Reduction of *Escherichia coli* O157:H7 and *Salmonella* after Application of Various Sanitizing Treatments to Harvesting Knives

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ABSTRACT

Consumption of food contaminated with *Escherichia coli* O157:H7 and *Salmonella* can cause enteric disease in consumers. If not properly sanitized, knives used during animal harvest can spread these and other pathogens. This study evaluated the reduction of *E. coli* O157:H7 and *Salmonella* on harvesting knives after nonthermal sanitation. Knives were inoculated in cocktails of *E. coli* O157:H7 or *Salmonella* and treated by 30-s immersions in ambient-temperature solutions (unless temperature was specified) of 1.1% sodium metasilicate (SMS), 200 ppm of quaternary ammonium compounds (QAC), 200 ppm of chlorine (Cl₂), 5% lactic acid (LA), 82.2°C water, and 21°C water. Initial and treated counts were determined by plating onto MacConkey and xylose lysine desoxycholate for *E. coli* O157:H7 and *Salmonella*, respectively. Initial counts were determined by sampling one side of the knife blade, while treated counts were sampled from the opposite side. Plates were incubated for 24 to 48 h at 37°C. Mean attachment of *E. coli* O157:H7 and *Salmonella* was 4.51 and 5.09 log CFU/cm², respectively. Mean log reductions on knives inoculated with *E. coli* O157:H7 were 1.16, 3.51, 3.38, 1.38, 3.82, and −0.41 CFU/cm² after treatment in SMS, QAC, Cl₂, LA, 82.2°C water, and 21°C water, respectively (P ≤ 0.05). Knives inoculated with *Salmonella* showed reductions of 0.78, 3.42, 3.40, 2.91, 4.12, and 0.36 log CFU/cm² after treatment in SMS, QAC, Cl₂, LA, 82.2°C water, and 21°C water, respectively (P ≤ 0.05). Results indicate that some ambient-temperature sanitizing agents have the potential to significantly reduce *E. coli* O157:H7 and *Salmonella* populations on knives used during animal harvest.

*Escherichia coli* O157:H7 and *Salmonella* are foodborne pathogens that can cause severe gastroenteritis; they commonly reside in the gastrointestinal tract, as well as on the skin and hides of many animals (10). Essentially, internal muscles are considered to be sterile. The hide-removal step of harvest can serve as a point of entry for pathogenic contamination, resulting in exposure of the external muscles of the carcass, thus allowing for contamination from the environment. Each successive step throughout harvest can result in microbial contamination of the carcass. Moreover, improperly sanitized knives can result in the transfer of contamination to subsequent carcasses. Previously, regulations required that harvesting knives be sanitized by immersion in 180°F (82°C) water (7, 19). However, hot water is not fully utilized in all areas of the world (20), making sanitation difficult during harvest. In 2000, regulations were implemented to allow for alternative sanitation procedures that provide equivalent microbial reduction (7, 19), and these should be considered when hot water is not accessible.

The Centers for Disease Control and Prevention estimates 35 outbreaks of *E. coli* O157:H7 occur in the United States each year (1). Infection with *E. coli* O157:H7 can result in watery to bloody diarrhea, abdominal cramps, and/or kidney failure, with severe cases progressing to hemolytic uremic syndrome or thrombotic thrombocytopenic purpura (12). Hemolytic uremic syndrome leads to kidney failure in children, while thrombotic thrombocytopenic purpura can cause renal or neurological abnormalities in adults (7). Of particular concern to public health is *E. coli* O157:H7, given the low infectious dose (<100 cells) necessary for infection (11, 12). *E. coli* O157:H7 and other Shiga toxigenic *E. coli* (STEC) are found throughout the world (2). However, illnesses derived from STEC are more common in developed countries (2, 3), particularly in the Northern Hemisphere (2).

