



## Incidence of *Salmonella* spp. in animal derived-protein in Egypt

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### ABSTRACT

This work aimed to determine the incidence of *Salmonella* in various sources of meat. One hundred and twenty random samples of different meat samples represented by chilled minced beef, meat block, poultry cuts (breast and thigh), and fish flesh (*T.niloticus*, *M.cephalus*) (20 of each) were collected from different supermarkets and butcher's shops in Menoufia governorate, Egypt. The results showed that the incidence of salmonella in the examined samples of minced beef, meat block, breast meat, thigh meat, flesh of *T.niloticus* and flesh of *M.cephalus* were 35%, 25%, 20%, 30%, 15% and 10% respectively. While serotyping of *Salmonellae* isolated from the examined samples were *S.enteritidis*, *S.typhimurum* and *S.virchow* in block meat while in minced meat, they were *S.enteritidis*, *S.typhimurum*, *S.virchow* and *S.heidelberg*, in addition to in chicken meat samples. They were *S.enteritidis*, *S.typhimurum*, *S.virchow* and *S.kentucky* in breast meat and *S.enteritidis*, *S.typhimurum* and *S.kentucky* in thigh meat. *Salmonella* strains isolated from *Mugil cephalus* were *S.enteritidis*, *S.typhimurum* from *T.niloticus* were *S.enteritidis*, *S.virchow* and *S.infantis*. Also enterotoxin gene were isolated from *S.enteritidis*, *S.typhimurum*, *S.kentucky* and *S.virchow* also could isolate *h1A* gene from *S.enteritidis*, *S.typhimurum*, *S.kentucky*, *S.virchow* and *S.heidelberg*. *FimH* were isolated from *S.enteritidis*, *S.typhimurum*, *S.kentucky*, *S.Virchow*, *S.infantis* and *S.heidelberg*. The presence of this bacterial species in the examined samples is a result of contamination during the slaughtering, defeathering or offal removal, transportation, distribution, storage, slicing, packaging, and retail sale of the products.

**Keywords:** *Minced beef, meat block, poultry meat (thigh, breast), fish flesh (T.niloticus, M.cephalus), salmonella.*

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## 1. INTRODUCTION

Generally, meat has exerted a crucial role in human evolution with its different forms as red meat, poultry meat and fish meat due to its role in maintenance balanced diet (Reyad, 2015 and Paula, 2013).

Poultry meat has an urgent demand in the consumer markets due to many advantages as easy digestibility and acceptance helping by

low price so it become worldwide need especially in developing countries with low income (Lotfy-rehab, 2014).

Otherwise, fish meat always seen as food necessary for good health since ancient times, fish considered as being a "brain food" due to its importance in development of healthy brain so that play an important role in fighting

hunger and mal nutrition also fish considered as delicious proteinous part in diet for all people, fish meat not only source of proteins and healthy fats but also unique source of omega-3 fatty acids with many vitamins and minerals (Fahmy-eman, 2016). So some studies mentioned that fish has great role in development and maintenance of eyes, skin and nervous system (Vladau, 2008).

Food borne pathogens cause many acute and life threatening diseases and most of these food borne illnesses are caused by pathogens originating from animal itself or animal products. These pathogens varied according to the method of manufacture, quality of used non-meat ingredients, and contamination level during the processing chain, packaging and storage. One of the most important bacteria causing food poisoning is salmonella. Salmonella is Gram negative bacteria, facultative aerobic rods, detected as major food borne pathogen so raw meat is considered an important source of bacteria (Wagner, 2001, Mahmoud, 2006). So eating such infected meat cause enteric illness that may be mild gastroenteritis or sever systemic illness as septicemia (Speedy, 2003).

Salmonella are well-known pathogens, highly adaptive and potentially pathogenic for humans and/or animals. Salmonella infections are capable of producing serious infections that are often foodborne and present as gastroenteritis. However, a small percentage of these infections may become invasive and result in bacteremia and serious extra intestinal disease (Fluit, 2005).

