

Disinfection of eggshells contaminated with *Salmonella enteritidis*

HARUN AKSU, KAMİL BOSTAN, ALİ AYDIN, MURAT YILDIRIM*, OYA KELEŞ*

Department of Food Hygiene and Technology, *Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Istanbul University, 34320, Avcilar, Istanbul, Turkey

Aksu H., Bostan K., Aydın A., Yıldırım M., Keleş O.

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Summary

The aim of the study was to investigate the effectiveness of various disinfectants on *Salmonella enteritidis* inoculated on eggshells. The contaminated eggs were treated with two different disinfectant solutions (benzalkonium chloride (BAG) and benzalkonium chloride / glutaraldehyde combination (BAC/G)) for 5 and 15 minutes. Following the treatment and storage at room temperature, the shells and their content were examined for *S. enteritidis* on days 7, 14, and 21. The results indicate that *S. enteritidis* may remain viable on the shells of non-disinfected eggs for a long period of time, and may penetrate into the edible portions of the egg during this period. Treating the eggs with a combination of benzalkonium chloride / glutaraldehyde for 15 minutes may probably safely eliminate the danger of *S. enteritidis* contamination.

Keywords: egg, *Salmonella enteritidis*, disinfection

Salmonella spp., which belong to the family of *Enterobacteriaceae*, play an important role in food poisoning, and are commonly found in food of animal origin. Eggs are considered to be one of the most popular food for *Salmonella* contamination. Analysis on eggs frequently reveal the presence of *S. enteritidis* (16, 20, 26, 27, 29). In most of the *S. enteritidis* infections of food-origin seen in humans, eggs and egg-containing foods are found to be responsible (1, 2, 25, 28).

The most important route of contamination of the eggshells with *Salmonella* or other pathogenic agents is the fecal route (16). Immediately after the eggs are laid, or at later stages, there is the chance that the eggs become contaminated with fecal material. The microorganisms found in the fecal material may penetrate the shell and the membrane due to the vacuum-effect resulting from the loss of heat after the lay, or as a result of unfavorable storage conditions and time, and may reach the egg's contents (7, 12, 19, 23, 26, 30). This may lead to serious health risks when these eggs are consumed raw or undercooked, or when food products containing these eggs are ingested. As the transfer of the microorganisms from the shell into the inner portions of the egg results in the death of the embryo, it may also lead to important problems for chick producers.

Various methods are used to remove and/or prevent the growth of both *Salmonella enteritidis* as well as other pathogenic agents on the eggshells (8, 13, 14, 17, 18, 22, 24, 30). Among these methods, antimicrobial agents added to the cleaning waters are commonly used. Some of the chemicals, however, may damage the shells and cuticula, and thus, may sensitize the eggs for subsequent recontamination (14, 21).

Generally, benzalkonium chloride (BAC) has surfactant properties, and displays its bactericidal effect by coagulating the proteins, especially the enzymes, of the bacterial cell. Because of its good diffusion capacity, it may be used to eliminate *Salmonellas* from pored surfaces and eggshells. Their lack of toxicity and their effectiveness at low concentrations provide an important advantage. Davison et al. (11) have investigated the effect of various disinfectants on *S. enteritidis*, and suggested the use of quaternary ammonium compounds (QAC), either alone or in combination with other disinfectants in coops. Glutaraldehyde (G), on the other hand, is a potent broad-spectrum disinfectant, which preserves its effectiveness in the presence of organic substances (6, 11). Different concentrations are recommended for the use of several commercial products containing benzalkonium chloride (BAC) and glutaraldehyde (G) combinations with potent biocidal activity, which are sold for the disinfection of eggshells.

This study aims to investigate the effectiveness of benzalkonium chloride (BAC), and benzalkonium chloride / glutaraldehyde (BAC/G) combination on the viability and number of *S. enteritidis* found on eggshells, when applied for different periods of time.

Material and methods

Eggs. The study was repeated three times, and with 110 eggs for each test, a total of 330 *Salmonella*-free eggs were used. The eggs were obtained from a special farm on a daily basis. Ten eggs were randomly chosen among the eggs used in the tests, and analyzed for the presence of *Salmonella* spp.

Test organism. A *Salmonella enteritidis* (ATCC 4931) strain obtained from the Microbiology Department of the Cerrahpaşa Medical Faculty, Istanbul University was used as test organism.

