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MOLECULAR EPIDEMIOLOGY OF SALMONELLA IN EGYPTIAN POULTRY FARMS

By

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ABSTRACT

This study was designated to investigate the distribution of *Salmonella* in different chicken farms broiler, layer and breeder of different ages through bacteriological examination for different types of samples and to detect zoonotic serotypes of *Salmonella* by Polymerase Chain Reaction assay. A total number of 263 samples. (171 organ samples as fallow:82 livers, 51 yolk sac, 20 spleens, 16 ovaries, 2heart and 90 fecal swap and 2 litter samples) were obtained from 93 different poultry farms in different localities at6 governorates Qalubia, Sharkya, Minofia, Gharbya, Esmailia, Dakhlia and Giza during the period from 2013 to 2015. The samples were collected under complete aseptic condition from chickens suspected to be infected with salmonellosis. The incidence of *Salmonella* among chicken farms was 5.3% (14/263). It was (9.4%) among the broiler farms and (2.9%) among the layer farms by conventional culture methods. The results obtained showed that, the incidence of *Salmonella* in different organ samples were as follows: 7.47% among liver samples and it considered the highest incidence of *Salmonella* isolation followed by yolk sac 2% while the lowest rate of *Salmonella* isolation was from the spleen 0.21%, no isolation from heart, ovary, fecal swabs and litter samples and it is belonging to four serotypes. *S.* Enteritidis and *S.* Typhimurium indicated the highest incidence (42.85% and 28.57% respectively), will the other serovars *S.* Kentuky and *S.* Muenster were lower in incidence (21.42% and 7.14% respectively). The detection of (*invA*) gene provides that, all isolates were positive for it except two isolate.

INTRODUCTION

In poultry, which represents an important source of protein throughout the world, avian *salmonellosis* considered an important disease causing serious impediment to the development of poultry industry especially in developing countries of Asia and Africa **(Ramachandran Pillai and Mangattumuruppel, 2013)**. Outbreaks of *Salmonella* have been associated with wide variety of foods especially those of animal origin **(Hernandez *et al*., 2005)**. In many countries human salmonellosis is mainly due to consumption of eggs followed by poultry, pork, beef, and dairy products **(Carraminana and Yanguela, 1997). Akhtar *et al*. (2010)** revealed that overall serovars *S.* Enteritidis prevalence rate in 206 *salmonella* positive samples were 75.24% (155). Out of 58 isolates of *salmonella* recovered from human stool samples, 44 (75.86%) were *S.* Enteritidis. Isolation frequency of *S.* Enteritidis from total isolates (148/206) in poultry source was 111/148 (75%), which indicated the zoonotic potential of *S.* Enteritidis in Pakistan. The prevalence of *salmonella* from Egyptian poultry farms was reported in many studies **Ibrahim *et al.* (2013)** reported that, the prevalence of salmonella from poultry in 2009 and 2010 in Beni-sufe Governorate, Egypt. Cloacal swabs were collected from poultry (150 broilers, 50 breeders, 50 layers, 50 turkeys, 50 ducks and 30 litter samples). The recovered *salmonella* strains were found belonging to *S.* Kentucky, *S.* Typhimurium and *S.* Saint Paul. The obtained results demonstrated that, the occurrence of *Salmonella* spp. accounted for 16.66, 10.0, 2.0, 6.0 and 2.0% in broilers, breeders, layers, ducks and turkeys, respectively. The conventional technique for the detection of the microorganism includes the following steps: pre enrichment, selective enrichment, isolation and selection, biochemical characterization, serological characterization and final identification. This technique requires at least four days for a negative result and six to seven days for the identification and confirmation of positive samples **(Soumet *et al*., 1997).** New methodologies based on molecular biology such as PCR method which is rapid, specific and sensitive method are used for detection of food borne pathogens **(Olsen *et al*., 1999)**. Real time-PCR (RT-PCR) technology offers several advantages compared with classical bacteriology in terms of speed, detection limit, potential for automation, and cost **(Lofstorm *et al.*, 2009)**. **Ibrahim *et al.*, (2014)** compared between conventional culture isolation methods and real time polymerase chain reaction (RT-PCR) technique for the detection of *Salmonella* in broiler chicks. About

