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PREVALENCE OF *SALMONELLA* STRAINS ISOLATED FROM INDUSTRIAL QUAIL EGGS AND LOCAL DUCK EGGS, IRAN

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ABSTRACT

Salmonella is a worldwide public health issue as one of the reasons for foodborne illness for humans and animals. Eggs can be a significant source of this bacterium and the prevalence of salmonellosis. Thus, the control of contamination by *Salmonella* has become essential for the consumer. This study investigates the prevalence, and serotype distribution of *Salmonella* isolates recovered from industrial quail eggs and local duck eggs collected from Qazvin city, Iran, in 2020. In this cross-sectional study, 130 eggs were collected randomly (including 100 industrial quail eggs and 30 local duck eggs) from the retail and stores in Qazvin city, Iran. *Salmonella* was isolated from eggshells and egg contents using conventional culture methods for selective isolation of *Salmonella* and biochemical identification, suspect colonies confirmed by Real-Time PCR assay for the amplification and detection of *Salmonella* using specific primers. A 16.67% prevalence of *Salmonella* was observed from duck eggs; however, no *Salmonella* recovered from quail eggs. *Salmonella* was isolated from 0% (0 groups of 6 groups) and 16.67% (1 group of 6 groups) of eggshells and contents of duck eggs, respectively. Isolates from positive egg samples characterized as *S. Typhimurium*. Although *Salmonella* infection was low in this study, Continuous monitoring is required to prevent health hazards associated with poultry products in this area, and the presence of duck eggs can be a public health problem. The results of this study are essential for the government, consumers, regulators of poultry products, producers like poultry farmers.

1.Introduction

Salmonella is one of the significant foodborne enteric pathogens globally and causing enormous economic losses in the poultry industry. Non-typhoidal *Salmonella* causes 4.07 million Disability Adjusted Life Years (Huang et al., 2016; Kirk et al., 2015). The serotype is a phenotypic trait according to which *Salmonella* is divided into groups A, B, C, D and *Salmonella* with over 2600 serotypes is a widespread zoonotic pathogen (Abdel-Maksoud et al., 2015; Hai et al., 2020).

Salmonella enterica serovar *Typhimurium* and *Salmonella enterica* serovar *Enteritidis* are the most current causes of non-typhoidal salmonellosis throughout the world (Lee et al., 2015); *S. Typhimurium* and *S. Enteritidis* are

causing gastroenteritis and, severe systemic infections may occur in infants, the elderly, and immunocompromised individuals for an instant the HIV-positive, diabetics or rheumatoid arthritis cases (Bonny et al.; Ceyssens et al., 2015; Lee, Runyon, Herrman, PhillipsHsieh, 2015). *Salmonella* is more dangerous in people under the age of 20 (children) and over 70 (elderly) than in other ages (Danesh Ghohar et al., 2017; Nadi et al., 2020).

Salmonella serovars cause of foodborne has a different prevalence in the various times and regions. For example, *S. Typhimurium* caused an outbreak in Australia, while *S. Enteritidis* caused the spread in Europe and United States (J. R. Andrews et al., 2015; Chousalkar et al., 2017; El-Tayeb et al., 2017).

Contamination of egg contents by *Salmonella* can occur in two ways, including 1) contamination of the egg content or vertical transmission before shell formation laid by invasion to ovaries and oviducts, and 2) contamination of the shell surface or horizontal transmission after laid with *Salmonella* infiltration into eggshell membranes after infection oviposition (De Vylder et al., 2013; Gantois et al., 2009).

Salmonellosis is mainly related to the consumption of meat, poultry, eggs and milk, and therefore this organism is a pathogen transmitted through food (Khodadadipour et al., 2016).Eggs and egg products are a significant part of the human diet. Eggs such as Quail eggs are tasty and have much nutritional value, a little fat content (Mir et al., 2015), and they can be a source of foodborne diseases, such as salmonellosis outbreaks worldwide (Moffatt et al., 2016). Non-typhoidal *Salmonella* strains (NTS) separated from eggshell and egg contents of quail and duck in different countries like India, Nigeria, Egypt and other countries (Ashraf et al., 2013; Harsha et al., 2011; Nwaobi et al., 2016; Routhu, 2019; TURGAY, 2004). Duck eggs and duck products were associated with *S. enterica* serovar *Typhimurium* outbreaks in Germany, England (UK) and Northern Ireland at different times (Noble et al., 2012; Owen et al., 2016).