Experimental contamination. From the previously enriched *S. enteritidis* culture, inoculation was made to the Brain Heart Infusion Broth (Oxoid®) prepared in a 10 L container, and incubated overnight at 37°C to reach sufficient cellular density (10^8 cfu/ml). The eggs (100 for each test) were kept at 37°C for one night, and dipped for 5 s in an *S. enteritidis* culture with the same temperature. The average number of *S. enteritidis* in the culture sample collected during dipping was 5.9×10^8 cfu/ml. The contaminated eggs were drained at 37°C for 3 hours, and then, stored at ambient (approximately 20°C) temperature.

Disinfection of the eggs. For the disinfection, benzalkonium chloride (Sigma®), and glutaraldehyde (Sigma®) were used.

After 20 of the experimentally contaminated eggs were separated as controls, the remaining eggs were divided into 4 groups with 20 eggs each, and placed into BAC (250 ppm) and BAC/G (250 ppm + 400 ppm) solutions prepared with 5 liters of distilled water at room temperature, for 5, and 15 minutes. After the treatment, the eggs were placed into pre-sterilized egg cartons and stored at ambient temperature for 21 days.

Microbiological analysis. The contaminated eggs, including the control group, were analysed for the presence of *S. enteritidis* immediately after treatment and on days 7, 14, and 21 of the storage period. For analysis, five eggs from each group were used. The tests were carried out on both the shells (together with the membrane) as well as the edible portions (yolk + albumen). A small hole was then made with sterilized implements and the egg content transferred to a sterile sampling bag with a sterile pipette. The remaining eggshell and the membrane were transferred to a separate sampling bag. The samples in each group were thoroughly mixed by crashing, and 10 g samples were homogenized 1:10 (w/v) with sterile physiological saline to give a 1 : 10 suspension in another sampling bag and serially diluted up to 10^{-8} . The dilutions were inoculated on Brilliant Green Agar (Oxoid®) containing 20 mg/l novobiocin (Sigma®) using the inoculation loop, and the plates were incubated at 37°C. To determine the organism in uncountable level, the same sample were cultivated on Lactose Broth (Oxoid®) and Tetrathionate Broth (Oxoid®) and then plated on Brilliant Green Agar (Oxoid®) containing 20 mg/l novobiocin (Sigma®). Following incubation, the typical colonies were identified using biochemical and serological tests (3, 15).

Results and discussion

The numbers of *S. enteritidis* on the disinfectant-treated and untreated eggs immediately after the treatment and on different storage days are shown in tab. 1. The number of *Salmonella* on the shells and membranes of the eggs in the control group, which were dipped into the *S. enteritidis* culture but not treated with disinfectants, was 6.4×10^4 cfu/g. This number was decreased to 2.0×10^2 cfu/g on the day 21 of the storage period. With regard to elimination of *S. enteritidis* on the eggshells, the treatment with

BAC/G combination for 15 minutes was the most effective, and resulted in complete reduction. The least effective treatment, on the other hand, was obtained following disinfection with BAC for 5 minutes, and the *S. enteritidis* on the eggshells in this group remained viable for the entire storage period. Nevertheless, no *Salmonella* was detected in the contents of the eggs in the disinfection groups, in contrast to the control group.

Benzalkonium chloride alone failed to eliminate the *Salmonella* on the eggshells; and better results were obtained when it was used in combination with glutaraldehyde. Similarly, increasing the treatment duration from 5 to 15 minutes also increased the reduction rate. The best result among the test groups was obtained from the treatment with benzalkonium chloride/glutaraldehyde combination for 15 minutes where complete reduction was achieved.

Different results are reported in the studies involving the disinfection of eggs. The disinfectants used by Himathongkham et al. (17) failed to completely eliminate *S. enteritidis* from the eggshells. The number of *S. enteritidis* on the eggshells was 5.43 log cfu after the contamination, and could be reduced to only 1.4 log cfu after the treatment with 250 ppm QAC (Quaternary Ammonium Compounds) for 5 minutes. Other disinfectants (0.5% chlorhexidine, 10% lugol/ethanol mixture) resulted in much lower reduction. Wang and Slavik (30) found that, in eggs washed with water containing QAC (100 ppm), the rate of penetration of superficially contaminated *S. enteritidis* into the eggs is reduced. In the same study, examination of the surface of the eggshell using an electron microscope revealed that BAC formed a permanent film layer on the surface of the eggshell, but didn't damage its surface. They also reported, however, that problems involving residues might arise. Nevertheless, the Turkish Food Codex regulation, and the European Drug Assessment Agency, classifies benzalkonium chloride and glutaraldehyde as substances whose residues on food of animal origin don't constitute a threat for human health (4, 5).

Catalano and Knabel (9) studied the effect of the pH and temperature of the egg washing solution on *S. enteritidis*.

