Performance of Several Enrichment Media in the Isolation of Salmonellae from Liquid Egg Products

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ABSTRACT

Ninety-seven samples of raw liquid whole egg and egg yolk were analyzed for the presence of Salmonella; 51 samples (52%) were found positive. A comparative study was conducted on the performance of seven selective enrichment procedures in the isolation of Salmonella from liquid egg products: selenite-cystine broth incubated at 37°C and 43°C, Muller-Kauffmann tetrathionate broth at 43°C, modified Rappaport medium RIO/10 and RIO/10 also incubated at 43°C, the experimental broth of Greenwood et al. incubated at 37° and 43°C. The best results were obtained with tetrathionate broth which detected 96% of all positive samples. Differences in the rate of isolation by the tetrathionate broth, selenite-cystine broth, modified Rappaport medium RIO/10 and RIO/100 incubated at 43°C, were not significant as determined by paired x² test.

The association of Salmonella with poultry and eggs has been recognized for many years (6). Liquid egg products (raw whole egg, raw egg yolk) are susceptible to contamination with Salmonella.

Many studies were conducted to evaluate different isolation techniques for the detection of Salmonella (7,10). Selenite and tetrathionate broths are the recommended selective enrichment media for Salmonella in most official procedures (2,3,4). Due to inconsistencies, a number of investigations have been carried out in search of a new and better selective enrichment. Vassiliadis et al. (17) reported that a modification of Rappaport's enrichment medium (RIO) was more efficient in the detection of Salmonella from samples of pork sausages, minced meat, sewage and feces of healthy pigs (16) than tetrathionate broth. In another study Vassiliadis et al. (17) compared two volumes of inoculum from the preenrichment culture into Rappaport's broth: 10 and 100 ml of RIO medium inoculated respectively with 0.1 and 1 ml of BPW preenrichment culture (RIO/10 and RIO/100). The latter yielded more positive samples from feces of healthy pigs. In the current investigation the performance of RIO/10 and RIO/100 was evaluated for samples of raw liquid egg products.

Greenwood et al. (9) developed an experimental broth which contained 8 mg of sodium sulfadiazine/L in addition to the selective ingredients magnesium chloride and sodium cholate. The broth proved to be superior to conventional selective enrichment media in the isolation of Salmonella from naturally contaminated mechanically deboned poultry meat. The experimental broth of Greenwood et al. is included in the present comparative study. Elevated temperature incubation improves the selectivity of enrichment media; 43°C is frequently proposed. However, ISO recommend 37°C for the selenite-cystine broth (3); 37°C was also the incubation temperature in the study of Greenwood et al. (9). We investigated the influence of the incubation temperature on performance of the selenite-cystine broth and the experimental broth of Greenwood et al.

MATERIALS AND METHODS

Samples

Ninety-seven samples of unpasteurized liquid whole egg and egg yolk were taken at liquid egg processing plants in Belgium. Sampling was preferentially performed from bulk containers.

Selective enrichment media

Selenite-cystine and Muller-Kauffmann tetrathionate broths were prepared according to ISO (3). The composition of Rappaport's modified medium (RIO) was described by Vassiliadis et al. (17). Experimental broth 2 from Greenwood et al. was prepared by the addition of 1 ml of a filter sterilized (8 mg/ml) solution of sodium sulfadiazine (Federa Brussels) to 1 L of experimental broth 1 (9). Experimental broth 1 was prepared by mixing 825 ml of solution A, 125 ml of solution B, and 50 ml of solution C at room temperature. Solution A contained Myosate peptone (BBL), 10 g; mannitol 1.0 g; sodium chloride 3.5 g; dibasic potassium phosphate 3.68 g; and monobasic potassium phosphate 1.32 g in 825 ml of distilled water. The solution was sterilized at 121°C for 15 min. Solution B was 20% magnesium chloride (MgCl₂·6H₂O) sterilized at 121°C for 15 min. Solution C was prepared by dissolving 10 g of sodium cholate (Sigma) in 50 ml of sterile distilled water. The final pH of the experimental broth 1 was 6.5 ± 0.1. All media were distributed in 100-ml volumes. RIO/10 broth was distributed in test tubes in 10-ml quantities.

Analysis

About 25 g of the egg product were added in jars containing 225 ml of buffered peptone water (8) and thoroughly mixed. The jars were incubated at 37°C for 18-24 h. Ten ml of the preenrichment medium was inoculated respectively in duplicate sets of 100 ml of selenite-cystine broth, Greenwood broth, and a single jar with 100 ml of Muller-Kauffmann tetrathionate broth. One-tenth and 1 ml of BPW preenrichment culture were
RESULTS AND DISCUSSION

The current study was limited to samples of raw liquid whole egg and egg yolk. Samples of egg white were omitted because of the inhibition avian egg white exerts on the growth of gram negative bacteria (5).

TABLE 1. The incidence of Salmonella serotypes isolated in raw liquid egg products.

