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SOFT CHEESE AND GRILLED CHICKEN AS A POSSIBLE SOURCE OF HUMAN SALMONELLOSIS WITH SPECIAL ATTENTION TO HUMAN RISK FACTORS

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ABSTRACT

This work was designed to detect the presence of *Salmonella species* among patients with food poisoning manifestations in three central hospitals located in three cities (Sohag, Tema and Elmaragha) in Sohag Governorate and from foods consumed by the majority of patients as soft cheese and grilled chicken using microbiological, PCR method (using 16S rRNA specific primer for *Salmonella species*) and serological typing of *Salmonella species*, as well as risk factors related to *Salmonella* infection in human was identified based on data collected from the patients. The results illustrated that *Salmonella species* was detected in 9 (4.6%), 2 (2.2%) and 5 (4.2%) of the examined patients, soft cheese and grilled chicken respectively. *Salmonella Typhimurium* was the predominant serotype followed by *S. Infantis*, *S. Enteritidis* and *S. Kentucky*. Owing to the risk factors associated with infection; patients in age group ranged from15-25, males and peoples in contact with infected persons and animals were more susceptible to the infection. Public health education, rapid detection of infection, risk factors identification and collaboration between health and agriculture authorities are important to set a successful control strategy.

***Key words**: Salmonella species*, Soft cheese, Grilled chicken, Risk factors, PCR

INTRODUCTION

Infection with *Salmonella species* is considered the most common food borne contaminant and recognized globally in developed and developing countries causing high morbidity and economic losses (Ammari *et al.,* 2009). The clinical manifestations include fever, watery diarrhea, nausea, abdominal pain, headache and occasional constipation with hospitalization required in severe cases of infection (Joseph and Carlos, 2012). Identification of infection risk factors associated with human infection (animal contact, type of food consumed by patients as dairy products made from raw milk or raw meat, contact with infected persons and history of chronic diseases) will assist the health authorities to set a control strategy (Lapo *et al.,* 2014). Although some studies reported that direct contact with infected animals is the main risk factor for salmonellosis , the foodborne route is still regarded as the primary transmission route (Kevin *et al.,* 2012) through consumption of contaminated foods mainly those of animal origin (Hernandez *et al.,* 2005) such as milk, egg, beef and poultry meat (Alcaine *et al.,* 2007). The major pathogenic serovars of *Salmonella* which infect human from food of animal origin include *S. Enteritidis* , *S. Typhimurium*, *S. Heidelberg* (Jawale and Lee, 2012), *S. Infantis* and *S. Kentucky* (Wafaa *et al.,* 2012), this brought attention for sensitive and rapid detection assay of *Salmonella species* in food. Culture and biochemical methods are inexpensive but require at least three days for the negative result and five to seven days for positive result confirmation, in addition, the power of biochemical test affected by the environmental contamination (Jasson *et al.,* 2010), so it is important to reduce the detection time by applying molecular methods such as PCR by using 16S ribosomal RNA (16S rRNA) primer to detect and discriminate between *Salmonella* and non *Salmonella species* (Ziemer, and Steadham, 2003). This study focused on the presence of *Salmonella* species among food poisoning patients and in some food products consumed by the majority of patients as soft cheese and grilled chicken sold in three cities in Sohag Governorate and explore the factors associated with human infection with salmonellosis.

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MATERIALS AND METHODS

1- Study design and data collection

The study was performed from August 2015 to Septemper 2016 in three central hospitals located in three cities (Soahg, Tema and Elmaragha) in Sohag Governorate. The population under study comprised

195 patients with manifestations like food poisoning admitted to the three hospitals and from food consumed by the majority of patients such as soft cheese (90) and grilled chicken (120). Data was collected from patients using a form available at http://www.bccdc.ca/health-professionals/professional-resources/surveillance-forms with modifications including personal information, animal contact and contact with infected persons.