120 livers and intestinal contents samples were collected from 1800 day - old imported and local broiler chicks. The incidence of *Salmonella* among imported chicks was 11.67% compared to 21.67% among local chicks using conventional cultural isolation methods. *Salmonella* Newport (*S.* Newport) showed the highest incidence rate in imported chicks, while *Salmonella* Enteritidis and *Salmonella* Typhimurium were frequently detected in local chicks. The RT-PCR results for detection of *invA* gene of *salmonella* spp. were 58.33% and 66.67% positive samples in imported and local chicks, respectively. Results have confirmed that RT-PCR technique is rapid, robust, effective and reliable method for detection of *Salmonella* spp. in broiler chicken when compared to conventional cultural methods. However, RT-PCR should be performed parallel with conventional methods for more accurate detection results of different *Salmonella* serovars. **Amini *et al.* (2010)** carried a multiplex polymerase chain reaction (multiplex PCR) assay**)** for detection of *Salmonella* Enteritidis and presence of *invA* and *spv* genes. In the first stage of the study, 1001 poultry samples were collected from a slaughter house in Kerman province (southern Iran). Biochemical and serological tests were then performed for identification of *Salmonella* serovars and 6.79 % (68/1001) were positive for *Salmonella*. Multiplex PCR with three set primers was then applied to confirm serovar Enteritidis 51.4% (35/68). Simple-PCR was then applied to detect *spvA* (*Salmonella* plasmid virulence), and *spvB* genes. Finally, multiplex PCR assay was carried out to simultaneously detect and identify *invA* and *spvC* genes. The presence of *spvA*, *spvB* and *spvC* in *S.* Enteritidis was 88.6% for each gene. In the second stage of the study, thirty-three bovines (n=13) and human (n=20) *S.* Enteritidis strains were isolated from the culture collection in the Department of Microbiology, Faculty of Veterinary medicine, University of Tehran, Iran. The analyses of the samples revealed that *spvA*, *spvB* and *spvC* genes were present in 90 % of *S.* Enteritidis from human source as compared to 100 % in bovine sources. The study represents the first report in Iran about the genotypic diversity of *spvA*, *spvB* and *spvC* genes of *S.* Enteritidis. Once *Salmonella* has become established in a primary breeding flock, a cycle can be established by which the organism passes via the eggs to the progeny and even to chicks hatched from eggs laid subsequently by infected progeny **(Sharma, 2010).**

THE AIM OF THE WORK

1 - Surveillance study on *salmonella* in chicken farms.

2 - Isolation and identification of salmonella spp from chicken.

3- Biochemical identification of the isolated bacteria.

4- Serological identification of the isolate.

5- PCR typing of the isolate.

MATERIAL AND METHODS

Sampling:

A total of 263 samples were collected from broiler, layer and breeder chickens of different ages from 93 different poultry farms in different localities at6 governorates Qalubia, Sharkya, Minofia, Gharbya, Esmailia, Dakhelia and Giza.

Table (1): Illustrates the number and sources of the examined poultry farms for isolation of *salmonellae.*

species / governorate	Farms of different type of production			Total number of farms
	Broiler	Layer	Breeder	
Qalubia	29	19	0	48
Sharkya	5	7	0	12
Gharbya	14	3	0	17
Dakhelia	4	4	0	8
Esmailia	1	2	2	5
Giza	0	3	0	3
Total	53	38	2	93

Clinical signs and postmortem examination:

The diseased birds were examined clinically for recording the clinical signs and the freshly dead birds as well as sacrificed diseased birds were subjected to post mortem (PM) examination for recording of PM lesions.

Bacteriological examination:

Salmonella was isolated according to standard methods (ISO 6579, 2002) (Microbiology of

feed stuffs - horizontal method for detection of *Salmonellae* species). All collected samples were inoculated in buffer peptone water (25g sample + 225g buffered peptone water) and incubated at 37 ºC for 18 to 20 hours. Then 0.1ml culture was inoculated in selective enrichment broth [Rappaport-Vassaliadis soya broth (RVS broth) (MERCK),Muller-Kauffmn Tetrathionate Novobiocin broth (MKTTn) (Oxoid)] and incubated at 41.5±1co, 37±1co for 24 hours respectively.A loopful from each broth culture was inoculated onto selective plating medium Xylose Lysine desoxycholate agar (XLD) (Oxoid), Brilliant Green agar and MacConkey agar media and incubated at 37c◦ for 24 hours and for 24 hours further if necessary. Isolated colonies were identified morphologically, microscopically and biochemically according to **(Quinn *et al.* 2002).**

Serological identification:

Serological identification of *Salmonellae* was carried out according to Kauffman - White scheme **(Kauffman, 1974)** for the determination of somatic (O) and flagellar (H) antigens using *Salmonella* antiserum (DENKA SEIKEN Co., Japan).

