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INCIDENCE OF *SALMONELLAE* IN CHILLED CHICKEN CARCASSES IN RETAILS PORT-SAID CITY

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ABSTRACT

A total of 120 samples were collected from retail markets from November 2011 to February 2012**.** *Salmonella* spp. was detected in 14 (11.7 %) of the samples analyzed. Among the chicken samples examined giblets had higher contamination level of *Salmonella* 7 (5.8 %) while the meat samples had lower percent of contamination 3(2.5%). Out of 14 *Salmonella* isolates, 5 different serotypes were identified *S.* typhimurium (42.9) was the most frequent followed by *S. enteritidis* 21.4%, *S. virchow* 21.4%, *S.anatum* 7.1% and *Salmonella type Π* 7.1%. The results of the present study indicated that there was a high level of *Salmonella* contamination of chicken meat and giblets in retail markets, which could be considered as one of the major potential source of human salmonellosis in Port-Said, city.

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INTRODUCTION

Poultry meat constitutes a substantial portion of protein in the present day diets, hence it has an important share (30%) in the world's total meat consumption (Del Rio *et al.,* 2007). Poultry meat can be contaminated with a variety of foodborne pathogens (Mor-Mur and Yuste, 2010). The presence of pathogenic microorganisms, spoilage microorganisms or both in Poultry is undesirable but unavoidable (Goncalves *et al.,* 2004). Salmonellosis is an important global public health problem causing substantial morbidity, and thus also has a significant economic impact. In spite of the improvement in hygiene, food processing, education of food handlers and information to the consumers, foodborne diseases still dominate as the most important public health problem in most countries. Dominguez *et al.* (2002).

Poultry meat and its derivatives are among the food products that cause the most concern to public health authorities, owing to the associated risks of bacterial food poisoning. The modernization of chicken farms and globalization of the bird breeding trade also have played a role in infection. Velge *et al.* (2005).

In humans, *Salmonella* is the cause of two diseases called salmonellosis: enteric fever (typhoid), resulting from bacterial invasion of the blood stream, and acute gastroenteritis, resulting from a foodborne infection/intoxication. It has been reported that 10-20% of Salmonellosis outbreaks were related to poultry meat consumption (Bailey, 2002). Most people infected with *Salmonella* develop diarrhea, fever, and abdominal cramps 6 to 72 hours after infection. In most cases, the illness usually lasts 3 to 7 days - most affected people recover without treatment. However, in some people the diarrhea may be so severe that the patient becomes dangerously dehydrated and must be taken to a hospital.

Poultry and poultry products are usually incriminated in outbreaks of human salmonellosis. *Salmonella* often reach the carcasses from the intestinal tracts or faecal materials on feathers or feet. Particularly scalding, defeathering, evisceration and giblet operations are the major points of spread in poultry processing plants (Bryan and Doyle, 1995), (D'aoust, 1989) and Uyttendaele *et al.* (1998).

The cross-contamination of hands of workers, working equipment and utensils could also serve as a mean of spread of *Salmonella* to uncontaminated carcasses and giblets in which contamination could continue during subsequent handling, processing and preparations. (Scott, 1996) and Uyttendaele *et al*. (1998).

Previous works undertaken in Egypt indicated the presence and distribution of *Salmonella* in wild birds (Azza, 2003), poultry farms (El-Jakee *et al.,* 2010), laying farms (Mona, 2007), calves (Moussa *et al.,* 2010), House sparrows and Laughing doves (Helal, 2007) poultry meat and meat products (Raafat *et al.,* 2011) man (Weal *et al.,* 2011). Various serotypes of *Salmonella* were also identified from House sparrows and Laughing doves, calves, poultry meat and man in Egypt (Helal, 2007; Moussa *et al.,* 2010, Raafat *et al.,* 2011 and Weal *et al.,* 2011).

A periodic surveillance of the level of *Salmonella* contamination in the different food animals, food products and environment is necessary to control the

spread of the pathogen and infection of man (Arumugaswamy *et al.,* 1995 and Dawson, 1992). The knowledge on the prevalent *Salmonella* serotypes in a country is also important in order to understand the distribution and means of introduction into a country Jegathesan (1984). This study was, therefore, undertaken to determine the prevalence and distribution of serotypes of *Salmonella* in chilled chicken carcass obtained from retails Port-Said city.

MATERIALS and METHODS

1-Sampling:
One hundred and twenty samples of each chilled chicken carcass (40 meat, 40 giblets and 40 drip) were randomly collected from different retails in Port- Said city. Each sample was obtained aseptically and transported in iceboxes packed with ice to laboratory as soon as possible to be subjected to bacteriological examinations.