Collection and preparation of samples

a- Human samples

195 stool samples were collected from patients in sterile cups and sent immediately to the laboratory in the Faculty of Veterinary Medicine, Sohag University.

b- Food samples

Food samples were collected based on the data obtained from patients according to the type of food consumed by the majority of patients and if possible from the same seller. We cannot examine the same food consumed by all patients because they eat all food and no leftovers were found and several patients lives in villages far from the central hospital in addition; some patients can't remember which food caused their illness. Therefore, food samples was purchased from restaurants, cafeterias, grocery and market vendors in the three cities under study (Sohag,Tema and Elmaragha) including 90 soft cheese (30 from each city) and 120 grilled chicken samples (40 from each city). The collected samples were sent to the Food hygiene department laboratory in the Faculty of Veterinary Medicine, Sohag University and prepared for microbiological examination according to (APHA, 1992).

2- Bacteriological examination of samples (ISO 6579, 2002)

Stool and food samples were preenriched in buffered peptone water for 24 hours at 37ºC, then 1 ml was transferred to Rappaport Vassiliadis broth (BD, Germany) for selective enrichment and incubated at 42°C for 24 hours. A loopful from each incubated tube was streaked on Xylose-lysine desoxycholate agar (Himedia, India) and incubated at 37°C for 24 hours. The distinct colonies were streaked onto nutrient agar (Condalab) slants for identification by several biochemical tests as triple sugar iron (TSI), Indole, Methyl red, Simmon citrate and Urease.

3- Molecular identification of *Salmonella species*

QIAamp DNA mini kit, QIAGEN, Germany was used for DNA extraction from the suspected isolates. PCR was used for the detection of 16S rRNA gene specific for *Salmonella species* as recorded by Ziemer and Steadham (2003) with cycling condition including step of initial denaturation for 5 min at 94°C, 35 cycles of denaturation at 94°C for 1 min, annealing for 1 min at 55°C, extension for 7 min at 72°C using thermal cycler (Bio-Rad, USA). Electrophoresis was performed for PCR product by staining 1.5% agarose gel with ethidium bromide then photographed under transilluminator UV light (Biometra). A 100 pb DNA ladder (Norgen biotek, Canada) was used as a DNA marker. The primer sequence is F: TGT TGT GGT TAA TAA CCG CA and R: CAC AAA TCC ATC TCT GGA with product size 574 pb.

4- Serological identification of *Salmonella* species

Serological identification of *Salmonella* strains was performed in central laboratories of microbiology of Ministry of Health, Egypt based on flagellar (H) and somatic (O) antigens according to (Popoff *et al.*, 2004).

RESULTS

Table 1: Bacteriological identification of *Salmonella* species in human and food samples.

City	Human samples			Soft cheese			Grilled chicken		
	No. of patients	*Salmonella sp.*		No. of samples	*Salmonella sp.*		No. of samples	*Salmonella sp.*	
		No	%		No	%		No	%
Sohag	58	2	3.4	30	1	3.3	40	1	2.5
Tema	71	4	5.6	30	1	3.3	40	3	7.5
Elmaragha	66	3	4.5	30	0	0	40	2	5
Total	195	9	4.6	90	2	2.2	120	6	5

Table 2: PCR and serelogical identification of *Salmonella* species in human samples.

Locality	No. of patients	Positive 16S rRNA gene		Serological identification					
				S .Typhimurium		*S. Infantis*		*S. Enteritidies*	
		No	%	No	%	No	%	No	%
Sohag	58	2	3.4	1	1.7	1	1.7	0	0
Tema	71	4	5.6	2	2.8	1	1.4	1	1.4
Elmaragha	66	3	4.5	3	3.4	0	0	0	0
Total	195	9	4.6	6	3.1	2	1.02	1	0.5

Table 3: Patient characteristics and risk factors related to *Salmonella species* infection.

