Optimization of Iron Supplementation for Enhanced Detection of 
*Salmonella* Enteritidis in Eggs

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**ABSTRACT**

Mixed raw egg contents were inoculated with approximately 10 CFU of *Salmonella* Enteritidis and supplemented with 0 to 7 mg of FeSO₄ per g of egg contents. Egg contents were then incubated at 37°C, and *Salmonella* Enteritidis colonies were enumerated for up to 106 h. Iron supplementation significantly enhanced the growth of *Salmonella* Enteritidis. Within the first 24 h of incubation, the optimum iron level for *Salmonella* Enteritidis growth in egg contents was between 0.2 and 2 mg of FeSO₄ per g of egg contents. After 24 h of incubation at 37°C, *Salmonella* Enteritidis counts in eggs supplemented with 0.5 mg of FeSO₄ per g of egg contents consistently reached approximately 1 × 10⁹ CFU/ml, whereas *Salmonella* Enteritidis Enteritidis counts in eggs without iron supplementation varied from less than 5 CFU/ml to 8.4 × 10⁶ CFU/ml. A 3 by 3 factorial design was used to study the effect of type of preenrichment and level of iron supplementation on the growth of *Salmonella* Enteritidis in egg contents. No significant differences in *Salmonella* Enteritidis counts between preenrichment and nonpreenrichment treatments were observed when egg contents were supplemented with 0.5 mg of FeSO₄ per g of egg contents. It was concluded that preenrichment was not necessary for isolation of *Salmonella* Enteritidis from eggs. The effect of iron supplementation on the sensitivity of detection by the direct plating method was investigated. The direct plating method detected a significantly higher percentage of *Salmonella* Enteritidis in raw egg contents supplemented with 0.5 mg of FeSO₄ per g of egg contents (90%) than in raw egg contents without iron supplementation (63%).

Egg-associated *Salmonella* Enteritidis outbreaks have been a major cause of foodborne illness in the United States and several European countries (15, 20, 25). To control *Salmonella* Enteritidis outbreaks, it is recommended that eggs be sampled for *Salmonella* Enteritidis (29). The conventional cultural methods for isolation of *Salmonella* from foods include preenrichment, selective enrichment, selective plating, and confirmation (9, 31). However, to test such large numbers of eggs and reduce testing costs, the U.S. Department of Agriculture recommended using a direct plating method for detection of *Salmonella* Enteritidis in eggs (29). This method involves incubating mixed contents of 20 raw eggs at room temperature (20 to 24°C) for at least 72 h before streaking onto selective media. However, Gast (13) found that the direct plating method detected *Salmonella* Enteritidis in egg contents significantly less frequently than the method involving preenrichment and selective enrichment. When mixed raw egg contents were inoculated with approximately 2 CFU of *Salmonella* Enteritidis cells and incubated at 25°C for 4 days, the direct plating method detected only 47.1% of the contaminated pools, whereas preenrichment followed by selective enrichment detected 75% (13).

The detection of *Salmonella* Enteritidis in eggs using the direct plating method is constrained by the infrequent occurrence of *Salmonella* Enteritidis contamination in eggs, the very small numbers of *Salmonella* Enteritidis in *Salmonella* Enteritidis–positive eggs, and the inhibitors naturally present in eggs (2, 4, 10, 14, 23, 25, 27). The probability of eggs containing *Salmonella* Enteritidis is approximately 1 in 10,000 eggs (25). Humphrey et al. (23) surveyed the naturally infected flocks in the United Kingdom and found that approximately 0.6% of eggs were positive for *Salmonella* Enteritidis. Most contaminated eggs contained less than 10 cells of *Salmonella* Enteritidis per egg. Gast and Beard (14) orally inoculated hens with *Salmonella* Enteritidis and found that eggs positive for *Salmonella* Enteritidis generally contained less than 10 CFU of *Salmonella* Enteritidis per ml of egg contents. A number of factors, such as high pH, lysozyme, and ovotransferrin, have been considered as part of the egg’s defense system against contamination (4). Ovotransferrin inhibits the growth of gram-negative bacteria in eggs through chelation of iron (2, 10, 27). Therefore, the direct plating method may not be able to detect all of the contaminated eggs.

