Journal of Advanced Veterinary Research

https://advetresearch.com/index.php/avr/index

Incidence of *Salmonella* species in Table Eggs and some Egg-based Products

Enas El-Prince[1], Mahmoud F. Hussein[2], Amira M. Abd El-Rahman[2]

[1]Department of Food Hygiene, Faculty of Veterinary Medicine, Assiut University, Egypt
[2]Animal Health Research Institute, Assiut, Egypt

ARTICLE INFO

Original Research

Received:

09 October 2018

Accepted:
01 January 2019

Keywords:

Salmonella
Table eggs
Mayonnaise
Cream cake
Custard

ABSTRACT

The incidence of *Salmonella* species was determined in 1050 eggs including balady hen's eggs, farm hen's eggs and duck's eggs (350 eggs each represented by 70 samples as every 5 eggs constitute one sample) and in 90 samples of egg-based products including mayonnaise, cream cake and custard (30 each) were collected from different localities in Assiut city, Egypt. *Salmonella* was recovered from 8.58, 5.72% of balady hen's egg shells using Xylose Lysine Deoxycholate (XLD) agar and Salmonella Shigella (SS) agar, respectively and could be isolated from egg content in a percentage of 1.43% by XLD agar. Different serotypes of *Salmonella* were isolated from shells of balady hen's eggs including *S. typhimurium*, *S. anatum*, *S. infantis*, *S. kentucky* while, *S. enteritidis* was the only serotype that recovered from both shell and content. In case of farm hen's eggs, *S. kentucky* and *S. infantis* could be identified from positive shell and content samples, respectively at same percentage of 1.43% by using XLD agar. On the other hand, *Salmonella* could not be detected on SS agar from both shell and content of all examined samples. Concerning duck's eggs 4.29 and 1.43% of shell samples were contaminated with *Salmonella* by using XLD and SS, respectively. While, 2.86 and 1.43% of examined egg content samples were positive using XLD and SS agar, respectively and *S. typhimurium* was the predominant serotype which isolated from both shell and content samples. While, *S. infantis* was recovered from shell only and *S. kentucky* was isolated from content only. *Salmonella* species were existed in 2 (6.66%) and 1 sample (3.33%) of the examined cream cake using XLD and SS agar, respectively while, none of the examined custard and mayonnaise samples were positive for *Salmonella* on both media. *S. kentucky*, S. shubra and *S. enteritidis* were isolated from the positive cream cake samples with an equal incidence of 3.33 % for each. Although XLD agar was found to be comparatively better in recovering *Salmonella* species than SS agar, the two media were found to be complementary to each other for recovering different *Salmonella* serotypes. Detection of common *invA* gene in all isolated *Salmonella* serotypes by PCR assay showed positive amplification of 284 bp fragment specific for the *invA* gene with total percentage of 100%. Screening of 12 isolates of *S. typhimurium* and *S. enteritidis*, which were the most prevalent serotypes in the positive samples for *stn*, *hilA* and *fimH* virulence genes by multiplex PCR revealed varying distribution pattern. The public health hazards and the recommended measures required to prevent contamination of eggs and its based products by *Salmonella* were discussed.

J. Adv. Vet. Res. (2019), 9 (1),1-7

Introduction

For thousands of years, eggs and its products have represented an important part of human diet as they are easy to obtain and they are rich in nutrients, containing proteins, minerals, fats, vitamins and more. While eggs are highly nutritious for humans, they are also nutritious for other living organisms. Just as the yolk provides nutrients to a growing embryo, it is also a nutritional resource for bacterial organisms when they cross egg shells and membranes. Scientific Committee on Veterinary Measures relating to public health has identified eggs and egg-based products containing raw eggs as a food group, which pose a public health hazard (European Commission, 2003). Eggs and egg-based products that are improperly handled can be a source of foodborne microorganisms, such as *Salmonella*, which is one of the most prevalent causes of foodborne illness (Howard *et al*., 2012; Galis *et al*., 2013). It has been the second after *Campylobacter* most commonly recorded cause of zoonotic disease in Europe. In 2012, a total of 91,034 confirmed cases of human salmonellosis were reported in the European Union, the EU notification rate for confirmed cases was 22.2 per 100,000 populations and the case-fatality rate was 0.14% as 61 deaths due to non-typhoidal salmonellosis (European Food Safety Authority, 2014). The genus *Salmonella* comprises two species, the first one is *S. enterica*, which is divided into six subspecies. The second species named *S. bongori* (formerly called *S. enterica* subspecies *bongori V*). Species and subspecies can be distinguished on the basis of characters and antigenic formulae into 2501 serovars (Solari *et al*., 2003). However, a recent report from the Centre for Infectious Disease Research and Policy classifies members of the *Salmonella* species into more than 2541 serotypes

*Corresponding author: Enas El-Prince
E-mail address: enas6262@yahoo.com

ISSN: 2090-6277/2090-6269/

(serovars) according to their somatic (O) and flagellar (H) antigens (CIDRAP, 2006). People generally acquire salmonellosis through foodborne exposure and direct contact with infected animals. A variety of investigations of outbreaks and sporadic cases have indicated that the most common food vehicles of salmonella infections in humans are poultry and poultry products, including raw and uncooked eggs (Hennessy *et al*., 2004; Plym and Wierup, 2006). In the United States between 1985 and 2002 contamination of eggs was identified as the source of 53% of all cases of *Salmonella* reported to the Centre for Disease Control and Prevention (Food Drug Administration, 2009). The two most commonly identified causative agents of foodborne salmonellosis are *S. enterica* serotypes typhimurium and enteritidis (Galis *et al*., 2013).

