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BACTERIOLOGICAL STUDIES ON *SALMONELLA ENTERITIDIS* ISOLATED FROM DIFFERENT SOURCES IN DAKHLIA GOVERNORATE

(With 4 Tables)

By

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دراسات بكتريولوجية على سالمونيلا انتريتيدس المعزولة من مصادر مختلفة فى محافظة الدقهلية

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نظراً لتزايد ظاهرة التسمم الغذائى وما يترتب عليها من مشاكل صحية جسيمة ف ى كل دول العالم المتقدمة منها والنامية. ولما كان ميكروب السالمونيلا من أهم الميكروبات المدرجة ضمن مسببات التسمم الغذائ ى فقد خُططت هذه الرسالة بغرض استبيان التواجد المحتمل لميكروب السالمونيلا في بعض المنتجات الغذائية ذات الأصل الحيوانى مثل لحوم الدجاج واللحوم الحمراء المجمدة والبيض وكذا منتجات الألبان وايضا العاملين بالمستشفيات وكذلك الكشف عن الأجسام المضادة فى دم الأشخاص المصابون بالتسمم الغذائى بواسطة الاليزا. وكانت نسبة عترات السالمونيلا انتريتيدس المعزولة 14(14%) من 100 عينة دجاج حى، 2 (2%) من 100 عينة دجاج مجمد، 1(1%) من 100 عينة لحم مجمد، 1(2%) من 50 عينة بيض مائدة ،3(12%) من 25 عينة لبن غير مبستر، 2(8%) من 25عينة ايس كريم مصنع فى المنازل، 6(12.5%) من 48 مسحة شرجية من العاملين بالمطبخ، 4(13.3%) من 30عينة من المتعاملين فى نظافة واعداد الطعام، 8(16.67%) من 48 عينة سيرم من اشخاص يعانون من اعراض تسمم غذائى.

SUMMARY

Foodborne diseases are a widely spreading and growing public health problem, in both developed and developing countries. *Salmonella* microorganisms are one of the most important causative agents of food poisoning, so this study was planned to determine the incidence of

Salmonella enteritidis in 546 samples from different sources of animal origin as live poultry, eggs, poultry meat, beef meat and milk products, and also of human origin as food handlers and patients suffering from food poisoning. Detection of *Salmonella enteritidis* antibodies in patient serum was detected by ELISA .Fourteen strains (14%) of *S.enteritidis* were isolated from 100 samples of live poultry, 2 strains (2%) from 100 samples frozen poultry, 1 strain (1%) from 100 samples frozen meat, 1 strain (2%) from 50 egg samples, 3 strains (12%) from 25 unpasteurized raw milk samples, 2 strains (8%)from 25 home made ice-cream samples, 6 strains (12.5%) from 48 stool samples of food poisoning people and 4 strains (13.3%) from food handlers. The results of ELISA proved that 8 (16.67%) of 48 serum samples had *S.enteritidis* antibodies.

Key words: *Salmonella enteritidis, poultry, eggs, milk and milk products, ELISA, stool.*

INTRODUCTION

Salmonella is one of the primary causes of human food poisoning throughout the world (Fantasia and Filetici, 1994). Worldwide, salmonellosis is a serious medical and veterinary problem and raises great concern in the food industry. Poultry is the most potential source of Salmonella food poisoning in man. Moreover, contamination of the poultry meat with Salmonella in the poultry slaughterhouses is very important (Ashton, 1990). The dominant type of Salmonella food poisoning was *Salmonella typhimurium* but since 1982, *S.enteritidis* has been challenging for the dominance. *S.enteritidis* carried out by chickens and poultry products is the major source of human intestinal infections (Fantasia and Filetici, 1994). Several serological tests have been developed for the diagnosis of *Salmonella* infections, the enzyme linked immunosorbent assay (ELISA) had been used especially for the detection of *S.enteritidis* and *Salmonella typhimurium* carriers in chicken (Barrow, 1994).

