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Molecular Detection of *Salmonella* Isolated From Eggs and Egg-based Products

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ABSTRACT

Salmonellosis in human can be caused by consumption of infected eggs. This study aimed to investigate the prevalence of *Salmonella* spp. in eggs and some egg-based products in Sohag city, Egypt. A total of 120 samples of hen's eggs (Baladi & farm), mayonnaise and cream cake (Jatooh) were examined for conventional and molecular detection of *Salmonella* spp. Every 3 eggs were combined as one egg sample, which was then divided into an egg-shell sample and an egg-content sample. The 120 samples were divided into egg-shell Baladi, egg-content Baladi, egg-shell farm, egg-content farm, mayonnaise and cream cake (20 each). All of the samples were subjected for conventional enrichment culture and biochemical methods. A 25 g sample of each sample was added to 225 ml of 1% buffered peptone water and incubated at 37°C for 24 hours, one ml of the enriched media was then transferred to Rappaport-Vassiliadis selective broth and incubated at 40°C for 48 hours. A loopful was then streaked on Salmonella-Shigella (S.S.) agar to look for colorless colonies with a black center. Biochemical identification was then performed using triple sugar iron agar test, lysin iron agar test and urease test. The results of conventional culture showed that 30% of egg-shell Baladi, 10% of egg-content Baladi, 20% of egg-shell farm and 15% of egg-content farm samples were positive for *Salmonella.* However, all of the mayonnaise and cream cake samples were *Salmonella*-free. The *Salmonella* isolates were then molecularly identified using PCR. The results by PCR analysis showed that *Salmonella* spp. were detected from 30% of egg-

shell Baladi, 5% of egg-content Baladi, 15% of egg-shell farm and 10% of egg-content farm samples. *Salmonella Enteritidis* was isolated from only one sample of egg-shell Baladi and only one sample of egg-content farm, while no *Salmonella Typhimurium* was isolated from any of the samples. It is important to apply control measures at all the levels of food chain, including preventing cross-contamination by separating the raw and the cooked products.

INTRODUCTION

Eggs are proteinous food containing vitamins and minerals needed in human nutrition except vitamin C [1], while contaminated eggs have been associated with public health hazards like salmonellosis.

Salmonellosis is a foodborne disease all over the world, and the animal-derived foods, particularly poultry, poultry products and eggs, have been involved in human salmonellosis [2].

Contaminated eggs, whether the shell or the content, have been recognized as one of the important sources of *Salmonella* [3]. Contamination of eggs with *Salmonella* is affected by some factors and variables, requiring appropriate management strategies. The pathways for egg contamination with *Salmonella* were occurred by direct contamination during egg development in hen's ovary and oviduct; and then indirectly after egg laying [3,4]. The factors affecting the egg contamination can be production process, storage, handling and then food preparation [5,6,7,8].

Salmonella is an important organism that can cause food poisoning producing various clinical syndromes [9]. Salmonellosis in humans is occured by several serotypes through food ingestion [10]. The most common serotypes that involved in salmonellosis and involved by poultry meat and egg consumption are *Salmonella Enteritidis and Typhimurium* [11, 12].

Salmonella can grow in a wide range of temperature from minimum 5° C to maximum 47° C with an optimum at 37° C, and the same time, it is heat sensitive that readily destroyed at pasteurization [13].

The routes of *Salmonella* transfer into the egg are vertical that occured before ovulation through direct contamination of whites, yolks, membranes or shells; and horizontal through penetrating the infected intestine or stool [14].

The shift of egg production from ordinary battery cages into free-range poultry farms [15], in addition to the shift in consumption habits with raw foods [16,17]; and the increase of unprocessed food popularity that contain raw eggs like, certain sauces, mayonnaise and raw egg based desserts such as ice-cream, potentially elevates the salmonellosis risk [17,18,19,20].

Therefore, the current investigation was planned to study *Salmonella* contamination in eggs and some egg-based products with molecular identification.

MATERIALS AND METHODS

Collection of the samples:

A total number of 120 samples including egg-shell Baladi, egg-content Baladi, egg-shell farm, egg-content farm, mayonnaise and cream cake (20 each). Each an egg-shell Baladi sample and an egg-content Baladi sample were delivered from 3 Baladi eggs together (as every 3 eggs constituted for one sample). Also, the egg-shell farm samples and the egg-content farm samples were represented in the same manner.

The egg-based products' samples were represented in the mayonnaise and cream cake (Jatooh) samples; as mayonnaise for egg-based condiments and cream cake for egg-based desserts. All the samples were obtained in a random manner from different locations in Sohag city, Egypt.

