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Pathogen Prevalence and Microbial Levels Associated with Restricted Shell Eggs

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ABSTRACT

Restricted shell eggs that do not meet quality standards for retail but maintain acceptable quality for inclusion in further processed eggs are often diverted to further processing. A study was conducted to characterize the microbiological populations present on and in these eggs. On a single day, restricted eggs were collected from three shell egg processing plants a total of three times (replicates). Six shells or egg contents were combined to create a pool. Ten pools of shells and contents were formed for each plant per replicate. Shells and membranes were macerated in 60 ml of diluent. Contents were stomacher blended to form a homogeneous mixture. Total aerobic microorganisms and *Enterobacteriaceae* were enumerated. The prevalence of *Salmonella, Campylobacter,* and *Listeria* was determined by cultural methods. Average aerobic counts were 4.3 log CFU/ml for the shells and 2.0 log CFU/ml for the contents. There were plant × replicate differences for both ($P < 0.05$ and $P < 0.01$, respectively). The average *Enterobacteriaceae* level associated with the shell was 2.4 log CFU/ml and less than 0.1 log CFU/ml for the egg contents, with 36.7% of the samples being positive. One shell sample (0.5% of total samples) was *Campylobacter* positive. Two shell samples (1.1% of total samples) were *Salmonella* positive. Twenty-one percent of samples were positive for *Listeria* (33 shells and 5 contents). Although current pasteurization guidelines are based on *Salmonella* lethality, the results of this study reiterate the need to revisit the guidelines to determine the effectiveness for other pathogenic species.

Egg products are becoming a larger portion of the total egg sales each year in the United States. Egg product per capita consumption has more than doubled from 36.8 in 1984 to 76.5 in 2004 *(3).* The increase in egg product use can be linked in part to both the American desire for convenience in food preparation and food safety concerns in the 1990s associated with eggs. A variety of egg products are available for retail, food service, and food product manufacturing, including liquid, dried, frozen, and value-added. Eggs that do not meet quality standards to be marketed as shell eggs, but are still edible, can be utilized in the production of egg products in accordance with U.S. Department of Agriculture, Food Safety Inspection Service guidelines. Such eggs are known as "restricted eggs" *(24)* and include dirty eggs and checks (shells cracked but shell membranes still intact). Allowable restricted eggs are a small percentage of the total eggs utilized in further processing.

Salmonella has been detected in the shell egg–processing environment and on unwashed and washed shell eggs *(17).* A retail study of eggs in Trinidad found 1.1% of the sampled eggs to be contaminated with *Campylobacter (1).* When retail eggs and egg products in New York State were examined for the presence of *Campylobacter,* none was detected *(4).* Izat and Gardner *(9)* did not detect *Campylobacter jejuni* from unwashed or washed eggs or wash water. Shane et al. *(22)* did not detect *C. jejuni* in the egg processing plant, on equipment, or in the water within the facility. Properly processed shell eggs have been identified as an unlikely source of *C. jejuni (9),* whereas cracked eggs and those with flaws, such as cage marks, have been shown to be more easily penetrated by *Campylobacter (2).*

Listeria was not found to be associated with retail eggs in Trinidad *(1)* but was isolated from ready-to-eat Spanish potato omelets *(23)* and in 3.1% of egg-containing products in an Italian survey *(6). Listeria innocua* was detected in 36% of raw liquid whole egg samples taken before pasteurization in the United States *(12).* Eggs and wash water samples from Canada were found to contain *L. innocua (8).* Furthermore, Laird et al. *(11)* concluded *Listeria* spp. can survive common wash water procedures and can be found in the shell egg processing plant. *Listeria*-positive wash water has not been linked to *Listeria*-positive washed eggs *(8, 10).*

Regulations governing the pasteurization of eggs in the United States were put into effect in 1971 *(24).* These guidelines were based on the reduction of *Salmonella* in the product. Researchers have begun to question the efficacy of these requirements. Adjustments in product composition (e.g., salted, sugared, high solids) can alter the heat resistance of organisms during pasteurization *(5, 13, 19, 20).* Current pasteurization requirements are not adequate to ensure *Listeria*-free products according to previous research *(5, 14, 20).* Palumbo et al. *(20)* suggested the requirements be revisited to enhance product safety. The use of UV, irradiation, high pressure, and other alternate processing intervention strategies is being investigated for their ability to reduce microbial populations in egg products. Before ef-

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TABLE 1. *Effect of plant and replicate on total aerobic bacteria associated with the shells of restricted eggs*[a]

Plant	Rep 1	Rep 2	Rep 3
A	4.2 BC[b]	5.0 AB	5.1 AB
B	3.4 CD	2.4 D	4.3 ABC
C	5.6 A	4.2 BC	4.9 AB

[a] Values are expressed as log CFU per milliliter. Standard error = 0.4 log CFU/ml; $n = 10$. Rep, repetition.