Tab. 1. The number of *Salmonella enteritidis* after the treatment with disinfectants and during the storage period

Part of eggs	Group	After the treatment		Storage days					
		Growth	cfu/g	7		14		21	
				Growth	cfu/g	Growth	cfu/g	Growth	cfu/g
Egg shell and membran	Control	+	6.4×10^4	+	8.6×10^3	+	4.0×10^2	+	2.0×10^2
	BAC-5	+	3.2×10^4	+	1.2×10^3	+	< 10	+	< 10
	BAC-15	+	< 10	-	< 10	-	< 10	-	< 10
	BAC/G-5	+	< 10	-	< 10	-	< 10	-	< 10
	BAC/G-15	-	< 10	-	< 10	-	< 10	-	< 10
Egg yolk and egg white	Control	+	< 10	+	< 10	+	< 10	+	< 10
	BAC-5	-	< 10	-	< 10	-	< 10	-	< 10
	BAC-15	-	< 10	-	< 10	-	< 10	-	< 10
	BAC /G-5	-	< 10	-	< 10	-	< 10	-	< 10
	BAC/G-15	-	< 10	-	< 10	-	< 10	-	< 10

Explanations: + growth was detected on the inoculates from the enrichment media; - no growth was detected on the inoculates from the enrichment media

They found that a high pH (> 11) and a high temperature (> 37.7°C) significantly reduced the number of viable *S. enteritidis* in the egg-washing-solution, and suggested that these conditions might help to reduce the incidence of *Salmonella* on eggshells. The same researchers found similar results in another study (8); they concluded that in addition to the pH and temperature of the washing solution, rapid cooling of the washed eggs to under 7°C prevents the penetration of *S. enteritidis* into the eggs. In these studies, the incidence of *Salmonella* spp. on the eggshells treated with a washing solution with pH adjusted to 11 still was 18.3%, and moreover, transfer from the shells into the content was detected when the eggs were cooled slowly. In our study, on the other hand, the number of *S. enteritidis* on the shells were decreased to below 10 cfu/g in all eggs except the ones treated with benzalkonium chloride for 5 minutes, and no penetration into the egg contents was detected without any cooling process. Lack of penetration into the egg contents might be due to the much lower number of bacteria on the eggshells compared to the aforementioned studies.

Although the number of *S. enteritidis* on the eggshells that were contaminated, but not disinfected, gradually decreased in our study, they remained viable until the end of the 21-day storage period. Similarly, although the *S. enteritidis* level was uncountable level from the first day, the organism was able to isolate from the egg content by enrichment procedure. This indicates that the storage at room temperature might constitute a health threat, when the eggs are subject to fecal contamination. Schoeni et al. (26) also reported that when eggs are stored at 25°C, *S. enteritidis* is able to penetrate the eggshell and the membrane, can be found in the egg contents, even though in low numbers. In a study by Wang and Slavik (30), *Salmonella* penetration from the eggshell to the contents was found after storage at both 4°C, as well as 23°C. Himathongkham et al. (17), on the other hand, reported that during the storage period of 4 weeks, the number of *S. enteritidis* in the eggshell (including the membrane) was gradually decreased, but in contrast to our findings, no penetration to the egg contents took place.

When *S. enteritidis* penetrates the eggshell and the membrane and reaches the egg contents, it may survive, and if the environmental conditions are favorable, may even reach high numbers. Schoeni et al. (26) reported that the number of *S. enteritidis* directly inoculated to the yolk and albumen of the egg partially increases when stored at 4 and 10°C, and increases to 10⁸-10¹⁰ from 10²-10⁴ when stored at 25°C for 5-7 days. In our study, the *S. enteritidis* penetrating to the egg contents in the control group was able to survive, but did not increase to such high numbers. This difference might be due to the high inoculation level in the above mentioned study. In experimentally contaminated egg albumin (two cells per egg), Cogan et al. (10) did not observed growth in 97% of the eggs stored at 20°C for 8 days. However, they reported that the number of *Salmonella* increased when the amount of inoculums per egg was higher than 25 cells.

In conclusion, our findings indicate that treatment with benzalkonium chloride and glutaraldehyde combination for 15 minutes is sufficient to eliminate the *S. enteritidis* found on the eggshells, and that eggs contaminated with *S. enteritidis*, which are not treated with any disinfectant might constitute a health risk for the consumers.

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Author's address: Prof. Dr. Harun Aksu, Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Istanbul University, Avcilar, 34320, Istanbul, Turkey; e-mail: h.aksu@istanbul.edu.tr