer both pre and post lethality will reduce long-term health threats.

The materials of single-use gloves influence the transfer of microorganisms — the influence may come from texture differences and intrinsic characters of the materials (Moore et al., 2013; Whitehead & Verran, 2006). Nitrile glove and vinyl glove both have matte/textured surface comparing to the gloss surfaced poly glove and poly-coated sleeve, thus their surface areas were larger than the latter, which may assist the retention of bacterial cells. Intrinsic properties of glove materials may also contribute to microorganism migration. The ability of *Salmonella* to attach to a surface is influenced by cell surface structure, surface charge, and hydrophobicity (Dickson & Koohmaraie, 1989). Polarity of nitrile butadiene rubber and polyvinyl chloride may interfere with surface

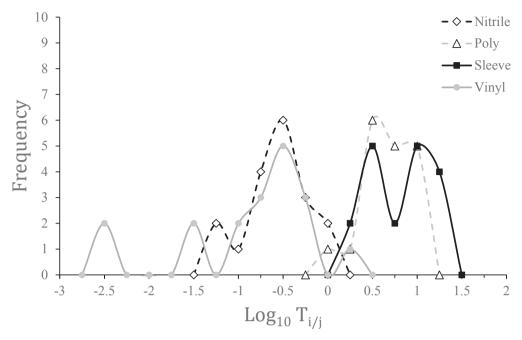


Fig. 3. Log percentage overall transfer rate from Salmonella inoculum to jerky slice via glove/sleeve contact. For each glove/sleeve type, 6 jerky slices were enumerated per trial, and triplicated trials were conducted (n = 18). Frequency indicates the number of samples where Salmonella was detected.

charge of *Salmonella* cells thus retain more cells on surface during the transfer. In contrast, polyethylene lacks this polarity thus we hypothesize that there was a weaker interaction with *Salmonella* cells or culture liquid. Moore et al. (2013) showed that aqueous drops on different glove materials had different surface tensions. The smaller contact angle of drops on nitrile rubber indicates greater adhesion with the material, which reduced the amount of liquid that was transferred during the contact (Moore et al., 2013). Similar trends were observed during our research, that the nitrile glove previously shown to have smaller contact angles were associated with lower transfer rate to jerky, while polyethylene material, previously shown to have greater contact angles were associated with higher transfer rates.

The current investigation was based on a worst-case assumption that the contamination of glove or sleeve has occurred and we used a small volume of liquid inoculum of *Salmonella* to standardize the contamination as presence of just a thin layer of liquid may enhance transfer. The transfer rate from other contamination sources (raw meat, food contact surfaces, other surfaces in facility that may be contacted with gloves, etc.) to different single-use gloves and sleeves was not evaluated, and it would be useful to quantify those transfer rates in future studies.

In summary, our results demonstrate that the transfer of *Salmonella* to dehydrated jerky slices via single-use gloves and sleeves is influenced by glove material type and contact pattern. A major contact between inoculated palm and dehydrated jerky slice resulted in a substantial transfer rate (0.36%–6.31%) of *Salmonella* even if appropriate PPE is applied. From this perspective, it reveals the importance of implementation of current good manufacturer practices (CGMP) and hazard analysis and critical control point (HACCP) system which regulate personnel operations to minimize the chance of cross-contamination. Besides, future research to quantify *Salmonella* transfer from contamination sources to gloves is also needed to better determine the glove types associated with lower cross-contamination risk in jerky processing.

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