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Positive after 24 h and 48 h</th>
<th>Positive after 24 h only</th>
<th>Positive after 48 h only</th>
<th>Total positive</th>
<th>% recovery of positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. enteritidis (9)</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>6</td>
<td>35.3</td>
</tr>
<tr>
<td>S. typhimurium (7)</td>
<td>11</td>
<td>2</td>
<td>3</td>
<td>16</td>
<td>94.1</td>
</tr>
<tr>
<td>S. mbandaka (7)</td>
<td>4</td>
<td>1</td>
<td>8</td>
<td>13</td>
<td>76.5</td>
</tr>
<tr>
<td>S. infantis (7)</td>
<td>6</td>
<td>16</td>
<td>13</td>
<td>35</td>
<td>52.9</td>
</tr>
<tr>
<td>S. agona (4)</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>S. virchow (3)</td>
<td>2</td>
<td>3</td>
<td>8</td>
<td>13</td>
<td>88.2</td>
</tr>
<tr>
<td>S. bareilly (3)</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>26</td>
</tr>
<tr>
<td>S. hadar (1)</td>
<td>16</td>
<td>1</td>
<td>1</td>
<td>18</td>
<td>64.7</td>
</tr>
<tr>
<td>S. menston (1)</td>
<td>16</td>
<td>1</td>
<td>2</td>
<td>19</td>
<td>79.4</td>
</tr>
<tr>
<td>S. brandenburg (1)</td>
<td>13</td>
<td>1</td>
<td>1</td>
<td>15</td>
<td>41.2</td>
</tr>
<tr>
<td>S. Newport (1)</td>
<td>13</td>
<td>1</td>
<td>1</td>
<td>15</td>
<td>88.2</td>
</tr>
<tr>
<td>S. senftenberg (1)</td>
<td>13</td>
<td>1</td>
<td>1</td>
<td>15</td>
<td>50.0</td>
</tr>
</tbody>
</table>

Unpasteurized liquid egg products seem to be frequently contaminated with *Salmonella*. About 50% of the samples were found positive for *Salmonella* 25 g by at least one selective enrichment broth. A total of 52 *Salmonella* strains belonging to 17 serotypes (Table 1) were isolated from the liquid egg samples.

In the first part of the study, 5 enrichment media were compared (Table 2): selenite-cystine broth at 37°C (SC-37), the experimental broth of Greenwood et al. at 37°C (Gr-37), Muller-Kauffmann tetrathionate broth at 43°C (MK-43), RIO/10 and RIO/100 broth incubated at 43°C. Differences in the rates of isolation by the MK-43, Gr-37 and RIO/100 media were not significant as determined by paired χ² test. The poor performance of SC-37 contrasts with earlier results on detection of *Salmonella* in eggs using SC broth (13). Identification of 8 of the 13 positive samples by the Gr-37 medium after 48 h of incubation is notable.

The comparative study was extended with selenite-cystine (SC-43) and the experimental broth of Greenwood (Gr-43), both incubated at 43°C. The sensitivity of SC-43 and Gr-43 compared favorably with that of the MK-43 medium, which identified the greatest number of samples (Table 3). Incubation of SC and Gr at 43°C markedly increased rates of isolation (Tables 2 and 3). These results are not inconsistent with earlier reports (7) on temperature-dependent isolation of *Salmonella* from selective enrichment broths.

Use of the experimental broth of Greenwood et al. on liquid egg products results in the formation of a white precipitation in the broth. MgCl₂·6H₂O (2.5% w/v) is included in the broth to enhance selectivity. It is possible that egg lipid compounds change the solubility of the magnesium salt. To what extent this phenomenon affects selectivity remains to be investigated.

 ISO (3) recommend use of selenite-cystine broth at 37°C. It is also stated in the guideline that in some instances another incubation temperature is favorable. The...
guideline doesn’t stipulate these instances. It results from our study that for raw liquid egg products 43°C is the preferred incubation temperature for the selenite-cystine broth. In many studies, the superiority of RIO/100 was highlighted. Vassiliadis et al. (16) reported on Salmonella isolations from pork sausages; the superiority of the RIO procedure over the Muller-Kauffmann tetrathionate broth was statistically highly significant. In the examination of 526 samples of minced meat, RIO was clearly more efficient than tetrathionate broth (18). A similar observation was made for the isolation of Salmonella from sewage (15), samples of feces of normal pigs (17). If data from Tables 2 and 3 are considered together, RIO/100 identified 41/51 (80%) positive samples; MK-43 identified 49/51 (96%) positive samples. This finding is inconsistent with literature data on the RIO/100 superiority over the Muller-Kauffmann tetrathionate broth.

RIO/100 proved to be more efficient than RIO/10. A similar result is reported by Vassiliadis et al. (17). It seems that at least 1 ml of cultured buffered peptone water is necessary. On the other hand, Rappaport media must not be inoculated with heavy inocula because they lose their selectivity (14).

None of the enrichment procedures was successful in isolating all the salmonellae identified by the 7 enrichment conditions combined. The closest was that of Muller-Kauffmann tetrathionate broth, which resulted in the isolation of Salmonella from 49 of 51 positive samples (96%). In the two instances where tetrathionate broth failed to give a positive result, Salmonella could be detected with only RIO/100 in the first instance, and with all the other procedures in the second instance. If a second enrichment broth is to be used for raw liquid egg products, which procedure should be preferred? The experimental broth of Greenwood et al. offers some disadvantages; the medium is not commercially available, the presence of non suspect colonies disturb the isolation of suspect colonies on XLD and Hektoen agar. According to the results from Table 3, and with regard to the disadvantages of the experimental broth of Greenwood et al., selenite-cystine broth at 43°C would be preferred as second enrichment medium. In any event, selective enrichment broths must be incubated at least for 2 d. Data from Tables 2 and 3 clearly indicate that some samples are defined positive only after 48 h of incubation.

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REFERENCES