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ORIGINAL ARTICLE

The Prevalence of *Salmonella Enteritidis* in Packaged and Tray Eggs Samples, Qazvin, Iran

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KEYWORDS

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ABSTRACT: *Salmonella* serotypes are considered as one of the most important foodborne pathogens. Eggs are a main source of the contamination caused by these pathogens and diseases in humans and the prevalence of the salmonellosis. This study was aimed to isolate *Salmonella* enteritidis from industrial eggs collected from different areas of Qazvin city, Iran in the year 2020.In this cross-sectional study, 200 eggs were collected randomly (including 100 industrial packaged eggs and 100 industrial tray eggs) from the retail and stores located in Qazvin city, Iran. After culturing of eggshells and egg contents according to the classic methods, suspected colonies were confirmed by PCR assay. *Salmonella* was detected in 10% (4/40) among the egg samples. *Salmonella* was isolated from 0% (0/40) and 10% (4/40) of eggshells and egg contents, respectively. Isolates from positive egg samples were characterized as S. Typhimurium.*Salmonella* Typhimurium is the most prevalent serotype of egg contamination in Qazvin city, Iran. It can be regarded as the risk evaluation of possible human foodborne diseases associated with the consumption of contaminated eggs.

INTRODUCTION

Egg is nutritious in the human's diet and it is in the food pyramid because of its protein content [1-2]. Microbial contaminated eggs are a known source of *Salmonella*, leading to foodborne diseases [3-4]. *Salmonella* with diversity in serovars has been isolated from eggs in developed and developing countries like, China, Nigeria, Cameroon, Ethiopia, Egypt, United States, Japan, Iran, and England [5-13].

Salmonella infection of eggs occurs in two ways, including 1) contamination of the egg content or direct contamination before shell formation laid by invasion to ovaries and oviducts, and 2) contamination of the shell surface or

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indirect contamination after laid with *Salmonella* infiltration into eggshell membranes after infection oviposition [14].

The CDC with WHO estimates that non-typhoid *Salmonella* causes 1.3 billion cases of acute gastroenteritis and 3 million mortalities each year [15].

S. enterica serovar Typhimurium and Enteritidis are the most common food-borne salmonellosis agents in the world. Typhoidal *Salmonella* is one of the reasons intense and life- menacing illness, but non-typhoidal *Salmonella* like *Salmonella* entritidis and typhimurium usually causes gastroenteritis. However, intense systemic infections may happen in infants, the elderly, and immunocompromised individuals [16-17]. *Salmonella* with over 2600 serotypes is a widespread zoonotic pathogen. Serotype is a phenotypic trait according to which *Salmonella* is divided into groups A, B, C, and D [15, 18].

Salmonella serovars have different outbreak in the diverse date or zone. Like, S. enteritidis cause of foodborne is more prevalence in eggs and egg products in the United States and Europe, while *Salmonella* typhimurium is more prevalent in Australia [19-21].

The conventional method, to detect *Salmonella* serovar, involves multiple periods and generally takes a few days [22]. PCR is the application to identify *Salmonella* serotype in egg and egg products as an assured and fast tool to evaluate the contaminated samples in the food chain [23]. *Salmonella* invA invasive gene in the mammalian epithelial cells is specific to *Salmonella*, and the DNA sequence in *Salmonella* spp is highly conserved. Thus, invA can act as a reliable and accurate gene for the detection of *Salmonella* by PCR [24].

According to the per capita consumption of eggs in the country, 200 eggs per person was announced, and they are trying to increase it to be 250 eggs by low egg cost. Due to low economic conditions of society and high egg consumption and, there is the possibility of egg contamination risk, to the strains of *Salmonella* for the community. So far, there has been little information about the distribution of strains in eggs. The aim of this study was to diagnose and isolate *Salmonella* enteritidis in packaged and tray eggs.

MATERIALS AND METHODS

Sample collection

We collected 200 eggs including 100 industrial packaged eggs and 100 industrial tray eggs. Samples were collected from retail and stores of Qazvin, Iran, in 2020 (100 eggs each represented by 20 samples, as every five eggs constitute one sample). Samples were placed in a separate sterile bag and immediately transferred to the laboratory in a cool box. The eggs were stored under sterile conditions at 4 °C until being analyzed.

