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Occurrence of *Salmonella spp*. in chicken eggs in Albania

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Abstract

Salmonella contamination of eggs and egg shells, has been identified as a public health concern worldwide. In this study, a total of 100 eggs samples, (produced in industrial farms) were collected from 12 regions of Albania during February - December 2017. The samples were transferred to the laboratory, in order to isolate *Salmonella.spp*. using conventional culture method including pre-enrichment, enrichment, selective plating and differential plating. Out of the overall samples, 48 eggs were collected from 4 large companies with 12 samples in each of them, while the other samples were collected from 8 small and medium-sized companies. The average prevalence of *Salmonella* was found to be 2%. Two egg samples were detected as contaminated with *Salmonella spp* and they belonged to small companies. This contamination was found to be present in egg shells but not on egg contents. The present study represents that poultry eggs produced in two small companies are potential reservoir of *Salmonella spp*. During consumption of contaminated poultry eggs with *Salmonella spp* pathogen it is possible to be present Human illnesses. Intervention strategies in poultry farms and overall chain are hence important to control *Salmonella* infection from farm to table.

Keywords: *eggs, Salmonella.spp, conventional method, public health*.

1. Introduction

Eggs are very important and complete foods not only for their nutritional aspects (e.g. high-quality proteins, vitamins A, B12, D and E), but also for their functional properties, i.e. the coagulant capacity of proteins, the foaming capacity of albumen proteins, the emulsifying capacity of the yolk, etc. These properties are used in different ways to produce and enrich many types of foods (e.g. bakery products including pastries, meat pies, sauces and dressings, sweets and pasta) and in several (homemade) dishes (e.g. mayonnaise, custard and ice cream). Eggs are often used raw or only lightly heat treated in such products. Eggs and egg products are used in many different locations (domestic kitchens, restaurants and catering outlets, food industry establishments) and can be vehicles for food-borne hazards. The storage conditions of the eggs, such as temperature and time, have an impact on the risk derived from these hazards [4]. Consuming eggs, however, has been associated with negative health impacts. Eggs that are improperly handled can be a source of food borne diseases, such as Salmonellosis. *Salmonella* species have been considered one of the most important food-borne pathogens, around the world [9]. A Wide range of foods has been implicated in such disease. However, foods of animal origin, especially poultry and poultry products, including eggs, have been consistently implicated in sporadic cases and outbreaks of human Salmonellosis [1, 5]. Poultry are widely acknowledged to be a reservoir for Salmonella. Egg contents may be contaminated with Salmonellae by 2 routes: Transovarian (vertical transmission) or trans-shell (horizontal transmission). In vertical transmission, Salmonella are introduced from infected reproductive tissues to eggs prior to shell formation. Salmonella serotypes associated with poultry reproductive tissues that are of public health concern include *Salmonella enteritidis*, *Salmonella typhimurium* and *Salmonella Heidelberg* [10]. Among the different serotypes, *Salmonella enteritidis* may be better able to achieve invasion, and as a consequence, may be found more frequently in reproductive tissues [13]. Horizontal transmission is usually derived from fecal

contamination on the egg shell. It also includes contamination through environmental vectors, such as farmers, pets and rodents. Many different serotypes of the genus Salmonella can be involved. They may be able to contaminate egg contents by migration through the egg shell and membranes. Such a route is facilitated by moist egg shells, storage at ambient temperature and shell damage [10, 3]. Globally, the annual incidence of foodborne salmonellosis is conservatively estimated at 80.3 million cases [8] but other estimates range from 200 million to 1.3 billion cases [2, 4]. In the United States alone it was estimated that non-typhoidal *Salmonella* spp. are responsible for 1 million cases of domestically acquired foodborne illness annually [14]. A study from the European Union estimated that only 1 out of every 57 cases of salmonellosis is reported. This study also demonstrated that the annual incidence of salmonellosis in each of the European Union member states varied between 16 and 11,800 per 100,000 people and that the incidence of salmonellosis in each country correlated significantly with the presence of *Salmonella enterica* serotype Enteritidis in laying hens, suggesting this was the primary source of infection [6]. Because of the typically low prevalence of contaminated eggs, the low numbers of organisms in such eggs and the bacteriostatic effect of the albumen, multiplication of these relatively few organisms is necessary to reach detectable levels in culture. This can be achieved using traditional three-step Salmonella culture methods [4]. Longer periods of pre-enrichment (48 hours rather than 24 hours) have been shown to further increase the sensitivity of detection in individual eggs or in pooled egg contents, as long as only a few competing organisms are present. In practice, in the EU, eggs are most likely to be tested as a foodstuff, therefore the full ISO 6579 method is used. Pre-enrichment at 41.5 °C may also be beneficial in some cases [12].