Salmonella toxins are known as enterotoxins because they are able to promote water loss from the small intestinal mucosa resulting in vomiting and diarrhea accompanied by dehydration (Martin et al., 2004). These heat stable toxins when allowed to increase for several hours in foods lead to food poisoning with or without causing any off odor, flavor or

abnormal color or texture (Reynolds et al., 2003).

Salmonellosis patients show clinical signs of diarrhea, cramps, nausea, vomiting, and may have bacteremia in severe cases. A reported isolation rate of salmonella in children with acute dysentery was 18 % (Bodhidatta et al. 2002).

So the aim of the present study was:

1-Isolation of salmonella in meat either minced or block, also in fish as tilapia and Mugil cephalus, in addition to breast and thigh of poultry meat.

2-Isolation and identification of e-coli in the same samples.

3-Isolation and identification of staphylococcus in addition to counting of staph aureus by traditional methods and also by recent techniques as PCR.

4-Screening of enterotoxigenic bacteria from meat block, minced meat, tilapia niloticus, Mugil cephalus, chicken breast and thigh meat by traditional methods and also by recent techniques as PCR.

## **Materials and methods**

### *2.1. Collection of samples:*

A total of 120 random samples (300 gram of each)of chilled minced beef, meat block, chicken breast meat, chicken thigh meat, flesh of *T.niloticus* and flesh of *M. cephalus* (20 of each) were purchased from different shops and supermarkets in El Menoufia government, Egypt. Each sample was packaged and weighed approximately 300 grams. All collected sample were transferred in an ice box to the laboratory of Animal Health Research Institute, Shibin-Elkom, under complete aseptic conditions without undue delay and examined. Bacteriologically for detection of salmonella and its enterotoxins.

### *2.2. Preparation of the samples (APHA, 2002):*

Ten grams of each sample was aseptically weighed into 90 ml of 0.1% peptone water in a sterile plastic bag, and then blended in a

Stomacher 400 Lab Blender (Seward Medical, London, UK) for 30 seconds. Accordingly, ten-fold serial dilutions were prepared for bacteriological examination.

2.3. Bacteriological examination:

1- Aerobic plate count and Enterobacteriaceae Count were carried out according to APHA, (2002).

2- Isolation and identification of Salmonellae were carried out according to ISO (2002).

3- Morphological examination: (Cruickshank et al., 1975).

4- Biochemical identification: (MacFaddin, 2000).

5- Serological identification of Salmonellae was applied according to Kauffman (1974).

6- Detection of salmonella enterotoxins: Oda et al. (1979).

2.4. Statistical analysis:

The evaluation and interpretation of obtained results were carried out using of Analysis of Variance (ANOVA) test according to Feldman et al. (2003).

3. RESULTS

It was evident from the results recorded in table (1) that 35%, 25%, 20%, 30%, 15% and 10% of the examined samples of chilled

minced beef, meat block, chicken breast meat, chicken thigh meat, flesh of T.niloticus and flesh of M.cephalus were positive for salmonella respectively.

Results obtained in table (2) declared that the suspected salmonella serovars were detected in serotyping of salmonellae isolated from the examined samples were S.enteritidis, S.typhimurum and S.virchow in meat block while in minced meat were S.enteritidis, S.typhimurum, S.virchow and S.Heidelberg in addition to in chicken meat samples, there were S.enteritidis, S.typhimurum, S.virchow and S.kentucky in breast meat and S.enteritidis, S.typhimurum and S.kentucky in thigh meat while S.enteritidis, S.typhimurum, S.virchow and S.infantis were isolated from the examined M.cephalus and T.niloticus.

The results in table (3) illustrated that enterotoxin gene were isolated from S.enteritidis, S.typhimurum, S.kentucky and S.virchow while hilA gene were isolated from S.enteritidis, S.typhimurum, S.kentucky, S.virchow and S.heidelberg. FimH were isolated from S.enteritidis, S.typhimurum, S.kentucky, S.Virchow, S.infantis and S.heidelberghe.