Salmonellosis manifests itself as diarrhea, abdominal cramps, vomiting, and fever. In the United States, the incidence of *Salmonella* foodborne infection has risen from 14 to 18 of 100,000 people between the years 2000 and 2010. Between 1998 and 2008, 1,490 outbreaks of *Salmonella* occurred in the United States, accounting for 72.7% of all foodborne illness outbreaks (1). Infection of foodborne *Salmonella* is a major concern across the world (16), though the incidence in developing countries is particularly difficult to estimate because of a common lack of epidemiological surveillance (5). *Salmonella* infections continue to plague public health worldwide, and efforts to improve *Salmonella* control warrant future investigation.
Because international regulations require harvest sanitation, knives are typically sanitized with short-term immersion (1 to 5 s) in a “knife sterilizer” containing hot water (≥82°C) (8). Previous studies aiming to validate different knife sanitizations have shown promising alternatives to standard thermal sanitation. Snijders et al. (17) showed that a 1-s thermal-water (82°C) treatment resulted in a 2.5 log-CFU/ml reduction of E. coli O157:H7. Alternatively, Taormina and Dorsa (18) observed a 3.02-, 2.38-, and 3.04-log CFU/cm² reduction of E. coli O157:H7 when treated for 15 s with hot water (82.2°C), 440 ppm of 4-alkyl quaternary ammonium compound (QAC), and 440 ppm of 4-alkyl acid QAC, respectively. Similarly, Salmonella Typhimurium DT104 was reduced by 2.39, 1.49, and 1.66 log CFU/cm² after a 15-s treatment with hot water (82.2°C), 440 ppm of 4-alkyl QAC, and 440 ppm of 4-alkyl acid QAC, respectively (18). Goulet et al. (8) evaluated E. coli O157:H7 and Listeria monocytogenes reductions on knives dipped in water for 42 time–temperature treatment combinations. The authors concluded that short dips in high-temperature water (i.e., 82°C water for 1 s) reduce pathogen populations equivalent to a dip in lower temperature water for a longer time. Furthermore, a knife prerinse in 40°C water increased reductions achieved by the subsequent dip treatments. Other research has suggested that short-term sanitizing treatments prepared with ambient-temperature tap water were not as effective as thermal sanitation during short-term immersion treatments (14, 17, 18). However, increasing the immersion time could improve the efficacy of such sanitizers, thereby providing an effective alternative for areas of the world not equipped with thermal sanitation capabilities. Therefore, the purpose of this study was to evaluate the effectiveness of various sanitizing agents prepared with ambient water to reduce E. coli O157:H7 and Salmonella on inoculated knives.

MATERIALS AND METHODS

Experimental design. The study was conducted as a completely randomized block design with three replications, each consisting of six sanitizing treatments applied for one time period. Each replication served as a block for data analysis.

Inoculants. Inocula of E. coli O157:H7 and Salmonella were prepared from stock cultures at the Texas Tech Food Microbiology Laboratory located in the Experimental Sciences Building. One milliliter of each strain (E. coli O157:H7 strains originally isolated from cattle: A4 966, 966, and A5 528; Salmonella strains: Typhimurium ATCC 14028, Anatum ATCC 9270, and München ATCC 8388) at an approximate concentration of 10⁶ CFU/ml was added separately to 1 liter of buffered peptone water (BPW; EMD, Gibbstown, NJ) and incubated for 24 to 28 h at 37°C, resulting in an approximate concentration of 10⁸ to 10⁹ CFU/ml of Salmonella and E. coli O157:H7 in each inocula. The three individual strains of each pathogen were mixed in a sterilized stainless steel container, yielding 3 liters of a three-strain cocktail for each pathogen. Each solution was agitated with a sterile scoopula to ensure adequate distribution of the cultures. The 3-liter volume was important, in allowing for total immersion of the knife blades during inoculation.

Knife inoculation. To closely simulate industry conditions, new boning knives (6 in [15.24 cm] curved; Dexter-Russell, Southbridge, MA) with an approximate surface area of 18 cm² were used in this experiment. The blade of each knife was dipped individually for 5 min in the inocula of either E. coli O157:H7 or Salmonella. Knives were allowed to dry for 30 min in a biological hood to allow for bacterial attachment. After 15 min of drying, the knives were flipped to facilitate uniform microbial attachment on both sides of the blade. This method allowed for an approximate 10⁴ to 10⁵ CFU/cm² attachment of E. coli O157:H7 and Salmonella.

Sanitizing treatments. Treatments consisted of immersion into 200 ppm of QAC (Birko Quadra-Quat, Olathe, KS), 200 ppm of chlorine (Clorox Company, Oakland, CA), 5% lactic acid at a pH of 3.5 (LA, 88% food grade; Birko, Olathe, KS), 1.1% sodium metasilicate with a pH of 11.0 and specific gravity of 1.02 (SMS; AvGard XP, Danisco, New Century, KS), hot-water (82°C), and room-temperature (21°C) water. All treatments were prepared with ambient-temperature tap water (21°C) unless temperature was specified. QAC test strips (Serim, Elkhart, IN), chlorine test strips (Serim) and a chlorine meter (model DT-DR, LaMatte, Chester-town, MD), pH strips (Sigma Aldrich, St. Louis, MO), and a specific-gravity hydrometer (VWR International, Radnor, PA) in addition to pH test strips (Sigma Aldrich) were used to measure concentrations of QAC, chlorine, LA, and SMS, respectively. A thermometer (NSF, Warren, MI) was used to monitor temperature of both hot- and ambient-temperature water treatments. These methods have been used in other studies to validate sanitizer concentrations (13).