Researchers have been attempting to develop a rapid and highly sensitive method for detecting *Salmonella* Enteritidis in eggs. It has been shown in numerous studies that supplementation of egg contents with iron can overcome the antimicrobial properties of ovotransferrin and enhance the growth and detection of *Salmonella* Enteritidis in eggs (3, 8, 11, 12, 16–18, 24). The U.S. Food and Drug Administration recommended adding 225 ml of Trypticase soy broth (TSB) supplemented with 35 mg/liter of FeSO₄ to 25-g egg contents (final FeSO₄ level = 0.0315 mg/ml) for isolation of *Salmonella* in eggs. Cudjoe et al. (8) recommended supplementing egg contents with 35 mg/liter of...
FeSO₄ to improve recovery of Salmonella Enteritidis from eggs. Gast and Holt (16–18) found that supplementing egg contents with 6 mg/ml of FeSO₄ significantly increased the detection rate of Salmonella Enteritidis in eggs. However, the optimum level of iron for multiplication of Salmonella Enteritidis in raw egg contents has not been determined.

TSB and buffered peptone water (BPW) are commonly used preenrichment media for isolation of Salmonella Enteritidis from eggs. Humphrey and Whitehead (22) found that addition of up to three volumes of BPW in egg contents did not significantly increase the growth of Salmonella Enteritidis. Gast (13) reported that preenrichment of egg contents with TSB supplemented with 35 mg/liter of FeSO₄ significantly increased the growth of Salmonella Enteritidis. Stephenson et al. (26) reported that TSB was the best preenrichment medium among the five preenrichment media evaluated (lactose broth, brain heart infusion broth, TSB, BPW, and nutrient broth) in recovery of Salmonella from raw egg contents. Gast and Holt (18) found that supplementing egg contents with concentrated TSB (10×) significantly increased the detection rate of Salmonella Enteritidis.

The objectives of this study were to determine the optimum level of iron for multiplication of Salmonella Enteritidis in raw egg contents, the effect of preenrichment with TSB or BPW on the growth of Salmonella Enteritidis, and the effect of iron supplementation on the detection rate of Salmonella Enteritidis by the direct plating method.

**MATERIALS AND METHODS**

**Eggs.** Nest-run eggs from The Pennsylvania State University Poultry Research Farm were used for all experiments. Eggs were candled. Any dirty eggs and eggs with visible cracks were removed. The remaining eggs were stored at 5°C and used within 4 days after they were laid.

**Preparation of Salmonella Enteritidis culture.** A mutant strain of Salmonella Enteritidis phage type 8 (Salmonella Enteritidis PT8NSR), which was resistant to 0.1 mg/ml of nalidixic acid (Sigma Chemical Co., St. Louis, Mo.) and 0.1 mg/ml of streptomycin sulfate (Sigma), was isolated as previously described (5) and used as an indicator organism. A loopful of Salmonella Enteritidis PT8NSR was transferred from a tryptic soy agar plus yeast extract (Difco Laboratories, Detroit, Mich.) plate containing 0.1 mg/ml of nalidixic acid and 0.1 mg/ml of streptomycin sulfate to tryptic soy broth plus yeast extract (TSBYE) (Difco) tubes containing the same concentration of both antibiotics. The TSBYE culture was then incubated for 8 h at 37°C and diluted in BPW (Difco), and Salmonella Enteritidis counts were determined. The Salmonella Enteritidis culture in BPW was stored at 5°C until used for the following experiments.

**Determination of the optimum iron levels for Salmonella Enteritidis growth.** Fifteen eggs were soaked in 200 ppm of Cl⁻ solution containing 0.1% sodium dodecyl sulfate (SDS) (Sigma) for 30 min (the Cl⁻/SDS solution was prepared by adding 8 ml of commercial bleach [5.25% sodium hypochlorite] to 992 ml of distilled water containing 1 g of SDS) (30) and then rinsed with sterile distilled water. Immediately, eggs were aseptically cracked and egg contents were aseptically put into a sterile blender (Model Osterizer 890-6J, Sunbeam Products, Inc., Boca Raton, Fla.) and blended for 30 s. Fifty grams of blended egg contents (the weight of an egg content was approximately 50 g) were put into a sterile jar containing a magnetic stir bar. One jar was retained as an uninoculated negative control. A 0.1-ml solution of BPW containing approximately 10 CFU of Salmonella Enteritidis was added to each jar (the Salmonella Enteritidis counts were determined by plating 0.1 ml of BPW onto each of five xylose lysine deoxycholate [XLD] [Difco] plates at the time of inoculation). The egg contents in each jar were supplemented with different amounts of FeSO₄ (Sigma). One jar was retained as an unenriched and inoculated control. Egg contents were mixed using the magnetic stir bar for 1 min and incubated at 37°C. Salmonella Enteritidis counts in egg contents were determined for up to 106 h by serial dilution in BPW, plating onto XLD, and incubation at 37°C for 48 h before counting colonies.