Isolation and detection of Salmonella

Egg processing

The eggs were prepared as described by Bacteriological Analytical Manual [25]. Briefly, a swab (Sterile cotton) technique was used to sample the shell surface of the intact eggs. Swabs dipped in 50 ml of trypticase soy broth (TSB) ((LIOFILCHEM, DIAGNOSTICI, ITALY) pre-enrichment) supplemented with ferrous sulfate (35 mg ferrous sulfate added to 1,000 mL TSB) and incubated at 37°C for 24 h. To investigate the *Salmonella* contamination in egg contents, each egg was first disinfected with disinfectant solution. The eggs dried in a sterile chamber for 10 min, then cracked with a sterile knife. Each egg's content was thoroughly mixed. Then, sample added to 500 ml of TSB and incubated at 37 °C for 24 h. For selective enrichment broth, 1 ml of the pre-enrichment solution was put in a tube containing 20 ml of RV (Scharlau, Spain) (incubated at 37°C for 24 h). The RV cultures were streaked onto xylose lysine deoxycholate (LIOFILCHEM, DIAGNOSTICI, ITALY) plates (Selective media) and incubated at 37 °C for 24 h. Presumptive colonies were stabbed into a triple sugar iron slant (Scharlau, Spain), urea agar (LIOFILCHEM, DIAGNOSTICI, ITALY) and then incubated at 37°C for 24h.

Preliminary identification of Salmonella by PCR

Primer synthesis

The specific primer InvA used to detect *Salmonella* [26]. Primers were used based on InvA gene sequence designed using Primer-BLAST software and NCBI gene bank. The primers were synthesized by SINACLON, Iran. Primers are listed in Table 1.

Table 1. Primers designed to detect *Salmonella*

Gene	Sequence of nucleotides	Primers size (bp)	Gene size (bp)
InvA	F-GCTGCTTTCTCTACTTAAC	19	95
	R-GTAATGGAATGACGAACAT	19	
SEN1392	F= GGATATGAGGTGCGTTTA	18	77
	R= CAGTGCCGGAATTATCTC	18	
STM4200	F-CACCTGATATAGAGTCCAA	19	101
	R- TATAGATGTTGTCGCCAA	18	

Real Time PCR

Total genomic DNA was extracted by using a pathway boiling. Polymerase chain reaction (PCR) method was used to verify and identification of isolates. *Salmonella* isolates examined and identified for invA genes in DNA extracted from isolate by multiplex quantitative PCR method was described by Raymond Heymans et al. with some modification [26]. For PCR, we used a Real time PCR (Rotor Gene Q) device with temperature cycle according to Table 2 in 46 cycles with the desired PCR mixture.

Table 2. PCR mixture, and cycling conditions for detection *Salmonella* isolates based on InvA genes

Composition	Stock	Content in final volume (20 µL)
Master mix	2 X	10 µL
Forward primer	10µM	0.5µl
Reverse primer	10µM	0.5µl
DDW	-	6µl
DNA	-	3µl (ng)
Stage	**Temperature (°C)**	**Time (s)**
Denaturation	95	15
Annealing	51.5	19
Extension	72	37

DNA electrophoresis with agarose gel

Confirmation of PCR product performed on 1.5% agarose gel (w/v) and TBE buffer with 100 volts at 60 minutes and finally specific bands were evaluated with gel dock eliminator (Figure 1)

Data analysis

All data analyses were performed using SPSS Statistical Software version 25. Dependent variable used in the study included the incidence of *Salmonella* and Independent variables included testing eggshell, egg content, and packaging type. The Chi- square test was used to compare the incidence of *Salmonella* to different variables. The results were considered significant with a P-value < 0.01.

RESULTS

Prevalence and serotypes

From two different groups collected randomly, there was no *Salmonella* infection in the packaged egg shell and tray egg shell, but 10% of the contents of the samples (4/40) were positive, (2/20) of the contents of packaged eggs and (2/20) of the contents of tray eggs, respectively. The isolates identified from positive egg samples were *Salmonella* typhimurium serotype (Figures 1 and 2). Shell contamination was significantly less than content contamination. There was no significant contamination difference between tray eggs and packaged eggs and there was a significant difference between the contamination in the shell and the contamination in the contents of both groups (Figure 3).

