INTRODUCTION

One of the first lines of defense for an egg from external bacterial contamination is the shell. Eggs with cracks in the shell surface pose greater food safety risks for consumers than intact eggs (Todd, 1996). Studies have determined that Salmonella Enteritidis, as well as Campylobacter jejuni, can penetrate, sustain, or grow in the contents of eggs with cracked shells (Chaudhary et al., 1989; Ernst et al., 1998; Hara-Kudo et al., 2001). The human eye can detect large cracks in the shell, especially in hand candling when enhanced with illumination. Microcracks in the shell, as defined by Bain et al. (2006), are much more difficult to detect. Microcracks are minute cracks in the shell surface (external or internal) that reduce the protective barrier properties of the shell. Studies using scanning electron microscopy have confirmed the presence of microcracks in the shell surface and have found greater bacterial penetration in eggs with a higher number of microcracks (Fajardo et al., 1993, 1995). Bain et al. (2006) assessed the microscopic formation of microcracks. They determined that the nature of microcrack formation made it unlikely that online crack detection in shell egg processing equipment would find microcracks because the technology is based on mechanical excitation.

Fresh microcracks are almost impossible to visually detect. Placing eggs in overnight refrigeration allows the microcracks to become more visible as a result of physical changes such as water migration and entrapment between the shell and shell membranes or enlargement caused by thermal stress on the shell during cooling. With the current US industry practice of high throughput machines and inline complexes, it is not plausible to regrade eggs 24 h after processing. A novel system for crack detection using modified pressure and imaging has been developed (Lawrence et al., 2008). The detection methodology allows for both large cracks from each treatment were used for cultural analysis of a shell rinse, shell emulsion, and contents sample for each egg. The ST levels were monitored on brilliant green sulfa agar with 200 mg/L of nalidixic acid. Egg contents were also enriched to determine the prevalence of ST in low levels. Salmonella Typhimurium was not detected on or in any of the control eggs, including the eggs imaged after the inoculated eggs. The highest level of ST was detected in inoculated shell emulsions (4.79 log cfu/mL). No differences in ST levels were found for any sample location between imaged and nonimaged inoculated eggs. Therefore, the modified-pressure imaging system for microcrack detection did not result in microbial cross-contamination or increase the level of microbial penetration in inoculated eggs. The imaging system can be used to assess eggs for cracks without negative food safety implications.

Key words: microcrack, detection, egg, Salmonella, modified pressure

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Research Note

Salmonella contamination in shell eggs exposed to modified-pressure imaging for microcrack detection

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ABSTRACT Microcracks in egg shells are a food safety risk and are difficult for professional human graders to detect. Modified-pressure imaging technology with 99.6% accuracy has been developed to detect microcracks. This study was conducted to determine whether the microcrack detection system would increase penetration of Salmonella into egg contents or lead to cross-contamination within the system. Thirty dozen grade A large white retail eggs were used for each of 3 replicates. Cracked eggs were removed and 72 eggs/replicate were dip inoculated in buffered peptone water containing 10⁵ cfu/mL of nalidixic acid-resistant Salmonella Typhimurium (ST), whereas 144 eggs were dipped in sterile buffered peptone water. All eggs were incubated overnight at 25°C before imaging. Forty-five eggs of each treatment were imaged in the following order: control, inoculated, control. Imaged and nonimaged eggs were refrigerated for 24 h. Fifty of each treatment were used for cultural analysis of a shell rinse, shell emulsion, and contents sample for each egg. The ST levels were monitored on brilliant green sulfa agar with 200 mg/L of nalidixic acid. Egg contents were also enriched to determine the prevalence of ST in low levels. Salmonella Typhimurium was not detected on or in any of the control eggs, including the eggs imaged after the inoculated eggs. The highest level of ST was detected in inoculated shell emulsions (4.79 log cfu/mL). No differences in ST levels were found for any sample location between imaged and nonimaged inoculated eggs. Therefore, the modified-pressure imaging system for microcrack detection did not result in microbial cross-contamination or increase the level of microbial penetration in inoculated eggs. The imaging system can be used to assess eggs for cracks without negative food safety implications.

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and microcracks to be seen by subjecting the eggs to quick, small negative pressure changes while digital images are collected. The modified-pressure imaging system had an overall accuracy of 99.6%, compared with 94.2% for trained, professional graders in a study comparing 1,000 eggs (Lawrence et al., 2009). Exposing eggs to the modified-pressure imaging system does not affect overall egg quality during storage (Jones et al., 2010).