Table1: Incidence of Salmonella isolated from the examined samples (n=20)

Type of sample		Positive samples	
		No	%
Chicken	Breast	4	20%
	Thigh	5	30%
	Meat block	3	25
Meat	Minced beef	4	35%
Fish	Mugil cephalus	2	10%
	Tilapia nilotica	3	15%

Table 2: Serotyping of Salmonellae isolated from the examined samples (n=20)

Samples	Chicken samples				Meat samples				Fish samples				Group	Antigenic structure	
	Breast		Thigh		Meat block		Minced meat		Mugil		Tilapia			O	H
Salmonella Strains	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%			
<i>S. Enteritidis</i>	1	5	2	5	1	5	1	5	1	5	1	5	D1	1,9,12	g,m : -
<i>S. Typhimurum</i>	1	5	1	5	1	5	1	5	1	5	-	-	E1	3,10,15,34	e,h : 1,6
<i>S. VIRCHOW</i>	1	5	-	-	1	5	1	5	-	-	1	5	E1	3,10,15,34	e,h : 1,5
<i>Salmonella Kentucky</i>	1	5	2	10	-	-	-	-	-	-	-	-	C3	8,20	i : Z6
<i>S.INFANTIS</i>	-	-	-	-	-	-	-	-	-	-	1	5	B	1,4,5,12	z <sub>10</sub> : 1,2
<i>S.Heidelberg</i>	-	-	-	-	-	-	1	5	-	-	-	-	E1	3,10,15,34	e,h : 1,5
Total	4	20	5	30	3	25	4	35	2	10	3	15			

Table 3: Occurrence of virulence genes of different Salmonella strains isolated from the examined samples

Virulence factors	<i>Salmonella</i> Serovars		
	<i>Stn</i>	<i>hilA</i>	<i>fimH</i>
<i>S. Typhimurium</i>	+	+	+
<i>S. Enteritidis</i>	+	+	+
<i>S. Kentucky</i>	+	+	+
<i>S. infantis</i>	-	-	+
<i>S. Heidelberg</i>	-	+	+
<i>S. Virchow</i>	+	+	+

stn: Enterotoxin gene. hilA: hyper-invasive locus gene. fimH: fimbrial gene.

#### 4. DISCUSSION

Salmonellosis is one of the major cause of human bacterial enteritis in many countries of the world. *Salmonella* frequently colonize the gastrointestinal track of animals without producing any clinical signs.

The following mechanisms for Salmonellosis had been detected:

a- Within the chromosome of *Salmonella* enterica, several virulence clusters known as *Salmonella* pathogenicity islands (SPI) are present, in particular, SPI 1 and SPI2 which play important roles in invasion and intracellular survival, respectively.

b- Orally ingested *Salmonella* surviving in the low pH of the stomach enters the small intestine and invades epithelial cells. Upon invasion, through type three secretion system (T3SS) or Injectosome (which is a protein appendage), *Salmonella* delivers its effector proteins across the host cell plasma membrane which leads to temporal reorganization of the host cell actin cytoskeleton and disturbance in cell membrane permeability. This induces uptake of the bacteria by a means of macropinocytosis (which is a form of endocytosis in which *Salmonella* were brought into the cell, forming an invagination, and then suspended within small vesicles where they survive and replicate with the help of SPI2 virulence genes.

c- *Salmonella* preferentially enters microfold cell, which transport them to the lymphoid cells (T and B cells) in the underlying peyer's patches across epithelial barriers and induce inflammatory reaction (Nabbut, H. N. (1993) and Hegazy and Hensel (2012).

According to table (1) Nearly similar results were recorded in chicken meat samples as Casarin et al 25% (2009), Antunes et al (2003) 26.6%, Ahmed et al (2014) 25%, Oz