	Food poisoning patients N/195		*Salmonella species* N/9	
1- Personal information	No	%	No	%
a- Age				
15-25	48	24.6	4	44.4
26-35	63	32.3	2	22.2
36-45	50	25.6	1	11.1
46-55	16	8.2	0	0
>55	18	9.2	2	22.2
b- Gender				
Male	133	68.2	6	66.7
Female	62	31.7	3	33.3
2- Animal contact	102	52.3	5	55.5
a- At home				
Chicken	31	30.4	0	0
Cattle and chicken	34	33.3	1	11.1
b- At work				
Poultry seller	13	12.7	1	11.1
Poultry farm worker	15	14.7	1	11.1
Food handler	4	3.9	1	11.1
c- Both at home and work	9	8.8	1	11.1
d- Not contact with animals	93	47.7	4	44.4
3- Contact with infected persons	7	3.6	2	22.2

Table 4: PCR and serelogical identification of *Salmonella* species in food samples**.**

Type of food	No. of the examined samples	Positive 16S rRNA gene		Serological identification					
				S .Typhimurium		*S. Infantis*		*S. Kentucky*	
		No	%	No	%	No	%	No	%
Soft cheese	90	2	2.2	1	1.1	1	1.1	0	0
Grilled chicken	120	5	4.2	2	1.7	2	1.7	1	0.8
Total	210	7	3.3	3	1.4	3	1.4	1	0.5

Table 5: Frequency distribution of *Salmonella species* in soft cheese in three cities in Sohag Governorate.

City	No. of the examined samples	Soft cheese							
		S .Typhimurium		*S. Infantis*		*S. Kentucky*		Total	
		No	%	No	%	No	%	No	%
Sohag	30	0	0	1	33.3	0	0	1	33.3
Tema	30	1	33.3	0	0	0	0	1	3.3
Elmaragha	30	0	0	0	0	0	0	0	0

Table 6: Frequency distribution of *Salmonella species* in grilled chicken in three cities in Sohag Governorate.

City	No. of the examined samples	Grilled chicken							
		S .Typhimurium		*S. Infantis*		*S. Kentucky*		Total	
		No	%	No	%	No	%	No	%
Sohag	40	1	2.5	0	0	0	0	1	2.5
Tema	40	1	2.5	1	2.5	0	0	2	5
Elmaragha	40	0	0	1	2.5	1	2.5	2	5

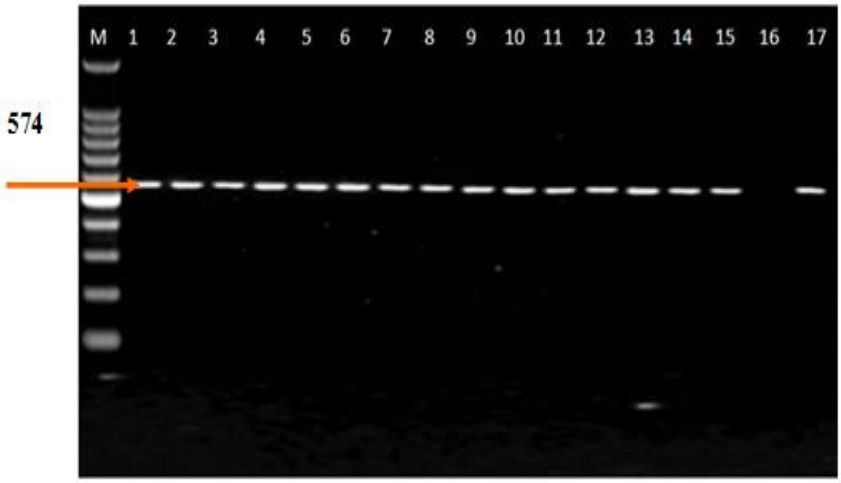

Figure 1: PCR result of 16S rRNA gene specific for *Salmonella species* in human and food samples. Lane M: 100 bp ladder, lane 1, 2, 3, 4, 5, 6, 7, 8, 9,10,11,12,13,14,15 and 17: positive 16S rRNA gene, lane 16: Negative 16S rRNA gene.