PCR procedures:

Extraction of DNA was according to QIAamp DNA mini kit instructions Temperature and time conditions of the primers during PCR were illustrated in (Table 2) according to Emerald Amp GT PCR mastermix (Takara) kit.

Table (2): Oligonucleotide primers sequences Source: Metabion (Germany).

Primer	Sequence	Amplified product	Reference
invA	**GTGAAATTATCGCCACGTTCGGGCAA**	**284 bp**	**Oliveira *et al*., 2003**
	TCATCGCACCGTCAAAGGAACC		

Table (3): Cycling conditions of *invA* primer during cPCR.

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
invA	**94˚C 5 min.**	**94˚C 30 sec.**	**55˚C 30 sec**	**72˚C 30 sec**	**35**	**72˚C 5 min.**

RESULTS

Diseased chickens of different ages showed signs of depression, anorexia, diarrhea, ruffled feathers, closed eyes and some cases of pasty vent. In adult bird sudden drop in feed consumption, ruffled feather and pale combs and fetid diarrhea in chronic carrier decrease fertility, hatchability and drop in egg production. Postmortem examination was performed to the freshly dead birds. All internal organs were thoroughly examined and gross lesions were recorded. Carcasses suspected to be suffering from avian salmonellosis were subjected to further bacteriological examination. Gross lesions of suspected cases were unabsorbed yolk sac, omphalities, and fibroins perihepatities with distention of gall bladder, cecal core, enteritis, prolaps, abnormal ova, mottled congested enlarged spleen, fibroins pericarditis and congestion. 171 pooled organ samples from liver, yolk sac, spleen and heart of the suspected birds and 90fecal swabs and 2 litter samples were collected for bacteriological examination. Bacteriological examination in the present study revealed that out of 263 samples (organs, fecal swabs and litter samples) obtained from 6 governorates subjected for *Salmonella* isolation, from which we could isolate *Salmonella* from 14 cases (5.3%) as shown in (Table 4). And it was serotyped to four serovar as shown in (Table 5)

Table (4): Shows the number and location of the positive *Salmonella* suspected samples.

No of samples governorates	Liver	Yolk sac	Spleen	ovary	heart	fecal swaps	Litter	Total
Qalubia	**5/47**	**3/31**	**0/9**	**0/8**	**0/2**	**0**	**0**	**8/97**
Sharkya	**1/7**	**¼**	**0/1**	**0/3**	**0**	**0/40**	**0**	**2/55**
Gharbya	**2/17**	**0/12**	**1/6**	**0/2**	**0**	**0**	**0**	**3/37**
Esmailia	**0/3**	**00/1**	**0**	**0**	**0**	**0/20**	**0**	**0/24**
Dakhlia	**1/8**	**0/3**	**0/4**	**0/3**	**0**	**0**	**0**	**1/18**
Giza	**0**	**0**	**0**	**0**	**0**	**0/30**	**0/2**	**0/32**
Total	**9/82**	**4/51**	**1/20**	**0/16**	**0/2**	**0/90**	**0/2**	**14/263**

Table (5): Shows the incidence of different isolated *Salmonella* serovar and serogroup.

serovar	Serogroup	No of isolates	%
***S.*Enteritidis**	**D1**	**6**	**42.85%**
***S.* Typhimurium**	**B**	**4**	**28.57%**
***S.* Kentucky**	**C3**	**3**	**21.42%**
***S.* Muenster**	**E1**	**1**	**7.14%**
-	**-**	**14**	**-**

Incidence of *Salmonella* was 9.4 %, 2.9 % in broiler and layer chicken farms respectively while there was no isolation from breeder farms.The incidence of *Salmonellae* was as follow: 7.47% from liver, 2% from yolk sac, and 0.21 from spleen samples.

-For liver samples: 9 isolates were found to be *Salmonella* from 83 liver samples and serotyped as *S.* Enteritidis (4). *S.* Typhimurium (3), *S.* Kentucky (2) for each.

