**Detection of Salmonella spp in commercial eggs in Iran**

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ABSTRACT

**Background and Objective:** Salmonellosis can be acquired through consumption of infected raw or undercooked eggs. The aim of this study was isolation and identification of *Salmonella spp* from the eggshells and the egg contents samples of Tabriz retails.

**Methods:** A total number of 150 samples of eggs were analyzed for the presence of *Salmonella spp* using conventional culture method and multiplex-PCR.

**Results:** Two (1.33%) out of 150 samples from eggshells were determined as contaminated with *Salmonella spp*. *Salmonella spp* was not isolated from the egg contents. *Salmonella* serovar was determined as *enteritidis* and *typhimurium*.

**Conclusion:** The results of the present study provide the recent dataset of the prevalence of *S. enteritidis* and *S. typhimurium* in eggs at retail shops in the northwest of Iran. It is important to remember that control is required at all levels in the food chain and by separating cooked and raw.

**Keywords:** Egg, *Salmonella*, Prevalence, Isolation, Iran

INTRODUCTION

*Salmonella* species have been considered one of the most important food-borne pathogens, around the world (1). *Salmonella enterica* serovar *typhimurium* and *Salmonella enterica* serovar *enteritidis* are the most frequently isolated serovars from foodborne outbreaks throughout the world. *S. enteritidis* and *S. typhimurium* usually induce self-limiting gastroenteritis or an asymptomatic carrier state in a wide variety of animal species (2). These serovars are also characterized by a wider geographical spread and they can be carried by a range of animal vectors (1). Human infections with *S. enteritidis* originate mainly from eggs and egg products (when consumed raw or undercooked), while *S. typhimurium* infections originate predominantly from pigs, cattle and poultry meat, as well as environmental contamination with companion animals or infected birds (1, 3). In the United States, about 80% (298 from 371) of the known-source *S. enteritidis* outbreaks from 1985 to 1999 were egg-associated (4). In European Union, *S. enteritidis* was identified as the cause of infection in 62.5% of the cases, and *S. typhimurium* in 12.9% (5).

Eggs are used as an inexpensive food source in the form of shell eggs, liquid, frozen, and/or dried products (6). The eggs and its products provide a reliable source of nutrition; as well as they serve a more functions in other products. Due to wide spread use of eggs as a food source, the safety of this product is important. Eggshells and egg contents
can be contaminated by the bacteria in a variety of routes, such as during egg formation in the hen reproductive system or the environmental conditions (7). Several outbreaks of salmonellosis have been reported where the eggs were the source of human infection; especially in the case of undercooked or raw eggs (8-9). Generally, there are two possible routes of egg contamination by Salmonella. Eggs can be contaminated by penetration of the bacterium through the eggshell from the colonized gut or from contaminated faeces during or after oviposition (horizontal transmission). The second possible route is by direct contamination of the yolk, albumen, eggshell membranes or eggshells before oviposition, originating from the infection of reproductive organs with Salmonella (vertical transmission) (1, 5).

In north west of Iran, little is known about the prevalence of this bacterium in foods especially in eggs. The aim of the present study was to isolate and identify Salmonella spp from the eggshells and egg contents of Tabriz retails.

MATERIALS AND METHODS

Study area and sampling. This study was conducted in the city of Tabriz, East-Azerbaijan province (northwest of Iran). The sampling area was divided into 30 clusters. Five stores were randomly selected from each cluster and an egg was randomly selected from each store. Sampled eggs had been produced in industrial farms. In total, 150 eggs were collected from 150 stores. All the samples were collected aseptically, placed into sterile bags and were transferred to the Food Microbiology Laboratory of Faculty of Veterinary Medicine, Tabriz University.

