

## A RESEARCH OF *SALMONELLA* SPP. IN EGG AND EGG PRODUCTS AND SURVIVAL OF *SALMONELLA* IN DIFFERENT TEMPERATURES

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### Abstract

*In this study, occurrence of Salmonella in egg and egg products and lifetime of Salmonella in refrigerator and boiling temperatures were studied. A total of 555 samples consisted of 250 chicken eggs, 180 quail eggs, 100 mayonnaise and 25 icing were collected from Ankara Region. The detection of Salmonella was investigated by the guidelines of the method recommended by International Standards Organization. As a result, Salmonella have not been isolated from quail eggs, mayonnaise and icing samples. However, Salmonella have been isolated and identified in 15 samples (6%) of 250 chicken eggs. These samples are a potential hazard for public health. Eggs were inoculated with Salmonella Enteritidis and cooked by recommended procedures for boiling. Boiling for 8 minutes was necessary for complete destruction of S. Enteritidis. After keeping the eggs in the refrigerator temperature up to 21 days no destruction of S. Enteritidis was observed.*

**Key words:** Egg, Egg product, Salmonella spp.

### Yumurta ve Yumurta Ürünlerinde *Salmonella* spp. Varlığı ve Farklı Sıcaklık Derecelerinde Yaşam Süresinin Araştırılması

*Bu çalışmada Salmonella'nın yumurta ve yumurta ürünlerinde varlığı ile buzdolabı ve kaynama sıcaklığında yaşam süresi araştırılmıştır. Araştırmada, Ankara piyasasından sağlanan 250 adet tavuk yumurtası, 180 adet bıldırcın yumurtası, 100 adet mayonez ve 25 adet krema örneği olmak üzere toplam 555 adet yumurta ve yumurta ürünü örneğinde çalışılmıştır. Örneklerde Salmonella'nın saptanmasında Uluslararası Standartlar Organizasyonu'nun (ISO) yöntemi kullanılmıştır. Sonuç olarak; bıldırcın yumurtalarında, mayonez örneklerinde ve kremalarda Salmonella izole edilememiştir fakat 250 tavuk yumurtasının 15'inde (%6) izole ve tanımlanmıştır. Bu örneklerin halk sağlığı açısından potansiyel zarar oluşturabileceği düşünülmektedir. Salmonella Enteritidis ile inokule edilen yumurtalar önerilen kaynatma prosedürlerine uygun olarak pişirilmiştir. S. Enteritidis'i tamamen yok etmek için sekiz dakika süre ile kaynatma işleminin gerekli olduğu saptanmıştır. Buzdolabı sıcaklığında 21 güne kadar bekletildikten sonra ise S. Enteritidis'in yaşamını devam ettirdiği gözlenmiştir.*

**Anahtar kelimeler:** Yumurta, Yumurta ürünleri, Salmonella spp.

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## INTRODUCTION

*Salmonella* is a facultative anaerobic, Gram-negative bacillus and a member of Enterobacteriaceae family. The members of *Salmonella* group was classified from A-Z like A, B, C1, C2, C3, C4, D1, D2, D3, E1, E2, E3, F, G1, G2. Except *Salmonella* Gallinarum and *S. Pullorum* all members of the genus are mobile. They have somatic (O), peripheral (Vi, M, Fimbria) and ciliar (H) antigens. Somatic O antigen is present in all *Salmonella*. Ciliar H antigen is present only in mobile *Salmonella*. Vi, M and Fimbria antigens are present only in some *Salmonella* types.

There are 2463 serotypes of *Salmonella*. The antigenic formulae of *Salmonella* serotypes are defined and maintained by the World Health Organization (WHO) Collaborating Center for Reference and Research on Salmonella at Pasteur Institute and new serotypes are listed in annual updates of the Kauffmann-White Scheme (1).

Infections caused by *Salmonella* spp. are among the most common zoonotic diseases worldwide, with *S. Enteritidis* as the dominating serovar in poultry flocks. Eggs and egg products still represent the main entrance of *Salmonella* spp. into the food chain (2). The food products most commonly identified as vehicles for transmission of *Salmonella* include raw eggs, under processed egg products, cheese, mayonnaise, baby food, meringue and ice cream (3).