food networking group (2003) 30% while lower results were obtained by Yousef-mervat (2003) 3%, Kozaciniski et al. (2006) 9.5%, Jordan et al (2006) 3.1%, Abo -samra (2013) 6% but higher results were obtained by Bonyadian et al. (2007) 52.2%, Hamada (2012) 65%, Abdel fattah- walaa (2014) 64%. Nearly similar results in meat samples were obtained by Abbassi-Ghozzi et al. (2011) 29.8%, Ahmed et al. (2014) 25% Adwan et al (2015) 25% while higher results obtained by Steven et al. (2006) 43%, Vanet (2007) 62%, Hejazy et al. (2012) 60% and Hobbs (2013) 60% but lower results obtained by Farid et al. (1987) 2.5%, WHO (2007) 10%, Nashid (1993) 16%, Arumugaswamy et al. (1995) 7.7%, Vanderlinde et al. (1998) 1.4%, Orji et al. (2005) 15.6%, Bahandare et al. (2007) 16.4%, Hassan et al. (2010) 9.68%, Sibhat et al (2011) 2%, Abdallah et al. (2009) 4%, Jalali (2008) 7.92%, Little et al. (2008) 1.1%, Majagaiya et al. (2008) 9.2%, Zakaria (2007) and ElDally (1994) failed to isolate salmonella.

Concerning fish meat samples lower results were obtained by Soliman et al. (2002) 3.3%, Basti et al. (2006) 2.7% but Dodds et al. (1992) and David et al. (2001) wasn't detect salmonella.

Results in table (4) showed that salmonella strains were isolated from examined samples of meat block were *S. enteritidis*, *S. typhimurum* and *S. virchow* While in minced beef, they were *S. enteritidis*, *S. typhimurum*, *S. virchow* and *S. heidelberg*. Hobbs (2013) that *S. typhi* (7.15%), *S. typhimurium* (46.43%), *S. dublin* (10.71%) and *S. enteritidis* (35.71%). Majagaiya et al. (2008). Different salmonella spp. was isolated from different types of meat samples belonging to Sero group D and Hassan et al (2010) found that The commonest bacterial isolates were non-typhoid salmonella (*S. Enteritidis* and *S. Typhimurium*).

Yousef-Mervat (2003) isolated two strains and identified as one belonged to *S. Enteritidis* and the other one belonged to *S. Typhimurium*.

But in chicken meat samples, there were *S. enteritidis*, *S. typhimurium*, *S. virchow* and *S. kentucky* in breast meat and *S. enteritidis*, *S. typhimurium* and *S. kentucky* in thigh meat.

Bonyadian *et al.* (2007) isolated *S. Typhimurium* as the main contaminant of the samples (52.2%) followed by *S. Enteritidis* (12.2%).

Yousef-mervat (2003) showed salmonella strains in examined fish meat samples that in mugil cephalus were *S. enteritidis*, *S. typhimurium* and in *T. niloticus* were *S. enteritidis*, *S. virchow* and *S. infantis*, while Abd El-Fatah and salem (2006) isolated *S. typhimurium* also Basti et al. (2006) isolated *S. dublin*.

Table (3) showed the incidence of virulence genes of different salmonella strains that *S. typhimurium*, *S. enteritidis*, *S. kentucky*, *S. virchow* were *Stn*, *hila* and *fimH* while *S. infantis* virulence gene is *fimH*, *S. heidelberg* virulence gene were *hila* and *fimH*. Beach et al. (2002) detected *hila* gene from isolated *S. typhi enteritidis*.

Oliveira – Sílvia *et al.* (2004) recorded that all *S. enteritidis* serotypes isolated from examined samples carried *invA* virulence gene.

Moussa *et al.* (2010) reviewed that *fimA* virulence gene was the most common expressed in isolated *S. enteritidis* and *S. typhimurium* serotypes followed by *invA* virulence gene in examined samples.

Fsanz (2013) stated that *Salmonella enterica* is a leading cause of human gastroenteritis in both developed and developing countries.

Ibekwe et al. (2008) stated that Salmonellosis is a cause for concern and a major public health

problem in developing countries due to poor sanitary conditions and lack of or inadequate portable water.

## 5. CONCLUSION

Meat selected samples are considered a good medium for the growth of salmonella and the production of toxins. The lowest contamination was in *M. cephalus* flesh meat but the highest contamination was in minced beef the presence of this bacterial species in such samples is a result of contamination along production lines include slaughtering, preparation, distribution, storage, packaging, and sale of the products. This subsequently contributes to health risks to the consumer. So, these samples need carefully control to protect consumers against food poisoning.

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