Neutralization of sanitizers. In order to neutralize the effects of residual sanitizers remaining on knives after treatment, the following neutralizers were utilized: Letheen broth (EMD) modified with Tween 80 (Acros Organics, Geel, Belgium) to neutralize QAC, Dey-Engley broth (BD, Franklin Lakes, NJ) for chlorine and SMS neutralization, and 1% peptone (BD) supplemented with 17 mM monophasic potassium phosphate (EMD) and 72 mM diphasic potassium phosphate (EMD) as the LA neutralizer. Neutralizing agents were evaluated to ensure that bacteria were not destroyed in their presence, and that neutralization of the sanitizer was achieved (data not shown). BPW was utilized for water treatments. Ten milliliters of these neutralizers, or BPW in the case of water treatments, was added to sterile spongesicles (World BioProducts, Mundelein, IL), which were used to sample knife blades before and after treatment. Likewise, dilutions were made using these neutralizers.

Microbiological analysis. After drying, one side of the knife (side A) was swabbed to determine initial pathogen counts of the knife. Knives were then immersed in a stainless steel container containing one sanitizer for 30 s. A total of three knives were treated by each sanitizer for every replication. After treatment, the opposing side of each knife (side B) was swabbed to quantify pathogen reductions achieved by each treatment. Swabbing was performed with a spongesicle hydrated with 10 ml of the appropriate neutralizing broth or BPW. The entire side of the knife blade was swabbed with 10 passes (5 passes per side of the swab). Sponges were aseptically returned to the spongesicle bag and stomached (Seward 400 Circulator, London, UK) at 230 rpm for 1 min. Serial dilutions were made with the appropriate neutralizers or BPW and spiral plated (AutoPlate 4000, Spiral Biotech, Watertown, MA) onto MacConkey (BD) or xylose lysine desoxycholate (BD) agars for E. coli O157:H7 and Salmonella samples, respectively. To measure reductions below the limit of detection of the spiral plater, all samples were also spread plated.
RESULTS AND DISCUSSION

As seen in Figure 1, the mean concentration of the *E. coli* O157:H7 and *Salmonella* cocktail was $8.730 \pm 0.102$ and $8.596 \pm 0.123 \log CFU/ml$, respectively. Previous work in our laboratory (data not shown) showed that lower cocktail concentrations ($\sim 10^6$) did not produce consistent attachment to a new stainless steel surface. The desired initial attachment of *E. coli* O157:H7 and *Salmonella* was approximately $10^5 \log CFU/cm^2$ for each pathogen. Mean pathogenic attachment onto the knives was $4.51 \pm 0.332$ and $5.09 \pm 0.154 \log CFU/cm^2$ for *E. coli* O157:H7 and *Salmonella*, respectively, for the three replications (Fig. 1). There was an approximately 3.5- to 4-log CFU/ml loss between the cocktail concentrations to the initial attachment values (CFU per square centimeter) onto boning knives. This was most likely because of poor bacterial attachment to the new stainless steel boning knives. Chimeleski and Frank (3) showed that various surface materials used throughout the industry have varying capacities to attach to pathogens. We hypothesized that used knives would have a rough texture with grooves, thereby leading to increased attachment. However, because the wear would not have been uniform for all knives, we would have introduced a degree of variability that could not have been controlled. Taormina and Dorsa (18) also evaluated alternatives to hot-water sanitation in pathogen reduction. These authors also desired the inoculum concentration to exceed $10^6$ CFU/cm$^2$ to ensure adequate attachment. Similarly, in the present study, a higher, more uniform attachment concentration was preferred, so that meaningful comparisons could be made about pathogenic reduction between various treatments.

Given the high concentration of attachment that was achieved, our experiment was designed to simulate a worst-case contamination scenario for *E. coli* O157:H7 and *Salmonella* contamination. Figure 2 shows all treatments except room-temperature water resulted in significant reductions of *Salmonella* on inoculated knives ($P \leq 0.05$). Aside from the hot-water treatment (which was statistically the most effective treatment in this study), chlorine, QAC, and LA treatments all produced the greatest statistically significant reductions of *Salmonella*. When initial and posttreatment populations were compared all treatments were significant ($P < .0001$), except for room-temperature water ($P = 0.2540$ against *Salmonella*) and 1.1% SMS ($P = 0.01416$ against *Salmonella*).