There are two pathways for eggs to become internally contaminated with *Salmonella*, direct contamination during egg formation in hen's ovary and oviduct (vertical transmission). Whereas, indirect contamination occurs after egg has been laid and *Salmonella* contaminating egg from colonized gut or from contaminated feces penetrates through the shell membrane (horizontal transmission) to egg. Such route is facilitated by moist egg shells, storage at ambient temperature and shell damage (De Reu *et al*., 2006; Howard *et al*., 2012). Both *S. enteritidis* and *S. typhimurium* have been demonstrated to have the ability to colonize the reproductive tract of hens (Gantois *et al*., 2008), however, *S. enteritidis* is more frequently isolated from the internal contents of eggs due to its ability to adhere better to reproductive tract mucosa compared to *S. typhimurium* (Wales and Davies, 2011). Although various bacterial pathogens have contaminated chicken's eggs, *Salmonella* accounts for the majority of documented cases (Spitzer, 2016). Certain food items have been specifically associated with cases of human salmonellosis and these include homemade desserts, ice cream and drinks containing raw eggs, shop-bought sandwiches containing mayonnaise or eggs, lightly cooked eggs and fried eggs "sunny-side up" (Molbak and Niemann, 2002). So that, *Salmonella* was isolated from eggs and egg-based products in different countries including Mexico, England, Yemen, Egypt, Japan, Turkey, Korea and Ethiopia by several investigators (Martinez *et al*., 2005; Little *et al*., 2007; Taha *et al*., 2010; Abd El Tawwab *et al*., 2013; Murakami *et al*., 2013; Can *et al*., 2014; Min Chan *et al*., 2015; Tsegaye *et al*., 2016). Considering the facts that emergence of egg associated salmonellosis as a leading foodborne disease, pandemic nature of *Salmonella* and globalized nature of trade and commerce, it is assumed important to study the incidence of *Salmonella* in various types of consumed eggs and its based products in Assiut governorate and to determine the risk associated with this commodity.

Materials and methods

Collection of samples

A total of 1050 eggs including balady hen's eggs, farm hen's eggs and duck's eggs (350 eggs each represented by 70 samples as every 5 eggs constitute one sample). Also, 90 samples of egg-based products including mayonnaise, cream cake and custard (30 samples each) were collected from different localities in Assiut city, Egypt.

Preparation of samples

Egg samples

Egg shells were tested as described by Moats (1980) and contents were prepared and evacuated according to Speck (1984).

Egg-based product samples

Samples were released aseptically from their containers and were prepared according to APHA (1992).

Isolation of Salmonella species

Salmonella species were isolated and identified biochemically according to Andrews and Hammack (2001) and ISO-6579 (2002).

Serological identification of Salmonella

According to Kauffman-White scheme (Kauffmann, 1974) for the determination of somatic (O) and flagellar (H) antigens using *Salmonella* antiserum (DENKA SEIKEN Co., Japan).

Polymerase Chain Reaction (PCR)

PCR was applied for detection of invasion A (*invA*) gene in all isolated *Salmonella* species and for identification of virulence factors including, enterotoxin (*stn*), hyper-invasive locus (*hilA*) and fimbrial (*fimH*) genes in both *S. typhimurium* and *S. enteritidis* by using primers (Pharmacia Biotech) as shown in Table 1.

Results and Discussion

The present study showed that *Salmonella* was recovered from 8.58, 5.72% of balady hen's egg shells using Xylose Lysine

Table 1. List of primers used for PCR amplification

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
InvA (F)	5′ GTGAAATTATCGCCACGTTCGGGCA '3	284	Shanmugasamy *et al.* (2011)
invA (R)	5′ TCATCGCACCGTCAAAGGAACC '3		
Stn (F)	5′ CTTTGGTCGTAAAATAAGGCG '3	260	Makino *et al.* (1999)
Stn (R)	5′ TGCCCAAAGCAGAGAGATTC '3		
HilA (F)	5′ CTGCCGCAGTGTTAAGGATA '3	497	Guo *et al.* (2000)
HilA (R)	5′ CTGTCGCCTTAATCGCATGT '3		
FimH (F)	5′ GGA TCC ATG AAA ATA TAC TC '3	1008	Menghistu *et al.* (2011)
FimH (R)	5′ AAG CTT TTA ATC ATA ATC GAC TC '3		

Deoxycholate agar (XLD) and Salmonella Shigella (SS) agar, respectively (Table 2).

Shell surfaces can be contaminated congenitally from carrier hens, which disseminate the organisms to widely distributed areas that receive the infected eggs. Also shells can be contaminated by excreta of farm animals live with balady chicken in the same place as a bad habit of Egyptian farmers, where Dhillon *et al*. (1974) showed that contaminated water, environment and intestinal tract are the main sources of shell contamination.

The egg shell incidence using XLD agar was in agreement with that indicated by Hussien *et al*. (2009) as 9% and by Rashwan (2013) as 7.83%. While, it was lower than that observed by Adil *et al*. (2012) (34.12%) and El Sherif and Hassan (2013) (26.67%). However, El-Prince (1988); Korashy *et al*. (2008) and El-Kholy *et al*. (2014) failed to detect *Salmonella* in all of examined egg shell samples. Moreover, *Salmonella* could be detected in balady hen's egg content in a percentage of 1.43% by using XLD agar only. Nearly similar result was recorded by El Jakee *et al*. (2016) (1.3%). While, highest percentages were obtained by Osman *et al*. (2011); Adil *et al*. (2012) and El Sherif and Hassan (2013). In the contrary, *Salmonella* species could not be isolated from balady hen's egg content by Msallam (2008) and Arif (2013). This variability may be referred to the health status of hens as transovarian transmission of *Salmonella* to eggs and the extent of shell contamination and the subsequent penetration of the shell (De Buck *et al*., 2004).

