Research Note

Recovery of *Salmonella* from Commercial Shell Eggs by Shell Rinse and Shell Crush Methodologies


*United States Department of Agriculture, Agricultural Research Service, Richard B. Russell Agricultural Research Center, 950 College Station Road, Athens, Georgia 30605; and †University of Georgia, Department of Food Science and Technology, Athens, Georgia 30602

**ABSTRACT** *Salmonella* is the most important human pathogen associated with shell eggs. *Salmonella Enteritidis* is the serotype most often implicated in outbreaks, although other serotypes have been recovered from eggs and from the commercial shell egg washing environment. Many sample methods are used to recover microorganisms from eggshells and membranes. A shell rinse and modified shell-and-membrane crush method for recovery of *Salmonella* were compared. Eggs were collected from 3 commercial shell-washing facilities (X, Y, and Z) during 3 visits. Twelve eggs were collected from each of 10 to 12 locations along the egg processing chain. After being transported back to the laboratory, each egg was sampled first by a shell rinse method and then by a shell crush method. For each technique (rinse or crush), 2 pools of 5 eggs per location sampled were selectively enriched for the recovery of *Salmonella*. Presumptive samples positive for *Salmonella* were confirmed serologically. Overall, there were 10.1% (40/396) *Salmonella*-positive pooled samples. *Salmonella* were recovered by the shell rinse and shell crush techniques (4.8 vs. 5.3%, respectively). Plant X yielded 21.5% *Salmonella* positives, whereas less than 5% of samples from plants Y and Z were found to be contaminated with the organism (4.2 and 4.5%, respectively). *Salmonella* was recovered more often from unwashed eggs (15.8%) than from washed eggs (8.3%). For some eggs, *Salmonella* was only recovered by one of the methods. Use of both approaches in the same experiment increased sampling sensitivity, although in most cases, crushing provided more sensitive *Salmonella* recovery.

(Key words: *Salmonella*, commercial shell egg, methods, rinse, crush)


**INTRODUCTION**

Many methods for the recovery of microorganisms from egg shells and membranes have been described (Board and Tranter, 1995) One of the simplest and most commonly used methods involves rinsing the shell of an intact egg with an isotonic diluent (Penniston and Hedrick, 1947; Gentry and Quarles, 1972). Rinsing, however, may not enable recovery of microorganisms that have become embedded in shell pores (Williams and Dillard, 1973). Crush methods may allow for the recovery of organisms from the surface of the shell and those that are located inside the pores or the membranes (Berrang et al., 1991).

An experiment was conducted in which a shell rinse method was used in conjunction with a technique in which shell and membranes were crushed together.

Eggs in various stages of the commercial shell egg process were sampled. Method efficacy for recovery of *Salmonella* was determined for both methods.

**MATERIALS AND METHODS**

**Shell Egg Sample Collection**

Eggs were collected from 3 commercial plants (X, Y, Z) in the southeastern United States at the following points of processing: accumulator, prewash wetting, first washer, second washer, sanitizer, drier, oiler, scales following check detection and candling, rewash belt entrance, rewash belt exit, and packaging (at 2 different packer lane belts). Eggs were collected after the line had been operating for at least 2 h and during the morn-morning break so as not to interfere with plant operations. Collection at this time also allowed for simultaneous collection at all sample sites. Twelve eggs from each collection site were aseptically placed into foam cartons, packed into half-cases, and transported back to the laboratory at ambient temperature. Plants were chosen based not only on willingness to participate and on their operational procedures but also on proximity to our...
facility so that eggs could be collected and analyzed quickly.

**Shell Egg Sampling Methodologies**

Ten of the 12 eggs collected at each site were sampled using a shell rinse technique. Each egg was placed into a sterile Whirl-Pak bag with 10 mL of sterile PBS and rinsed by shaking for 1 min. Before rinsing, PBS was warmed to 42°C to facilitate bacterial recovery. After a rinse sample was obtained, each egg was removed and transferred to a different sterile bag. Rinse samples were then stored at 4°C overnight. On the following morning, each egg was removed from the second bag and cracked open on the edge of a sterile beaker. Egg meats (yolk and albumen) were discarded, and the inside of the shell was rinsed using sterile PBS to remove most of the adhering albumen. An effort was made to eliminate as much of this material as possible because of the antimicrobial components of albumen. Shell and membranes from a single egg were crushed in a gloved hand and forced into a sterile 50-mL disposable centrifuge tube. After 20 mL of sterile PBS was added, a sterile glass rod was moved vertically in and out of the tube for 1 min. This allowed for a maceration of shells and membranes as well as a thorough mixing of the sample with the diluent.

**Salmonella Enrichment**

For each of the 12 collection sites, 2 pooled samples were formed by combining shell egg rinses or crushed shells and membranes from 5 eggs. Samples were preenriched in buffered peptone water at 35°C for 18 to 24 h, followed by enrichment in TT broth and Rapport-Vassiliadis broth overnight at 42°C. Enriched samples were plated onto brilliant green sulfa and XLT-4 agar plates and incubated at 37°C for 24 h. Presumptive positives were inoculated into lysine iron agar and triple sugar iron slants and incubated at 35°C for 18 to 24 h. Those samples with presumptive results indicative of *Salmonella* on these media were confirmed by serogrouping antisera.Confirmed isolates were then streaked for purity and stocked onto agar slants and ceramic beads in cryogenic protective media. A culture of each isolate was provided to the National Veterinary Services Laboratory of the USDA’s Animal and Plant Health Inspection Services (Ames, IA) for serotyping. A sample was recorded as positive if it was confirmed and serotyped from either the shell rinse or crushed shell and membrane composite samples. A comparison of recovery frequency was accomplished by chi-squared test of independence (Cochran, 1954).

