INFLUENCE OF DIDECYL DIMETHYL AMMONIUM BROMIDE ON THE SURVIVAL OF SALMONELLA ON EGGSHELLS

MIECZYSŁAW RADKOWSKI AND JAN URADZIŃSKI

Department of Veterinary Public Health, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, 10-957 Olsztyn, Poland
rad@uwm.edu.pl

Received for publication September 9, 2009

Abstract

The aim of the research was to determine the influence of selected concentrations of didecyl dimethyl ammonium bromide (DDAB) on the survival rate of Salmonella strains on eggshells. 1,260 chicken eggs on the day they were laid were used. Salmonella enterica serotypes Enteritidis, Typhimurium, and Agona were inoculated into 9 ml nutrient broth and incubated for 24 h at 37°C. Then 10-fold dilutions of the culture were made and 0.1 ml of each dilution was inoculated on the shell of each egg. Then, each egg was immersed in 200 ml of 0.025%, 0.05%, or 1.0% water solution of DDAB. The eggs were kept for 2 min in the 0.05% and 1% solution and for 2, 5, and 10 min in the 0.025% solution. The results proved that the detection of Salmonella depends on the inoculum of those bacteria on the egg surface. At the contamination rate of 10⁵ cells on the surface of the egg and immersing it for 2 min in 0.025% DDAB solution, there were no Salmonella found. At the contamination rate of 10⁶ CFU of Salmonella and immersing for 5 min in 0.025% DDAB solution, no Salmonella organisms were found. Previous research by the authors has indicated that it is possible to kill Salmonella on the surface of eggs by immersing them in 0.025% DDAB.

Key words: eggs, Salmonella, decontamination, didecyl dimethyl ammonium bromide.

The results of studies conducted around Poland by the Sanitary and Epidemiological Stations of cases of food poisoning caused by Salmonella show that the largest number of focal points of the disease are associated with the consumption of unprocessed chicken eggs, i.e. products such as mayonnaise, creams made from eggs, ice-creams, etc. (5, 8). Similar reasons for food poisoning have been found, even in countries with a high level of hygiene of food production and distribution, such as the USA, the Netherlands, Canada, Germany (2, 4, 6, 12). The EU veterinary service has issued regulations ordering the eradication of Salmonella infections transmitted by food (22).

Only a small portion of eggs for consumption are contaminated with Salmonella and they are usually contaminated on their shell surface. In the USA, one in 10,000 eggs is infected with Salmonella Enteritidis and in Great Britain one in 15,000 (2, 10, 13, 16). In addition, naturally and artificially infected egg contents are recorded very rarely (19, 21). Recently, a gradual decrease in the number of infections of eggs with Salmonella has been observed. Despite the low level of shell contamination (0.3% to 0.4%), an assessment based on the total amount of 1 million tonnes of eggs produces a figure of about 60-70 million contaminated eggs, which still make it to consumers in Germany (15).

Eggshells contaminated with Salmonella are often responsible for food poisoning (7, 4, 9, 14, 25). Eggshells are usually associated with salmonellosis if they had not been properly refrigerated, pooled, or then consumed raw or undercooked. This is mainly caused by the fact that Salmonella carriers actually show no symptoms of being infected - the veterinary services are, in fact, unable to prevent marketing authorisation for eggs with shells contaminated with Salmonella (20). In commercial egg processing, where large numbers of eggs are used, one egg is sometimes enough to contaminate the whole product. Disinfection procedures must then be applied (20).