Different serotypes of *Salmonella* were isolated from balady hen's egg shells including *S. typhimurium*, *S. anatum* and *S. kentucky* each of 1.43% using both agars. While, *S. infantis* could be recovered from 1.43% of balady hen's egg shell samples using XLD agar (Table 3). Moreover, *S. enteritidis* was recovered from balady hen's egg shells in percentages of 2.86 and 1.43% by using XLD and SS agar, respectively and was isolated from egg contents in a percentage of 1.43% by using XLD agar. *S. enteritidis* is known to have unusual ability to colonize in ovarian tissues of hens and transmitted vertically to be presents within the contents of intact shell eggs, therefore it is not surprising that it was isolated from shells and contents (Olsen and Hammack, 2000). Moreover, Shirota *et al*. (2001) suggested that *S. enteritidis* was more associated with human foodborne disease outbreaks than other *Salmonella* serotypes particularly those associated with egg and egg products which results in more deaths than any other pathogen. Lower *S. enteritidis* incidence (0.6%) from egg content was detected by El Jakee *et al*. (2016), however, Osman *et al*. (2011) showed higher value.

In the case of farm hen's eggs, the incidence of *Salmonella* in shell samples was 1.43% (by using XLD agar only) (Table 2). This indicates that eggs were contaminated either during its lays through the contaminated cloacae or from direct contact with contaminated nest, litter, trays and transport boxes after collection and delivered to markets.

This result agreed to some extent with those of Musgrove

Table 2: Incidence of *Salmonella* species in the examined egg and egg-based products sample

Samples		No. of examined samples	Positive samples			
			XLD		SS	
			No.	%	No.	%
Balady hen's egg	Shell	70	6	8.58	4	5.72
	Content	70	1	1.43	-	-
Farm hen's egg	Shell	70	1	1.43	-	-
	Content	70	1	1.43	-	-
Duck's egg	Shell	70	3	4.29	1	1.43
	Content	70	2	2.86	1	1.43
	Mayonnaise	30	-	-	-	-
	Cream cake	30	2	6.66	1	3.33
	Custard	30	-	-	-	-
Total		510	16	3.14	7	1.37

Table 3. Incidence of different *Salmonella* serotypes in the examined egg and egg- based products sample

Samples	Used media	*Salmonella* serotypes											
		S. typhimurium		*S. enteritidis*		*S. anatum*		*S. infantis*		*S. kentucky*		*S. shubra*	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Balady hen's egg shell	XLD	1	1.43	2	2.86	1	1.43	1	1.43	1	1.43	-	-
	SS	1	1.43	1	1.43	1	1.43	-	-	1	1.43	-	-
Balady hen's egg content	XLD	-	-	1	1.43	-	-	-	-	-	-	-	-
	SS	-	-	-	-	-	-	-	-	-	-	-	-
Farm hen's egg shell	XLD	-	-	-	-	-	-	-	-	1	1.43	-	-
	SS	-	-	-	-	-	-	-	-	-	-	-	-
Farm hen's egg content	XLD	-	-	-	-	-	-	1	1.43	-	-	-	-
	SS	-	-	-	-	-	-	-	-	-	-	-	-
Duck's egg shell	XLD	3	4.29	-	-	-	-	-	-	-	-	-	-
	SS	-	-	-	-	-	-	1	1.43	-	-	-	-
Duck's egg content	XLD	1	1.43	-	-	-	-	-	-	1	1.43	-	-
	SS	1	1.43	-	-	-	-	-	-	-	-	-	-
Cream cake	XLD	-	-	-	-	-	-	-	-	1	3.33	1	3.33
	SS	-	-	1	3.33	-	-	-	-	-	-	-	-

XLD: Xylose Lysine Deoxycholate agar, SS: *Salmonella* Shigella agar

et al. (2005) (1.19%) and Wilson (2007) (1.8%), while, Murchie *et al*. (2007) demonstrated lower incidence of shell contamination in island of Ireland (0.04%). However, the value of *Salmonella* in farm hen's egg shell is lower than that of Almario (2014) (10.7%); Min Chan *et al*. (2015) (17%); and Zubair *et al*. (2017) (4.85%). On the other hand, Favier *et al*. (2001) failed to isolate *Salmonella* from 122 egg shell samples.

In addition, one sample (1.43%) out of 70 examined egg content samples was contaminated with *Salmonella* by using XLD agar. Low incidence of content contamination may be attributed to the antimicrobial effect of egg albumen and also may be due to low contamination rate (Thammasuvimol *et al*., 2006; Van Immerseel, 2010). Also, *Salmonella* could not be isolated on SS agar from both shell and content of all examined farm hen's egg samples. However, *S. kentucky* (one strain from shell) and *S. infantis* (one strain from content) were identified (1.43% for each) as shown in Table 3.

Although, there are relatively low incidence of positive samples in farm hen's eggs the pathogens represent a potential risk to consumers on the basis that all *Salmonella* are potentially pathogenic (Zansky *et al*., 2002; Kabir, 2009).