Preparation of egg samples [21]**:**

Each 3 eggs (either Baladi or farm) were collected aseptically in an individual plastic bag, and handled as one egg sample for detection of *Salmonella* on egg-shells and then in the contents, in which, the 3 eggs were sunken in 10 ml of sterile buffered peptone water (BPW) in their bag, rubbed for 2 minutes and then taken as an egg-shell sample. After that, the 3 eggs were then flamed on their broad end and aseptically broken and their contents were taken into a sterile bag and thoroughly homogenized as an egg-content sample.

Isolation of *Salmonella* [22,23]**:**

An amount of 25 g was taken from the sample, followed by pre-enrichement in 225 ml of buffered peptone water (BPW) 1% and then incubation for 24 hours at temperature 37 °C. After that, 1 ml was transferred into 10 ml Rappaports-Vassiliadis (RV) selective enriched broth with incubation for 48 hours at temperature 40° C

A loopful from the incubated RV broth was streaked on Salmonella Shigella (S.S.) agar selective plates, followed by incubation for 24 hours at temperature 37° C for colorless colonies with black centres that were suspected to be *Salmonella*. The suspected *Salmonella* colonies were subcultured into nutrient slope for biochemical screening tests as sugar fermentation, H_2S production using triple sugar iron, lysine decarboxylation, urea splitting in Christensen's urea agar [24].

Molecular detection of *Salmonella*:

A multiplex PCR with 3 sets of primers was that used for *Salmonella* detection and for the most common serotypes of *Salmonella* enterica as *Sal. Typhimurium and Sal. Enteritidis*. This is a very rapid and simple molecular method for serotyping common *Salmonella*, the specific sequence could be detected in all *Salmonella* enterica serotypes.

Pure colonies were subjected to identification using m-PCR for *Sal. Enteritidis* and *Sal. Typhimurium* that was run in the Molecular Biology Unit of the Molecular Biology Researches and Studies Institute (MBRSI), Assiut University, Egypt.

Table 1. Primers for m-PCR identification of the most frequent *Salmonella*

Primer	Length (nucleotides)	Primer sequence (from 5 to 3)	Size	Reference
ST 11-F (1)	24	F: 5′ GCCAACCAT TGCTAAATT GGCGCA 3′	429	[25]
ST 15-R (1)	25	R: 5′ GGTAGAAAT TCCCAGCGG GTACTGG 3′		
Fli15-F (2)	22	F: 5′ CGGTGTTGC CCAGGTTGGTAAT 3′	559	[26]
Tym (2)	22	R: 5′ ACTCTTGCT GGCGGTGCG ACTT 3′		
S1-F (2)	20	F: 5′ GCCGTA CACGAG CTTATAGA 3′	250	[25]
S4-R (2)	20	R: 5′ ACCTAC AGGGGCACA ATAAC 3′		

The three Primers' sets that used in this study were (ST11-F and ST15-R) primer as an universal primer for detection of all *Salmonella* species, (Fli15-F and Tym-R) primer for *Salmonella Typhimurium* identification and (S1-F and S4-R) primer for *Salmonella Enteritidis* identification, according to Soumet *et al*. (1999) [25, 26] (Table 1).

The protocol used for boiling extraction of genomic DNA from the cells was applied according to Schmitt and Pawlita (2009) [27]**:**

During the extraction of DNA, the samples should be protected from exogeneous contaminations as well protect the staff and workers from being infected with dangerous substance. For this purposes, All steps of DNA ectraction must be done in DNA Cabinets (containing UV light and circulating air filtered systems) and also we must use pipette tips with aerosol resistant filters and change the tips at each step of pipetting. Boiling extraction of genomic DNA is done as following:

a. By using (1.5 ml) labelled Eppendorf tubes, the cells that was planned to extract DNA from it (either or trypsinated adherent cells or suspension) must be counted (10^6 to 10^7) and centrifugated (600×g for 5 minutes) in tabletop centrifuge, the cells become in the form of pellet.
b. By using 100 µl of Phosphate Buffered Saline, the pellet is resuspended.
c. Tubes were placed at temperature 95° C for 15 minutes.
d. Recentrifugation at (>10.000×g) for 5 minutes to pellet the cellular debris.
e. The lysate (supernatant) is transferred into a correctly labelled (with sample name) 1.5 ml Eppendorf tubes.
f. Lysate is keeped at temperature (-20 to 4° C).

According to soumet *et al*. (1999), multiplex-PCR was applied in 22 microliter volume which contained the following: 11 microliter of PCR master mix, 1 microliter from each primer and also 5 microliter of the target DNA. The thermocycler settings up were initial denaturation for 5 minutes at temperature 95º C, then 35 cycles of the following: one minute at 95º C, one minute at 48º C and one minute at 72º C, then the final extension for 10 minutes at 72º C. After amplification, electrophoresis was done by using agarose (1%) [25, 26].