Efficient, rapid and specific methods like DNA methods for detecting and identifying various pathogens such as *Salmonella* species with 95 bp products in a different kind of food are significant for clinical and reporting aims (Adzitey et al., 2012). Polymerase chain reaction (PCR) is a molecular biology technique with high sensitivity. The invA gene of *Salmonella* in the mammalian epithelial cells contains sequences unique to this genus, and it is an appropriate PCR target by a potential diagnostic application (Ashraf, Ahmed, Aisha, FatmaMohammed, 2013).

Due to the low economic conditions of society, high egg consumption, the possibility of egg contamination risk of *Salmonella* for the community, a little information about the distribution of strains in eggs. In this regard, this study aimed to evaluate the prevalence of *Salmonella* contamination and characterization of serotypes in industrial quail eggs and local duck eggs supplied from retail markets of Qazvin, Iran.

2. Materials and methods

2.1. Materials

We collected 130 eggs, including 100 industrial quail eggs and 30 local duck eggs. Samples were collected from retail and stores of Qazvin, Iran, in 2020 (every 100 eggs represented by 20 samples, five eggs constitute one sample). Samples were placed in a separate sterile bag and immediately transferred to the laboratory in cool boxes. The eggs were stored under sterile conditions at four °C until being analyzed.

2.2. Methods

2.2.1. Isolation and detection of Salmonella

The eggs were prepared as described by Bacteriological Analytical Manual (W. H. Andrews et al., 2011). 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, DIAGNOSTIC, 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. The egg was

disinfected with a disinfectant solution to investigate the *Salmonella* contamination under eggshell and egg contents. The disinfection solution Prepared by adding 250 ml iodine/potassium iodide solution to 750 ml 70% alcohol solution and mixed well. Submerge eggs in disinfection solution for 10 seconds (ensured not less than 10 seconds). The eggs were removed from the solution and air-dried in a sterile chamber, then cracked with a sterile knife. Each egg contents were thoroughly mixed. Then, the sample was 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 Rappaport Vassiliadis (Scharlau, Spain) (incubated at 37°C for 24 h). The RV cultures were streaked onto xylose lysine deoxycholate (LIOFILCHEM, DIAGNOSTIC, ITALY) plates (Selective media) and incubated at 37 °C for 24 h. In the XLD cultures, Salmonella has colonies with black centres. Presumptive colonies were processed and identified by biochemical tests like triple sugar iron agar slant (Scharlau, Spain) and urea agar (LIOFILCHEM, DIAGNOSTIC, ITALY) then incubated at 37 °C for 24 h. (Figure 1). A selective medium of TSIA slant was used to detect the lactose, saccharose and dextrose fermenters and determine the organisms to produce H2S. Black slant and yellow butt or pinkish slant and yellow butt were recorded as the positive reaction for *Salmonella*. The colour of urea agar was yellow (Negative result).

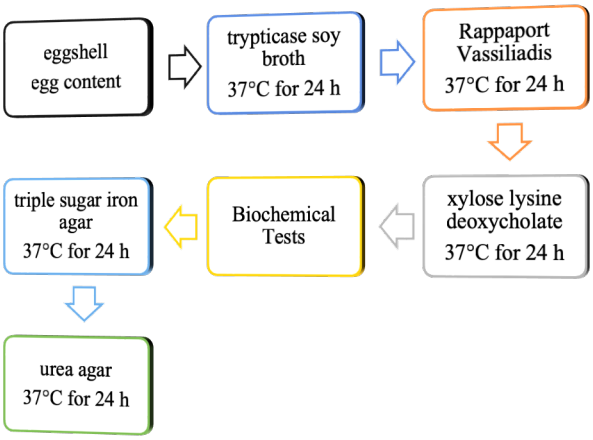

Figure 1. Isolation and identification of *Salmonella*.

2.2.2. Preliminary identification of Salmonella by PCR

The specific primer InvA was used to detect *Salmonella* (Heymans et al., 2018). Primers were used based on the InvA gene sequence designed using Primer-BLAST software and NCBI gene bank. SINACLON, Iran, synthesized the primers. Primers listed in Table 1.

Table 1. Primers designed to detect *Salmonella*.

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

2.2.3. Real Time PCR

Total genomic DNA was extracted by using a pathway boiling. PCR method was used to verify and the 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 (Heymans, Vila, van Heerwaarden, Jansen, Castelijn, van der VoortBiesta-Peters, 2018). A Real-time PCR (Rotor-Gene Q) device was used by a 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

2.2.4. Data analysis

All data analyses were performed using SPSS Statistical Software version 25. The dependent variable used in the study included the incidence of *Salmonella*. Independent variables included testing eggshells and egg content. The Chi-square test was used to compare the incidence of *Salmonella* to different variables. The results were considered significant by a P-value < 0.05.