The incidence of *Salmonella* in examined duck's egg shell samples was 4.29% on XLD agar and 1.43% on SS agar (Table 2). Nearly similar result was obtained by Harsha *et al*. (2011), while, higher values were conducted by Korashy *et al*. (2008) and Suksangawong (2008), however, Rezk and Saleh (2008); Adzitey *et al*. (2012); Nor Faiza *et al*. (2013) and Sedeek and Aioub (2014) could not detect *Salmonella* in all of examined duck's egg shell samples. In case of duck's egg contents, 2.86 and 1.43% of samples were contaminated with *Salmonella* by using XLD and SS agar, respectively. Bad habits of ducks as laying eggs near dirty and damp places, in addition to rapid deterioration of the antibacterial activity of albumen on storage give the chance to raise the rate of contamination in duck's eggs. There is also a practice of eating raw eggs among the villagers as they consider it more nutritious. Hence the prevalence of Salmo¬nella in duck's eggs poses definite threat to unwary consumers.

Concerning *Salmonella* serotypes it is noticeable that, the predominant serotype in duck's eggs was *S. typhimurium* which isolated from 3 shell samples (4.29%) on XLD agar and isolated from content with equal incidence of 1.43% by using each of both media. Also, one isolate of *S. infantis* was recovered from egg shell on SS agar, while, *S. kentucky* was isolated from one egg content sample by using XLD agar (Table 3).

Regarding to egg-based products, none of examined custard and mayonnaise samples were pos¬itive for *Salmonella* on both media. While, *Salmonella* species were existed in 2 (6.66%) and 1 sample (3.33%) of the examined cream cake using XLD and SS agar, respectively. *S. kentucky* and S. shubra were isolated from the positive cream cake samples with an incidence of 3.33% for each using XLD agar. While, by using SS agar *S. enteritidis* could be isolated in percentage of 3.33% (Tables 2 and 3). Unlike this study, Gumus *et al*. (2005); Meldrum *et al*. (2006) and Siriken *et al*. (2009) did not find *Salmonella* in any of cream cake samples. While, higher percentage (16%) was indicated by Can *et al*. (2014) in cream cake samples produced in Turkey.

In the present study, XLD agar was found to be comparatively better in recovering *Salmonella* than SS agar as *Salmonella* species were isolated from 16 (3.14%) out of 510 egg and egg-based product samples using XLD agar. While, by using SS agar they were isolated from 7 (1.37%) (Table 2). This result is in harmony with that postulated by Rall *et al*. (2005); Rashwan (2013) and Taha *et al*. (2013).

In this study PCR assay was carried out for detection of *invA* gene (common gene) in all of the 23 isolated *Salmonella* strains. All isolates showed positive amplification of 284 bp fragment specific for the *invA* gene with total percentage of 100% as shown in Table 4 and Fig. 1. Therefore, PCR method based on *invA* gene is a rapid and reliable method for detection and confirmation of *Salmonella* as a complementary to conventional culture methods (Rodulfo *et al*., 2012). This result is in conformity to previous studies observed by Karmi (2013); Ezzat *et al*. (2014) and Osman *et al*. (2014).

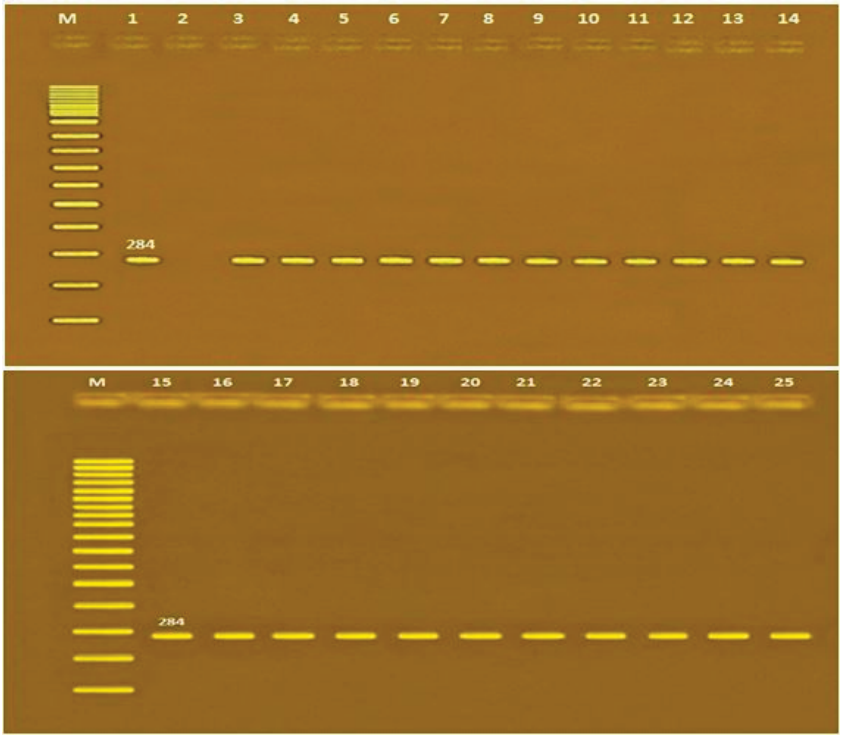

Fig. 1. Agarose gel electrophoresis of PCR of *invA* (284 bp) gene for demonstration of *Salmonella* species isolated from the examined samples of eggs and egg-based products. Lane M: 100 bp ladder as molecular size DNA marker. Lane 1: Control positive *Salmonella* strain for *invA* gene. Lane 2: Control negative. Lanes from 3 to 25: The 23 positive *Salmonella* strains for *invA* gene.

Table 4. Results of PCR of the isolated *Salmonella* species from the examined egg and egg-based products samples.

