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Evaluation of an Alcohol-based Sanitizer Spray's Bactericidal Effects on *Salmonella* Inoculated onto Stainless Steel and Shell Egg Processing Equipment

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Abstract: Improved sanitation procedures during shell egg processing may reduce the risk of food-borne illness. Experiments were conducted to determine the ability of an alcohol-quaternary ammonium sanitizer or water to reduce *Salmonella* inoculated onto stainless steel and shell egg processing equipment. A nalidixic acid-resistant *Salmonella typhimurium* (ST) isolate was grown on agar plates at 37°C for 18-24 h; cells were harvested and added to Phosphate Buffered Saline (PBS) to generate 8.5 *Salmonella* cells/mL inoculum in two experiments. Both experiments were repeated twice. In the first experiment four stainless steel beakers were contaminated by spraying 10 mL of inoculum, respectively. Beakers were allowed to dry for 15 min. Two of the beakers were sprayed with 20 mL of water and the other two were sprayed with a sanitizer solution (70% isopropyl alcohol and 200 ppm quaternary ammonium). After 5 min and 24 h each beaker was swabbed with a PBS moistened sponge and plated onto BGS supplemented with 200 ppm nalidixic acid to enumerate surviving ST. In the second experiment, inoculum was sprayed onto two brushes from shell egg packing machinery. After 15 min, one brush was sprayed with water for five min and the other was sprayed with the sanitizer for 30 s. Each brush was sampled by swabbing three times after 5 min and 24 h. After 5 min, 5.5 and 0.6 log CFU/mL ST and after 24 h, 2.8 and 0.0 log CFU/mL ST were recovered from stainless steel, respectively. Packer head brush average results were 4.7 and 3.1 log CFU/mL ST after 5 min and were 4.0 and 0.00 log CFU/ml ST after 24 h. This sanitizer solution and delivery system were 100 to 10,000 times more effective than water in reducing *Salmonella* numbers.

Key words: Eggs, sanitation, *Salmonella*, stainless steel, packer head brushes

INTRODUCTION

Safety regulations are being drafted for the shell egg industry. Good Manufacturing Practices (GMPs) and Sanitation Standard Operating Procedures (SSOPs) are important precursors to HACCP documentation (National Advisory Committee for Microbiological Criteria of Foods, 1992). *Salmonella* is the pathogen most often associated with egg-borne outbreaks of salmonellosis (Ricke *et al*., 2001). If eggs are handled appropriately and consumed after adequate cooking and served without recontamination outbreaks do not occur (Cogan and Humphrey, 2003). Developing effective sanitation procedures that will reduce *Salmonella* contamination of equipment or other surfaces in the processing environment may help to reduce consumer exposure even if eggs are not handled or cooked properly (Carrasco *et al*., 2012).

Earlier work indicated that sanitation in shell egg processing facilities could be improved (Jones *et al*., 2003; Musgrove *et al*., 2004). Cleaning and sanitizing procedures for most equipment have not been validated for the shell egg industry. Experiments were conducted to determine the ability of an alcohol-quaternary ammonium sanitizer delivered in a mist to reduce *Salmonella* inoculated onto stainless steel and shell egg processing equipment.

MATERIALS AND METHODS

A marker strain, of *Salmonella typhimurium* resistant to nalidixic acid was placed on BGS agar (Accumedia Manufacturers Inc., East Lansing, MI, USA) plates supplemented with 200 ppm nalidixic (BGS + Nal) acid and incubated at 37°C for 18-24 h. Cells were harvested and added to phosphate buffered saline to generate an inoculum. Cell density was estimated spectrophotometrically and then confirmed by plating serial dilutions onto BGS + Nal incubating as described. Final inoculum was determined to be ~8.4 $\log_{10}$ *Salmonella* cells/mL in each of two repetitions (8.5 and 8.4, respectively). The inoculum was then placed in a sterile spray bottle with 90 mL Phosphate Buffered Saline (PBS) for application onto beakers and brushes. Final inoculums concentration was 7.4 $\log_{10}$ *Salmonella* cells/mL.

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In the first experiment the inside of four sterile stainless steel 1 L beakers was inoculated by spraying 10 mL of inoculum, respectively. Excess liquid was decanted and the beakers were allowed to dry for 15 min. Using a spray bottle, two of the beakers were sprayed with 20 mL of water and the other two were sprayed with a sanitizer solution (70% isopropyl alcohol and 200 ppm quaternary ammonium) using an automated sprayer. The sanitation system chosen for comparison with water and testing in this study, used carbon dioxide to aerosolize the sanitizer mixture (70% ethanol and 200 ppm quaternary ammonium) in such a fine spray that it can be applied directly to water sensitive components. The device used is portable and a 91.4 cm wand is used to apply the solution. The sanitizing solution is approved for non-porous food contact surfaces (Biomist, 2012).

