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ORIGINAL ARTICLE

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 food-borne 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

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can be contaminated by the bacteria in a variety of routs, 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 H_2S 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

Table 1. Primers used for the detection of *S. typhimurium.*

Primer	Target gene	Length (bp)	Sequence (5´-3´)	Amplification product (bp)
ST141	*inv*A	26	GTGAAATTATCGCCACGTTCGGGCAA	284
ST139	*inv*A	22	TCATCGCACCGTCAAAGGAACC	
Rfbj	*rfb*J	24	CCAGCACCAGTTCCAACTTGATAC	663
Rfbj	*rfb*J	24	GGCTTCCGGCTTTATTGGTAAGCA	
Flic	*fli*C	23	ATAGCCATCTTACCAGTTCCCCC	183
Flic	*fli*C	24	GCTGCAACTGTTACAGGATATGCC	
Fljb	*flj*B	24	ACGAATGGTACGGCTTCTGTAACC	526
Fljb	*flj*B	24	TACCGTCGATAGTAACGACTTCGG	

distilled water, and 1.25 µL of each primer (10 µM), in addition to buffer, dNTPs, and $MgCl_2$ 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)
ST11	*Random sequence**	24	GCCAACCATTGCTAAATTGGCGCA	429
ST14	*Random sequence**	25	GGTAGAAATTCCCAGCGGGTACTGG	
S1	*Spv***	20	GCCGTACACGAGCTTATAGA	250
S4	*Spv*	20	ACCTACAGGGGCACAATAAC	
SEFA2	*sefA****	20	GCAGCGGTTACTATTGCAGC	310
SEFA4	*sefA*	20	TGTGACAGGGACATTTAGCG	

* Randomly cloned sequence specific for the genus *Salmonella*
** *Salmonella* plasmid virulent gene
*** *S. enteritidis* fimbrial antigen gene

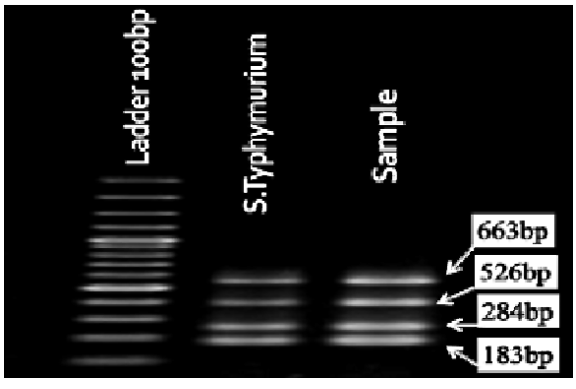

Fig. 1. Multiplex polymerase chain reaction for detection of *S. typhimurium.* Four genes included *rfbJ (663 bp), fljB (526 bp), invA (284 bp), and fliC (183 bp)* amplified to the *S. typhimurium.*

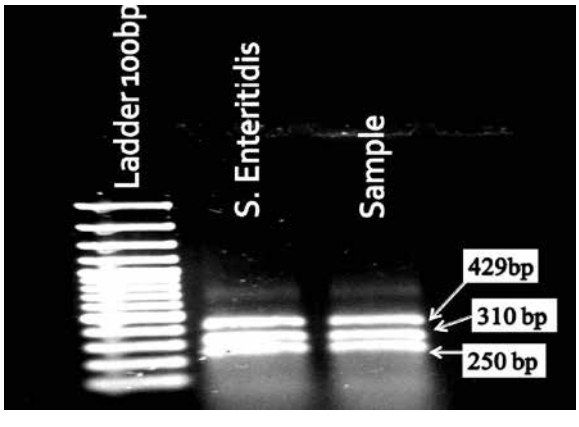

Fig. 2. Multiplex polymerase chain reaction for detection of *S. enteritidis.* Three genes included genus ***Salmonella*** (429 bp), sefA (310 bp), and ***spv*** (250 bp) amplified to the S. enteritidis.

poultry products and salmonellosis occurrence in humans. It was demonstrated in this study that 1.33% of eggs was contaminated by *Salmonella*. In similar studies conducted in Iran, contamination rate of commercial eggs with *Salmonella* has been reported 1.6% in Mashhad (North-eastern Iran, 2008) (11) and 1.61% in the city of Isfahan (Central Iran, 2011) (12). However, *Salmonella* has not been isolated from commercial eggs in other studies (13, 14). The rates for contamination of eggs with *Salmonella* in other countries are very different: 9.35% in Uruguay (15), 3.85 to 7.93% in India (16, 17), 0 to 1.11% in USA (18, 19), 1.05% in France (20), 0.4% in Canada (21), 0.25% in Japan (22), and zero in Poland (23).

Although, *Salmonella* was isolated from eggshells in this study, but none of the contents were contaminated by *Salmonella*. In similar studies in Iran, *Salmonella* 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 represent 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. enteritidis* (11) or *S. typhimurium* (12) was isolated from eggs, but in our study, both serovars of *enteritidis* and *typhimurium* were identified. *S. enteritidis* is the most prevalent serovar in the world (1, 15, 17, 20, 22). Few eggs related outbreaks of salmonellosis caused by *S. typhimurium* are reported in humans in the European Union (3.5% against 77.2% caused by *S. 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. enteritidis* and *S. typhimurium* in eggs at retail shops in the northwest of Iran. Although, *Salmonella* was isolated from eggshells in this study, but *Salmonella spp* was not isolated from the egg contents. It is important to remember that control is required at all levels in the food chain and by separating cooked and raw.

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