The isolated strain	No. of isolates	Positive isolates for *invA* gene
S. typhimurium	7	7
S. enteritidis	5	5
S. anatum	2	2
S. infantis	3	3
S. kentucky	5	5
S. shubra	1	1
Total	23	23

To assess the virulence of *Salmonella* isolated from the examined egg and egg-based product samples, the distribution of 3 virulence genes namely *stn*, *hilA* and *fimH* genes was determined in the isolates of *S. typhimurium* and *S. enteritidis* because of their higher prevalence in the examined samples and also they are the most commonly isolated *Salmonella* from foodborne outbreaks. For this purpose, multiplex polymerase chain reaction (MPCR) technique was used which uses pairs of primers that allow the simultaneous detection and identification of different specific DNA sequences in the same sample at the same time (Maciorowski *et al*., 2005).

Varying distribution pattern of these virulence genes was observed among *S. typhimurium* and *S. enteritidis* isolates. Incidences of *stn*, *hilA* and *fimH* in *S. typhimurium* isolates were 42.86, 71.43 and 57.14%, respectively. Only one isolate of 7 *S. typhimurium* isolates was positive for the three virulence genes evaluated, 3 isolates carried single gene and the other 3 isolates carried 2 genes. The distribution of *stn*, *hilA* and *fimH* in *S. enteritidis* isolates was found to be 40, 60 and 40%, respectively. 2 of the 3 genes were found in 2 isolates of *S. enteritidis*, while, the other 3 isolates were carried only one gene as shown in Table 5 and Fig. 2.

Table 4. Results of PCR of the isolated *Salmonella* species from the examined egg and egg-based products samples.

The isolated serotypes	Virulence genes		
	Stn	*HilA*	*FimH*
1st *S. typhimurium*	-	+	-
2nd *S. typhimurium*	+	+	+
3rd *S. typhimurium*	-	-	+
4th *S. typhimurium*	-	+	+
5th *S. typhimurium*	+	-	-
6th *S. typhimurium*	+	+	-
7th *S. typhimurium*	-	+	+
1st *S. enteritidis*	+	+	-
2nd *S. enteritidis*	-	-	+
3rd *S. enteritidis*	-	+	-
4th *S. enteritidis*	+	-	-
5th *S. enteritidis*	-	+	+

It was clear that gene pattern was not the same for all isolates, the variety in number and distribution of different virulence markers among screened *Salmonella* serovars suggests that within those serovars there are different pathotypes potentially responsible for different clinical syndromes in the host.

Therefore, to improve the quality of eggs and its based products and to safeguard the consumer from being infected with *Salmonella* all precautions showed be adopted during their handling and production.

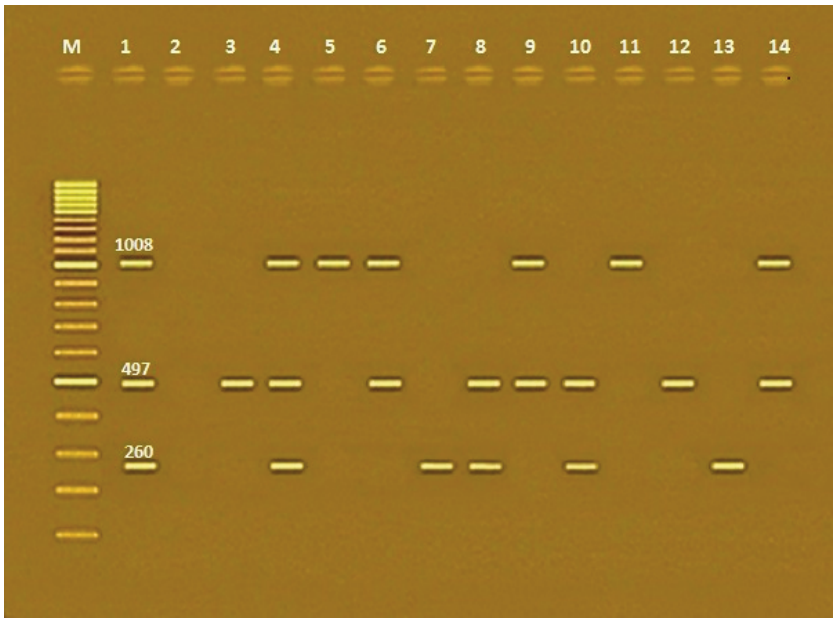

Fig. 2. Agarose gel electrophoresis of multiplex PCR of *stn* (260 bp), *hilA* (497 bp) and *fimH* (1008 bp) virulent genes for characterization of both *S. typhimurium* and *S. enteritidis*.

Lane M: 100 bp ladder as molecular size DNA marker. Lane 1: Control positive for *stn*, *hilA* and *fimH* genes. Lane 2: Control negative. Lane 3: Positive *S. typhimurium* strain for *hilA* gene. Lane 4: Positive *S. typhimurium* strains for *stn*, *hilA* and *fimH* genes. Lane 5: Positive *S. typhimurium* strain for *fimH* gene. Lane 6: Positive *S. typhimurium* strains for *hilA* and *fimH* genes. Lane 7: Positive *S. typhimurium* strain for *stn* gene. Lane 8: Positive *S. typhimurium* strains for *stn* and *hilA* genes. Lane 9: Positive *S. typhimurium* strains for *hilA* and *fimH* genes. Lane 10: Positive *S. enteritidis* strains for *stn* and *hilA* genes. Lane 11: Positive *S. enteritidis* strain for *fimH* gene. Lane 12: Positive *S. enteritidis* strain for *hilA* gene. Lane 13: Positive *S. enteritidis* strain for *stn* gene. Lane 14: Positive *S. enteritidis* strain for *hilA* and *fimH* gene.

References

Abd El Tawwab, A.A., Ammar, A.M., Ali, Aisha, R., El Hofy, Fatma, I., Ahmed, M.E., 2013. Detection of common (*invA*) gene in *Salmonella* isolated from poultry using polymerase chain reaction technique. Benha Vet. Med. J. 25, 70-77.