3.Results and discussions

3.1. Prevalence and serotypes

A 16.67% prevalence of *Salmonella* was observed from duck eggs, and no *Salmonella* was recovered from quail eggs of two different groups collected randomly. *Salmonella* was isolated from 0% (0 groups of 6 groups) and 16.67% (1 group of 6 groups) of eggshells and contents of duck eggs, respectively. The isolate identified from positive egg samples were *Salmonella typhimurium* serotype (Figures 2). Shell contamination was significantly less than

content contamination (Table 3). There was a significant relationship between contamination in duck and quail samples and there was a significant relationship between contamination in shell and contamination in contents of duck eggs.

Table 3. Distribution and sources of *Salmonella* isolated.

Sample			Quail egg shell	Quail egg content	Duck egg shell	duck egg content	Total
positive	**pos**	Count	0	0	0	5	5
		% Within egg	0.0%	0.0%	0.0%	16.7%	1.9%
	not	Count	100	100	30	25	255
		% Within egg	100.0%	100.0%	100.0%	83.3%	98.1%
Total		Count	100	100	30	30	260
		% Within egg	100.0%	100.0%	100.0%	100.0%	100.0%
Mean			1.9808				
Std. Deviation			.1376				

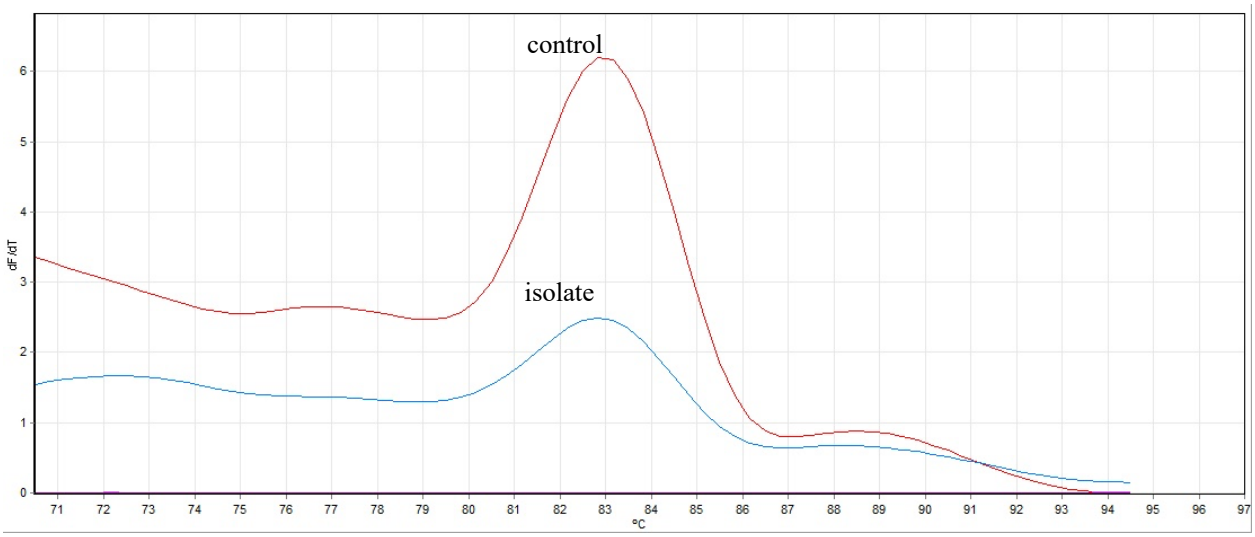

Figure 2. Melting curve analysis of SYBR Green real-time PCR product of *Salmonella Typhimurium*.

3.2. Egg contamination with different *Salmonella* strains and serotypes

Salmonella is one of the reasons foodborne diseases in humans, and salmonellosis is caused by eggs infected with this pathogen and is a significant public health issue worldwide (Rahman et al., 2019). Liquid egg products are implicated in an extensive range of foods that may not receive enough heat treatment' like pasteurization, thus causing contamination of the latest production (TURGAY, 2004).

Salmonella has been isolated from quail eggshells from Indonesia and India (Erina et al., 2019; Harsha, Reshmi, Varghese, Divya, Rahiman Hatha, 2011). also, it has been isolated from quail egg content from different regions, including Nigeria, Iran, and Turkey (Atere et al., 2015; Nwaobi, Kwaga Okolocha, 2016; Hamid Staji et al., 2012; TURGAY, 2004). The prevalence in these studies was higher than in our study. This contamination could be due to environmental contamination and poor storage conditions within the study areas. *Salmonella* has not been isolated from quail eggs from Nigeria, India and Iran which these results agree with our studies (Badouei et al., 2012; Bose et al., 2020; Sangeetha et al., 2019). Quail are reported to be more resistant to infectious diseases than chicken, so it effectively reduces the prevalence of infection and even the absence of infection in eggs (Routhu, 2019). No contamination observed in this study may be due to increased knowledge on the prevention and control of poultry illness.