- For yolk sac samples: 4 isolates were found to be *Salmonella* from 50 yolk sac samples and serotyped as *S.* Enteritidis (2). *S.* Typhimurium (1), *S.* Kentucky (1) for each.

-For spleen samples: only one sample was isolated from 21 spleen samples and serotyped as *S.* Muenster.

-There were no isolates obtained from the other organs like heart and ovary and there were no isolates obtained from the fecal swabs and litter samples.

The results revealed that highest percentage of isolation was from El-Qalubia followed by El Gharbya and El-Dakhlia then El- Sharkya The data illustrated in (Table 6) which show that *S.* Enteritidis was isolated from El-Qalubia, El-Gharbya and El-Dakhliawhile *S.* Typhimurium was isolated from El - Qalubia and El - Gharbya while *S.* Kentucky was isolated from El- Qalubia and El - Sharkya. *S.* Muenster was isolated from El - Gharbya.

Table (6): Types of *Salmonella* serovars isolated from the examined samples in 6 governorates.

Governorate	No. of examined samples	Positive		Types of isolated *Salmonella* serovars	No. of positive samples
		No.	%		
Qalubia	97	8	8.2%	*S.* Enteritidis	4
				S. Typhimurium	3
				S. Kentucky	1
Sharkya	55	2	3.6%	*S.* Kentucky	2
Gharbya	37	3	8.1%	*S.* Enteritidis	1
				S. Typhimurium	1
				S. Muenster	1
Esmailia	24	0	0	-	-
Dakhlia	18	1	5.5%	*S.* Enteritidis	1
Giza	32	0	0	-	-
Total	263	14	5.3%	-	14

Serological identification of *salmonellae*:

Table (7). Illustrated the antigenic structure of the isolated *Salmonella* species from different samples examined and their antigenic structures according to Kauffman - White scheme **(Kauffman, 1974).**

Table (7): Antigenic structural of *Salmonella* isolates.

Key No.	Identified strains	Group	Antigenic structure	
			O	H
7 L-68 a-71 b-81	*S.* Kentucky	C3	8,20	i : Z6
12 b- 32 a-74 a	*S.* Typhimurium	B	1,4,5,12	i : 1,2
69 a-70 a-72 a- 72 b- 73 b- 76	*S.* Enteritidis	D1	1,9,12	g,m : 1,7
70 c	*S.* Muenster	E1	3,10,15,34	e,h : 1,5

Result of PCR:

By using conventional PCR for the detection of *invA* gene in the isolated *Salmonella* species showed that, all isolated *Salmonella* serovars contained this gene except two samples as showed in (Table 8) and Fig. (1).

(Table 8): Result of detection of *Salmonella invA* gene by PCR.

***Salomella* serovar**	***invA***	
	Positive	**Negative**
***S.* Enteritidis**	**5 (69a-70a-72a-73b-76)**	**1 (72b)**
***S.* Typhimurium**	**3 (12b-32a-81)**	**1 (74a)**
***S.* Kentucky**	**2 (7b-68a)**	**1 (71b)**
***S.* Muenster**	**1 (70c)**	**0**