Adil, S., Muhammad, S.M., Iftikha, H., Faisal, S., Rao, Z.A., 2012. Prevalence of *Salmonella* species in hen eggs and egg storing–trays collected from poultry farms and marketing outlets of Faisalabad, Pakistan. Pakistan. J. Agri. Sci. 49, 565-568.

Adzitey, F., Rusul, G., Nurul H., 2012. Prevalence and antibiotic resistance of *Salmonella* serovars in ducks, duck rearing and processing environments in Penang, Malaysia. Food Res. Int. 45, 947–952.

Almario, J.A.N., 2014. Prevalence of *Salmonella* on laying hen farms and control of colonization in poultry through egg yolk antibodies. M.V.Sc. Thesis, Fac. Sci., Maryland Univ., USA.

Andrews, W.H., Hammack, T.S., 2001. *Salmonella*. In: Bacteriological Analytical Manual. 8th ed., Chapter 5. USFDA, White Oak, MD.

APHA (American Public Health Association), 1992. Standard Method for Examination of Dairy Products. 13thed. Washington DC., USA.

Arif, A.D., 2013. Isolation and identification of *Salmonella* species from the table eggs in Sulaimani province. AL-Qadisiya J. Vet. Med. Sci. 12, 24-27.

Can, H.Y., Elmali, M., Karagöz, A., Öner, S., 2014. Detection of *Salmonella* spp., *S. enteritidis*, S, typhi and S, typhimurium in cream cakes by polymerase chain reaction (PCR). Med. Weter 70, 689-692.

CIDRAP, 2006. Centre for Infectious Disease Research and Policy, Academic Health Centre, University of Minnesota. http://www.cidrap.umn.edu/cidrap/ contents/fs/food disease/causes/salmoview.html.

De Buck, J., Van Immerseel, F., Haesebrouck, F., 2004. Colonization of the chicken reproductive tract and egg contamination by *Salmonella*. J. Appl. Microbiol. 97, 233–245.

De Reu, K., Grijspeerdt, K., Messens, W., Heyndrickx, M., Uyttendaele, M., Debevere, J., Herman, L., 2006. Egg shell factors influencing egg shell penetration and whole egg contamination by different bacteria, including *S. enteritidis*. Int. J. Food Microbiol. 112, 253–260.

Dhillon, A.S., Maurer, A.J., Deibel, R.H., Haller, R.W., 1974. Feeding of different levels of *Salmonella*e to chickens. Indian J. Poult. Sci. 9, 103-107.

El Jakee, J., Khelfa, D.G., El-Safty, M.M., Seida, A.A., Marouf, S., Hahne, J., Mahmood, Z., Nagy, Sarah (2016. Multiplex PCR-based detection of *S. typhimurium* and *S. enteritidis* in specific pathogen free (SPF) and commercial eggs. Clin. Microbiol. 5, 241-245.

El-Kholy, A.M., Hassan, G.M., Mohammed, Dalia (2014. Microbiological quality of poultry farm table eggs in Beni-Suef City, Egypt. Assiut Vet. Med. J., 60, 10-13.

El-Prince, E.M., 1988. Microbiological quality of hen's eggs. M.V.Sc. Thesis, Fac. Vet. Med., Assiut Univ., Egypt.

El Sherif, W.M.A., Hassan, A.M., 2013. The impact of temperatures to reduce the risk of S. arizona and *S. enteritidis* in table eggs. Assiut Vet. Med. J. 59, 146-154.

European Commission (2003. Opinion of the Scientific Committee for Veterinary Measures relating to Public Health on *Salmonella*e in Foodstuff. Available at: http://europa.eu.int/comm/food/fs/sc/scv/out66_en.pdf. Accessed 21 February 2006.

European Food Safety Authority, 2014. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2012. EFSA J. 12, 35-47.

Ezzat, M.E., Shabana, I.I., Esawy, A.M., Elsotohy, M.E., 2014. Detection of virulence genes in *Salmonella* serovars isolated from broilers. Anim. and Vet. Sci. 2, 189-193.

Favier, G.L., Escudero, M.E., de Guzman, A.M.S., 2001. Effect of chlorine, sodium chloride, trisodium phosphate, and ultraviolet radiation on the reduction of Yersinia enterocolitica and mesophilic aerobic bacteria from egg shell surface. J. Food Prot. 64, 1621-1623.

Food Drug Administration, 2009. Prevention of *S. enteritidis* in shell eggs during production, storage and transportation. Final rule. Fed. Regist. 74, 33030–33101.

Galis, A.M., Marcq, C., Marlier, D., Portetelle, D., Van, I., Beckers, Y., Théwis, A., 2013. Control of salmonella contamination of shell eggs preharvest and postharvest methods: A review. Compr. Rev. Food Sci. Food Saf. 12, 155–182.

Gantois, I., Eeckhaut, V., Pasmans, F., Haesebrouck, F., Ducatelle, R., Van Immerseel, F.A., 2008. Comparative study on the pathogenesis of egg contamination by different serotypes of *Salmonella*. Avian Pathol. 37, 399–406.

Gumus, T., Daglıoglu, O., Konyalı, A.M., 2005. Microbiological quality of cream cakes sold in Tekirdag province. J. Tekirdag Agric. Fac. 2, 215-220.

Guo, X., Chen, J., Beuchat, L., Brackett, R., 2000. PCR detection of *S. enterica* serotype montevideo in and on raw tomatoes using primers derived from *hilA*. Appl. Environ. Microbiol. 66, 5248-5252.