2- Isolation and identification of *Salmonella*:
Salmonellae were isolated and identified according to the techniques recommended by the International Organisation for Standardisation (ISO, 2002).

2-1- Pre-enrichment in non selective medium:
Briefly, 25 g of each sample of meat and giblet was weighed and cut into smaller fine pieces with sterile scalpel blades. Each sample was put in a sterile stomacher bag and 225 ml of buffered peptone water (BPW; Difco, USA) was added, homogenized using a stomacher. In case of drip samples, pre-enriched in BPW in a ratio of 1ml of the sample to 9 ml of BPW. The pre-enriched Samples were incubated for 16 to 20 hours at 37°C.

2-2- Selective enrichment:
One ml and 0.1 ml of the pre-enrichment broth was transferred aseptically into each of 10 ml of Mauller Kouffmann Tetrathionate novobiocin broth (Difco, Detroit, USA) and 10 ml of Rappaport Vassiliadis soy broth (RVS; Oxoid) and incubated for 18 to 24 hours at 37°C and 42°C respectively.

2-3-Plating out on selective agar media:
Each enrichment culture was streaked on two selective agar media, xylose lysine deoxycholate agar (XLD; Oxoid) and Hektoen enteric agar (HE; Difco)and incubated at 37°C for 18 to 24 hours.

2-4- Selection of colonies for purification:
From each selective media plate, at least one colony considered being typical or suspect was picked and streaked onto the surface of nutrient agar plate for further identification.

2-5- Identification of the isolates:

2-5-1- Microscopical identification of the isolates:
Smears from the purified colonies wear prepared and stained with Gram's stain method and examined microscopically for the morphological character of *salmonella* according to (Quinn *et al.,* 2002).

2-5-2- Biochemical identification:
Salmonella spp. isolates were identified biochemical by Triple sugar iron agar, Lysine iron agar, and urea then by Microbact-12A test kit.

2-5-3- Serotyping of *salmonella* isolates:
Colonies with biochemistry profile of *Salmonella* were serologically confirmed using diagnostic polyvalent (O, H) and monovalent *Salmonella* anti-sera. Suspected *salmonella* were cultured on TS agar slants for 24 h at 37°C. A loopfull from the culture was suspended in drop of phosphate buffer saline (pH 7.4) on a slide to make a homogenous suspension and then a drop of *Salmonella* anti-sera was added to the suspension and thoroughly mixed to bring the organisms in close contact with anti-sera. Positive agglutination occurred with in a minute. A delayed or partial agglutination was considered as negative or false reaction. (Kauffmann and Das- Kauffmann 2001)

RESULTS

Of the total of 120 samples examined, 11.7 % were contaminated with *salmonella*e (Table 1). A high level of *salmonella* contamination was found in giblet (5.8%) followed by drip (3.3%) and meat (2.5%).

Table 1: Prevalence of *Salmonella* isolated from chilled chicken carcasses.

Type of samples	*No. of samples (no.)*	prevalence of *Salmonella*			
		Negative		Positive	
		No	%	No	%
Meat	40	36	30	3	2.5
Giblet	40	33	27.5	7	5.8
Drip	40	37	30.8	4	3.3
Total	120	106	88.3	14	11.7

Out of the total 14 *salmonella* isolates, 5 different serotypes were identified of which *S.* Typhimurium 42.9 was the most frequent followed by *S. virchow* 21.4%, *S. enteritidis* 21.3%, *S.anatum* 7.1% and *Salmonella type Π* 7.1%. (Table 2). *S.*Typhimurium and *S. enteritidis* were isolated from all sample types (chicken meat, giblets and drip) while other serotypes isolated from giblets and drip. Most of the isolates belonged to the sero-group B. (42.9%) while the lowest number of the isolates belonged to sero-group E1 and type Π. (Table 3)

Table 2: Prevalence of different *Salmonella* serovars recovered different samples.

Type of samples	*No. of samples*	*Salmonella* Typhimurium		*Salmonella enteritidis*		*Salmonella virchow*		*Salmonella anatum*		*Salmonella type Π*	
		No	%	No	%	No	%	No	%	No	%
Meat	40	2	14.3	1	7.1	-	-		-	-	-
Giblet	40	3	21.4	1	7.1	2	14.3	1	7.1	-	-
Drip	40	1	7.1	1	7.1	1	7.1	-	-	1	7.1
Total	120	6	42.9	3	21.3	3	21.4	1	7.1	1	7.1

[n]Total number of isolates. [n=14]

[%] calculated according to the total number of isolates.

Table 3: Antigenic formula of the isolated serovar.