The current study was undertaken to determine whether exposure to the modified-pressure imaging system would result in an increase in egg content contamination of *Salmonella* Typhimurium in experimentally inoculated shell eggs. Furthermore, cross-contamination of eggs within the grading system was examined.

**MATERIALS AND METHODS**

**Egg Collection**

For each replicate, 360 USDA grade A large white eggs (USDA, 2000) were collected as they exited the egg processing line at a commercial facility. The eggs were processed under continuous inspection in accordance with the voluntary shell egg surveillance program (USDA, 2005). Eggs were immediately transported to the laboratory and placed on clean, 30-egg pulp flats and stored at 4°C until initiation of the study. Cracked eggs were removed and discarded. Three replicates were conducted for this study.

**Inoculation**

A nalidixic acid-resistant strain of *Salmonella* Typhimurium (ST) from in-house cultures was grown overnight on brilliant green sulfa agar (Acumedia Manufacturers, Lansing, MI) with 200 mg/L of nalidixic acid (BGSNA; Sigma N-4382, Sigma-Aldrich, St. Louis, MO) at 37°C. An inoculation suspension was prepared in 25 mL of sterile PBS and the optical density was assessed (540 nm; Spectronic 20D+, Thermo Electron Corp., Madison, WI) to determine approximate concentration.

The shell egg inoculation methods of Jones and Musgrove (2005) were used with slight modifications. Twenty-four eggs at a time were immersed in inoculated buffered peptone water (BPW; Acumedia Manufacturers) or sterile BPW. The approximate concentration of inoculum was 10⁵ cfu/mL, as confirmed through plating on BGSNA. During each replicate, 72 eggs were inoculated with ST and 144 eggs were exposed to sterile BPW. All eggs were stored on sterile plastic egg flats at 25°C for 24 h.

**Shell Egg Imaging**

Eggs were imaged according to the methods of Lawrence et al. (2009). Briefly, a 15-egg modified-pressure imaging system (Figure 1) was used. During analysis the eggs were exposed to 4 short negative pressure cycles (approximately 193 mm Hg each). For each imaging set, the 15 eggs loaded into the system came from a single inoculation dip. The remaining 9 eggs (of 24 total eggs/flat) were retained as nonimaged controls. Eggs were aseptically removed from the microcrack detection system and placed in individual sterile sample bags. Nonimaged controls were also aseptically placed in individual sterile sample bags. The order of imaging for each replicate was 3 sets of the following: controls, ST inoculated, postinoculated controls. Between replicates, the system was sanitized with 70% ethanol. Swabs were collected and plated on BGSNA to ensure no residual ST was present.

**Microbial Assessments**

Individual eggs were assessed for all microbial comparisons. From each inoculation set, 9 (of the 15) imaged eggs and 6 (of the 9) nonimaged eggs were used. This provided the following total number of eggs assessed per replicate: control = 27 imaged, 18 nonimaged; inoculated = 27 imaged, 18 nonimaged; postinoculation control = 27 imaged, 18 nonimaged. Shell rinses were conducted according to the methods of Jones et al. (2002) using 10 mL of 42°C sterile PBS. Eggs were then dipped in 70% ethanol and allowed to air dry. Shell matrix and membrane contamination was determined using the shell crush method of Musgrove et al. (2005). Briefly, the shell and corresponding membranes from a single egg were placed in a sterile conical 50-mL centrifuge tube with 10 mL of 42°C PBS and macerated for 1 min with a sterile glass rod. The contents of each egg were placed in a sterile sample bag and stomacher blended for 1 min at normal speed (Stomacher 400 Circulator, Seward Ltd., London, UK).

All samples were plated in duplicate (0.1 mL for diluents, 0.25 mL for egg contents) on BGSNA and incubated for 18 to 24 h at 37°C before enumeration. Because of the natural antimicrobial properties of the egg contents, enrichment procedures were also used to...
determine the prevalence of ST at levels below those detected from direct plating. Each egg contents was preenriched with BPW and incubated at 37°C for 24 h. Subsequently, 1 mL from each sample was transferred to Rappaport-Vassiliadis broth (Becton Dickinson, Sparks, MD) and incubated at 42°C for 24 h. The enriched samples were then struck onto BGSNA and incubated at 37°C for 24 h before the prevalence of positive samples was assessed.

**Statistical Analysis**

Microbial counts were subjected to log transformation and analyzed for significance through the GLM procedure of SAS (SAS Institute, 2002). Inoculation method (control, ST exposed, post-ST control), imaging (imaged and nonimaged), and replicate were the main effects. Means were separated by the least squares method. Prevalence of ST in the egg contents was analyzed by the chi-square method.