*Salmonella enterica* serotype Enteritidis (*S. Enteritidis*) is the most common serotype associated with food borne *Salmonella* infection worldwide (4). Enterocolitis is the most common manifestation of *Salmonella* infection and *S. Typhimurium* and *S. Enteritidis* are predominant in this infection. After ingestion of *Salmonella* there is nausea, headache, vomiting and profuse diarrhea. Inflammatory lesions of the small and large intestine are present (5).

Eggs may be a source of infecting people with *Salmonella* spp. and thus a cause of food poisoning (6). Salmonellosis is an important public health problem (7-10). According to Turkish Food Codex in 25g of egg and egg products *Salmonella* should not be isolated (11).

Egg is a very rich food source for all organisms and microorganisms also. Eggs that are supplied from healthy chickens are sterile but then they are contaminated with the feces, water and hands (12, 13, 14). Egg contamination by *S. Enteritidis* can be caused by penetration through the eggshell from contaminated feces after or during oviposition, by direct contamination of yolk, albumen, eggshell membranes/eggshells before oviposition originating from the infection of reproductive organs with *S. Enteritidis* or during transporting and storing (6, 15).

Transmission to hens may originate from contaminated food or water or by contact with wild animals. But the main concern with this bacterium is the existence of silent carriers, i.e. animals harbouring *S. Enteritidis* without expressing any visible symptoms. These animals can, in turn, transmit the bacterium to their flock-mates through horizontal transmission or to their offspring by vertical transmission (16).

Three types of boiled eggs are generally distinguished: soft-, medium- and hard-boiled. The risk of microbial contamination is minimal for hard-boiled eggs, but medium- and certainly soft-boiled eggs are a potential risk factor for Salmonellosis (17). Many outbreaks have resulted from the misuse or mishandling of eggs (18). In our country eggs are stored in refrigerators or cold storages while in some other countries besides cold storage, drying or freezing is also used (14, 19).

Our aim was to identify and detect *Salmonella* in egg and egg products in Ankara Region and lifetime of *Salmonella* in refrigerator and boiling temperatures.

## EXPERIMENTAL

### *Samples Collection*

The samples consisted of 250 chicken eggs, 180 quail eggs, 100 mayonnaise and 25 icings, which were collected from local markets and pastry shops and different districts of Ankara, respectively were used in this study. The detection of *Salmonella* in egg and egg products were investigated by the guidelines of the method recommended by International Standards Organization (20).

Mayonnaise and icings were collected in a sterile cup and transferred to laboratory in cold chain. Eggs and egg products were kept at +4°C.

### *Analyses of Samples*

Investigation of penetration of *Salmonella* spp. to eggshells of eggs was held by taking samples from the eggshells by sterile swabs. Swab materials were inoculated into Selenite Cystine Broth (SC) (Merck) and incubated at 37°C for 24-27 hours. After incubation a loopfull of the cultures were transferred to Salmonella-Shigella Agar (SS Agar) (Merck) and Xylose Lysine Deoxycholate agar (XLD Agar)(Merck) and incubated at 37°C for 24-27 hours. After incubation they were investigated in case of *Salmonella* spp. occurrence.

After taking the swab samples, the eggshells were disinfected by iodize tincture and broken into sterile cups. 25 g of these egg samples, mayonnaise samples and icing samples were then weighed under sterile conditions and transferred into 225 ml buffered peptone water. After incubation at 37°C for 18 ± 2 hours, a volume of 0.1 ml of the preenriched samples were used to inoculate 10 ml of the Rappaport-Vassiliadis medium (RV)(Merck) and 10 ml of the Selenite-Cystine medium (SC) and they were incubated at 42°C and 37°C for 24 ± 3 hours respectively. After incubation a loopfull of the cultures were transferred to Salmonella-Shigella Agar (SS Agar) and Xylose Lysine Deoxycholate agar (XLD Agar) and incubated at 37°C for 24-27 hours. The typical and atypical colonies that were isolated from the samples were passaged to McConkey Agar and the pure colonies that were isolated from McConkey Agar plates were used in biochemical tests. Conventional biochemical tests that are mentioned in International Standards Organization (20) were applied to the tested samples.