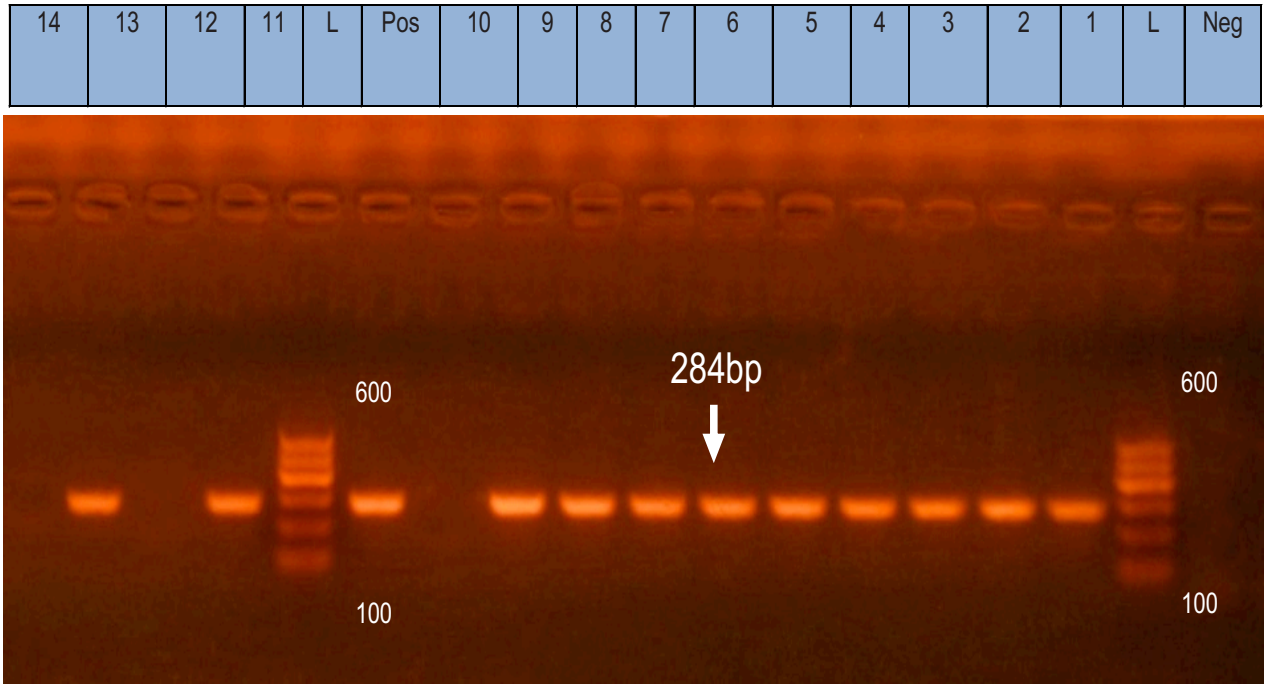

Fig. (1): Agarose gel electrophoresis showing the result of PCR amplification for detection of *Salmonella* (*invA*) gene showing 284 bp DNA fragment Positive samples: (69a-70a-72a-73b-76-12b-32a-81-7b-68a-70c).

Negative samples: (72b-74a-71b).

DNA Molecular weight marker Gel Pilot 100 bp ladder (cat. no. 239035) supplied from QIAGEN (USA).

Number of bands: 6.

Size range: 100-600 bp.

DISCUSSION

Salmonellosis is an important socioeconomic problem in several countries, mainly in developing countries, where this etiological agent is reported as the main cause responsible for food born disease outbreaks **(Alves *et al.,* 2001)**. It is one of the most problematic zoonosis in terms of public health all over the world not only because it is highly endemic, but also because of the difficulties in its control the disease in addition to significant morbidity and mortality rates **(Tessari *et al.,* 2013)**. *Salmonella* detection from poultry meat has been performed by standard bacteriological procedures such as ISO 6579 **(Anon, 2002)** in Europe and in USA **(Wallance *et al.,* 1999)**. However, efforts have been made to reduce the time required for diagnosis and to increase the sensitivity and the accuracy of the methods to detect *salmonella* in poultry samples **(Mandrell and Wachtel, 1999).** In the present investigation ISO 6579 **(Anon, 2002)** method for isolation of *Salmonella* was used among the examined samples and employ pre-enrichment and selective enrichment broth, then plating on XLD agar. BG agar uses the dye brilliant green to select for Gram-negative enteric bacteria and lactose fermentation to indicate various non-salmonellae **(David *et al.,* 1984)**. XLD agar uses the ability of *salmonella* to ferment xylose, decarboxylase lysine, and produce hydrogen sulfide in addition to the selective activity of the bile alt (detergent), deoxycholate**.** In the present study the presence of *Salmonellae* in broiler, layer and breeder farms (fecal swabs, different organs liver, spleen, yolk sac, heart and litter samples) was investigated and result were reported in (Tables 4). *Salmonellae* were isolated from different samples with incidence of 8.6 % by **Wales *et al.* (2006)**. Contaminated poultry products are widely accepted as a major source of *salmonella* infections **(Cogan and Humphrey, 2003)**. In the present study the incidence of *salmonella* among chicken farms was (5.3%). It was (9.4%) among the broiler farms and (2.9%) among the layer farms by conventional culture methods. The result obtained from bacteriological methods less agreed with **Molbak and Neimann (2012), Kimmura *et al.* (2004), Trawinska *et al.* (2008) and Rabie *et al.,* (2012)**. **Snow *et al.* (2008)** who isolated *Salmonella* in a rate of (10.7%) in the United Kingdom, while **Ibrahim *et al.* (2013)** reported that, the incidence of *salmonella* in broiler was (16.66 %) in Beni-Suef Governorate, Egypt. The percentage of isolation of *salmonella* spp. from broiler chickens in this study was more or less similar to that published by several previous authors as **Cardinal *et al.,* (2004), Saad *et al.* (2007), Badr and Abd El Monaem (2008) and Muhammad *et al.***