**RESULTS AND DISCUSSION**

No ST was detected during enumeration from the shell rinses, shell emulsions, or egg contents of control eggs (both pre- and post-ST inoculated eggs; Table 1). The detected level of ST was significantly greater (P < 0.0001) for the inoculated eggs compared with both control treatments in all instances. Very low levels of ST were detected in the contents and shell rinses of inoculated eggs (0.38 and 0.59 log cfu/mL, respectively). The highest degree of ST contamination was found in the shell matrix (shell and accompanying membranes) of inoculated eggs (4.79 log cfu/mL).

Mean levels of ST enumerated in imaged and nonimaged inoculated eggs are presented in Table 2. No significant differences were found based on whether eggs were exposed to the modified-pressure imaging system. Enriching the contents of all eggs for ST did not produce results differing from those found when ST was directly enumerated. Neither of the control treatments resulted in ST-positive egg contents. Of the 135 inoculated eggs evaluated, 132 were positive for ST (98% prevalence). The 3 samples that were not positive after enrichment were nonimaged, inoculated eggs.

Pressure changes in the egg have been a concern for microbial contamination for many years. The concern is establishing a negative pressure within the egg, thus increasing the likelihood of microorganisms associated with the shell being drawn into the egg contents (Haines and Moran, 1940; Pritsker, 1941; Stuart and McNally, 1943; Funk, 1948; Lorenz and Starr, 1952; Forsythe et al., 1953; Brant and Starr, 1962, 1966). Although eggs in the current study were exposed to 4 short negative pressure cycles, a negative pressure gradient was maintained across the shell and shell membranes. In other terms, the pressure changes associated with the microcrack detection technology exert a negative pressure on the outside of the eggs, thus opening any cracks in the shell (Lawrence et al., 2008). It is the change in pressure that opens the cracks. If the negative pressure was held for a longer period of time, the pressure inside the egg would equilibrate with the pressure outside and the crack would close. However, the system was designed to capture the negative-pressure image while the cracks are opening and then quickly release the pressure before the pressure can decrease inside the egg. Thus, the pressure changes are very short (about 350 ms) and do not affect the egg contents. The results of the current study confirm that *Salmonella* Typhimurium present on and within the shell did not migrate into the contents of the eggs at a greater rate because of exposure to the modified-pressure microcrack detection system. The potential existed for *Salmonella* Typhimurium present on and within the shell to be transferred within the imaging system because of the pressure modifications exerted on the eggs. However, no instances occurred in which ST was transferred to noninoculated control eggs imaged after inoculated eggs.

**Table 1.** Levels of nalidixic acid-resistant *Salmonella* Typhimurium detected on and in eggs exposed to the modified-pressure microcrack detection system

<table>
<thead>
<tr>
<th>Sample site</th>
<th>Control (log cfu/mL)</th>
<th>Inoculated (log cfu/mL)</th>
<th>Postinoculated Control (log cfu/mL)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contents</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.0001</td>
</tr>
<tr>
<td>Shell rinse</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.0001</td>
</tr>
<tr>
<td>Shell emulsion</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>Means within a row with different letters are significantly different (P < 0.0001).

<sup>1</sup>ND = none detected.

<sup>2</sup>Eggs were immersed in either buffered peptone water inoculated with a nalidixic acid-resistant strain of *Salmonella* Typhimurium (inoculated) or sterile buffered peptone water (controls).

**Table 2.** Effect of exposure to the modified-pressure microcrack detection system on the detection of nalidixic acid-resistant *Salmonella* Typhimurium on and in inoculated shell eggs

<table>
<thead>
<tr>
<th>Sample site</th>
<th>Imaged (log cfu/mL)</th>
<th>Nonimaged (log cfu/mL)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contents</td>
<td>0.37</td>
<td>0.39</td>
<td>NS</td>
</tr>
<tr>
<td>Shell rinse</td>
<td>0.54</td>
<td>0.66</td>
<td>NS</td>
</tr>
<tr>
<td>Shell emulsion</td>
<td>4.76</td>
<td>4.84</td>
<td>NS</td>
</tr>
</tbody>
</table>

<sup>1</sup>NS = P > 0.05.
Thus, the modified-pressure microcrack detection system can be used to accurately evaluate shell eggs for the presence of cracks without increasing the likelihood of *Salmonella* contamination or cross-contamination. This method is therefore a safe option for effectively screening eggs for cracks, thus increasing the safety of eggs reaching consumers.

**ACKNOWLEDGMENTS**

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