**A Research Note**

**Immersion in Boiling Water to Disinfect Egg Shells Before Culturing Egg Contents for *Salmonella enteritidis***

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**ABSTRACT**

Culturing egg contents has become an essential part of efforts to reduce the frequency of egg-associated transmission of *Salmonella enteritidis* (SE) to human consumers. Accurate and dependable results from such testing can be achieved only if the exterior surfaces of eggs are effectively disinfected to prevent any SE present on shells from contaminating the contents when eggs are broken for culturing. The present study determined that brief (5 s) immersion of eggs in boiling water was highly effective in reducing both the incidence and level of SE on the shells of experimentally contaminated eggs. This treatment did not, however, affect the recovery of SE added to the interior contents of eggs and was successfully applied to the isolation of SE from the contents of eggs laid by experimentally infected hens.

**MATERIALS AND METHODS**

**Effect of brief immersion in boiling water on *Salmonella enteritidis***

In each of two replicate trials, 50 visibly clean eggs were collected on the day of use from our laboratory’s specific-pathogen-free flock, individually dipped in an overnight tryptone soya (TS) broth (Oxoid USA, Ogdensburg, NY) culture containing approximately 10⁶ CFU/ml of a phage type 13a SE isolate (5) and air-dried for 30 min at room temperature. Thirty eggs were then transferred to a plastic egg flat and immersed in a boiling water bath for 5 s. 10 eggs were individually dipped for 5 s in 2% tincture of iodine, and the remaining 10 eggs (retained as positive controls) were not subjected to either disinfection treatment. After drying, the shell of each egg was broken against a hard surface covered with sterile aluminum foil to release the contents. Each shell was placed in a separate sterile bag, 30 ml of distilled water containing 0.1% bacto peptone (Difco Laboratories, Detroit, MI) was added, and the shell was manually crushed and massaged for 30 s. Aliquots (0.1 ml) from each sample were then spread onto brilliant green agar (Difco) plates and incubated for 24 h at 37°C. SE colonies on these plates were counted as an indication of the level of shell contamination.

**Effect of brief immersion in boiling water on *S. enteritidis* in egg contents**

In each of two replicate trials, the contents of 24 eggs were inoculated with SE by inserting a syringe needle through and then parallel to the shell so that the inoculum was injected into the albumen at the interior shell surface. Each egg was injected with approximately 10⁴ SE cells in a 0.1-ml inoculum, prepared by diluting an overnight TS broth culture of a phage type 13a SE isolate (5) in 0.85% saline. The holes in the shells were sealed with cellulose nitrate-based adhesive compound (Steven Industries, Bayonne, NJ), which was allowed to harden for 2 h at room
temperature. Twelve eggs were then transferred to a plastic egg flat and immersed in a boiling water bath for 5 s. The other 12 eggs (retained as positive controls) were not subjected to a disinfection treatment. After drying, the shell of each egg was broken against a hard surface covered with sterile aluminum foil and the contents were released into a sterile bag. The egg contents were mixed for 15 s in a Stomacher Model 80 Lab Blender (Tekmar Co., Cincinnati, OH), an aliquot (0.5 ml) from each sample was spread onto brilliant green agar, and the agar plates were incubated for 24 h at 37°C. SE colonies on these plates were counted as an indication of the level of contents contamination.

**Application of immersion in boiling water to eggs from experimentally infected hens**

In each of two replicate trials, 30 Single Comb White Leghorn hens from our laboratory’s specific-pathogen-free flock were housed in single-bird laying cages in a disease-containment isolation building. All hens were orally inoculated with an overnight TS broth culture containing approximately 10⁹ cells of a phage type 14b SE isolate (4). The contents of all eggs laid by each hen during the first 15 d postinoculation were pooled and sampled for SE at 3-d intervals. At each sampling interval, eggs from 15 hens (either odd or even cage numbers) were disinfected before sampling by immersion in boiling water for 5 s, and eggs from the other 15 hens were disinfected by dipping for 5 s in 2% tincture of iodine. Assignment of odd and even cage numbers to the two shell disinfection treatments alternated with successive sampling intervals. The egg pools were cultured for SE by previously described methods (4).

**Statistical analysis**

Significant differences (P < 0.05) between treatment groups or replicate trials in the frequency of isolation of SE were determined by applying the chi-square test (13). A one-way analysis of variance (13) was applied to assess the significance (P < 0.05) of differences between groups or trials in the numbers of SE colonies counted. As no significant variation was observed between replicate trials within an experiment, the results were combined for analysis.

**RESULTS**

**Effect of brief immersion in boiling water on S. enteritidis on egg shells**

SE was detected on the shells of all 20 positive control eggs, with a mean colony count of 153.2 CFU per plate. Dipping eggs in 2% tincture of iodine reduced the detectable incidence of SE contamination of shells to 45.0% (9/20 eggs) and the level of contamination to 3.3 CFU per plate (P < 0.005 in both instances). None of the 60 eggs examined after immersion in boiling water yielded any SE colonies from their shells. Both the incidence and the level of SE contamination on the shells of eggs immersed in boiling water were significantly (P < 0.005) lower than among either positive control or iodine-dipped eggs.