(2010) which ranged from 1.7% to 28.6 %. Poultry are the most important reservoir for salmonella with prevalence in chicken carcasses ranging from 20 -70% in most countries **(D'Aoust, 1989)**. The results of the European baseline survey where the prevalence of *salmonella* in broiler flocks in 2005-2006 indicated was 27.9% positive flocks in Ireland, compared to 23.7% in the EU overall **(EFSA, 2007)**. The prevalence of *Salmonella* in egg-laying flocks was 1.4% in Ireland according to the European baseline study, compared to 30.7% in the EU overall **(EFSA, 2006)**. In the present study, the incidence of *salmonella* in different organ samples was as follows: 7.47% among liver samples and it considered the highest incidence of *salmonella* isolation followed by yolk sac 2% while the lowest rate of salmonella isolation was from the spleen 0.21%, no isolation from heart, ovary, fecal swabs and litter samples. Comparing with **Mohamed (1998)** the rate of *salmonella* isolation from liver was 12%. While **Putturu *et al.* (2012)** reported that 50 % rate of *salmonella* isolation from liver and 40% from spleen and kidney samples. In Ireland in 2004 shows that of the 7,616 raw poultry meats sampled at processing level, 245 (3.2%) were positive for *salmonella* with the most common serovar isolated being Enteritidis, Kentucky, Bredeney and Mbandaka **(FSAI, 2004)**. The annual cost of medical treatment for salmonellosis. In addition to loss of productivity, imposes a significant financial burden on many countries. More than 2,500 serotypes of *Salmonella* are known, serotypes Enteritidis and Typhimurium accounted for the majority of cases of human salmonellosis **(O'Regan *et al.,* 2008)**. Out of 160 samples tested by **Shah and Korejo (2012)**, 78 (48.75%) were found positive for various species of *Salmonella*, out of the positive samples, *S.* Enteritidis was found in 38 (48.71%), *S.* Typhi in 16 (20.51%), *S.* Pullorum in 16 (20.51%) and *S.* Typhimurium in 8 (10.25%) samples. It was noticed that out of 263 samples from chicken farms, 14 samples (5.3%) were positive for isolation of *Salmonella*. *S.* Enteritidis and *S.* Typhimurium indicated the highest incidence (42.85% and 28.57% respectively), will the other serovars *S.* Kentuky and *S.* Muenster were lower in incidence (21.42% and 7.14% respectively).Regarding the incidence of *Salmonella* serovars that isolated from chicken farms in the present study, 6 *S.* Enteritidis (42.85%) were isolated similarly the serovar *S.* Enteritidis was diagnosed more frequently as recorded by **Abd-Allah, (1995)** who detecated 10 (40 %) serovars of *S.* Enteritidis out of 25 isolated salmonella strains. **Herikstad *et al.,* (2002)** considered *S.* Enteritidis is the most common species of *Salmonella* that isolated worldwide.Also, **AbdElghany *et al.,* (2012)** recorded that