Harsha, H.T., Reshmi, R., Rinoy, V., Divya P.S., Mujeeb, R., Mohamed, H., 2011. Prevalence and antibiotic resistance of *Salmonella* from the eggs of commercial samples. J. Microbiol. Infect. Dis. 1, 93-100.

Hennessy, T.W., Cheng, L.H., Kassenborg, H., Ahuja, S.D., Mohle-Boetani, J., Marcus, R., 2004. Egg consumption is the principal risk factor for sporadic S. serotype heidelberg infections: a case-control study in Food Net sites. Clin. Infect. Dis. 38, 237–243.

Howard, Z.R., O'Bryan, C.A., Crandall, P.G., Ricke, S.C., 2012. *S. enteritidis* in shell eggs: Current issues and prospects for control. Food Res. Int. 45, 755–764.

Hussien, M.F., Sayed, M., Abdel-Azeem, M., Hassanein, R., 2009. Incidence of *Salmonella* species in table eggs sold in Assiut City. Zagazig Vet. J. 73, 131-140.

ISO 6579, 2002. Microbiology of food and animal feeding stuffs-horizontal method for the detection of *Salmonella* species. International Standard., 4th ed.

Kabir, S.M.L., 2009. The role of probiotics in the poultry industry. Int. J. Mol. Sci. 10, 3531-3546.

Karmi, M., 2013. Detection of virulence gene (*inva*) in *Salmonella* isolated from meat and poultry products. Int. J. Gen. 3, 7-12.

Kauffmann, G., 1974. Kauffmann white scheme. J. Acta. Path. Microbiol. Sci. 61, 385.

Korashy, E.A., Wahba, N.M., Hassanein, R., 2008. Public health hazards of some bacterial pathogens associated with consumption of eggs and studying the best cooking methods for their destruction. Assiut Vet. Med. J. 54, 59-75.

Little, C., Surman-Lee, S., Greenwood, M., 2007. Public health investigations of *S. enteritidis* in catering raw shell eggs, 2002–2004. Lett. Appl. Microbiol. 44, 595-601.

Maciorowski, K.G., Pillai, S.D., Jones, F.T., Ricke, S.C., 2005. Polymerase chain reaction detection of foodborne *Salmonella* spp. in animal feeds. Crit. Rev. Microbiol. 31, 45–53.

Makino, S., Kurazono, H., Chongsanguam, M., Hyashi, H., Cheun, H., Suzuki, S., Shirahata, T., 1999. Establishment of the PCR system specific to *Salmonella* species and its application for the inspection of food and fecal samples. J. Vet. Med. Sci. 61, 1245–1247.

Martinez, A., Navarrete, J., Corpus M., 2005. Identification of *S. enteritidis* in table eggs in Mexico City. Tecnica Pecuaria En Mexico. 43, 229-337.

Meldrum, R.J., Smith, R.M.M., Ellis, P., Garside, J., 2006. Microbiological quality of randomly selected ready-to-eat foods sampled between 2003 and 2005 in Wales, UK. Int. J. Food Microbiol. 108, 397-400.

Menghistu, H.T., Rathore, R., Dhama, K., Agarwal, R.K., 2011. Isolation, identification and polymerase chain reaction (PCR) detection of *Salmonella* species from field materials of poultry origin. Int. J. Microbiol. Res. 2, 135-142.

Min Chan, I., Jeong, S.J., Kwon, Y.K., Jeong, O.M., Kang, M.S., Lee, Y.J., 2015. Prevalence and characteristics of *Salmonella* spp. isolated from commercial layer farms in Korea. Poult. Sci. 94, 1691–1698.

Moats, W.A., 1980. Classification of bacteria from commerical egg washers and washed and unwashed eggs. J. Appl. Environ. Microbiol. 4, 710–714.

Molbak, K., Niemann, J., 2002. Risk factors for sporadic infection with *S. enteritidis*, Denmark, 1997 – 1999. Amer. J. Epidemiol. 156, 654–661.

Msallam, A.K., 2008. Occurrence of *Salmonella* spp. in hens eggs and their environment in selected farms in Gaza strip. M.V.Sc. Thesis, Fac. Science, Gaza Univ., Palestine.

Murakami, K., Noda, T., Onozuka, D., Sera1, N., 2013. *Salmonella* in liquid eggs and other foods in Fukuoka prefecture, Japan. Int. J. Microbiol., Vol 2013, Article ID 463095,1-5.

Murchie, L., Whyte, P., Xia, B., Horrigan, S., Kelly, L., Madden, R.H., 2007. Prevalence of *Salmonella* in grad A whole eggs in the island of Ireland. J. Food Prot. 70, 1238-1240.

Musgrove, M.T., Jones, D.R., Northcutt, J.K., Harrison, M.A., Cox, N.A. Ingram, K.D., Hinton, A.J., 2005. Recovery of *Salmonella* from commercial shell eggs by shell rinse and shell crush methodologies. Poult. Sci. 84, 1955-1958.

Nor Faiza, S., Saleha, A.A., Jalila, A., Fauziah, N., 2013. Occurrence of *Campylobacter* and *Salmonella* in ducks and duck eggs in Selangor, Malaysia. Tropical Biomedicine 30, 155–158.

Olsen, A.R., Hammack, T.S., 2000. Isolation of *Salmonella* spp. from the housefly, Musca domestica and the dump fly, *Hydrotaea aenescens* (Wiedemann) (Diptera: Muscidae), at caged-layer houses. J. Food Prot. 63, 958-960.