Microbiological analysis. The eggs were transferred aseptically to beakers containing 225 ml of Buffered Peptone Water (BPW, Merck, Germany). They were then incubated at 37 °C for 24 h followed by transferring of 1 ml to the selenite–cystine broth (SC, Merck, Germany) and 0.1 ml to the Rappaport–Vasiliadis medium (RV, Merck, Germany) with incubation for 24 h at 37 °C (SC) and 41.5 °C (RV) (23). From the broths one loopful was subcultured on Brilliant Green and Phenol Red agar (BGA, Merck, Germany) and Bismuth Sulphite Agar (BSA, Merck, Germany). The media were inoculated at 37 °C for 24 h (BGA) or 48 h (BSA). Then, suspected colonies were transferred onto Salmonella-Shigella agar (Merck, Germany) plates, and incubated at 37 °C for 24 h. The plates were observed for typical Salmonella-like colonies, randomly, two colonies from each plate were picked, purified and subjected to primary biochemical screening tests, which involved reactions on Triple Sugar Iron agar (Merck, Germany), Lysine Iron agar (Merck, Germany), motility and Indole and H₂S production in Sulfide-Indole-Motility (SIM, Merck, Germany) and urea splitting ability in Christensen’s Urea agar (Merck, Germany). Slide agglutination test were performed using polyvalent H serum and the group sera (Difco, USA).

Detection of Salmonella in egg contents: After treatment of the eggs in BPW, they were taken out of the beakers and the eggshells were sterilized by immersing for 12 seconds in water at 100 °C. Then the eggs were broken in the sterile beaker and 225 ml of BPW was poured over them. Salmonella was then isolated as described above.

DNA preparation and Multiplex-PCR assay. The suspected Salmonella colonies were subjected to Multiplex-PCR assay for final confirmation as Salmonella spp. and identification of typhimurium or enteritidis serovars. Isolated strains were cultured onto Luria Bertani agar (LBA, Merck, Germany) and incubated at 37 °C for 24 h. For DNA extraction, 1-2 colonies of each sample from LB agar was suspended in 250 μL of sterile distilled water. In order to have uniform turbidity the samples were vortexed, and then were boiled for 10 min and centrifuged at 6000×g for 7 min. Supernatants were collected and saved for multiplex PCR analysis (10).

Multiplex PCR was performed with 2 independent sets for DNA amplification of S. typhimurium and S. enteritidis. Four sets of primer pairs specific for rfbJ (663 bp), fljB (526 bp), invA (284 bp), and flIC (183 bp) were used in the case of S. typhimurium (Table 1), and three sets of primer pairs designed for a random sequence specific for the genus Salmonella (429 bp), sefA (310 bp), and spv (250 bp) for S. enteritidis (Table2). Both reactions were performed in a final volume of 25 μL that contained 4 μL of template DNA, 2.5 μL of reaction buffer (10X), 0.8 μL of dNTPs (10 mM), 1 μL of MgCl₂ (50 mM), 0.3 μL of Taq polymerase (5 U μL⁻¹), 8.4 μL of sterile distilled water, and 1 μL of each primer (10 μM) for S. typhimurium, and for S. enteritidis, 3 μL of template DNA, 0.6 μL of Taq polymerase, 9.9 μL of

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distilled water, and 1.25 μL of each primer (10 μM), in addition to buffer, dNTPs, and MgCl₂ at the same volume and stock concentrations as mentioned.

Amplification was carried out using a Techne TC-512 thermocycler (Techne, UK), as follows: 35 cycles of 30 s for denaturation at 94 °C, 90 s for annealing at 56 °C, and 30 s for primer extension at 72 °C, followed by a terminal extension at 72 °C for 10 min in the case of S. enteritidis. Target genes for ST were amplified using the same thermocycler, as follows: 30 cycles of denaturation at 95 °C for 1 min, annealing at 65 °C for 1 min, primer extension at 72 °C for 30 s, followed by 7 min at 72 °C for terminal extension. For both amplifications, initial denaturation at 95 °C for 5 min was used.

The amplification products were analyzed by agar gel electrophoresis. Electrophoresis of the amplification products was performed on 1.2% and 1.8% agarose gel for S. typhimurium and S. enteritidis samples, respectively. In both reactions, a 100-bp ladder was used as a molecular weight marker. The gels were stained with ethidium bromide (2 µg/mL) to visualize fluorescent bands while using UV in the gel document system (Bio-Rad, UK).

**Statistical analysis.** The data were analysed by SPSS software (version 16). P-value less than 0.05 were considered as statistically significant.

**RESULTS**

Two samples (1.33%) out of 150 samples from eggshells were determined as contaminated with Salmonella spp. Salmonella serovar was determined as enteritidis and typhimurium (Fig. 1 and 2). salmonella spp. was not isolated from the egg contents. However, there was no statistical difference between eggshells and the egg contents with Salmonella contamination (p= 0.25).