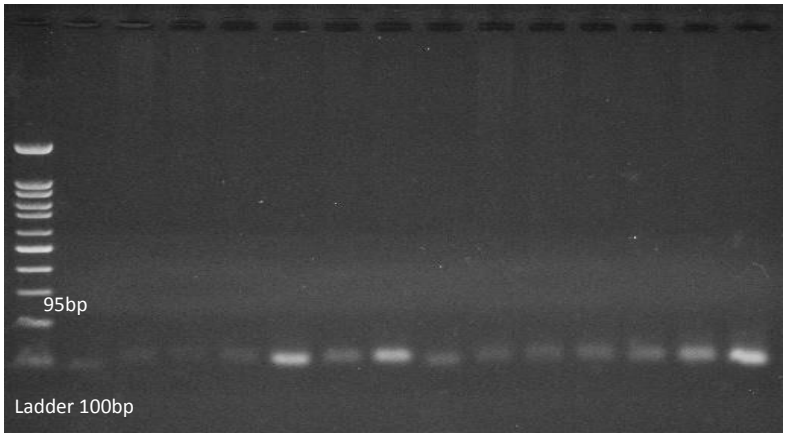

Figure 1. PCR PCR screening of *Salmonella* isolates (STM gene)

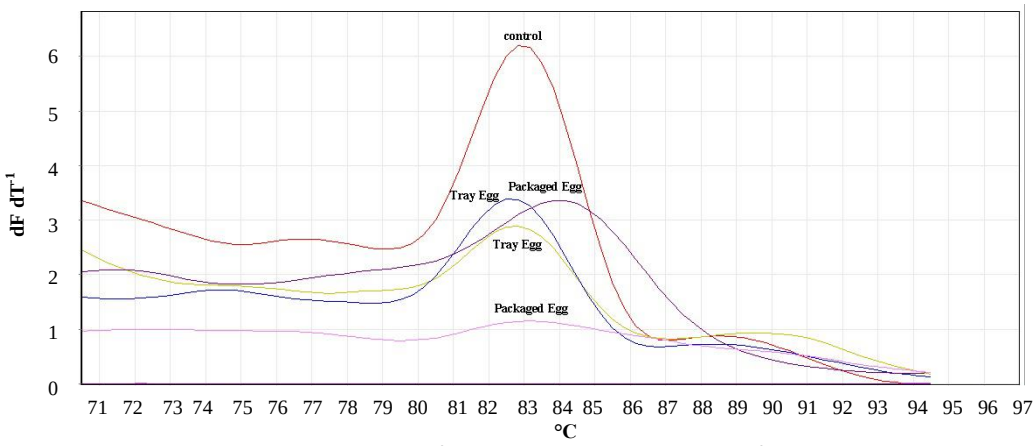

Figure 2. Melting curve analysis of SYBR Green real-time PCR product of *Salmonella* Typhimurium

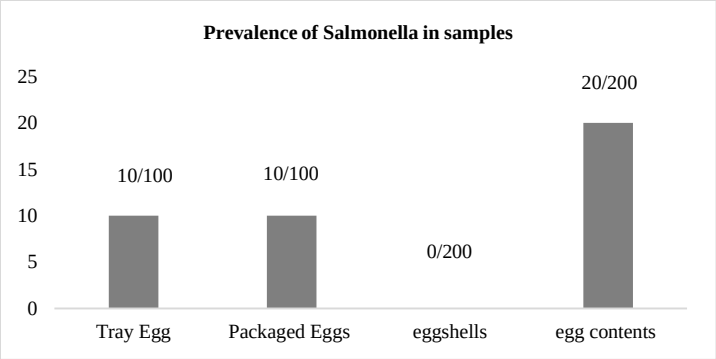

Figure 3. Prevalence of *Salmonella* in samples

DISCUSSION

Salmonella is one of the leading causes of foodborne illness in the world; the contamination rate of *Salmonella* in poultry's egg had led to many outbreaks and sporadic cases in humans [27].