2. Material and Methods

2.1. Study area and sampling.

This study was conducted in the 12 regions of Albania during February - December 2017. Sampled eggs had been produced in industrial farms. In total, 100 eggs were collected aseptically, placed into sterile bags and were transferred to the Food Microbiology Laboratory of Food Safety Veterinary Institute, Tirana - Albania.

2.2. Media and reagents.

All the culture media and reagents were commercial products. Tryptic soy broth, tryptic soy agar (TSA), buffered peptone water (BPW), Muller-Kauffmann tetrathionate broth (M-KTB), desoxycholate citrate lactose agar as modified by Hynes (DCL), and Kligler iron agar,. Selenite cystine broth (SCB) and brilliant green agar (BGA) were from Biolife Italiana S.r.l.. Biochemical identification of cultures was made by the API 20E system (bioMerieux), and serological identification was made with Salmonella polyvalent and monovalent anti-O and anti-H sera (either Pasteur Diagnostics or Biogenetics).

2.3. Sample Processing

The sterile plastic bags containing selected eggs were opened with scissors and the samples processed immedatelly. Swab technique was used to sample the shell surface of the intact eggs. Sterile cotton swabs dipped in sterile buffered peptone water (BPW) were used to swab the entire surface area of the eggshell. The swabs were directly inoculated into 10 ml BPW in screwcapped bottles [15]. The same eggs from which shell sample was collected were used for interior (egg content) sampling. The eggs surface were sterilized by immersing in 70% alcohol for 2 min, air dried in a sterile chamber for 10 min and then cracked with a sterile knife. Each egg's content was mixed thoroughly and 25ml of the mixed egg content was inoculated into 225 ml of BPW and homogenized for two minutes with shaker. The mixture then homogenized using a laboratory blender (Stomacher VWR Star blender LB 400) for 30 seconds

2.4 Isolation and Identification of Salmonella

The isolation was conducted utilizing the conventional methods for the detection of Salmonella following the standard guidelines from ISO 6579: [7] (Microbiology of food and animal feeding stuffs horizontal method for the detection of Salmonella spp.)

2.5 Non-Selective Pre-Enrichment

The swabs were directly inoculated into 10 ml BPW in screw capped bottles and incubated at 37°C for 16-18 hrs. Each egg's content was mixed thoroughly and 25 ml of the mixed egg content was inoculated into 225 ml of BPW and incubated at 37°C for 16-18 hrs [15].

2.6 Selective Enrichment

The pre-enrichment broth after incubation was mixed and 0.1 ml of the broth was transferred into a tube containing 10 ml of Rappaport Vassiliadis medium (RV broth). Another 1 ml of the preenrichment broth was transferred into a tube containing 10 ml of Muller-Kauffmann tetrathionate broth (MKTT broth). The inoculated RV broth was incubated at 41.5 °C ±1°C for 24 ± 3 hours and the inoculated MKTT broth at 37 °C ± 1 °C for 24 ± 3 hours [15].

2.7 Plating out and Identification

Xylose lysine desoxycholate (XLD) agar (Biolife Italiana S.r.l.) and HEA Salmonella - Shigella (SS) agar (Biolife Italiana S.r.l.) plates were used for plating out and identification purpose. A loop-full of inoculums from the RV broth and MKTT was transferred and streaked separately onto the surface of xylose lysine deoxycholate agar (XLD agar) and salmonella-shigella agar (SSA) separately. The plates were incubated at 37°C ± 1°C for 24 ± 3 hours. After proper incubation, the plates were examined for the presence of suspected Salmonella colonies which XLD agar is pink with a darker pink center whereas lactose-positive salmonellae are yellow with or without blackening. Confirmation was done by using biochemical test according to ISO 6579 [7].

3. Results and Discussion

Out of the 100 eggs that were collected from 12 regions of Albania, egg samples were detected as contaminated with *Salmonella spp* and they belonged to small companies. This contamination was found to be present in egg shells but not on egg contents. The present study represents that poultry eggs produced in two small companies are potential reservoir of *Salmonella spp*. The egg is considered as ideal food and major source of protein in Albania. But the contamination of Salmonella spp. can causes major public health burden by consuming raw or under cooked eggs and egg products. In addition, the contamination of egg by salmonella involves major health expenses. From 2005 to 2014, according to the Institute of Public Health, every year in Albania are registered 200 to 600 cases with Salmonella. So it is necessary to monitor the infection level of egg and egg production.

4. Conclusions

Continuous monitoring and control methodologies, which should be applied in poultry farms for the control of spread and eradication of this pathogen, where possible, are strongly recommended [11]. Efforts including critical control point programs in food production are needed to reduce the incidence of *Salmonella* in food. Consumers-awareness efforts would protect public health

5. References

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