P1-57 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

Safety regulations are being drafted for the shell egg industry. Sanitation standard operating procedures are an important precursor to HACCP regulations. *Salmonella* is the pathogen was most often associated with egg-borne outbreaks. 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. Experiments were conducted to determine the ability of a alcohol-quaternary ammonium sanitizer delivered in a mist to reduce Salmonella inoculated onto stainless steel and shell egg processing equipment. A nalidixic acid-resistant *Salmonella* Typhimurium was grown on agar plates at 37°C for 18−24 h. Cells were harvested and added to phosphate buffered saline to generate inoculum with a density of ~100 billion cells/mL in each of two repetitions. Inoculum was added to a sterile spray bottle. Each of two experiments were repeated twice. In the first experiment the inside of four stainless steel beakers was inoculated by spraying 10 mL of inoculum, respectively. Excess liquid was decanted and the beaker was 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). Sanitizer was delivered in a mist fine enough to spray onto water sensitive equipment. After five min and 24 h each beaker was swabbed with a sponge moistened with phosphate buffered saline. After swabbing, sponge diluent was enumerated by plating serial dilutions onto BGS supplemented with 200 ppm nalidixic acid. In the second experiment, the 10 mL of inoculum was sprayed onto two brushes used to transport washed shell eggs into cartons or flats. After drying for 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 at the same time intervals as described previously. After 5 min, on average 4.2 and 2.0 log CFU/mL *Salmonella* were recovered from stainless steel sprayed with water and sanitizer, respectively. After 24 h, 3.2 and 1.2 log CFU/mL were recovered, respectively. Packer head brush average results were 4.7 and 3.1 log CFU/mL *Salmonella* after 5 min and 4.0 and 0.00 after 24 h. Significance: Often, spraying with water is the sanitizing treatment after eggs are washed. This sanitizer solution and delivery system were 100 to 10,000 times more effective than water in reducing *Salmonella* numbers. similar (95%).

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 (*5*). *Salmonella* is the pathogen most often associated with egg-borne outbreaks of salmonellosis. If eggs are handled appropriately and consumed after adequate cooking and served without recontamination outbreaks do not occur (*2*). 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. Cleaning and sanitizing procedures for most equipment have not been validated for the shell egg industry. Experiments were conducted to determine the ability of a 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, resistant to nalidixic *Salmonella* Typhimurium was placed on BGs agar plates supplemented with 200 ppm nalidixic acid and incubated at 37°C for 18−24 h. Cells were harvested and added to phosphate buffered saline to generate inoculum. Cell density was estimated spectrophotometrically. Final inoculum was determined to be ~100 billion *Salmonella* cells/mL in each of two repetitions. Inoculum was added to a sterile spray bottle. Each of two experiments were repeated twice.

In the first experiment the inside of four stainless steel beakers was inoculated by spraying 10 mL of inoculum, respectively. Excess liquid was decanted and the beaker was 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). The water was applied using a spray bottle. Sanitizer was atomized with a device that generates small mist-like droplets; the mist generated was fine enough to spray onto water sensitive equipment. The device (Biomist, Inc. Wheeling, IL) uses CO_2 as a carrier to spray the sanitizer (Figure 1). Surfactants continue sanitizing after the alcohol evaporates. The sanitizing solution is approved for food contact surfaces.

After 5 min and 24 h, each beaker was swabbed with a sponge moistened with phosphate buffered saline. After swabbing, sponge diluent was enumerated by plating serial dilutions onto BGS supplemented with 200 ppm nalidixic acid and incubated at 37°C for 18-24 h. After incubation, plates were observed and *Salmonella* colonies were counted.

In the second experiment, the 10 mL of inoculum was sprayed onto each of two brushes used to transport washed shell eggs into cartons or flats. After drying for 15 min, initial *Salmonella* numbers were determined. Brushes were sprayed with water for 2 min. 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.

TABLE 1. Results (Log CFU *Salmonella*/mL rinsate) for swabs of *Salmonella* inoculated stainless steel beakers 5 min and 24 h after rinsing with water or a sanitizing solution.

Rep/ treatment	5 min	24 h
1-Water	5.8	3.1
2-Water	5.2	2.4
1-Sanitized	0.0	0.0
2-Sanitized	0.0	0.0

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RESULTS AND DISCUSSION

As shell egg processing regulations shift in focus from quality to safety, more attention is given to improving sanitation (*5)*. In surveys conducted in 2002, it was determined that sanitation made no significant decrease to aerobic microorganism or *Enterobacteriaceae* (*3*, *4*). If food contact surfaces are not adequately cleaned and sanitized, bacterial biofilms may form (*2*). Experiment 1 results appear in Table 1. After 5 min, on average 5.6 and 0.0 log CFU/mL *Salmonella* were recovered from stainless steel sprayed with water or sanitizer, respectively. After 24 h, 2.8 and 0.0 log CFU/mL were recovered, respectively. The sanitizing solution applied in this experiment was very effective in reducing *Salmonella* contamination on stainless steel.