**RESULTS**

*Salmonella* recovery rates by rinse or crush method are summarized in Table 1. Data were pooled for the 3 plants by stage of processing: before (accumulator, prewash rinse, rewash entrance, and rewash exit), during (washer 1, washer 2, sanitizing rinse, drier, and oiler), or after processing (scales and packer head lanes). Samples composed of eggs collected at the accumulator, prewash, or rewash belts yielded the most *Salmonella* positive results, although a composite from each of the 12 sites was positive at least once during the 9 plant visits (3 for each of 3 plants). *Salmonella enterica* serovars isolated were identified as Typhimurium, Typhimurium (Copenhagen), Heidelberg, Kentucky, and 4,12.

**DISCUSSION**

*Salmonella* is considered the most important human enteropathogen associated with shell eggs (Baker and Bruce, 1995). S. Enteritidis is the serotype most often implicated in egg-borne outbreaks of salmonellosis although product temperature abuse followed by consumption of raw or undercooked eggs are usually risk factors. This serotype occurs at a low frequency (1 in 20,000 eggs) even when flocks are known to be colonized by S. Enteritidis. However, all serotypes of *Salmonella enterica* are potential human pathogens, and their presence on eggshells is of interest (Ricke et al., 2001). This experiment was performed to explore how various sampling factors could affect method efficacy in recovering this important human pathogen from commercial shell eggs.

In our study, we obtained 40 *Salmonella* isolates from eggshell rinse and shell-and-membrane crush samples. Tap water (1) and wash water (1) samples were also occasionally positive for the organism. An in-depth discussion of how wash water temperature, wash water pH, tap water potability, and other conditions affected *Salmonella* prevalence were addressed in other publications (Musgrove, 2004; Northcutt et al., 2005).

On first review of the data, it would appear that rinsing egg shells or crushing egg shells and membranes together were equally effective in their ability to recover *Salmonella*. When combining data from all 3 plants and plant visits (X1, X2, X3, Y1, Y2, Y3, Z1, Z2, and Z3) 19 rinse samples (4.8%) and 21 crush samples (5.3%) were *Salmonella* positive. However, the first visit to plant X yielded results that were atypical compared with the other plant visits. There were 23 separate *Salmonella* positive samples for X1, more than all the other plant visits combined, including X2 and X3. Several factors contributed to the *Salmonella* prevalence observed for X1. On this particular plant visit, wash water temperatures were below average (43°C), pH values were also lower than average (pH 10.3), excessive foaming was observed in both washers, and a tap water sample tested positive for *Salmonella*. Visit X1 was also yielded the most rinse samples that were positive.

When data from visit X1 were removed, there were only 4 samples positive by the rinse method (23.5%) and 13 (76.5%) samples positive by the crush method. After separation by stage of process, rinse methods recovered 30, 25, and 0% for composite samples collected before,
during, or after processing, whereas crushing yielded 70, 75, and 100% \textit{Salmonella} positives. Based on these results, it becomes clear that under normal processing conditions, crushing of shells and membranes allows for greater \textit{Salmonella} recovery, particularly for eggs that have been processed. These results confirm results published by Rizk et al. (1966). After washing and sanitizing inoculated eggs, no \textit{Salmonella} were recovered by a surface sampling method, but cells were recovered by blending of shells and membranes.

Visit X2, from which the lowest wash water pH values were encountered in the study (pH 9.1), was the only other plant visit that recovered \textit{Salmonella} by the rinse method. Wash water temperature was 40°C in the first washer during this visit, and a wash water pH reading of 9.1 was recorded from the second washer. Milder wash water conditions may have contributed to greater \textit{Salmonella} populations on the shell exterior, allowing for recovery by the shell rinse method. In a study conducted by Jones et al. (1995), \textit{Salmonella} was recovered from 1/90 egg shells using the rinse method of Gunaratne and Spencer (1973). This single positive was obtained during a sampling when wash water pH was 10.19, the lowest recorded in the study by Jones et al. (1995).

Shell rinse and crush methods were used to recover aerobic microorganisms and \textit{Enterobacteriaceae} from commercial shell eggs (Musgrove, 2004). In this work, it was determined that stage of processing influenced which sampling approach was best suited for each of the populations. Shell rinsing recovered higher numbers of aerobes, especially from eggs that had not been washed. However, crushing was more effective at recovering \textit{Enterobacteriaceae} from eggs collected after processing. In the current study, \textit{Salmonella} was recovered at a higher rate than in previous studies (Cox and Davis, 1968; March, 1969; Jones et al., 1995). Solowey et al. (1946) recovered \textit{Salmonella} from 5% of 123 washed-dirty eggs by rinsing but 13% by grinding and compositing shell samples. In our study, during sampling for plant X, one of the sampling approaches (rinse or crush) recovered \textit{Salmonella} missed by the other method, allowing for increased recovery. When \textit{Salmonella} occurred at a higher prevalence as was the case at plant X, rinse and crush were comparable. Rinsing is a simpler, faster method for recovering microbial populations from egg shells. However, when eggs were washed under optimal processing conditions and organism prevalence was lower, the crush method was more effective at recovering \textit{Salmonella}.

### ACKNOWLEDGMENTS

The authors gratefully acknowledge the technical assistance of Patsy Mason, Susan Akins, Sherry Turner, Manju Amin, Jerrie Barnett, Jordan Shaw, and Taylor Jones. This work was assisted in part by a grant from the United States Egg and Poultry Association (RE project no. 501), and their support is greatly appreciated.

### REFERENCES