Suspect colonies on XLD plates were randomly picked and used to inoculate triple sugar iron agar (Difco) and lysine iron agar (Difco) slants containing 0.1 mg/ml of nalidixic acid and 0.1 mg/ml of streptomycin sulfate. The slants were incubated at 35°C for up to 48 h. Group D factor 9 slide-agglutination reactions were conducted on the colonies that had positive reactions in triple sugar iron agar and lysine iron agar slants.

Initial studies were conducted with the following FeSO₄ concentrations: 1, 2, 3, 4, 5, 6, and 7 mg of FeSO₄ per g of egg contents. The concentrations of 1 and 2 mg of FeSO₄ per g of egg contents yielded the highest Salmonella Enteritidis counts. Therefore, more experiments were run in this range to determine the optimum FeSO₄ level. One milliliter of FeSO₄ solution was added to each jar to get the following FeSO₄ concentrations: 0.5, 1, 1.5, and 2 mg of FeSO₄ per g of egg contents. Since there were no differences among these four treatments with FeSO₄ supplementation, further studies were conducted with the following FeSO₄ concentrations to determine the lowest optimum iron level: 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5 mg of FeSO₄ per g of egg contents. This experiment was replicated twice.

**Effect of preenrichment and iron supplementation on Salmonella Enteritidis growth in egg contents.** Fifteen eggs were disinfected using Cl⁻/SDS solution and then rinsed with sterile distilled water. Immediately, eggs were aseptically cracked and egg contents were aseptically put into a sterile blender and blended for 30 s. Fifty grams of blended egg contents was put into a sterile jar containing a magnetic stir bar. One jar was retained as an uninoculated negative control. A 0.1-ml solution of BPW containing approximately 10 CFU of Salmonella Enteritidis was added to each jar (the Salmonella Enteritidis counts were determined by plating 0.1 ml of BPW onto each of five XLD plates at the time of inoculation). Each jar was randomly assigned to each of the nine treatments as shown in Table 1. Then appropriate preenrichment broth (BPW or TSB) and/or FeSO₄ was added to the jars according to the treatments.

**TABLE 1. Experimental design to study the effect of type of preenrichment and level of iron supplementation on the growth of Salmonella Enteritidis in egg contents**

<table>
<thead>
<tr>
<th>FeSO₄ (mg/g)</th>
<th>Egg*</th>
<th>Egg* plus 50 ml of TSB</th>
<th>Egg* plus 50 ml of BPW</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>0.0315&lt;sup&gt;a&lt;/sup&gt;</td>
<td>D</td>
<td>E</td>
<td>F</td>
</tr>
<tr>
<td>0.5</td>
<td>G</td>
<td>H</td>
<td>I</td>
</tr>
</tbody>
</table>

<sup>a</sup> A total of 50 g of egg contents.

<sup>b</sup> Concentration recommended by the Food and Drug Administration for isolation of Salmonella Enteritidis in eggs (30).
Forty eggs were disinfected using the Cl Enteritidis in egg contents using the direct plating method. Enteritidis colonies were randomly picked and confirmed biochemically and serologically as described above. Three replicates were performed.

FIGURE 1. Growth of Salmonella Enteritidis in raw egg contents at 37°C without iron supplementation. A total of 50 g of egg contents was inoculated with approximately 10 CFU of Salmonella Enteritidis and incubated for 106 h. For the third replicate, data missing at 12, 16, 24, 44, 64, and 106 h indicated that Salmonella Enteritidis counts at that time were less than 5 CFU/ml.

Egg contents were mixed using the magnetic stir bar for 1 min and incubated at 37°C for 72 h. At 24, 48, and 72 h, Salmonella Enteritidis counts in egg contents were determined by serial dilution in BPW, plating onto XLD plates, and incubation at 37°C for 48 h before counting colonies. The suspect Salmonella Enteritidis colonies were randomly picked and confirmed biochemically and serologically as described above. Three replicates were performed.