Salmonella serovars	No of strain	Sero-group	Antigenic structure		
			[O]	[H]	
				Phase (1)	Phase(2)
Salmonella Typhimurium	6	B	1,4,[5],12	i	1,2
Salmonella enteritidis	3	D1	9	g,m	------
Salmonella anatum	1	E1	3,10 [15] [15,34]	e,h	1,6
Salmonella virchow	3	C1	6,7,14	r	1,2
Salmonella type Π	1	type Π	6,7	g,m,s,t	------

DISCUSSION

Among the foodborne pathogens the genus *Salmonella* is one of the most common causes of foodborne infections worldwide (Baird-Parker, 1990). Characteristic feature of this organism is its wide host range, which comprises most animal species including mammals, birds and cold-blooded animals in addition to humans. It has been reported that *Salmonella* is one of the most important pathogens responsible for human food poisoning in the developed world, and chicken products are widely acknowledged to be a significant reservoir for *Salmonella*. Therefore, this organism has been isolated from a range of foods in almost every country in which it has been investigated Rajashekara *et al.* (2000). Chickens are commonly infected with a wide variety of *salmonella* serovars. Infections are generally sub-clinical and one serovar may be a predominant isolate in a country for several years before it replaced by another serovar (Wray *et al.,* 1996). Bacteriological examination is the traditional mean to obtain accurate data about the prevalence of infected host of *salmonellae* (Commission of the European Communities, 1992).

In the present study, a total number of 120 chicken samples representing (40 meats, 40 giblet and 40 drip) were collected from different retails in port-said city for bacteriologically and serologically examination to detect the presence of *salmonella* species.

The level of *Salmonella* contamination of chilled chicken carcasses observed in our study (11.7%) was relatively high and confirms the findings of previous studies on *Salmonella* contamination in poultry and poultry products in Egypt (El-Jakee *et al.,* 2010; Raafat *et al.,* 2011).

A number of authors in different countries have reported different prevalence rates of *Salmonella* in poultry and poultry products 21.1 % in Ethiopia (Molla and Mesfin 2003), 22.8 % in UK (Plummer *et al.* 1995), 35.5 % in Malaysia (Rusul *et al.,* 1996), 35.8 % in Spain (Dominguez *et al.,* 2002) and 36.7% in Belgium Uyttendaele *et al.* (1998).

The variation in the prevalence of *Salmonella* contamination could be partly due to differences in sample type, sampling techniques, distribution of *salmonellae* in a lot examined and the detection methods employed (Bryan and Doyle 1995; Dominguez *et al.,* 2002; Rusul *et al.,* 1996 and Uyttendaele *et al.,* 1998).

In the present study out of the total 120 samples 40 were giblets (liver, heart and gizzard) had higher contamination level of *Salmonella* (5.8 %) while the meat samples had lower percent of contamination 2.5%. (Table 1). This was in agreement with the findings of (Jerngklinchan *et al.,* 1994; Molla *et al.,* 1999 and Boniphace 2001) who reported that prevalence rates of *Salmonella* in chicken giblets more than of the carcass samples.

On the other hand a high level of *Salmonella* contamination was detected in retail chicken meat and giblets (Arumugaswamy *et al.,* 1995; Rusul *et al.*, 1996 and Carraminana *et al.,* 1997).

Cross-contamination of *Salmonella* from giblets to carcass could occur during handling, processing,

packing and distribution. The packing of giblets with the carcass observed in this study at processing plants could have also contributed to increase *Salmonella* cross-contamination. In addition to these, scalding water can become contaminated with *Salmonella* from faeces, plucking equipment, cages and floors. Workers can spread the contamination during retailing (Arumugaswamy *et al.,* 1995 and Uyttendaele *et al.,* 1998). Rupture of the intestine could also occur during evisceration and pooling giblets might lead to cross-contamination of carcasses and other chicken parts.

Out of the total 14 *Salmonella* isolates, 5 different *Salmonella* serotypes were identified (Table 2). The most prevalent serotypes were *S.* Typhimurium, followed by *S. virchow* and *S. enteritidis*. Our results come in agreement with Wafaa *et al.* (2012) who reported that *S.* Typhimurium and *S. enteritidis* were the most prevalent serotypes isolated from diseased and apparently healthy broiler chicken flocks in Egypt.

Other researchers have reported some of these serotypes in poultry meat and poultry products Carraminana *et al.* (1997); (Boniphace, 2001); Molla and Mesfin (2003). It should also be noted that the presence and distribution of *Salmonella* serotypes could vary from region to region (Uyttendaele *et al.,* 1998 and Dominguez *et al.,* 2002). While some serotypes emerge and decrease over time, others maintain their dominant role for many years with widespread distribution. The rapid international trade in agricultural, aquacultural and food products has also facilitated the introduction of new *Salmonella* serotypes into importing countries (D'aoust, 1994).