Salmonella contamination in eggs has had different prevalence rates in Iran and around the world. Examining the contamination in the shell and contents of the eggs from Zanjan and evaluating egg yolk in Tabriz showed contamination of *Salmonella* equal to 0% in the industrial eggs [28-29]. In the first study, the absence of *Salmonella* contamination in the eggshell is in line with this study; however, the absence of *Salmonella* contamination in egg content in both studies is not consistent with to the results of this study. The contamination rate of *Salmonella* from egg (white and yolk) in Chinese stores was 3.2%, less than the results of our study [30]. Examination of eggshells and contents of egg in Duhok showed a prevalence of 4.85% *Salmonella* in eggshells, which is not consistent with the results of our study [31]. Some studies have reported infection with *Salmonella* serotypes in eggshells and contents [32-34].In these studies There are no significant differences in variables such as eggshell condition, type of packaging, sampling location, or sampling season; but there are significant differences between provincial areas. The different geographical areas used for sampling and the environmental and breeding conditions as well as the relationship of birds with large animals can be the main reasons for the difference in the level of contamination.

Salmonella contamination of eggs occurs in two ways: 1) vertical transmission - In this study, there was no contamination in the eggshell, but in 2 groups of samples contamination was observed in the egg contents which *Salmonella* contamination was observed in the ovary and oviduct of poultry and direct contamination of eggs during formation in the reproductive tract. 2) Horizontal transmission through contaminated intestines or feces, environmental factors such as farmer, insects, and rodents; wet eggshells, storage at room temperature, and shell damage facilitate this contamination [35].

There is an epidemiological link between *Salmonella* enteritidis and eggs, but some other *Salmonella* serotypes may have colonies in the ovary and oviduct of laying hens that contaminate egg contents during formation [36]. *Salmonella* typhimurium causes a high frequency of systemic and colonial contamination in the reproductive tissues of poultry [37]. In our study, *Salmonella* isolates from egg contents were *Salmonella* typhimurium.

The invA enhancer gene has been offered as an international standard for the distinction of *Salmonella*. PCR was used to identify the InvA gene of *Salmonella* serotypes with 95 bp products and it showed that the isolates were *Salmonella* typhimurium [38]. In the study from Bangkok, they detected *Salmonella* from the eggshell using the invA gene [39]. In this study, the invA gene was also used to detect *Salmonella*.

Salmonella enteritidis and *Salmonella* typhimurium have been detected in poultry in all European countries [32]. In

the study from Cameroon, they reported the prevalence of *Salmonella* in tray eggs was 88.6% and the prevalence of *Salmonella* enteritidis and *Salmonella* typhimurium were 75.7% and 4.3%, respectively [8]. In the research from Duhok, reported that 4.85% of the eggshell samples were contaminated with *Salmonella* spp. None of the egg contents samples was infected with *Salmonella*, and *Salmonella* enteritidis and *Salmonella* typhimurium were 10 and 5 strains, respectively [31]. The main serotypes have different prevalence in different countries. In a study from Australia, they showed *Salmonella* typhimurium was as the main factor for egg-borne disease [22]. In a research from Mashhad, It was confirmed that the isolated colonies are *Salmonella*, and their serovar was determined as Typhimurium and showed that the most common egg *Salmonella* isolate in Iran is *Salmonella* typhimurium [40]. In this study, all the isolates were identified as *Salmonella* typhimurium and the reason for the different prevalence of serovars depend on different geographical areas.

This study provides valuable preliminary information on the prevalence of *Salmonella* in eggs and it can be used to assess the risk of human food contamination associated with the consumption of contaminated eggs

CONCLUSIONS

Human disease caused by *Salmonella* enterica due to egg consumption is a long-recording issue. Our study analyzes the prevalence of *Salmonella* from industrially tray eggs and packaged eggs, an important foodstuff. The results showed that the rate of *Salmonella* contamination was 10%, detected from the egg contents. All isolates belonged to *Salmonella* typhimurium serovar. These results provide useful information for improving *Salmonella* control strategies in eggs as well as laying hens and reducing *Salmonella* contamination in laying hens will be effective in reducing egg contamination.

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ETHICAL CONSIDRATION

This paper is the result of the MA thesis of the Department of Food Health and Safety of Qazvin University of Medical Sciences with the code of ethics IR.QUMS.REC.1398.291.

Conflict of interests

The authors declare that there are no competing interests associated with the manuscript.

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