It has been demonstrated that sanitizers are less effective on belting than on stainless steel surfaces (*1*). This was the case in the current experiment (Table 2). After eggs are washed, graded, and weighed, they are mechanically sorted. Transporters pick the eggs up from the scales and release them over a set of packer head brushes that reduce the chances of shells breaking as they reach the packer lane belt. Each brush is made up of bristles that are approximately 20 cm long, providing a great deal of surface area. On most of the equipment used in commercial facilities, the packer head brushes are very difficult to reach. Spraying with tap water may be the only cleaning or sanitizing step that is observed daily for packer head brushes (*6*). In 2006 *(4*), packer head brushes and eggs (unwashed, washed but not sorted, washed and sorted) were sampled in two commercial processing facilities. Aerobic microorganisms and *Enterobacteriaceae* were enumerated and enriched for *Salmonella*, *Campylobacter*, and *Listeria*. Packer head brushes from the less hygienic facility were found to have higher numbers of the enumerated populations and each of the pathogens were recovered at least once. Eggs from this facility were more contaminated than from the more hygienic facility. In this experiment, sanitizing reduced Salmonella within 5 min and eliminated within 24 h.

TABLE 2. Average results (Log CFU *Salmonella*/mL rinsate) for two repetition of swabs of *Salmonella* inoculated packer head brushes 1 min, 5 min, and 24 h after rinsing with water or a sanitizing solution.

Treatment	1 min	5 min	24 h
Control	5.2	NS	NS
Water	4.9	NS	NS
Sanitized	3.1	3.3	0.0

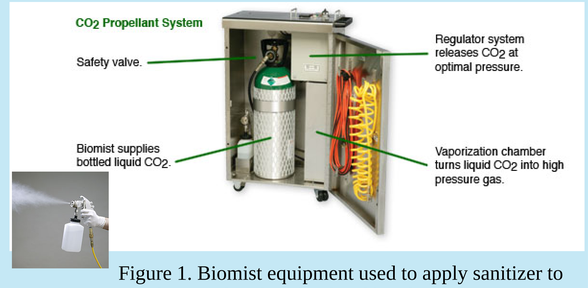

Figure 1. Biomist equipment used to apply sanitizer to stainless steel and packer head brushes.

CONCLUSIONS

1. Sanitizing reduced *Salmonella* numbers on stainless steel by 99.9% compared to rinsing with water and by 99% on packer head brushes.
2. Reducing *Salmonella* numbers on egg contact surfaces may decrease the risk that egg shells will become recontaminated after being washed.

Figure 2. Eggs being directed into foam cartons via packer head brushes and packer lanes in a commercial shell egg facility.

REFERENCES

1. Bremer, P.J., I. Monk, R. Butler. 2002. Inactivation of *Listeria monocytogenes/Flavobacterium* spp. biofilms using chlorine: Impact of substrate, pH, time and concentration. Lett .Appl. Microbiol. 35:321-325.
2. Cogan, T.A. and T.J. Humphrey. 2003. The rise and fall of *Salmonella* Enteritidis in the UK. J. Appl. Microbiol. 94:114S-119S.
3. Jones, D.R., J.K. Northcutt, M.T. Musgrove, P.A. Curtis, K.E. Anderson, D.L. Fletcher, and N.A. Cox. 2003. Survey of shell egg processing plant sanitation programs: effects on egg contact surfaces. J. Food Prot. 66:1486-1489.
4. Musgrove, M. T. and D. R. Jones. 2006. Sanitation sampling in US shell egg processing plants. Proceedings of the 35th Annual United States Japan Cooperative Program in Natural Resources Food and Agricultural Panel. FS13-FS18. 2006.
5. Musgrove, M.T., D.R. Jones, J.K. Northcutt, P.A. Curtis, K. E. Anderson, D.L. Fletcher, and N.A. Cox. 2004. Survey of shell egg processing plant sanitation programs: effects on non-egg contact surfaces. J. Food Prot. 67:2801-2804.
6. USDA. Plant sanitation and good manufacturing practices program for processing eggs. 1999. Washington, D.C.