[b] Means within the table with differing letters are significantly different ($P < 0.05$).

fectively developing new pasteurization technologies, it is important to understand the microbiological status of the raw product. This study was conducted to examine the levels of bacteria present and the prevalence of *Salmonella, Campylobacter,* and *Listeria* associated with restricted eggs destined for further processing.

MATERIALS AND METHODS

On a single day, three shell egg–processing facilities were visited. All three facilities participate in the U.S. Department of Agriculture Agricultural Marketing Service voluntary grading program *(25),* which requires set guidelines for shell egg processing. At each plant, two 30-egg pulp flats of restricted eggs were collected and transported to the laboratory on ice. This process was repeated for 3 consecutive weeks (replicates). After arriving at the laboratory, all flats were placed in a single cardboard half case and stored at 4°C overnight.

The next morning, the eggs were cracked on the edge of a sterile glass beaker. The contents of six eggs were combined into a single sterile laboratory sample bag. The inside surface of the shells was rinsed with warm, sterile phosphate-buffered saline (PBS) to remove any adhering albumen. Six shells were pooled into a sterile specimen cup, 60 ml of PBS was added, and shells were macerated with a glass stirring rod for 1 min according to the methods of Musgrove et al. *(16).* Contents pools were homogenized in a laboratory stomacher (Stomacher 400 Circulator, Steward Ltd., London, UK) at normal speed for 1 min. Ten pools each of contents and shells were formed for each plant during a replicate.

Total aerobic microorganisms were enumerated by spiral plating (Autoplate 4000, Spiral Biotech, Norwalk, Mass.) 0.1 ml of shell diluent onto duplicate plate count agar plates (Difco, Becton Dickinson, Sparks, Md.). Aerobic counts were enumerated for contents by spread plating 0.25 ml of homogenized contents onto duplicate plate count agar plates. After 48 h at 37°C, colonies were counted and recorded as CFU per milliliter.

TABLE 2. *Effect of plant and replicate on total aerobic bacteria present in the contents of restricted eggs*[a]

Plant	Rep 1	Rep 2	Rep 3
A	1.5 D[b]	2.7 AB	2.9 A
B	2.0 BCD	2.4 ABC	2.0 BCD
C	1.8 CD	1.3 D	1.4 D

[a] Values are expressed as log CFU per milliliter. Standard error = 0.2 log CFU/ml; $n = 10$. Rep, repetition.

[b] Means within the table with differing letters are significantly different ($P < 0.01$).

TABLE 3. *Effect of plant and replicate on* Enterobacteriaceae *associated with the shells of restricted eggs*[a]

Plant	Rep 1	Rep 2	Rep 3
A	2.3	2.4	2.5
B	2.8	1.9	3.1
C	2.6	1.8	1.9

[a] Values are expressed as log CFU per milliliter. Standard error = 0.3 log CFU/ml; $n = 10$. Rep, repetition.

Enterobacteriaceae were enumerated by duplicate plating 1 ml of either shell diluent or homogenized contents into violet red bile glucose agar with overlay (Difco, Becton Dickinson). Plates were incubated at 37°C for 20 to 24 h. *Salmonella, Campylobacter,* and *Listeria* prevalence was determined according to the methods described in Jones et al. *(10).* Presumptive *Listeria* isolates were biochemically identified with Microgen *Listeria* ID strips (Microbiology International, Frederick, Md.).

Bacterial counts were subjected to log transformation *(21)* and analyzed for significance through the General Linear Models procedure of SAS. Means were separated by the least-square method. Prevalence data were subjected to chi-square frequency analysis *(21)* to determine significance.

RESULTS

Total aerobic counts associated with the shells of the restricted eggs can be found in Table 1. Detected levels ranged from 2.4 to 5.6 log CFU/ml. The average for all samples was 4.4 log CFU/ml. Plants A and C had similar average aerobic shell counts (4.8 and 4.9 log CFU/ml, respectively) compared with 3.4 log CFU/ml for plant B. Aerobic counts in the egg contents ranged from 1.3 to 2.9 log CFU/ml (Table 2). The average plant values for all repetitions were within 1 log of each other. The overall average of all pools was 2.0 log CFU/ml.

There were no differences between plants and replicates for levels of *Enterobacteriaceae* on the surface of the restricted eggs (Table 3). Detected levels ranged from 1.8 to 3.1 log CFU/ml. The average detection was 2.4 log CFU/ml. Very low levels of *Enterobacteriaceae* were found in the egg contents pools (Table 4). Although a significant difference exists between plants and replicates, this significance occurs because of the consistently low levels detected in positive contents pools. The prevalence of *Enterobacteriaceae* was 36.7% in all of the contents pools.