Effect of iron supplementation on detection of Salmonella Enteritidis in egg contents using the direct plating method. Forty eggs were disinfected using the Cl/SDS solution and then rinsed with sterile distilled water. Immediately, each egg was put into an individual sterile jar containing a magnetic stir bar and cracked using a sterile tong. Eggshells were removed and the egg contents were mixed for 2 min, and 0.05 ml of BPW containing approximately 2 CFU of Salmonella Enteritidis was added to each of the 40 jars and each of the 20 XLD plates. (Because of the low numbers of Salmonella Enteritidis used, some samples might not receive Salmonella Enteritidis cells. Therefore, the purpose of adding Salmonella Enteritidis inoculum onto XLD plates was to see how many samples did not receive Salmonella Enteritidis cells so that the effect of iron supplementation on percentage of detection of Salmonella Enteritidis could be evaluated.) Half the egg samples were supplemented with 25 mg of FeSO₄ per egg to achieve a FeSO₄ concentration of around 0.5 mg of FeSO₄ per g of egg contents.

Egg contents were mixed for 1 min and then incubated at 37°C for 120 h. A loopful of egg contents from each jar was streaked onto XLD plates containing 0.05 mg/ml of nalidixic acid and 0.05 mg/ml of streptomycin sulfate at 24, 48, 72, 96, and 120 h. The XLD plates were incubated at 37°C for up to 72 h. The suspect Salmonella Enteritidis colonies were randomly picked and confirmed biochemically and serologically as described above. Three replicates of the above experiment were performed. The percentage detection of Salmonella Enteritidis was calculated as follows: Percentage Detection of Salmonella Enteritidis = Number of Salmonella Enteritidis–Positive Jars × 100/Total Number of Jars.

FIGURE 2. Effect of iron supplementation on Salmonella Enteritidis counts in raw egg contents incubated at 37°C for 24 h. A total of 50 g of egg contents was inoculated with approximately 10 CFU of Salmonella Enteritidis and supplemented with 0 to 7 mg of FeSO₄ per g of egg contents.

Statistical analysis. Statistical analysis was conducted using Minitab 11.2 (Minitab Inc., University Park, Pa.). One-way analysis of variance (ANOVA) was used to compare significant differences between treatments (Figs. 4 through 6) (Fisher's pairwise comparisons, individual error rate = 0.05). For the 3 by 3 factorial design (Fig. 6), it was first analyzed as a factorial design using the General Linear Model to study the main effects of preenrichment and iron supplementation and their interaction. Then it was treated as nine individual treatments and analyzed using one-way ANOVA to compare differences between treatments.

RESULTS

Determination of the optimum iron levels for Salmonella Enteritidis growth. The growth curve of Salmonella Enteritidis in egg contents without FeSO₄ supplementation at 37°C is shown in Figure 1. Salmonella Enteritidis counts in egg contents without FeSO₄ supplementation were extremely variable, varying from less than 5 CFU/ml to 8.4 × 10⁶ CFU/ml (data not shown) after 24-h incubation and from less than 5 CFU/ml to 4.2 × 10⁶ CFU/ml after 106-h incubation. However, supplementation of egg contents with 0.05 to 7 mg of FeSO₄ per g of egg contents promoted the rapid growth of Salmonella Enteritidis after 24 h of incubation (Fig. 2). Egg contents supplemented with 0.2 to 2 mg of FeSO₄ per g of egg contents had the highest Salmonella Enteritidis counts (approximately 1.0 × 10⁹ CFU/ml) after 24 h of incubation.

The effect of selected FeSO₄ levels on Salmonella Enteritidis growth in egg contents during 106-h incubation at 37°C is shown in Figure 3. Supplementation of egg contents with up to 3 mg of FeSO₄ per g of egg contents promoted the growth of Salmonella Enteritidis in egg contents during the 106-h incubation. The Salmonella Enteritidis counts in egg contents supplemented with 0.05 to 3 mg of FeSO₄ per g of egg contents were around 2 × 10⁹ CFU/ml at the end of 106-h incubation. Compared with no iron supplementation, supplementation with 4 to 7 mg of FeSO₄ per g of egg contents promoted the growth of Salmonella Enteritidis within 24 h (approximately 10⁸ CFU/ml) and then inhibited...
its growth. The *Salmonella* Enteritidis counts in egg contents supplemented with 4 to 7 mg of FeSO₄ per g of egg contents were approximately 10⁷ CFU/ml after 106-h incubation.