Electrophoresis by agarose gel:

Positive amplification of PCR products were examined by using agarose gel electrophoresis and suitable molecular weight markers [28].

RESULTS

Table 2. *Salmonella* incidence in the examined Baladi hen's eggs

The examined samples	The samples No.	Presumptive isolated *Salmonella*		PCR confirmed *Salmonella*		*Sal. Enteritidis*		*Sal. Typhimurium*		Other *Salmonella* spp.	
		No.	%	No.	%	No.	%	No.	%	No.	%
Egg-shell	20	6	30	6	30	1	5	0	0	5	25
Egg-content	20	2	10	1	5	0	0	0	0	1	5
Total	40	8	20	7	17.5	1	2.5	0	0	6	15

Table 3. *Salmonella* incidence in the examined farm hen's eggs

The examined samples	The samples No.	Presumptive isolated *Salmonella*		PCR confirmed *Salmonella*		*Sal. Enteritidis*		*Sal. Typhimurium*		Other *Salmonella* spp.	
		No.	%	No.	%	No.	%	No.	%	No.	%
Egg-shell	20	4	20	3	15	0	0	0	0	3	15
Egg-content	20	3	15	2	10	1	5	0	0	1	5
Total	40	7	17.5	5	12.5	1	2.5	0	0	4	10

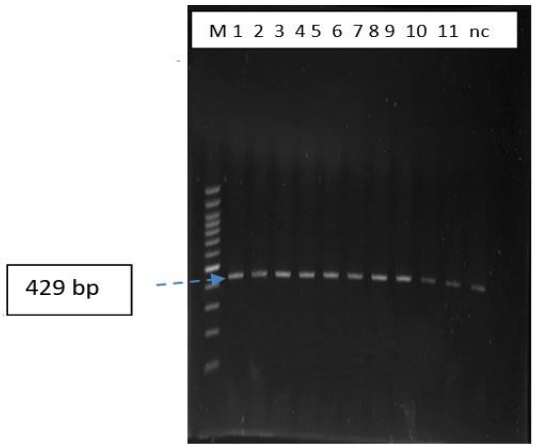

Figure 1. Electrophoresis by using agarose gel (1%) to detect PCR product for the isolated *Salmonella* spp.

M= (100 bp) for DNA marker

Lanes from 1 to 11: positive for *Salmonella* spp. at (429 bp)

Negative control= nc

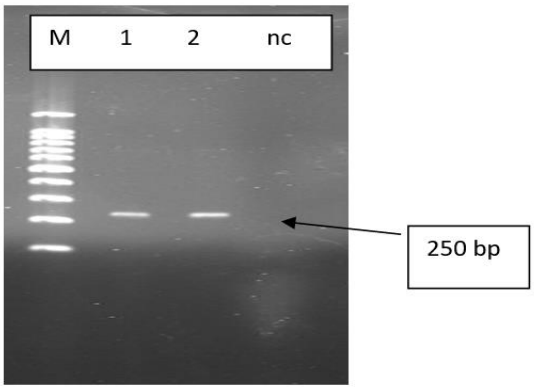

Figure 2. Electrophoresis by using agarose gel (1%) to detect PCR product for the isolated *Sal. Enteritidis*

M= (100 bp) for DNA marker

Lanes 1 & 2: positive for *Sal. Enteritidis* at (250 bp).

Negative control= nc

DISCUSSION

Epidemiologically, there was a link between *Salmonella* presence in poultry products and salmonellosis in humans [29].

Eggshell surfaces might be contaminated from carrier poultry. Furthermore, as a result of poor management by Egyptian farmers, shells can be contaminated by the excreta of farm animals living alongside Baladi chickens.

Dhillon *et al*. (1974) [30] reported that contamination the surrounding area, environment, water and intestine are the main cause of shell contamination.

In the present study, the obtained results cleared that *Salmonella* spp. was isolated from 30 and 5% of the egg-shell and egg-content samples of the examined Baladi hen's egg, respectively (Table 2).

The results showed by El-Prince *et al*. (2019) [31] revealed that *Salmonella* was detected in percentages of 8.58 and 1.43% in Baladi hen's egg-shell and content respectively, and *Sal. Enteritidis* and *Sal. Typhimurium* were recovered from egg-shell in percentages of 2.86 and 1.43%, respectively, while in egg-content *Sal. Enteritidis* was isolated from 1.43% and no *Sal. Typhimurium* was isolated.

Lower result was by El Jakee *et al*. (2016) [32] that *Salmonella* spp. were isolated from 1.3% of samples. *Sal. Enteritidis* and *Sal. Typhimurium* were isolated in a percentage of 0.6 and 0.7%, respectively.