Of all 180 shell and contents pools, only two shell samples were positive for *Salmonella.* Both samples were

TABLE 4. *Effect of plant and replicate on* Enterobacteriaceae *present in the contents of restricted eggs*[a]

Plant	Rep 1	Rep 2	Rep 3
A	ND	0.05 BC[b]	0.36 AB
B	0.24 ABC	ND	0.33 ABC
C	0.46 A	0.01 C	ND

[a] Values are expressed as log CFU per milliliter. Standard error = 0.12 log CFU/ml; $n = 10$. Rep, repetition; ND, none detected.

[b] Means within the table with differing letters are significantly different ($P < 0.05$).

TABLE 5. *Prevalence of* Listeria *spp. in the contents and associated with the shells of restricted eggs*[a]

Plant	% positive	
	Contents	Shells
A	10.0	66.7
B	ND	13.3
C	6.7	30.0
P values	NS	0.0001

[a] ND, none detected; NS, not significant.

from a single replicate at plant B. After serotyping (APHIS, Ames, Iowa), it was determined that both isolates were *Salmonella* Heidelberg. A single *Campylobacter* isolate was found in a shell sample from plant B. *Listeria* was detected in 21% of the total samples. Approximately 37% of the shell pools were positive for *Listeria,* whereas only 6% of the contents pools were positive. There were no differences in the prevalence of *Listeria* in the contents pools from the three plants (Table 5). Plant A had a greater frequency of *Listeria* associated with the shells of the restricted eggs (66.7%) than did plants B (13.3%) and C (30.0%). Of the *Listeria* isolates recovered (38 total), the following identifications were made: *L. grayi* (2.6%), *L. welshimeri* (13.2%), and *L. innocua* (84.2%). *L. innocua* was the only isolate found in the positive egg contents pools.

DISCUSSION

The restricted eggs tested in this study had been washed in the shell egg–processing facility from which they were collected before being diverted to further processing. This diversion could have been because of quality defects or because the eggs did not meet cleanliness standards after washing. Musgrove et al. *(17)* found 4.6 log CFU/ml aerobic microorganisms on the shell surface before washing and 4 log CFU/ml on egg shells from the rewash belt. The present study found a similar level of aerobic organisms associated with the shell. *Enterobacteriaceae* numbers found in the current study were greater than those previously reported for unwashed eggs or those collected from rewash belts *(17).* The present study utilized a shell crush and rub technique initially described by Musgrove et al. *(16),* found to be superior to rinses in the recovery of *Enterobacteriaceae* and other microorganisms from shells. This could account for some of the differences in recovery. Furthermore, the restricted egg samples contained checked eggs (shells cracked but membranes intact). This represents a breakdown in one of the natural antimicrobial defenses of the egg.

Salmonella prevalence was low (1%), and *Salmonella* Enteritidis was not isolated. Shell eggs are generally not considered a reservoir for *Campylobacter*; therefore, the detection of *Campylobacter* on restricted eggs was of note. Allen and Griffiths *(2)* reported cracked and flawed eggs were more easily colonized by *Campylobacter.* The ability of *Campylobacter* to survive in the egg after penetration has been found to be limited *(18).* Consequently, Clark and Bueschkens *(7)* found *C. jejuni* was able to grow in liquid yolk and yolk-albumen combinations. The presence of *Campylobacter* on restricted eggs poses another potential hurdle for pasteurization techniques to overcome and should be included in studies considering the effects of common additives on the lethality of organisms during pasteurization.

Listeria has been detected in further egg-processing facilities and products over the years. Moore and Madden *(15)* sampled in-line filters in a pasteurization plant and found approximately 72% to be positive for *Listeria.* The genera isolated were *L. innocua* (62%) and *Listeria monocytogenes* (38%). In another study, approximately 36% of raw liquid whole egg samples from further processing plants were found to be positive for *L. innocua (12). L. innocua* has also been isolated from egg samples and wash water in shell egg processing plants in Canada *(8).* The authors further suggest that *L. innocua* can better withstand the shell egg washing environment than other *Listeria* species. The isolation of *L. innocua* in the current study is therefore not surprising, but the detection of *L. grayi* and *L. welshimeri* has not been commonly reported in eggs.

Although at a low rate of prevalence, *Campylobacter* and *Listeria,* as well as *Salmonella,* contaminated egg shells or contents. The findings of this study indicate that organisms other than *Salmonella* need to be considered when devising intervention strategies for pathogen contamination of further processed egg products. It is not known if current guidelines for pasteurization are sufficient to combat the diversity of pathogenic organisms detected on and in eggs entering further processing, especially when the product is adjusted (e.g., salted, sugared, high solids). As new technologies are developed for further egg processing, a broader vision of pathogen reduction, including diversity of organisms and processing additives, should be considered.

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