Sterile sponges and bags were hydrated with Phosphate Buffered Saline (PBS) (Whirlpak, Inc., Detroit, MI, USA) After 5 min and 24 h, each beaker was swabbed in triplicate using a separate moistened sponge for a section the length of the sponge (5 cm) from top to bottom (inside the beaker). Rinsate was recovered from each sponge sample by squeezing, which caused the liquid sample to pool in the bottom of the bag. After swabbing, sponge rinsate was enumerated by plating serial dilutions onto BGS + Nal and incubated at 37°C for 18-24 h. After incubation, plates were observed and typical *Salmonella* colonies, pink with halos, were counted and converted to $\log_{10}$/mL rinsate.

In the second experiment, the 10 mL of inoculum was sprayed onto each of two brushes. These brushes are part of machinery used to transport washed shell eggs into cartons or flats. Brushes which had never been used were inserted into a device designed in house to spin them as they would in a commercial processing facility (~60 rpm). After drying for 15 min, initial *Salmonella* numbers were determined. Brushes were sprayed with water for 2 min using a hose and a municipal water supply; followed by a 30 s sanitizer application. Swab samples were taken 1 min after spraying with water and 1 min, 5 min and 24 h after being sprayed with sanitizer. *Salmonella* numbers were determined as described previously.

RESULTS

Average results for the two repetitions of the stainless steel experiment are presented in Table 1. A two-tailed heteroscedastic t-test was used to analyze the beaker data. There was a significant difference between water and the sanitizer at 5 min and 24 h sampling ($p \leq 0.05$, $p \leq 0.05$). Though there was a significant difference between water after 5 min and 24 h ($p \leq 0.05$); none existed for the same time periods for sanitizer treated stainless steel.

Table 1: Results from two repetitions ($\log_{10}$ cfu *Salmonella typhimurium*/mL rinsate)[1] for swabs of stainless steel beakers inoculated with *Salmonella typhimurium*[2] 5 min and 24 h after rinsing with water or a sanitizing solution

Treatment	5 min	24 h
Water	$5.5^{A,X}$	$2.8^{B,X}$
Sanitizer	$0.6^{A,Y}$	$0.0^{A,Y}$

[1]Results were determined by plating serial dilutions of rinsate onto BGS agar supplemented with 200 ppm nalidixic acid and incubated at 37°C for 18-24 h.
[2]*Salmonella typhimurium* was resistant to 200 ppm nalidixic acid.
[A,B]Values within a row with different superscripts are significantly different ($p<0.0001$).
[X,Y]Values within a column with different superscripts are significantly different ($p<0.0001$)

Table 2: Average results (log cfu *Salmonella*/mL rinsate)[1] for two repetitions of swabs of *Salmonella typhimurium*[2] inoculated packer head brushes 1 min, 5 min and 24 h after rinsing with water or a sanitizing solution (70% ethanol and 200 ppm quaternary ammonium)

Treatment	1 min	5 min	24 h
Control	5.2^{X}	NS	NS
Water	4.7^{X}	NS	NS
Sanitizer	$3.1^{A,Y}$	3.3^{A}	0.0^{B}

[1]Results were determined by plating serial dilutions of rinsate onto BGS agar supplemented with 200 ppm nalidixic acid and incubated at 37°C for 18-24 h.
[2]*Salmonella typhimurium* was resistant to 200 ppm nalidixic acid.
[A,B]Values within a row with different superscripts are significantly different ($p<0.0001$).
[X,Y]Values within a column with different superscripts are significantly different ($p<0.0001$)

Average results for the two repetitions of the packer head brush experiment are depicted in Table 2. A two-tailed paired t-test was used to analyze the packer head brush data. There was no significant difference between 1 min for the control and the water treatment; however the sanitizer treatment was significantly different ($p \leq 0.05$). For the sanitizer, no difference was detected between 1 and 5 min but the 24 h value was significantly different ($p \leq 0.05$).

DISCUSSION

When eggs are handled and cooked properly, they are unlikely to contribute to salmonellosis outbreaks (Ricke *et al*., 2001). However, prevention of cross-contamination and recontamination of foods by *Salmonella* during processing helps to assure food safety (Carrasco *et al*., 2012). Inadequate sanitation and poor equipment design can contribute to cross-contamination (Forsythe and Hayes, 2000). As shell egg processing regulations shift in focus from quality to safety, more attention is given to improving sanitation.

In earlier studies, 11 facilities were sampled before and after sanitation practices were performed. It was determined that sanitation made no significant decrease in aerobic microorganism or *Enterobacteriaceae* numbers on any of the surfaces sampled, including

packer head brushes (Jones *et al*., 2003; Musgrove *et al*., 2004). As a result, further work was conducted on nest run cart shelves, vacuum loaders and packer head brushes (Musgrove and Jones, 2006; Jones and Musgrove, 2008).