**Effect of brief immersion in boiling water on S. enteritidis in egg contents**

SE was detected in the contents of all 24 positive control eggs and all 24 eggs immersed in boiling water. No significant differences were observed between the two treatment groups in the level of SE contamination of contents (means of 169.9 CFU per plate for positive control eggs and 172.5 CFU per plate for eggs immersed in boiling water).

**Application of immersion in boiling water to eggs from experimentally infected hens**

No significant effects on SE recovery from egg pools were associated with the two disinfection treatments. SE was isolated from 19 of 108 (17.6%) pools of egg contents from infected hens after disinfection of shells by dipping in 2% tincture of iodine and from 18 of 108 (16.7%) pools after immersion of eggs in boiling water.

**DISCUSSION**

Brief immersion of eggs in boiling water was investigated in earlier studies as a means of diminishing or postponing bacterial spoilage of egg contents (2,9). In the present study, this treatment was found to be highly effective for reducing the numbers of SE on egg shells artificially contaminated with large numbers of SE cells. Dipping eggs in tincture of iodine also reduced the number of contaminating SE on shells but left small numbers of SE on the shells of nearly half of the treated eggs. The method for enumerating viable SE cells used in this study involved direct plating of samples on selective media, without prior resuscitation of any damaged cells. Accordingly, the results obtained should not be interpreted to imply unequivocally that either disinfection method achieved the complete elimination of all SE cells from the surfaces of inoculated eggs. The comparative efficacies of other disinfection methods (such as dipping or swabbing with alcohol) have not been established.

Immersion of eggs in boiling water did not, moreover, diminish the number of SE cells recovered from the contents of experimentally inoculated eggs. Any negative effect of a shell disinfection method on the viability of SE in egg contents could significantly reduce the sensitivity of egg culturing procedures, as most available evidence indicates that naturally contaminated eggs generally contain rather small numbers of SE cells (6,8). Immersion in boiling water did not reduce the frequency of isolation of SE from egg pools produced by infected hens in the current study, so this disinfection method evidently does not affect SE in natural internal contamination sites.

Because of the reported low incidence of SE contamination in eggs laid by infected commercial flocks (7), accurate testing requires pooling and culturing large numbers of eggs. Immersion in boiling water could easily be adapted to permit efficient disinfection of large quantities of eggs. Disinfection by immersion in boiling water also eliminates any concerns about inadvertently transferring disinfectant chemicals into culture media. Consistently thorough and dependable shell disinfection before culturing is essential to ensure that the frequency at which positive results are obtained is an accurate indication of the frequency of contents contamination. Results from eggs laid by experimentally infected hens in the present study reinforce earlier indications that contents contamination can indeed occur independently of shell contamination (5,7).

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The explication of this anomalous behavior of the galactose could be that this monosaccharide will be released as a result of the hydrolysis of lactose and will be consumed in condensation reactions with amino acids that are involved in the set of Maillard reactions (4,6,14). It could be that in the first phase of formation there is a balance between galactose released and consumed, which at higher temperatures is decanted by the release process. In intensive treatments, a sufficient amount of lactulose has already been formed for there to be considerable degradation of the released galactose. At high heating temperatures and times, the two galactose formation processes would have an accumulative effect.

The graph, showing the theoretical equivalent thermal deterioration of the process (Fig. 6), also shows a set of parallel lines with two clearly defined slopes which allow the two Z values for the process to be calculated. In the range of temperatures lower than 120°C, it is 127.3°C which gives an idea of the slowness of the formation. At higher temperatures, in the range of thermal sterilization treatment, the Z value becomes normal at 33.12°C. As this graph shows, the galactose formation has clearly defined values for UHT processes (shaded in the diagram), as well as for classical sterilization and pasteurization; this is shown as a heating parameter that gives a clear standard for classification of heat-treated milk.

The values of $Q_{10}$ obtained for the formation of galactose during thermal treatment were:

$$Q_{10}(90-120°C) = 1.19,$$
$$Q_{10}(120-150°C) = 2.98.$$

These values are in consonance with the works of Reuter (12) and Burton (2), which gave a range of 2 to 5 in the value of $Q_{10}$ as correct for the chemical formation processes. As a comparison, the data given for lactulose can also be used, namely, $Z = 28.6°C$ and $Q_{10} = 2.23$ (6).

In brief, it can be stated that galactose behaves as a reliable heating parameter for heat-treated milk, although in the near future the effectiveness of this parameter will be tested on commercial samples of pasteurized, UHT, and sterilized milk.

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REFERENCES


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