**DISCUSSION**

Epidemiological evidence suggests that there is a direct link between the presence of Salmonella in

| Table 2. Primers used for the detection of S. enteritidis. |
|----------------|----------------|-------------|-------------------|------------------|
| Primer       | Target gene   | Length (bp) | Sequence (5'–3')                      | Amplification product (bp) |
| ST141        | invA          | 26          | GTGAAATTATCGCCACGTTCCGCGCA             | 284               |
| ST139        | invA          | 22          | TCGCAGCTCAAGGGAACC                      |                   |
| Rfbj         | rfbB          | 24          | CCAGCACCAGTTCCAACTTGATAC               | 663               |
| Rfbj         | rfbB          | 24          | GGCTTCCGCGCTTTAATTGTAAGCA              |                   |
| Ficc         | fibC          | 23          | ATAGGCAATCTTACGTTCCCGCC                | 120               |
| Ficc         | fibC          | 24          | GCTGCAACTGTACAGGATATGCG                |                   |
| F1b          | f1B           | 24          | ACGATGCTCGGCTTCGTAACC                  | 526               |
| F1b          | f1B           | 24          | TACCGTCGATAGTAAACGACTTGG               |                   |

* Randomly cloned sequence specific for the genus Salmonella
** Salmonella plasmid virulent gene
*** S. enteritidis fimbrial antigen gene
were isolated only from commercial eggshells (11, 12), which are consistent with the findings of our study. In most reports from other countries, Salmonella has been isolated only from shells (17, 18, 22), but there are also reports of contamination of the egg contents (15, 16). Contamination of eggshells represents a risk for the consumers, as they can directly infect and cross-contaminate the egg contents or other foodstuffs (1). Salmonellosis outbreak through contaminated eggshells with Salmonella in USA (2010) showed the importance of shell contamination, which this outbreak caused illness in 1939 persons (24).

In previous reports from Iran, only one of $S. \text{enteritidis}$ (11) or $S. \text{typhimurium}$ (12) was isolated from eggs, but in our study, both serovars of $S. \text{enteritidis}$ and $S. \text{typhimurium}$ were identified. $S. \text{enteritidis}$ is the most prevalent serovar in the world (1, 15, 17, 20, 22). Few eggs related outbreaks of salmonellosis caused by $S. \text{typhimurium}$ are reported in humans in the European Union (3.5% against 77.2% caused by $S. \text{enteritidis}$) (1, 3).

In general, egg-related outbreaks result from breakdowns in controlling measurements along the farm to fork continuum. International poultry control programs in developed countries have resulted in significant decreases in egg-related salmonellosis. These programs included: on-farm monitoring, diverting contaminated eggs for processing, culling infected flocks, cleaning and disinfection of sheds, maintaining cold chain of eggs, and vaccination of flocks (25). It is recommended that these controlling measurements should be done carefully in all countries including Iran.

Results of the present study provide the recent dataset of the prevalence of $S. \text{enteritidis}$ and $S. \text{typhimurium}$ in eggs at retail shops in the northwest of Iran.