The effect of low iron levels (from 0.05 to 0.5 mg of FeSO₄ per g of egg contents) on *Salmonella* Enteritidis counts after 24-h incubation at 37°C is shown in Figure 4. When egg contents were supplemented with 0, 0.05, 0.1, or 0.2 mg of FeSO₄ per g of egg contents, *Salmonella* Enteritidis counts with a higher level of iron supplementation were significantly higher than those with a lower level of iron supplementation. There were no significant differences in *Salmonella* Enteritidis counts between supplementation with 0.2, 0.3, 0.4, and 0.5 mg of FeSO₄ per g of egg contents. However, 0.5 mg/g of FeSO₄ supplementation was used in the following experiments to account for bigger eggs and the possibility that some eggs may have had higher levels of ovotransferrin.

**Effect of preenrichment and iron supplementation on *Salmonella* Enteritidis growth in egg contents.** The effect of iron supplementation and preenrichment with TSB or BPW on *Salmonella* Enteritidis growth during 72-h incubation at 37°C is shown in Figure 5. For 24-h incubation, FeSO₄ supplementation and preenrichment with TSB or BPW significantly increased the growth of *Salmonella* Enteritidis in egg contents. The *Salmonella* Enteritidis counts in treatment A were around 10⁵ CFU/ml, whereas the *Salmonella* Enteritidis counts in treatments B and C were approximately 10⁷ CFU/ml after 24-h incubation. For treatments with iron added (D, E, F, G, H, I), preenrichment with TSB or BPW did not significantly increase *Salmonella* Enteritidis counts. No significant interactions between iron and TSB or between iron and BPW were observed. All treatments reached approximately 10⁹ after 48-h incubation and stayed almost stable afterward (Fig. 5). No significant differences in *Salmonella* Enteritidis counts were found between treatments at 48 and 72 h.

**Effect of iron supplementation on detection of *Salmonella* Enteritidis in egg contents using the direct plating method.** *Salmonella* Enteritidis was detected in 43.3% of the unsupplemented egg contents inoculated with approximately 2 CFU of *Salmonella* Enteritidis after 24-h incubation at 37°C (Fig. 6). Extending the incubation time significantly increased the percentage of detection. The percentage of detection of *Salmonella* Enteritidis in unsupple-
Enteritidis was detected in 90% of the supplemented samples after 24-h incubation at 37°C. The direct plating method detected a significantly higher percentage of Enteritidis in raw egg contents by the direct plating method. Each of 40 egg samples and 20 XLD plates were inoculated with approximately 2 CFU of Salmonella Enteritidis. Half of the egg samples were supplemented with 0.5 mg of FeSO₄ per g of egg contents. All egg samples were incubated at 37°C for up to 120 h and cultured for Salmonella Enteritidis by direct plating onto XLD plates each day. Within each day, bars labeled with different letters are significantly different (P < 0.05). Pooled standard deviations for days 1 through 5 were 6.2, 6.9, 6.9, 5.5, and 5.5, respectively.

Emmeted egg contents after 120-h incubation was 63.3%. However, the direct plating method detected a significantly higher percentage of Salmonella Enteritidis in egg samples supplemented with 0.5 mg of FeSO₄ per g of egg contents than in unsupplemented egg samples (Fig. 6). Salmonella Enteritidis was detected in 90% of the supplemented samples after 24-h incubation at 37°C. Extending the incubation time after 24 h did not increase the percentage of detection in egg samples supplemented with iron.

**DISCUSSION**

The growth of low numbers of Salmonella Enteritidis cells in eggs without iron supplementation was extremely variable. Salmonella Enteritidis counts in some egg samples reached 10⁶ CFU/ml after 24-h incubation, whereas counts were less than 5 CFU/ml in other samples after 106-h incubation. Iron in egg contents is bound to ovotransferrin and as such would be unavailable for Salmonella Enteritidis. We speculate that initially the egg mixture was aerobic and that iron was in the Fe²⁺ state, which promoted the growth of Salmonella Enteritidis during the 106-h incubation period. However, when Salmonella Enteritidis grew to a higher level, it may have reduced the medium and converted Fe³⁺ to the Fe²⁺ state, which reacts with hydrogen peroxide via the Fenton reaction to form the extremely toxic hydroxyl radical (1). This hypothesis is supported by the finding that the addition of pyruvate (a hydrogen peroxide decomposer) to the egg contents supplemented with high levels of iron allowed the Salmonella Enteritidis to reach approximately 10⁹ CFU/ml during the 106-h incubation (data not shown). Preliminary experiments indicated that the inhibitory effect of high iron concentrations on the growth of Salmonella Enteritidis in the egg contents might also be due to the drop in pH caused by the interaction of egg contents with high FeSO₄ concentration and the growth of Salmonella Enteritidis during incubation at 37°C (data not shown). The inhibitory effect at high iron concentrations may be due to the combined effect of both hydroxyl radical formation and a decrease in pH during the growth of Salmonella Enteritidis in the egg contents. There was no significant effect of preenrichment on the growth of Salmonella Enteritidis in treatments with iron supplementation.