DISCUSSION

Salmonella species were detected by the microbiological methods in 9 (4.6%) out of 195 patients suffering from food poisoning manifestation admitted to three central hospitals in Sohag Governorate, 2 (2.2%) of 90 soft cheese and 6 (5%) of 120 grilled chicken samples purchased from groceries, restaurants, cafeterias and market vendors in the three cities under study (Sohag, Tema and Elmaragha) (Table 1). Although bacteriological methods are inexpensive, it is consuming time, so early detection of *Salmonella species* in human and food is important for human health and food industry (Aida *et al.*, 2012). Therefore rapid and sensitive PCR method was used for confirmation of the obtained bacteriological strains using *Salmonella species* specific 16S rRNA primers and found that human and soft cheese samples give the same results obtained by culture methods while in grilled chicken samples, only one sample was negative by PCR (Table 2,4 and Figure 1). In comparison between PCR and conventional microbiological methods, PCR is more successful especially in *Salmonella* outbreaks because it is rapid and requires limited manpower (Van der *et al.*, 2000).

The results shown in Table 2 revealed that among 195 patients suffering from food poisoning manifestation, 9 (4.6%) were positive for *Salmonella species*, this results goes parallel with Gharieb *et al.* (2015) and Hassan *et al.* (2016). Higher results were obtained by

Nader *et al.* (2015) and lower results were reported by Mayada and Adel (2015), this diversity may be related to the geographical distribution, seasonal variation, food habits and the hygienic practice taken during food handling, transportation, and contact with animals and infected patients. According to the regional distribution; Tema reported the highest infection rate 4 (5.6%) followed by Elmarahga 3 (4.5%) and Sohag 2 (3.4%), this may be clarified by the lack of personal hygiene, bad eating habits and unrestricted control measures taken by the authorities for food safety and health inspection in this locality.

Salmonella serotyping is important in the epidemiological investigation of *Salmonella* infection; identifying *Salmonella* serotype is helpful in providing the information about the source and the severity of infection (Molbak *et al.*, 2006). As reported by Thomas *et al.* (2012); the most prominent *Salmonella* serovar is *Salmonella Typhimurium* which detected in 6 (3.1%) patients and *Salmonella Infantis* was detected in two (1.02%) patients while only one (0.5%) patient represent *Salmonella Enteritidis* opposite to Ammar *et al.* (2010) who detected *Salmonella Enteriridis* as a predominant serovar. *Salmonella Infantis* has previously been found in chicken eggs, poultry, cattle and animal feed but human infections has been increased around the world, also it considered one of the main *Salmonella* serotypes causing human gastroenteritis (Najjar *et al.*, 2012).

Table 3 illustrated the patient characteristics and risk factors related to *Salmonella species* infection among nine salmonellosis patients, the results revealed that the highest infection rate 4 (44.4%) was detected in the age group from15-25 years while the older age 46-55 gave negative results for *Salmonella species*, and infection in males 6 (66.7%) was higher than females 3 (33.3%), this may be due to that people in this age (15-25) and males spend most of their time outside homes and buy their meals from restaurants, markets and grocery which may lack the hygienic measures. Exposure to animals either occupational or at home increase the probability of *Salmonella species* infection as 5 (55.5%) patients were found to be in contact with animals. As shown in our results; two patients (22.2%) out of nine patients having a history of contact with infected persons were harbored *Salmonella species* in their stool, therefore detection of *Salmonella species* is important for diagnosis of infected patients and identification of carriers, hence; contact with infected patients with gastrointestinal manifestations especially inside the household considered a risk factor and source of infection among the family member (Lapo *et al.*, 2014).

Cheese is the most ready to eat milk product consumed by the Egyptian people and widely distributed in groceries and vendors in local markets without any observations from the health authorities. It is manufactured by traditional methods on small scale in farmer houses under unhygienic measures using raw milk or milk heated below the pasteurization temperature with high microbial load (Robinson and Tamime, 2002). So it is considered a risk food and consumption of this cheese leading to *Salmonella* outbreaks and other pathogens such as *Staphylococcus aureus* and *Listeria monocytogens* (Hall and French, 2011).