Nevertheless, treatments of chlorine, QAC, and LA were each statistically similar to one another. Treatment of 1.1% SMS resulted in a statistically significant reduction; however, the mean reduction was not as high as reductions observed with the other treatments. Taormina and Dorsa (18) reported reductions of *Salmonella* Typhimurium DT 104 equal to 1.49, 1.66, and 1.34 log CFU/cm$^2$ after a 15-s dip treatment in 440 ppm of QAC, 440 ppm of acid QAC, and 165 ppm of peroxyacetic acid–700 ppm of H$_2$O$_2$, respectively; however, when these sanitizers were applied to knives for 1 s, no nonthermal sanitizer achieved a 1-log reduction. The increased treatment time was used in our study to achieve improved pathogen reductions as compared with the reductions observed with the shorter treatment durations of other studies. For example, Taormina and Dorsa (18) reported log reductions of 0.61 and 1.66 CFU/cm$^2$ with 440 ppm of acid QAC after dip treatments of 1 versus 15 s, respectively, on knives contaminated with *Salmonella* Typhimurium DT 104, indicating improved reductions by extending treatment exposure time.

Figure 3 illustrates all treatments, with the exception of room-temperature water, resulted in significant reductions of *E. coli* O157:H7 on inoculated knives ($P \leq 0.01$). Room-temperature water ($P = 0.1416$) was not considered significant for control against *E. coli* O157:H7. These reductions were similar to those observed for *Salmonella*-contaminated knives. Aside from hot-water treatment, Cl$_2$...
and QAC were statistically the most effective at reducing E. coli O157:H7. Unlike control seen against Salmonella, LA treatment was less effective at controlling E. coli O157:H7, which is most likely because of the acid tolerance associated with this pathogen. As observed with Salmonella, immersion in 1.1% SMS resulted in a statistically significant reduction of E. coli O157:H7, though the reduction was not as great as the other treatments, excluding ambient water. Taormina and Dorsa (18) reported E. coli O157:H7 reductions of 2.38, 3.04, and 1.52 log CFU/cm² after a 15-s immersion treatment in 440 ppm of QAC, 440 ppm of acid QAC, and 165 ppm of peroxyacetic acid–700 ppm of hydrogen peroxide, respectively. Alternatively, Rodrigues (14) observed reductions of 2.28 and >4.86 log CFU/cm² on knives after a 5- and 30-s immersion in 100 ppm of Cl₂, respectively. These studies showed that alternative sanitation treatments do exist, and these treatments can produce greater reductions after an increased treatment interval (14, 18). Snijders et al. (17) observed reduction of E. coli O157:H7 after treatment of LA, also showing that treatment time interval can impact the magnitude of reduction. This research illustrated that LA could be a possible hot-water sanitation alternative. In contrast to hot-water alternatives, Goulter et al. (8) observed a greater reduction of E. coli O157:H7 after a longer treatment interval.

In conclusion, ambient-temperature sanitizing treatments of QAC and Cl₂ achieved the greatest reductions of E. coli O157:H7 and Salmonella populations, with LA also achieving statistically similar control of Salmonella. Sanitizers were mixed with room-temperature water (21°C) prior to treatment. Significant reductions were achieved with these ambient-temperature sanitizing solutions, implying that our alternative knife sanitation procedure has potential for pathogen reduction on harvesting knives. However, chlorine forms carcinogenic compounds and is thought to have decreased efficacy when organic matter is introduced into the solution (15), and LA is less effective against E. coli O157:H7. For these reasons, we recommend a 30-s immersion in QAC as the most acceptable knife-sanitation alternative for facilities not equipped with hot water.

With a one-knife process that is generally used, a 30-s dip treatment would prove unfeasible. Therefore, we recommend a three-knife rotation rather than the two-knife rotation, as suggested by previous literature (6), which allows each knife to undergo sanitation for the necessary 30-s time period. This cycle of knife use and dip treatment will allow for adequate time in the sanitizing treatment, thereby preventing cross-contamination to subsequent animals during harvest. To ensure efficacy in the presence of continuously introduced organic material (i.e., blood), fresh

FIGURE 2. Log-transformed least-square mean values for initial versus treated levels of Salmonella on knife blades inoculated with Salmonella. Bacterial populations are expressed in CFU per square centimeter. Least-square means without a common letter differ (P ≤ 0.05) statistically.

FIGURE 3. Log-transformed least-square mean values for initial versus treated levels of Escherichia coli O157:H7 on knife blades inoculated with E. coli O157:H7. Bacterial populations are shown as CFU per square centimeter. Least-square means without a common letter differ (P ≤ 0.01) statistically.
sanitizer and knives should be provided every 30 to 45 min. Knives must be vigorously cleaned and sanitized prior to additional use.

Previous studies have suggested alternative methods to reduce pathogens on knives, but the effects of fat and meat residue has not been fully elucidated. Fat content of a food product can alter the pathogenetic reduction observed after cooking, sanitizing, etc. (9, 12). Fat can protect pathogens from stomach pH as well as temperature treatments (12). The present study has demonstrated the potential of nonthermal sanitizers to significantly reduce the populations of E. coli O157:H7 and Salmonella on knives. Additional research should focus on the ability of these nonthermal sanitizing treatments to reduce microbial populations on contaminated knives harboring meat and fat residues.

REFERENCES