When egg contents were supplemented with 0.5 mg of FeSO₄ per g of egg contents, the three treatments (G, H, I) reached approximately 10⁶ CFU/ml after 24-h incubation at 37°C. Salmonella Enteritidis counts in egg contents supplemented with 0.5 mg of FeSO₄ per g of egg contents were even slightly higher than in TSBYE after 24-h incubation at 37°C (data not shown). These results clearly demonstrated that supplementation of egg contents with 0.5 mg of FeSO₄ per g of egg contents stimulates the rapid growth of Salmonella Enteritidis and that preenrichment with TSB or BPW may not be necessary.

The direct plating method only detected Salmonella Enteritidis in 63.3% of unsupplemented egg contents after

**FIGURE 6. Percentage of detection of Salmonella Enteritidis in raw egg contents by the direct plating method. Each of 40 egg samples and 20 XLD plates were inoculated with approximately 2 CFU of Salmonella Enteritidis. Half of the egg samples were supplemented with 0.5 mg of FeSO₄ per g of egg contents. All egg samples were incubated at 37°C for up to 120 h and cultured for Salmonella Enteritidis by direct plating onto XLD plates each day. Within each day, bars labeled with different letters are significantly different (P < 0.05). Pooled standard deviations for days 1 through 5 were 6.2, 6.9, 6.9, 5.5, and 5.5, respectively.**
120-h incubation at 37°C. However, Salmonella Enteritidis was detected in 90% of the supplemented samples after only 24-h incubation at 37°C.

Because of the low numbers of Salmonella Enteritidis (approximately 2 CFU per egg) used to inoculate egg contents, it was very possible that a small portion of egg samples did not receive any Salmonella Enteritidis cells. This was supported by the fact that 13.3% of the 20 XLD plates did not have any Salmonella Enteritidis colonies. Since there was no significant difference in percentage of detection between iron supplementation treatment and XLD plates ($P < 0.05$), it was concluded that iron supplementation gave 100% detection of Salmonella Enteritidis.

Gast and Holt (16) reported that direct plating demonstrated 100% detection when the number of Salmonella Enteritidis in eggs was greater than $2 \times 10^4$ CFU/ml (assuming volume of an egg content was 50 ml). Salmonella Enteritidis counts in eggs supplemented with 0.5 mg/g of FeSO$_4$ reached $10^8$ CFU/ml after only 24-h incubation at 37°C, far beyond this detection threshold. After 24-h incubation of iron-supplemented egg contents, typical Salmonella Enteritidis responses were observed on XLD plates after only 20-h incubation of XLD plates at 37°C; however, typical responses were only observed after 40 h for the unsupplemented eggs.

The percentage of detection results using the direct plating method in this study were comparable with the results obtained by Gast (13) and Gast and Holt (16). Gast (13) found that the direct plating method without iron supplementation detected Salmonella Enteritidis in 47.1% of egg samples when mixed raw eggs were inoculated with approximately 2 CFU of Salmonella Enteritidis cells and incubated at 25°C for 4 days, whereas preenrichment with TSB followed by selective enrichment detected 75% of the egg samples. Gast and Holt (16) found that the direct plating method detected Salmonella Enteritidis in 77% of egg samples supplemented with 6 mg/ml of FeSO$_4$ and 54% of unsupplemented egg samples when mixed raw eggs were inoculated with less than 10 CFU of Salmonella Enteritidis cells and incubated at 37°C for 24 h. Based on our results, direct enrichment of Salmonella Enteritidis in raw egg contents supplemented with an optimum concentration of iron (0.5 mg of FeSO$_4$ per g of egg contents) significantly enhances detection of Salmonella Enteritidis in eggs.

The direct plating method with iron supplementation is rapid and highly sensitive for detection of Salmonella Enteritidis in raw eggs. Because of the low cost of FeSO$_4$, this method is practical in large-scale detection of Salmonella Enteritidis in eggs. Moreover, the contents of clean and intact eggs usually contain pure cultures of Salmonella Enteritidis (21). Based on the results of this study, direct plating of egg contents supplemented with 0.2 to 2 mg of FeSO$_4$ per g of egg contents should be used in place of preenrichment and selective enrichment for consistent and rapid detection of low levels of Salmonella Enteritidis in eggs.

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