One of the possible solutions might be the use of an appropriate disinfectant. Effective and safe agents for egg disinfection have been sought around the world for many years. The number of chemical agents used to disinfect eggs is limited as a result of their adverse effects on the human body and because they are difficult to dissolve and apply directly. According to current trends, quaternary ammonium bases, commonly regarded as safe, are used for egg disinfection. Among many commercially-available disinfectants, didecyl dimethyl ammonium bromide (DDAB) can largely meet the requirements. Its use in the food production industry was authorised by a Announcement of the Minister of Agriculture and Rural Development of Poland on 30 June 2000 listing the pharmaceuticals and medicinal materials, whose use is authorised in animals in domestic trade in disinfecting eggs for reproduction and sanitising drinking water (3). DDAB is not allowed in the EU - and also not in Poland – for the
decontamination of eggshells. Hence, the use of a solution of DDAB for surface disinfection of eggs for consumption seems justified (3). The available literature does not contain any information about the effect of DDAB on the quantitative contamination of eggshells with *Salmonella*.

In recent years, a continuous increase in salmonellosis cases in man has been noted in Poland: most of them were caused by three serotypes of *Salmonella enterica*: Enteritidis, Agona, and Typhimurium (5). Therefore, this study aimed at finding the effect of selected concentrations of DDAB on the survivability of the above-mentioned serotypes on eggshells.

**Material and Methods**

One thousand, two hundred and sixty chicken eggs, obtained from six laying flocks from 5,000 to 200,000 layers on the day they were laid, were used. The flocks have had been surveyed serologically by the authors and found to be free of *S. Enteritidis* (Flodkscreen Salmonella Enteritidis Antibody Elisa Kit No. V010, UK).

The following serotypes of *Salmonella enterica* were used: Enteritidis No. 14/93, Typhimurium No. 227/84, and Agona No. 1408. The strains of *S. Enteritidis* and *S. Typhimurium* were obtained from the collection of the National Veterinary Research Institute in Pulawy, while the strain *S. Agona* was obtained from the National Centre of *Salmonella* in Gdynia. The strains were inoculated in 9 ml nutrient broth and incubated for 24 h at 37°C. Ten-fold dilutions of the culture were prepared and 0.1 ml each dilution was inoculated on shell of each egg. The initial inoculum for the control samples was determined for each series. The bacterial suspensions were spread with a special wide loop on the biggest possible egg surface and the eggs were then left at room temperature for 2 h, until the suspension had dried completely. Each contaminated egg was subsequently immersed in 200 ml of 0.025%, 0.05%, or 1.0% water solution of DDAB (ABIC Ltd, Israel). The eggs were kept in the solution for 2 min in 0.05% and 1.0% solutions and for 2, 5, and 10 min in 0.025% solution. The temperature of the solutions was 25°C, pH 7.8-8.0. After the appropriate time, each egg was immersed in sterile distilled water in order to remove DDAB on the quantitative contamination of eggshells. Hence, the use of a solution of DDAB for surface disinfection of eggs for consumption seems justified (3). The available literature does not contain any information about the effect of DDAB on the quantitative contamination of eggshells with *Salmonella*.

The results obtained are given in Table 1. The initial inoculum, determined on the agar medium, was the following: *S. Enteritidis* - 4.6 x 10⁵ CFU/mL, *S. Typhimurium* - 2.4 x 10⁶ CFU/mL, and *S. Agona* - 5.5 x 10⁵ CFU/mL. The analysis of the results obtained from eggs immersed in the DDAB solution showed that the *Salmonella* identification rate was determined by the bacteria inoculum spread over the eggs' surface. The data shown in the Table indicate that along with the increase in the time of immersion in DDAB, the number of eggs on which *Salmonella* was found decreased. However, not all *Salmonella* serotypes survived in the same way. *S. Enteritidis* was inactivated the earliest. *S. Enteritidis* and *S. Agona* were not found on the egg surface when immersing for 2 min in 0.025% solution of DDAB with a contamination of 10⁵ CFU.
When eggs were immersed for 5 and 10 min in 0.025% solution of DDAB, the effect was much stronger. The immersion of eggs contaminated with 10^6 CFU of Salmonella sp. in 0.025% solution of DDAB for 5 min completely eliminated S. Enteritidis and S. Agona.

It should be added here that investigations carried out on eggs artificially contaminated with Salmonella and immersed in DDAB never revealed the penetration of these bacteria through the shell.