Contamination of foods especially grilled chicken depends on the level of *Salmonella* on poultry meat used as a raw product beside storage temperature and cross contamination at retail during slaughtering, scalding, evisceration and distribution. Also incomplete heat treatment during preparation and unhygienic practice during handling and preparation (El-Leithy and Rashad, 1989).

The results in Table 4 revealed that two (2.2%) out of 90 soft cheese and 5 (4.2%) out of 120 grilled chicken samples were positive for *Salmonella species*, the obtained results is similar to those obtained by Arif (2012) and Effimia (2015) and lower than that reported by Mayada and Maha (2014), and opposite to Ortolani et al. (2010) who cannot detect *Salmonella species* in soft cheese. The presence of *Salmonella species* in food may be resulted from that food not freshly prepared and left for long time at room temperature which enhances the multiplication of bacteria. Regarding to *Salmonella* serotypes, *Salmonella Typhimurium*, *Salmonella Infantis* and *Salmonella Kentucky* was detected in food samples with percentages of 1.4, 1.4 and 0.5, respectively. The occurrence of the same *Salmonella species* in human stool proved that the contaminated food is a major source of salmonellosis infection (Hernandez, 2005) and the detection of *Salmonella Enteritidis* in human stool and their absence in the examined food samples explained by presence of other source of infection such as contact with animals or infected persons or other type of food.

Results in Table 5 and 6 explained that grilled chicken considered as a possible source of *Salmonella species* infection in the three examined localities, while soft cheese was detected only in two clusters, their absence in Elmaragha does not preclude the possibility of its existence with illegal distribution and marketing in poor hygienic measures (Molla *et al.*, 2003). Therefore, education of the public about the food safety is required parallel with restricted control measures taken by the health and veterinary authorities.

CONCLUSION

Salmonellosis is transmitted to human through several pathways including food and non food sources, risk factor identification will reflect the

epidemiological picture which is not clear in Egypt and help the health and veterinary authorities to set a control strategy to reduce the infection.

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الجبن الطرى والفراخ المشوية كمصدر محتمل لعدوى السالمونيلا فى الانسان مع الاهتمام بعوامل الخطورة

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صممت هذه الدراسة لتحديد مدى تواجد أنواع السالمونيلا بين مرضى التسمم الغذائى فى ثلاث مستشفيات مركزية فى ثلاث مدن بمحافظة سوهاج (سوهاج، طما ، المراغة) ومن الأطعمة التى يستهلكها غالبية المرضى مثل الجبن الطرى والدجاج المشوى باستخدام عدة طرق مثل الميكروبيولوجيا والبيولوجيا الجزيئية من خلال استخدام برايمر 16S RNA المحدد لجين السالمونيلا واختبار السيرولوجى لتحديد أنواع السالمونيلا. وكذلك تحديد عوامل الخطورة ذات الصلة بعدوى السالمونيلا فى الانسان من خلال البيانات التى تم جمعها من المرضى. وقد أوضحت النتائج أن السالمونيلا وجدت فى ٩ (٤.٦%) من المرضى، ٢ (٢.٢%) من الجبن الطرى و ٥ (٤.٢%) من الدجاج المشوى، وكانت السالمونيلا تايفميورم هى النوع السائد. بالاشارة الى عوامل الخطورة; تبين ارتفاع نسبة الاصابة بين المرضى فى الفئة العمرية من ١٥-٢٥ سنة ، الذكور، والذين على اتصال باشخاص مصابين أو حيوانات. التثقيف الصحى، الكشف السريع عن العدوى، تحديد عوامل الخطورة والتعاون بين السلطات الصحية والزراعية مهمة لوضع استراتيجية ناجحة لمكافحة العدوى.