The high prevalence of *S.* Typhimurium observed in the present study reflects the presence of this serovar in the intestinal tract of live broilers, contaminating carcasses during slaughter and processing.

This study on poultry meat sale points revealed that most of the retails do not operate in a safe and clean environment, and rarely practice the appropriate covering for displayed carcass. Moreover, the isolation of different *Salmonella* sero-groups B (42.9%), D1and C1 (21.3%) and (21.4%), E1 and type Π (7.1%) were identified. **(**Table 3**)**

These indicate the presence and distribution of various serotypes of *Salmonella* of animal and human origin, which are of significance in the veterinary and public health sectors in Port-said city. The isolation of invasive *Salmonella* serotypes such as *S.* Typhimurium and other pathogenic *salmonella* in our study indicates the public health significance of these serovars as contaminated chicken meat and meat products may pose health hazards. This risk may further be higher if chicken meat or giblets are consumed undercooked or cross-contamination in the kitchen with *Salmonella* during meal preparation (Scott, 1996 and Uyttendaele *et al.,* 1998). The risk increases if the drip contains *salmonella* which may reach to the consumer due to improper handling of these products. The importance of some of the basic instructions regarding storage temperature, cooking and prevention of *Salmonella* contamination and cross-contamination is not appreciated by many consumers (Scott, 1996 and Uyttendaele *et al.,* 1998). Therefore, efforts should be made to enhance public awareness and consumer education to prevent the horizontal spread of *Salmonella*. Some of the measures in controlling *Salmonella* and other foodborne pathogens in the food chain include the introduction of good manufacturing practices (GMP) and hazard analysis critical control point (HACCP) concepts together with stringent control of all aspects of chicken meat production, preparation and distribution (Dawson, 1992). Education of personnel involved in food preparation and microbiological monitoring of broiler chicken and rejection of infected flocks from food production is also required (Arumugaswamy *et al.,* 1995). The high level of contamination of chicken meat and giblets with *Salmonella* observed in our study indicates the need for an improvement in the microbiological quality of retail chicken. There is also a need for a comprehensive epidemiological study and control of *Salmonella* contamination at various levels of chicken production and processing plants in Egypt.

It could be concluded that there are different *Salmonella* serotypes including *S.* typhimurium, *S. enteritidis*, *S.virchow, S. anatum* and *Salmonella type Π* circulating in broiler chicken farms in Port said city, Egypt and the most prevalent ones are *S.Typhimurium* and *S.enteritidis*. The prevalence of both *Salmonella* serotypes in our broiler chicken farms constitutes an important problem due to their zoonotic importance and consequently the adverse effect on the human health.

Foodborne illness usually arises from improper handling, preparation, or food storage. Good hygiene practices before, during, and after food preparation can reduce the chances of contracting an illness. There is a consensus in the public health community that regular hand-washing is one of the most effective defenses against the spread of foodborne illness.

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مدى تواجد ميكروب السالمونيلا فى الدجاج المبرد فى محلات مدينة بورسعيد

نهله طـه قرشى ، جيهان محمد عمر محمد

تم جمع ١٢٠ عينة من محلات بيع الدجاج المبرد من مدينة بورسعيد في الفترة بين نوفمبر ٢٠١١ وفبراير ٢٠١٢ كالاتى: ٤٠عينة لحم دجاج &٤٠ عينة أحشاء تؤكل &٤٠ عينة من السائل المنفصل وتم عزل السالمونيلا من ١١,٧% من أجمالي العينات التي تم فحصها بينما بينت الدراسة إلى أن أعلي نسبة عزل كانت لعينات أحشاء تؤكل بنسبة ٨, ٥ % بينما كانت أقل نسبة للعزل في عينات السائل المنفصل٠ تم عزل عدد ١٤ عترة وتصنيفها بيوكيميائيا وسيرولوجيا باستخدام أجسام مناعيه. وبتصنيف الانواع المعزولة من العينات موضع الدراسة وجد ان العترات المعزولة كانت من السالمونيلا تيفيميوريم والسالمونيلا انتيريتيدس والسالمونيلا فيرشو والسالمونيلا اناتيم والسالمونيلا من النوع ٢. تم مناقشة تاثير عترات السالمونيلا المعزولة علي صحة الانسان والاحتياطات الذي يجب عملها للحد من أنتشار هذا الميكروب.