Osman, Kamelia, M., Amin, Zeinab, M.S., Bakry, M.A., El Safty, M. M., 2011. Identification of the *Salmonella* genes Yafd and Xtha: a step into the control of salmonella food poisoning associated with consumption of eggs in Egypt. Global Veterinaria 7, 625-631.

Osman, K.M., Marouf, S.H., Mehana, O.A., Al-Atfeehy, N., 2014. *S. enterica* serotypes isolated from squabs reveal multidrug resistance and a distinct pathogenicity gene repertoire. Rev. Sci. Tech. Off. Int. Epiz. 33, 997-1006.

Plym, L.F., Wierup, M., 2006. *Salmonella* contamination: a significant challenge to the global marketing of animal food products. Rev. Sci. Tech. 25, 541–554.

Rall, V.L.M., Rall, R., Aragon, L.C., De Silva, M.G., 2005. Evaluation of three enrichment broth and five plating media for salmonella detection in poultry. Brazillian J. Microbiol. 36, 147-150.

Rashwan, R.S., 2013. Detection of *invA* gene in non typhoidal *Salmonella* isolated from food products and clinical cases. M.V.Sc., Thesis, Fac. Med., Assuit Univ., Egypt.

Rezk, M.S., Saleh, O.A., 2008. Microbiological investigation of duck eggs with special reference to *Salmonella* in Bohira province. Assiut, Vet. Med. J. 54,118. 96-107.

Rodulfo, H., De Donato, M., Luiggi, J., Michelli, E., Millánand, A., Michelli, M., 2012. Molecular characterization of *Salmonella* strains in individuals with acute diarrhea syndrome in the state of Sucre, Venezuela. Revista da Sociedade Brasileira de Medicina Tropical 45, 329-333.

Sedeek, E.K., Aioub, K.H., 2014. First record for isolation and identification of *S. enterica* (serovar westpark) from eggs retailed at Alexandria Governorate. 2nd Conference of Scientific Association of Animal Health Research Institute, pp. 255-263.

Shanmugasamy, M., Velayutham, T., Rajeswar, J., 2011. InvA gene specific PCR for detection of *Salmonella* from broilers. Vet. World 4, 562-564.

Shirota, K., Katoh, H., Murasa, T., Ho, T., Otsuki, K., 2001. Monitoring of layer feed and eggs for *Salmonella* in eastern Japan 1993 and 1998. J. Food Prot. 64, 734-737.

Siriken, B., Cadirci, O., Inat, G., Pamuk, S., 2009. Microbiological examination of meatball, cream cake and turkish delight (lokum). J. Anim. Vet. Adv. 8, 2049-2054.

Solari, C.A., Mandarino, J.R., Panizzutti, M.H.M., Farias, R.H.G., 2003. A new serovar and a new serological variant belonging to *S. enterica* subspecies diarizonae. Memórias do Instituto Oswaldo Cruz. 98, 501-502.

Speck, M.L., 1984. Compendium Method for Microbiological Examination of Food. American Public Health Association, Washington, D.C.

Spitzer, H., 2016. An analysis of bacterial contamination of chicken eggs and antimicrobial resistance. College of Saint Benedict and Saint John's University, all College Thesis Program. Paper 27. Available at http://digitalcommons.csbsju.edu/honors_thesis/27.

Suksangawong, C., 2008. Survey study on *Salmonella* spp. in eggs in Muang District. Chiang Mai. Proceedings, The15th Congress of FAVA, FAVA - OIE Joint Symposium on Emerging Diseases. Bangkok, Thailand.

Taha, Rihab; Alghalibi, S.M., AL-Ammari, Yasmin, N., 2010. Incidence and distribution of *Salmonella* serogroups in some local food in Sana'a- Yemen. Authorized by Al-Nasser University's Research Office. www.al-edu.com.

Taha, Rihab; Alghalibi, S.M., Saeed, S.M.G., 2013. *Salmonella* spp. in patients suffering from enteric fever and food poisoning in Thamar City, Yemen. Eastern Mediterranean Health J. 19, 88-93.

Thammasuvimol, G., Seo, K.H., Song, K.Y., Holt, P.S., Brackett, R.E., 2006. Optimization of ferrioxamine E concentration as effective supplementation for selective isolation of *S. enteritidis* in egg white. J. Food Prot. 69, 634-638.

Tsegaye, S., Beyene, W., Tesfaye, B., Tessema, T.S., Feleke, A., 2016. Prevalence and antimicrobial susceptibility pattern of *Salmonella* species from exotic chicken eggs in Alage, Ziway and Shashemene, Ethiopia. African J. Basic and Appl. Sci. 8, 180-184.

Van Immerseel, F., 2010. Stress-induced survival strategies enable *S. enteritidis* to persistently colonize the chicken oviduct tissue and cope with antimicrobial factors in egg white: A hypothesis to explain a pandemic. Gut Pathogens 2, 23.

Wales, A., Davies, R., 2011. A critical review of *S. typhimurium* infection in laying hens. Avian Pathol. 40, 429–436.

Wilson, M.W., 2007. Survey of retail eggs for *Salmonella*. Client Report FW0779 Client Report FW0420 for the New Zealand Food Safety Authority. ESR Christchurch Science Centre, Christchurch, New Zealand.

Zansky, S., Wallace; Schoonmakerbopp, D., Smith, P., Ramsey, F., Painter, J., Gupta, A., Kalluri, P., Noviello, S., 2002. Outbreak of multidrug resistant S. newport. USA: JAMA. 288, 951-953.

Zubair, A.I., Al-Berfkani, M.I., Issa, A.R., 2017. Prevalence of *Salmonella* species from poultry eggs of local stores in Duhok. Int. J. Res. Med. Sci. 5, 2468-2471.