The development of effective vaccines against *S.enteritidis* for chickens has been hindered by lack of knowledge concerning the immune responses against *Salmonella* in chickens. In general, the mucosal immune system of the intestine, including mucosal immunoglobulin A "secretory IgA" and associated lymphocytes and leukocytes, forms the first line of defense against *S.enteritidis* infection. Systemic immune responses, including humoral and cell-mediated responses play important roles in the resistance and clearance of *S.enteritidis* infection (Zhu *et al.*, 2009).

This study was planned as attempt to spotlight on estimation of the incidence of *Salmonella* organisms isolated from different sources of animal origin as live&frozen poultry, eggs, frozen beef meat, milk and milk products, and also of human origin as food handlers and patients suffering from food poisoning, serotyping of the isolated *Salmonella* species and identifying the prevalent serotypes causing food poisoning, with a special concern to ELISA in detection of *S.enteritidis* antibodies and Throwing light on antimicrobial sensitivity for strains of *S.enteritidis*.

MATERIALS and METHODS

Table 1: Number, sources and clinical condition of samples collected from Dakahlia governorate

Source	Number	Condition of samples
Stool samples from patients with food poisoning signs	48	Apparently sick
Blood samples from same patients	48	Apparently sick
Food handlers' hand swabs	30	Apparently normal
Live poultry	100	Apparently normal
Frozen poultry before cooking	100	Apparently normal
Frozen meat before cooking	100	Apparently normal
Table eggs	50	Apparently normal
Milk and milk products Raw milk Ice-cream Soft cheese Yoghurt	 25 25 25 25	 Apparently normal Apparently normal Apparently normal Apparently normal
Total	576	

Media: Nutrient agar, MacConkey's agar, XLD agar and sugar media were used for isolation of *Salmonella* species and identification of the bacterial isolates according to Cruickshank *et al.* (1975).

Serological typing of *Salmonella*: The isolates that were preliminarily identified as *Salmonella* were subjected to serological identification according to Kauffman-White Scheme (Kauffman, 1974).

ELISA: ELISA technique was done to detect *S.enteritidis* antibody in serum samples from patients suffering from food poisoning using *S.enteritidis* ELISA kits (IDEXX USA) with *S.enteritidis* coated ELISA plate according to Hassan *et al.* (1990).

Antibiotic susceptibility test: Determination of the susceptibility of the isolated strains to antibiotic discs (oxoid) was adopted using the disc diffusion technique according to Finegold and Martin (1982).

RESULTS

Table 2: Incidence of *Salmonella enteritidis* from the examined samples

Samples	Total samples	*Salmonella enteritidis*	
		No.	%
Stool samples	48	6	12.5
Food handlers	30	4	13.3
Live poultry	100	14	14
Frozen poultry samples	100	2	2
Frozen meat samples	100	1	1
Egg samples	50	1	2
Raw milk	25	3	12
Ice-cream	25	2	8
Soft cheese	25	-	-
Yoghurt	25	-	-
Total	528	33	64.8%

Table 3: Antimicrobial sensitivity test in the examined samples

Antibiotics	Conc. µg	Percentage of effect on isolated *S.enteritidis* strains							
		Human	Food handlers	Live poultry	Frozen poultry	Frozen meat	Egg	Raw milk	Ice cream
Enrofloxacin	5	91.9%	85.4%	83%	81%	83.8%	83.5%	77.4%	77%
Trimethoprim-sulfamethoxazole	25	24%	19%	12.3%	18.7%	15.4%	21%	9.4%	17.5%
Ampicillin	20	6%	9%	8%	14.2%	5%	10%	13.6%	12.1%
Chloramphenicol	30	52%	49%	33.7%	65%	43%	41%	54%	54.2%
Ciprofloxacin	5	93.8%	86%	87.5%	93%	83%	95.4%	88%	90.2%
Neomycin	30	61.2%	58.3%	43.4%	48%	62.4%	73.7%	67.8%	50.2%
Gentamicin	10	68.6%	62.4%	73.4%	68.5%	53.7%	68.2%	45.7%	67.9%
Tetracycline	30	55.4%	50.8%	52%	57.5%	43%	48.7%	8%	40.6%

Table 4: Serodiagnosis of *Salmonella enteritidis* using ELISA.