### Discussion

Diverse results are reported in the studies involving the disinfection of eggshells. Aksu et al. (1) examined the effectiveness of various disinfectants on Salmonella Enteritidis inoculated on eggshells. The contaminated eggs were treated with two disinfectant solutions: benzalkonium chloride and benzalkonium chloride/gluteraldehyde combination, for 5 and 15 min. These authors indicated that treatment with these compounds for 15 min is sufficient to eliminate the S. Enteritidis found on the eggshells, and that eggs contaminated with S. Enteritidis and immersed in DDAB never revealed the penetration of these bacteria through the shell.

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time of action (min)</th>
<th>Dilution / inoculum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E^a T^b A^c E T A E T A E T A E T A E T A</td>
</tr>
<tr>
<td>Unwashed (control 0.0)</td>
<td>2-10^a</td>
<td>10 10 10 10 10 10 10 10 10 10 10 10 10</td>
</tr>
<tr>
<td>Water wash (control 0.0)</td>
<td>2-10^a</td>
<td>10 10 10 10 10 10 10 10 10 10 10 10 10</td>
</tr>
<tr>
<td>0.025%</td>
<td>2</td>
<td>4 8 8 0 2 0 0 2 0 0 2 0 0 2 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>0.5%</td>
<td>2</td>
<td>0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>1.0%</td>
<td>2</td>
<td>0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
</tr>
</tbody>
</table>

^a Salmonella Enteritidis  
^b Salmonella Typhimurium  
^c Salmonella Agona

The eggshell surface is frequently contaminated with Salmonella in the cloaca or after egg laying in the henhouse, or in transport or storage. Disinfection is therefore necessary, applied commonly to eggs for reproduction (24). The most commonly used methods in hatching centres include washing eggs in water at 20-25°C, washing in solutions of various chemical agents, treating with formaldehyde, and UV radiation. However, eggs for consumption are not washed or disinfected, which may result in their microbiological contamination, with resultant food poisoning in humans. Washing eggs greatly reduces the risk of infecting the contents while breaking them. Washing has been found to reduce the risk of infecting the egg contents from several to several dozen. In view of this, all eggs are washed in modern egg-processing plants. The production of eggs and marketed egg products is defined in the Regulation of the Minister of Agriculture and Rural Development of Poland of 12 March 2004 (23).

Eggs can be disinfected in physical or chemical processes. Among the physical methods the most commonly used are thermal methods, or radiation, which are effective, but which cannot be used everywhere. They are energy- and time-consuming. For this reason, eggs are disinfected by chemical methods.

Treatments applied in food production are thought to ensure sufficient safety to the consumer if they reduce the number of Salmonella by 6 log (26). In view of this, the effects of disinfecting eggs observed after immersing them for 10 min in a 0.025% solution of DDAB can be regarded as satisfactory. The compound inhibiting the multiplication of Salmonella can be used to reduce the infection of embryos and chicks with Salmonella sp., but also with other bacteria, fungi, viruses, mycoplasma, and protozoa. It has neither toxic nor irritant properties (3). The use of DDAB in the disinfection of eggs for reproduction seems fully justified. However, it is debatable whether it should be used in the disinfection of eggs for processing or direct consumption.
References


2. Anonymous. Salmonella annual summary 2002. US. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Infectious Diseases, Division of Bacterial and Mycotic Disease, Foodborne and Diarrheal Disease Branch, Atlanta, 2002.


23. Regulation of the Minister of Agriculture and Rural Development of 12 March 2004 on the requirements concerning the production of eggs and marketed egg products. O J 2004 No. 52, Item 521.

24. Regulation of the Minister of Agriculture and Rural Development of 12 March 2004 on the detailed veterinary requirements concerning the poultry and hatched eggs. O J 2004 No. 219, Item 2225.


26. Ziółowski C., Skwarek P., Pawiak R.: Microbiological methods used for investigation of chemicals disinfectants applied for veterinary purposes. Edited by the National Veterinary Research Institute, Pulawy, Poland, 1989.