there were different *Salmonella* serotypes including *S.* Enteritidis, *S.* Infentis, *S.* Chiredzi *S.* Kentucky, *S.* Typhimurium and *S.* Tsevie circulating in broiler chicken farms in Qalubia Governorate, Egypt and the most prevalent ones were *S.* Enteritidis and *S.* Typhimurium in the present study, a total of 4 (28.57%) *S.* Typhimurium serovars were isolated these results were nearly relative to that obtained by **Oh and Choi, (1996) and Chiu *et al.,* (2010),** while opposite to **Snow *et al.* (2008)** who isolated *S.* Typhimurium in a rate (0.2%). **EFSA. (2010)** reported that, the most frequently isolated *Salmonella* serovars in broiler chickens carcass samples were, respectively in decreasing order, *S.* Infants (29.2%), *S.* Enteritidis (13.6%), *S.* Kentucky (6.2%) and *S.* Typhimurium (4.4%). As noticed in the present study for liver samples: 9 isolates were found to be *Salmonella* and serotyped as *S.* Enteritidis (4). *S.* Typhimurium (3), *S.* Kentucky (2) for each. While from yolk sac samples: 4 isolates were found to be *Salmonella* and serotyped as *S.* Enteritidis (2). *S.* Typhimurium (1), *S.* Kentucky (1) for each. while spleen samples: only one isolate was obtained and serotyped as *S.* Muenster there were no isolates obtained from the other organs like heart and ovary and there were no isolates obtained from the fecal swabs and litter samples. As shown in (Table 6) the highest rate of salmonella isolation was from El-Qalubia (8.2%), then from El-Gharbya (8.1%) and El- Dakhlia (5.5%) and the lowest rate from El-Sharkya (3.6%). Since *Salmonella* is closely related to both public and animal health, more rabid and sensitive methods for the identification of this bacterium were required **(Whyte *et al.,* 2002).** *Salmonella* spp. in poultry includes nonselective pre-enrichment followed by selective enrichment and plating on selective and differential agars. These methods take approximately 4-7 days.Conventional culture method has some disadvantages, it was laborious and time consuming, generally requiring 3-4 days to obtain a negative result and up to 7 days to confirm a positive result **(Andrews *et al.,* 2001).** Development of rapid and accurate detection methods for *Salmonella* spp. has been increased due to the higher incidence of salmonellosis in industrialized countries over the past decades **(Lewis, 1997)**. In the present study, Polymerase chain reaction (PCR) for detection of *invA* gene of *Salmonella* spp. was carried out after incubation in an enrichment broth **(Oliveira *et al.*, 2003 and Lin and Tsen, 1999; Soumet *et al.,* 1999 and Luke *et al.,* 2002)**. In the present study detection of *Salmonella* using targeting *invA* gene, giving PCR product of 284 bp size with all strains except three strain with a percentage of (78.5 %) and this agreed with **Turki *et al.*, (2014)** who found that 3out of 48 *salmonella* strains were

negative for *invA* gene, similar results were observed in other studies **(Malorny *et al.,* 2003; Turki *et al.,* 2014)** and nearly to **Osman *et al.,* (2014)** with percentage (100 %), **and Eckmann *et al.,* (1997)**, Amplification of invA gene now has been recognized as an international standard for detection of *Salmonella* genus **(Ochman *et al.,* 1996; Malorny *et al.,* 2003).** *InvA* gene encoded a protein in the inner membrane of bacteria which is responsible for invasion to the epithelial cells of the host **(Darwin and Miller, 1999 and Jennifer *et al.,* 2003).** Development of a PCR system remains a suitable molecular tool to diagnose *Salmonella* on the basis of invA amplification **Bisi - Johnson *et al.,* (2011).** No amplified DNA fragments were obtained from non-*Salmonella* species. The invA gene is conserved among *Salmonella* serovars and is a useful marker for molecular detection of *Salmonella* by PCR **(Jenikova *et al.,* 2000; Olivieira *et al.,* 2003; Salehi *et al.,* 2005).**

CONCLUSION

The present study showed that, the incidence of *salmonella* among chicken farms was (5.3%). It was (9.4%) among the broiler farms and (2.9%) among the layer farms by conventional culture methods. The incidence of *salmonella* was differing according to different organ samples it was 7.47% among liver samples and it considered the highest incidence of *salmonella* isolation followed by yolk sac 2% while the lowest rate of *salmonella* isolation was from the spleen 0.21%, no isolation from heart, ovary, fecal swabs and litter samples. It was concluded that, the highest rate of *salmonella* isolation was from El-Qalubia (8.2 %), then from El-Gharbya (8.1%) and El- Dakhlia (5.5%) and the lowest rate from El-Sharkya (3.6%). *S.* Enteritidis constituted the highest incidence (42.85%) in chicken farms followed by *S.* Typhimurium (28.57%) will other serovars *S.* Kentucky and *S.* Muenster were lower in incidence (21.42% and 7.14% respectively). PCR for detection of *Salmonella* Spp. using invA gene was rapid, accurate, and more sensitive and greatly reduced the time and manpower required when compared with conventional culture methods, although this technique is actually much more expensive.

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