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REFERENCES

1. Martelli F, Davies RH. Salmonella serovars isolated from table eggs: An overview. Food Res Int 2012; 45:745-754.
2. Uzzau S, Brown DJ, Wallis T, Rubino S, Leori G, Bernard S, et al. Host adapted serotypes of Salmonella enterica. Epidemiol Infect 2000; 125:229-255.
3. EFSA. Scientific opinion on a quantitative estimate of the public health impact of setting a new target for the reduction of Salmonella in laying hens. EFSA J 2010; 8:1546.
4. Patrick ME, Adcock PM, Gomez TM, Altekruse SF, Bernard S, et al. Host adapted serotypes of Salmonella enterica enteritidis infections, United States, 1985–1999. Emerg Infect Dis 2004; 10:1.
5. Gantois I, Ducatelle R, Pasmans F, Haesebrouck F, Holland BH, Tauxe RV, et al. Salmonella enteritidis and antimicrobial resistance in retail egg products: Spain, 2002–2003. Food Microbiol 2006; 23:294-298.
6. Downes FP, Ito K. Compendium of methods for the microbiological examination of foods (4th ed.) American Public Health Association, Washington, DC 2001:473-81.
7. Howard ZR, O'Bryan CA, Crandall PG, Ricke SC. Salmonella Enteritidis in shell eggs: Current issues and prospects for control. Food Res Int 2012; 45:755-764.
8. Gillespie I, O'Brien S, Adak G, Ward L, Smith H. Foodborne general outbreaks of Salmonella Enteritidis phage type 4 infection, England and Wales, 1992-2002: where are the risks? Epidemiol Infect 2005; 133:795-801.
9. Crespo PS, Hernández G, Echeita A, Torres A, Ordóñez P, Aladueña A. Surveillance of foodborne disease outbreaks associated with consumption of eggs and egg products: Spain, 2002–2003. Euro Surveill 2005; 10:E050616.
10. Mirzaie S, Hassanzadeh M, Ashrafi I. Identification and characterization of Salmonella isolates from captured house sparrows. Turk J Vet Anim Sci 2010; 34:181-186.
11. Jamshidi A, Kalidari G, Hedayat M. Isolation and identification of Salmonella Enteritidis and Salmonella Typhimurium from the eggs of retail stores in Mashhad, Iran using conventional culture method and multiplex PCR assay. J Food Safety 2010; 30:558-568.
12. Miranzadeh H, Zahravi Salehi T, Karimi V. The count of aerobic mesophilic bacteria and isolate Salmonella spp on egg in Isfahan. J Veterinary Journal (Pajouhesh-va-Sazandegi), 2012; 25:31-36.
13. Mahdavi M, Jalali M, Safaei H, Shamloo E. Microbial quality and prevalence of Salmonella and Listeria in eggs. Int J Environ Health Res 2012; 48:16-21.
14. Ghasemian Safaei H, Jalali M, Hosseini A, Narimani T, Sharifzadeh A, Raheimi E. The prevalence of bacterial contamination of table eggs from retail markets by Salmonella spp., Listeria monocytogenes, Campylobacter jejuni and Escherichia coli in Shahrekord, Iran. Jundishapur J Microb 2012; 4: 249-253.
15. Betancor L, Pereira M, Martinez A, Gossia G, Foakes M, Flores K, et al. Prevalence of Salmonella enterica in poultry and eggs in Uruguay during an epidemic due to Salmonella enterica serovar Enteritidis. J Clinical Microbiol 2010; 48:2413-2423.
16. Singh S, Yadav AS, Singh SM, Bhati P. Prevalence of Salmonella in chicken eggs collected from poultry farms and marketing channels and their antimicrobial resistance. Food Res Int 2010; 43:2027-30.
17. Suresh T, Hatha A, Sreenivasan D, Sangeetha N, Lashmanaperumalsamy P. Prevalence and antimicrobial resistance of Salmonella enteritidis and other Salmonellas in the eggs and egg-storing trays from retail markets of Coimbatore, South India. Food Microbiol 2006; 23:294-299.
18. Jones D and Musgrove M. Pathogen prevalence and microbial levels associated with restricted shell eggs. J Food Protect 2007; 70:2004-2007.
19. Jones F, Rives D, Carey J. Salmonella contamination in commercial eggs and an egg production facility. Poultry Sci 1995; 74:753-757.
20. Chemaly M, Huneau-Salaun A, Labbe A, Houdayer C, Petetin I, Fravalo P. Isolation of Salmonella enterica in laying-hen flocks and assessment of eggshell contamination in France. J Food Protect 2009; 72:2071-2077.
21. Poppe C, Duncan CL, Mazzocco A. Salmonella contamination of hatching and table eggs: a comparison. Can J Vet Res 1998; 62:191-198.
22. Sasaki Y, Tsujiyama Y, Asai T, Noda Y, Katayama S, Ashrafi I. Identification and characterization of Salmonella isolates from captured house sparrows. Turk J Vet Anim Sci 2010; 34:181-186.
23. Radkowski M. Occurrence of Salmonella spp. in consumption eggs in Poland. Int J Food Microbiol 2001; 64:189-191.
24. Lou M, Keelara S, Thakur S. Molecular characterization of Salmonella enterica serotype Enteritidis isolates from humans by antimicrobial resistance, virulence genes, and pulsed-field gel electrophoresis. Foodborne Pathog Dis 2012; 9:232-238.
25. Moffatt C, Musto J. Salmonella and egg-related outbreaks. Microbiol Aust 2013; 34:94-98.