Stainless steel is the preferred and most often used material for food-contact surfaces. It is resistant to corrosion and more easily cleaned than other construction materials (Forsythe and Hayes, 2000). Egg spray wash machinery and transfer cups are constructed from stainless steel. Sanitation procedures vary from facility to facility however; spraying equipment with water using a nozzle sprayer is commonly employed. The scales and transfer cups on older equipment may be water sensitive which limits the sanitation procedures that can be used on them.

Results for the stainless steel experiment are in Table 1. In the current study there was a 3 $\log_{10}$/mL rinsate difference between numbers of ST recovered from beakers sprayed with water compared to those treated with the sanitizer after 5 min. Longer contact time would have made it more difficult to affect attached *Salmonella* (Bae *et al*., 2012), however given the short duration of contact time with water or the sanitizer, the reduction observed is promising. Valeriano *et al*. (2011) reported a significant reduction in *Salmonella enteritidis* inoculated onto stainless steel medallions after treatment with essential oils of peppermint (*Mentha piperita*) or lemon balm (*Cymbopogon citratas*), but 10 min of contact time were required. After 20 min, viable *S. enteritidis* were not recovered. Given the design of egg processing equipment, soaking the components for extended periods of time is not practical. Drying can be detrimental to bacterial survivability, even for *Salmonella* (Bell and Kyriakides, 2002) so lower numbers are expected with a longer drying time. The alcohol has an initial bactericidal effect and quickly evaporates while the quaternary ammonium, which is more effective at killing gram negative bacteria, remains as a residue (Forsythe and Hayes, 2000; Biomist, 2012). No viable *Salmonella* were recovered after 24 h from sanitizer-treated beaker swabs. Beakers used in the study were new. Over time, stainless steel can become scratched which makes it more difficult to produce a bactericidal effect (Lomander *et al*., 2004).

In the US, automated equipment can wash, grade, weigh and package 70,000 to 180,000 eggs/hour (DiamondMobaUS, 2012). Shells eggs enter the facility via belts with in-line facilities (hens are housed in buildings which are physically connected to the processing facility) or loaded onto the belt if the eggs are from an off-line source. Eggs transfer to rollers and pass through a detergent spray wash. Excess moisture is reduced by blowing hot air over the shells. Finally, they are checked for cleanliness and defects such as blood spots, meat spots, bloody yolks, abnormal shapes, cracks in shell or membranes and cleanliness. Eggs of acceptable quality are then weighed and picked up by transfer cups. Cups deposit the eggs on a packer head lane, which has been programmed to assemble eggs into flats or cartoons by size. There is a 35.6 cm drop from where the eggs are released to the packer head lane belt below. On each packer head lane, there are two brushes which are 91.4 cm in length and 27.9 cm in diameter. These brushes turn toward each other at ~60 rpm. There purpose is to transfer the egg onto the packer lane belting without damaging the shell or membranes. These brushes are composed of thousands of plastic bristles which are 22.9 cm long.

It has been demonstrated that sanitizers are less effective on belting than on stainless steel surfaces (Bremer *et al*., 2002). Joseph and Otta (2001), reported that *Salmonella* biofilms were more difficult to remove on plastic compared to concrete or stainless steel when sodium hypochlorite and iodophor sanitizers were used. This was the case in the current experiment (Table 2). The thousands of 22.9 cm long bristles which make up each of the packer head brushes provide a great deal of surface area, in contrast to the stainless steel beakers. USDA (1999) regulations stipulate that eggs are to be packed as dry as possible. Given the rate of washing, grading and packing possible with modern machinery, it is understandable that shells will still be slightly damp when they contact the brushes. This moisture increases the chances that *Salmonella* can survive and the large surface area decreases the likelihood of the cleaning or sanitizing procedure to be effective in reducing bacterial numbers (Forsythe and Hayes, 2000).

On most of the equipment used in commercial facilities, the packer head brushes are very difficult to reach, requiring disassembly of machinery to access the entire set of brushes. Spraying with tap water may be the only cleaning or sanitizing step that is observed daily for packer head brushes, though some facilities remove and soak an alternate set of brushes on a weekly basis (Davis, 2010). The sanitation system used in this study would allow reaching the brushes without disassembly; making it possible to sanitize daily.

In 2006 (Musgrove and Jones), packer head brushes and eggs were sampled in two commercial processing facilities. Aerobic microorganisms and *Entero-bacteriaceae*, whose relative numbers are indicators of sanitation, were highest on facility Mixed Operations (MO) packer head brushes compared to an off-line facility (OL). Though washing reduced *Entero-bacteriaceae* numbers on shell eggs to <0.2 $\log_{10}$ cfu/mL at both facilities, there was greater number of contaminated eggs at the MO facility. In the present experiment, sanitizing reduced *Salmonella* within 5 min and eliminated it within 24 h, which would eliminate the brushes as a cross-contamination or recontamination source.

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