No. of samples	Optical density	S/P	No. of samples	Optical density	S/P
1	0.982	0.4069	25	0.772	0.2136
2	1.210	0.6169	26	1.285	0.6860
3	1.740	1.1049	27	0.848	0.2836
4	1.325	0.7228	28	1.395	0.7872
5	2.085	1.4226*	29	1.842	1.1988
6	0.985	0.4097	30	1.487	0.8720
7	0.762	0.2044	31	2.358	1.674*
8	1.562	0.9410	32	1.185	0.5939
9	1.443	0.8314	33	2.796	2.0773*
10	1.035	0.4558	34	1.096	0.5119
11	1.854	1.2099	35	1.384	0.7771
12	1.635	1.0082	36	0.694	0.1418
13	1.214	0.6206	37	1.862	1.2173
14	1.465	0.8517	38	1.692	1.0608
15	2.472	1.7790*	39	2.395	1.7081*
16	0.652	0.1031	40	1.427	0.8167
17	1.821	1.1795	41	1.396	0.7882
18	1.689	1.0580	42	0.874	0.3075
19	0.796	0.2351	43	1.625	0.9990
20	2.647	1.9403*	44	2.235	1.5607*
21	1.523	0.9051	45	1.487	0.8720
22	1.326	0.7237	46	1.742	1.1068
23	0.984	0.4088	47	2.452	1.7605*
24	1.694	1.0626	48	1.884	1.2375

Number of seronegative samples (%) = 40 (83.33)
Number of seropositive samples (%) = 8 (16.67%).
S/P $\geq$ 1.35 positive, < 1.35 negative
* Indicates positive result.

DISCUSSION

Salmonellosis is considered one of the dangerous zoonotic diseases causing severe economical losses in both human and animal sources. Food poisoning resulting from consumption of food from animal origin contaminated with *Salmonella* microorganisms, is being one of the emphasizing needs to spotlight upon its prevalence among human and feed.

The obtained results in Table (2) showed that the *S.enteritidis* was isolated from human stool samples with an incidence of (12.5%). This result was nearly similar to the result recorded by Das *et al.* (1990)

who recorded that a percent (12%) of *S.enteritidis* was isolated from human stool samples. On the other hand, the obtained results were higher than the results recorded by Danish Zoonosis Center (1997) which recorded an incidence (0.07%) of SE, this low incidence was mainly due to the higher hygienic measures and lower than the incidence recoded by Kwabata *et al.* (2006) (70%), this high incidence because all samples are taken from human suspected salmnellosis outbreaks.

The incidence of *S.enteritidis* isolated from 30 hand swabs of food handlers was (13.3%), this incidence agreed with incidence of Dryden *et al.* (1994) which was (12.3%), this proved that improper sanitary measures, and inadequate hand washing or clean may be the cause. The incidence was higher than results obtained by Smith *et al.* (2009) (5.3%) in food handlers, their low incidence was good indication for high sanitary measures of these workers. Our result was lower than that recorded by Bailey and Gosby (2005) which was 31%, and was mainly due to the very low hygienic measures of the workers.

The obtained data revealed that the incidence of *S.enteritidis* from 100 cloacal swabs was (14%). This result was of a similar range of results reported by Osman (1992) who isolated *S.enteritidis* from broiler farms with an incidence (17.8%). These results may came from contamination during sampling and also may be due to massive use of antibiotic treatment beginning from day-1 of age, which leads to presence of carrier individuals which may harbour the organism with no expressing of signs of a disease. On the other hand, *S.enteritidis* was isolated with lower incidence by Hui and Das (2001) (4.28%), Murugkar *et al.* (2005) (3.64%) and El-Zeedy *et al.* (2007) (1.54%) from live poultry samples. These low incidences of isolation may be due to recently used hygienic control measures used in poultry farms, and the developed methods of rearing of flocks.

The incidence of *S.enteritidis* in poultry meat was (2%). Higher results were reported by Jalali *et al.* (2008) (17.9%) and Duarte *et al.* (2009) (9.6%) in broiler carcasses. This may be due to that poultry meat and its products are liable for contamination from different sources at slaughtering, during its production, handling, packing and storages.