After biochemical analyses the bacteria that were identified as *Salmonella* spp. were serotyped with polyvalent *Salmonella* antisera in Refik Saydam National Hygiene Center, The Ministry of Health. Antisera were supplied from Refik Saydam Hifzıssıhha Institute, Antigen - Antisera Laboratory.

*S. Enteritidis* RSKK 91 and *S. Typhi* RSKK 96031 standard strains were used as positive controls. McFarland 0.5 density of the overnight cultures of these standard strains were prepared and 10µl of these cultures were applied to egg, mayonnaise and icing samples and every step of the study were applied to these samples for positive control.

For the determination of survival of *Salmonella* in refrigerator and boiling temperatures, eggs were inoculated with 0.1 ml of a *S. Enteritidis* culture. Before injection, eggs were disinfected by iodize tincture and after injection the injection hole was closed with glue to discard contamination. Culture suspension was prepared from an overnight standard *S. Enteritidis* culture with McFarland 0.5 turbidity. After injection, three eggs for each group were held and for boiling temperature, the eggs were added directly to boiling water for no more than 10 minutes. After boiling 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 minutes the eggs were broken and for the isolation of *Salmonella* the method described above was performed. For determining the survival in refrigerating temperature eggs were kept in the refrigerator up to 21 days. Also the

eggs in the refrigerator were broken everyday up to 21 days and the examination above was repeated.

## RESULTS AND DISCUSSION

As a result, *Salmonella* spp. has not been isolated from eggshells, quail eggs, mayonnaise samples and icings, which were collected from Ankara Region. However, *Salmonella* have been isolated and identified in 15 samples (6 %) of 250 chicken eggs. The isolated *Salmonella* were serotyped with polyvalent *Salmonella* antisera in Refik Saydam Hygiene Center and the isolated 15 *Salmonella* were serotyped as *S. Enteritidis* 9, 12:g, m. *Salmonella* were isolated from the eggs of A (3 eggs) and B (12 eggs) firms.

**Table 1.** Percent of *S. Enteritidis* in the examined samples

<i>Samples</i>	<i>Number of Samples</i>	<i>Number of Samples Isolated S. Enteritidis</i>	<i>Percent of S. Enteritidis %</i>
<i>Chicken egg samples from local markets and open markets</i>	250	15	15 (6%)
<i>Quail egg samples from local markets</i>	180	No colonies observed	0 %
<i>Mayonnaise from local markets and sideboards</i>	100	No colonies observed	0 %
<i>Icing from pastry-shops</i>	25	No colonies observed	0 %
<b>Total</b>	<b>555</b>	<b>15</b>	<b>% 2.7</b>

We have inoculated eggs with *S. Enteritidis* and eggs were cooked by recommended procedures for boiling. Boiling for 8 min was necessary for complete destruction of *S. Enteritidis*. After keeping the eggs in the refrigerator temperature up to 21 days no destruction of *S. Enteritidis* was observed.

Burow has investigated 1443 egg samples and 0.42% of them had *Salmonella* contaminated shells (21). Lodetti and Zavanella have studied with 105 shell egg samples and they have determined that 1 sample (0.95%) was contaminated with *Salmonella* spp. (22). In our study *Salmonella* were not isolated from eggshells but 15 *S. Enteritidis* were isolated from liquid part of the egg samples. One possible explanation of this situation is that *S. Enteritidis* might have a higher tropism and affinity for the reproductive organs of the hen than other serovars. Of six serovars, *S. Enteritidis* and *S. Typhimurium* are the only serovars that are able to colonize the reproductive organs. The association between reproductive tract colonization and egg contamination is also found in *S. Gallinarum* biovar Pullorum (15). Therefore, our results indicate that the isolated *Salmonella* were not transmitted to the egg by contamination but they were possibly transferred from the reproductive organs of the hen to the egg. De Buck et al., have indicated that most studies show the percentage of infected eggs to be below 0.03% but artificially the percentage of infected hens can range from 0 to 27.5% (15). This indication also supports our findings.

According to the obtained results, proportion of *Salmonella* in 555 samples, which were collected from Ankara region, was 2.7%. The occurrence of *Salmonella* spp. in egg and egg product samples has been investigated in several countries.