Some studies have reported infection with *Salmonella* serotypes both in duck eggshells and contents for instance, in a study in India, *Salmonella* contamination was detected in duck egg content (16.66%) and duck eggshell (6%) in which contamination of duck eggshell was higher than the prevalence rates of our study (Harsha, Reshmi, Varghese, Divya, Rahiman Hatha, 2011). These results show that *Salmonella* contamination in retail duck eggs in India is a severe public health concern at that time. Some studies have not reported infection with *Salmonella* serotypes in duck eggshells and contents like in Malaysia and Iran (Adzitey, RusulHuda, 2012; Badouei, GhalejooghiMadadgar, 2012; Sarif et al., 2012).

At the same time, the prevalence of *Salmonella* contamination in duck eggs identified in this study is higher than that reported in duck liver and Fecal swabs in Egypt by a prevalence of 8.3% and 4%, respectively also, the different prevalence of *Salmonella* contamination in local and industrial eggs reported in Asia (Hai, Yin, Lu, Lv, Zhao Bie, 2020; Rahman, Ahmad, Mahmud, Barman, Haque, Uddin Ahmed, 2019; Xie et al., 2019). The differences in the prevalence of *Salmonella* contamination in these studies is due to the different geographical areas, differences in the environmental and breeding conditions, C&D (Cleaning and disinfection), nonmetal duck houses, regardless of the production cycle, rodent control, a relationship of birds with large animals, health and safety of stores and retail, methods of isolation, humidity and temperature associated with the climate although flock size (Small, Medium, Large), dog presence, avian influenza history, and distance to the nearest poultry farm did not significantly affect Salmonella prevalence (Kim et al., 2021). Strategies that are effective for food safety and ultimately the protection of public health are monitoring and good hygiene practices like the development of the management system of breeding places and shops and retail (places, tools and methods of regular cleaning and disinfection) and the use of environmental health standards (dry and clean place of breeding and sales (Chen et al., 2020).

The prevalent serotypes detected in our study was *S. Typhimurium*, which is similar to research that has been done on eggs, duck, duckling and duck eggs around the world (Adzitey, RusulHuda, 2012; Ashraf, Ahmed, Aisha, Fatma Mohammed, 2013; Chen, Bai, Wang, Zhang, Zhan, Shen, Zhang, Wen, Gao Liao, 2020; Lenchenko et al., 2020; Sodagari et al., 2019; H Staji et al., 2017; Wang et al., 2020). Due to geographical location, climatic conditions and type of test method, the distribution of serotypes in the world is different (Han et al., 2020).

The real-time PCR assay is being used as a rapid and reliable tool for controlling and detecting contaminated *Salmonella* samples along the food production chain. The invA invasive gene has been offered as an international standard for detecting *Salmonella* in egg and egg products or food chains by PCR. Thus, invA can act as a reliable and accurate gene for diagnosing *Salmonella* by PCR, and PCR showed that the isolates were *Salmonella typhimurium* (Cheng et al., 2008; Gole et al., 2014; Malorny et al., 2003; Malorny et al., 2004). The study from Bangkok detected *Salmonella* from the eggshell using the invA gene (Loongyai et al., 2010). In this study, *Salmonella* invA invasive gene was also used to detect *Salmonella.*

Like other bird species, healthy ducks can be a source of *Salmonella*, and their gastrointestinal tract and faeces are infected with this pathogen (Adzitey, RusulHuda, 2012), show no signs of infection, and transmit the pathogen to other humans and animals. Also, the consumption of raw or almost cooked eggs can lead to salmonellosis, and this disease causes economic losses through disease and death (Muhammad et al., 2010).

Contamination of egg contents by *Salmonella* can occur in two ways, including 1) direct contamination before shell formation laid, and 2) indirect contamination after laid along the storage time like lack of hygiene at the layer farms, improper washing, grading and packing operations (Pärn et al., 2017). In this study, surface contamination of eggshells was not seen in two sample groups, but internal contamination of egg contents was seen in local duck egg samples, which shows direct contamination of eggs during formation in the reproductive tract.

4. Conclusions

In summary, our findings showed that *Salmonella* was identified in a group of duck eggs. A positive sample infected with *Salmonella* was detected to *Salmonella typhimurium*, and *Salmonella* was not identified in quail eggs. This study can be helpful in risk management options, risk assessment, regulators about food safety.

Our study has a limitation. One of the reasons for the lack of duck eggs was the beginning of autumn, so sampling in early summer and the tropical months of the year is recommended that the more samples, the more accurate the conclusion will be.

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