Reported data indicated that incidence of *S.enteritidis* isolates from 100 frozen beef meat samples were (1%). This obtained result was low than that reported by Mosupye and Holy (1999) (3.3%). These results may be due to no proper evisceration occurred to the carcasses or an external contamination occurred from food handlers or an unhygienic handling of meat during its preparation and storage. The incidence of

S.enteritidis from egg samples was (2%). This obtained result was to some extent lower than that obtained by Molbak and Neimann (2002) who recorded similar results (3.4%) from raw eggs and (2.5%) from fried eggs. These results can be explained as collected eggs had transovarian transmission from carrier birds which had no signs of a disease but shedding of *S.enteritidis* in eggs have been occurred. The incidence of *S.enteritidis* isolated from raw unpasteurized milk was (12%). This result was supported by Karns *et al.* (2005) who recorded a nearly similar incidence (11.8%) Also, Nero *et al.* (2009) isolate (9.2%) from raw unpasteurized milk. This similarity may be due to that the raw milk had been contaminated from utensils, handlers or milkers. However, this obtained incidence was higher than the incidence obtained by Jayarao and Henning (2001) (6.1%), and this lower incidence may be due to more hygienic measures application during milking and handling. Some other researchers failed to isolate *S.enteritidis* from milk at all, such as EKici *et al.* (2004) and Abd El-Aal (2008).

The obtained data showed that incidence of *S.enteritidis* isolated from ice-cream samples was (8%). This obtained result was within range but quite higher than obtained incidence by El-Sharef *et al.* (2005) who recorded an incidence of (5%) from ice-cream samples. This incidence may be due to that processing and manufacturing of ice-cream was affected by contamination during processing or from food handlers' contaminated hands or contaminated utensils. Our results were higher than the results of Thomas *et al.* (1996) who recorded a (3%) incidence as they collected samples from ice-cream manufactured from pasteurized milk and this incidence of isolation may have come from post-manufacture contamination. The reported results showed that *S.enteritidis* could not be isolated from either yoghurt or soft cheese (Zero %). These results agreed with results indicated by Borelli *et al.* (2006); Abd El-Aal (2008) who failed to isolate any *Salmonella* strain from yoghurt or soft cheese. These results may be due to that the processing of soft cheese occurs when the acidity of milk is increased towards the acid pH (decreased pH value) nearly to pH (4), and this low pH is a more killer and not suitable for *S.enteritidis* growth or multiplication. Also, the same manner occurs in yoghurt as high acidity of yoghurt leads to death of the microorganism. On the other hand, some investigators could isolate *S.enteritidis* such as Yagoub *et al.* (2006) (6%) from yoghurt and Colak *et al.* (2007) (1.5%) from cheese.

The enzyme linked immunosorbent assay (ELISA) is now widely used to detect antibodies in a variety of test system such assays are

specific, sensitive and relatively inexpensive to set up. It can offer rapid screening of samples with a negative result available in 24 hours and an early indication of a potential positive result. Results of ELISA revealed that from 48 serum samples of patients suffering from food poisoning, 8(16.67%) of the examined serosamples were sero-positive (Table 4). The obtained result was somewhat lower than the results of Schneid *et al.* (2009) who recoded (26%).

Regarding the antimicrobial sensitivity tests Table(3) using disk diffusion method, to the isolated *S.enteritidis* strains from the previous sources, showed that ciprofloxacin was the drug of choice followed by enrofloxacin, gentamicin, then neomycin, tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole and the lowest sensitivity was to ampicillin. This results was assured by the results of some researchers such as Duarte *et al.* (2009) who isolated *S.enteritidis* from patients with food poisoning symptoms, Murugkar *et al.* (2005) who showed that all *S.enteritidis* isolated from poultry, Yagoub *et al.* (2006) who found that all isolated *S.enteritidis* from milk and its products, Simth *et al.* (2009) who found that all isolated *S.enteritidis* from food handlers was highly sensitive to ciprofloxacin followed by enrofloxacin then chloramphinicol and low sensitive to gentamicin, trimethoprim- sulfamethoxazole but highly resistant to ampicillin.

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