Telo et al. have studied with seventy-nine shell egg lots imported into Albania and investigated them for the presence of the *Salmonella* spp. *Salmonella* was detected in 1 out of

79 analyzed pooled samples (1.26%). The *Salmonella* strain was isolated only from the eggshell, but not from the liquid part (23). Radkowski has studied a total of 1200 eggs in 40 local markets in Poland and *Salmonella* was not found on the shell or inside the eggs (6). Yun Hee has conducted a study with 135 dozen-shell eggs and determined that none of the egg yolks were found to contain *Salmonella* organisms (24). Schutze et al. have conducted a study to determine whether poultry shell eggs are a major reservoir of *S. Enteritidis* in Arkansas. One hundred dozen commercially purchased shell eggs were cultured for the presence of *Salmonella* spp. One dozen of the 100 dozen egg shells cultured were found to be externally contaminated with *S. Heidelberg*, while none of the liquid parts of the 100 dozen eggs were found to contain *Salmonella* organisms (25). Camilleri has studied with 900 eggs and determined that none of the eggs examined was infected with *Salmonella* spp. (26).

There are also several reports stating the lack of *Salmonella* spp. among the chicken egg samples in Turkey. Erol has studied with 150 chicken eggs in Ankara and did not isolate *Salmonella* spp. in any of the samples (27). İnal and Özyer have also reported that they have examined 400 chicken eggs in case of *Salmonella* occurrence and no *Salmonella* spp. was isolated (28). Çakıroğlu et al. have conducted a study in order to assess the microbiological quality of chicken eggs, in terms of the presence of *Salmonella* spp., in Ankara and among 882 chicken egg samples they did not isolate *Salmonella* spp. (29). These findings have lower rates in comparison with our study in case of *Salmonella* presence in the egg yolk.

Some researchers reported that even higher isolation ratios were detected compared to the results of this study. Little et al., have studied with 16 971 eggs and they have reported that *Salmonella* were recovered from 3.4%. *Salmonella* was isolated from 5.5% and 6.3% of Spanish and eggs of unknown origin, respectively, used in catering premises linked to outbreaks, a level significantly higher than that (1.1%) found in non Lion Quality UK eggs sampled (30).

In Turkey, Erdoğan, has investigated the presence of *Salmonella* in 123 liquid whole quail eggs and found seven *S. Enteritidis* in the samples (31). This finding has a higher ratio than our findings.

In egg and egg products there are limited numbers of reports. Akgün et al., have examined microbiologically from the aspect of *Salmonella* in the cream pastry samples and did not find *Salmonella* in any of the samples (32). This finding is compatible with our findings. However, Elmalı have reported that they have isolated *Salmonella* spp. in 6 of the 75 cream pastry samples (8%) and this finding does not support our findings (33). This shows that all processes are operated from production to consumption in hygiene rules in the pastries that we have supplied our samples. There are no reports of investigating *Salmonella* spp. in commercial mayonnaise samples in case of hygienic purposes with traditional methods. In our study *Salmonella* spp. was not detected in commercial mayonnaise samples.

Baker et al. have inoculated shell eggs with *S. Typhimurium* and they were cooked by recommended procedures for boiling. Boiling for 7 min was necessary for complete destruction of *S. Typhimurium* (34). Our findings indicate the results in the liquid part of the egg and we have found that after boiling for 8 min was necessary for complete destruction of *S. Enteritidis*. The 1 minute difference between the egg shells and liquid parts are thought to be because of the later heating of the liquid part than the egg shell so our findings are not uncoordinated with the findings of Baker et al. And after 21 days at 4°C no destruction was observed in case of *Salmonella* in the liquid parts of the eggs.

Isolation of the microbial agents such as *Salmonella* spp, *Yersinia enterocolitica*, *Listeria monocytogenes* is an important matter of fact in the treatment of infectious diseases because these bacteria may become resistant to the drugs (35, 36). In recent years, the numbers of life-threatening infections caused by multi-drug resistant Gram positive and Gram negative bacteria have reached an alarming level in many countries around the world (37). Since, egg and egg

products are consumed so much, detection of *Salmonella* and identification of its serotypes is a very important concern either epidemiologic or therapeutic. So, determining the potential food poisoning agents will be useful in gaining time and prevent the economic loses. Food borne outbreaks have been reported in recent years and this has attracted our attention in terms of public health consequences of microbial contamination in eggs.

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