El Sherif and Hassan (2013) [33] revealed that *Salmonella* spp. was isolated from Baladi egg-shell and content with percentages of 10 and 8%, respectively. *Sal. Typhimurium* was detected in 3.33 and 6.66% in egg-shell and content while *Sal. Enteritidis* was detected in 3.33% for both. On the other hand, *Salmonella* failed to be isolated from Baladi hen's egg-content by Msallam (2008) [34], Arif (2013) [35].

This variability may be due to hens health as transovarian transmission of *Salmonella* to eggs and degree of shell contamination and consequently penetration of the shell [36]. This indicated that eggs were contaminated either during laying or from contact with the contaminated surrounding [31].

In the present work, the obtained results revealed that in the farm hen's eggs, *Salmonella* was isolated from 15 and 10% of the egg-shell and egg-content samples, respectively (Table 3).

Min Chan *et al*. (2015) obtained similar results [37], in which *Salmonella* was isolated from 17.2% of egg-shell and 5.2% from internal content and no *Sal. Typhimurium* or *Sal. Enteritidis* were isolated. Almario (2014) [38] revealed that *Salmonella* spp. was recovered from 10.7% of farm hen's egg-shell and all isolates were identified as *Sal. Typhimurium*.

Zubair *et al*. (2017) [39] showed that *Salmonella* incidence was 4.85% in egg-shell while could not isolated from egg-content, and out of 17 positive egg-shell 3 different serotypes were identified as *Sal. Enteritidis* (ten strains), *Sal. Typhimurium* (five strains), *Sal. Typhi* (two strains).

Shah *et al*. (2021) [40] revealed that *Salmonella* contamination in eggs-shell was found as 4.5%, while in eggs-content was 8%. *Salmonella enterica Enteritidis* was found as 3.5% from both egg-shell & albumin contents and 3% from yolk-content; whereas,

Sal. Typhimurium was found as 1% from egg-shell, 1.5% from albumin contents. *Sal. Typhimurium* could not be isolated from egg-yolk.

In another study by Shahzad *et al*. (2012) [41], *Salmonella* prevalence was recorded as 34.12 and 12.69% in eggs-shell and in egg-content, respectively.

When through the light towards the egg-based products, none of the examined mayonnaise and cream cake (Jatooh) samples were positive for *Salmonella*. Similar results were showed by Gumus *et al*. (2005) [42]. Meldrum *et al*. (2006) [43] and Siriken *et al*. (2009) [44] as *Salmonella* spp. could not be detected in any of cream cake.

Higher results were detected by Toni (2020) [45] in which *Salmonella* spp. was found in 5 samples (10%) of cream cake and *Sal. Enteritidis* was identified in 2 samples and *Sal. Infantis*, *Sal. Malade* and *Sal. Tamale* were detected in the rest 3 positive samples (1 each).

El-Prince *et al*. (2019) [31] found only one sample of cream cake was contaminated with *Sal. Enteritidis*, and all mayonnaise was *Salmonella* free.

Higher incidence was showed by Can *et al*. (2014) [46] as *Salmonella* spp. was in 16% of cream cake produced in Turkey.

According to NSW Food Authority and HPA (2009) [47], all positive samples were unsatisfactory with high microbiological risk and unfit for human consumption as *Salmonella* spp. should not be detected in 25 g of tested samples.

Because cream cake is one of the egg-based products, presence of *Salmonella* spp. may be attributed to contaminated eggs either from the shell or the contents [48]. Moreover, cream layer or cross-contamination and subsequent keeping of cakes at ambient temperatures for long period before consumption could play a role in contamination with *Salmonella* [49].

El-Gendi and Amin (2019) [50] revealed that there no *Salmonella* was isolated from commercial mayonnaise while in small producers mayonnaies 3.75% was positive for *Sal. Enteritidis*.

In heat treatment absence, adding more vinegar represents the primary safety factor, which lowering the pH. Garlic addition also can lower total bacterial content [51]. Lemon (citric acid) also may have an important role in lowering *Salmonella* in commercial mayonnaise [52]. Lock and Board (1996) reported that adding vegetable oil may also affect the survival of *Salmonella* in mayonnaise [53].

It should be throw the light towards low *Salmonella* concentrations can still cause infection [54].

Conventional bacterial methods for the identification of *Salmonella* are time-consuming and do not provide information on serotypes. Because PCR-based approaches use primers that specific to *Salmonella* , DNA sequences could not be amplified if it was from other species. As a result, molecular approaches have been particularly successful and beneficial for detecting *Salmonella* strains in foods [46].

CONCLUSION

Salmonella was isolated from egg-shells and egg-contents of both Baladi and farm hen's eggs, but could not detected in both of mayonnaise and cream cake (Jatooh). *Sal. Enteritidis* was detected in egg-shells and contents, while *Sal. Typhimurium* could not detected at all.

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