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Salmonella enteritidis isolated from different sources in Sohag Governorate.

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Abstract

Foodborne diseases are a widely spreading and growing public health problem, in both undeveloped and developing countries. *Salmonella* microorganisms are one of the most important causative agents of human food poisoning. In this study, 200 samples collected 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 and examined bacteriologically for detection of *S. enteritidis*. Results showed that six strains (15%) of *S. enteritidis* were isolated from 40 samples of live poultry meat, 2 strains (5%) from 40 samples frozen poultry meat, 2 strain (5 %) from 40 samples frozen beef meat, 3 strains (15%) from 20 unpasteurized raw milk samples, 2 strains (10 %) from 20 home made ice-cream samples, 4 strains (20 %) from 20 stool samples from people suffered from food poisoning, 10 serum samples from the same patient where had *S. enteritidis* antibodies by ELISA technique.

Key words: *Corona virus, ELISA, Direct fluorescent anti-body technique (FAT).*

Introduction

Food–born diseases are an important cause of morbidity and mortality worldwide .The worldwide incidence of non-typhoidal Salmonellosis is estimated at 1-3 billion cases and 3 million deaths annually **(Tassios *et al*., 1997)**. Although Salmonela gastroenteritis is generally self–limiting illness, sever cases require antimicrobial therapy. 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)**. Systemic immune responses, including humoral and cell – mediated responses play important roles in the resistance and clearance of *S.interitidis* infection **(Zhu *et al.*, 2009)**.

Food contamination with antibiotic–resistance determinates can be transferred to other pathogenic bacteria, potentially compromising the treatment of sever bacterial infections. The prevalence of antimicrobial resistance has increased during recent decades

(Chiu *et al.*, 2002). This increase attributed to the selection pressure created by using antimicrobials in food-producing animals, in addition to the unregulated use of antibiotics by humans in developing countries **(Bywater, 2004).**

This study was planned as attempt to spotlight on estimation of the incidence of *Salmonella enteritidis* 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 symptoms, 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* isolates.

Materials And Methods

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 su20bjected 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 *S.enteritidis* strains to antibiotic discs (Oxoid) was adopted using the disc diffusion technique according to **Finegold and Martin (1982)**.

Table (1). Number, sources and clinical condition of samples collected from Sohag governorate

Source	Number	Condition of samples
Stool samples from patients with food poisoning signs	20	Apparently sick
Blood samples from same patients	20	Apparently sick
Live poultry meat (fresh)	40	Apparently normal
Frozen poultry before cooking	40	Apparently normal
Frozen meat before cooking	40	Apparently normal
Milk (Raw milk) and milk products Ice-cream	20 20	Apparently normal Apparently normal
Total	200	

RESULTS

Table (2). Incidence of *Salmonella enteritidis* in the examined samples.

Samples	Total samples	*Salmonella enteritidis*	
		No.	%
Stool samples	20	4	20
Live poultry	40	6	15
Frozen poultry samples	40	2	5
Frozen meat samples	40	2	5
Raw milk	20	3	15
Ice-cream	20	2	10
Total	180	19	15 .6

Table (3). Antimicrobial sensitivity test for *S.enteritidis* isolates from human and live poultry in the examined samples.

antibiotics	Conc. ug	Percentage of effect on isolated *S.enteritidis* strains					
		Human	Live poultry	Frozen poultry	Frozen meat	Raw milk	Ice cream
Enrofloxacin	5	91.9%	83%	81%	83.8%	77.4%	77%
Trimethoprim-sulfamethoxazole	25	24%	12.3%	18.7%	15.4%	9.4%	17.5%
Ampicillin	20	6%	8%	14.2%	5%	13.6%	12.1%
Cholramphenicol	30	52%	33.7%	65%	43%	54%	54.2%
Ciproflexacin	5	93.8%	87.5%	93%	83%	88%	90.2%
Neomycin	30	61.2%	43.4%	48%	62.4%	67.8%	50.2%
Gentamicin	10	68.6%	73.4%	68.5%	53.7%	45.7%	67.9%
Tetracycline	30	55.4%	52%	57.5%	43%	8%	40.6%

Table (4). Serodiagnosis of *Salmonella enteritidis* using ELISA.

No. of samples	Optical density	S/P
1	1.210	0.6169
2	1.740	1.1049
3	2.085	1.4226*
4	0.762	0.2044
5	1.443	0.8314
6	1.035	0.4558
7	1.854	1.2099
8	1.635	1.0082
9	1.465	0.8517
10	0.652	0.1031
11	1.821	1.1795
12	1.689	1.0580
13	0.796	0.2351
14	2.647	1.9403*
15	0.984	0.4088
16	1.694	1.0626
17	0.694	0.1418
18	1.862	1.2173
19	1.523	0.9051
20	1.326	0.7237

Number of seronegative samples (%) = 18 (90 %)
Number of seropositive samples (%) = 2(10 %)
S/P ≥ 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 (20%). This result was nearly similar to the result recorded by **Das *et al.* (1990)** who recorded that a percent of (12%) of *S.enteritidis* was isolated from human stool samples. Also, the obtained results were higher than those results reported 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 recorded by **Kawabata *et al.* (2006)**, (70%), this high incidence because all samples are taken from human suspected salmonellosis outbreaks.

The incidence of *S.enteritidis* in poultry meat was (15%). Nearly similar results (13.2%) were reported by **Van Polt (2003), Ammar *et al.* (2010)** and (21%) obtained by **Phan *et al*. (2006)**from 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 40 frozen beef meat samples were (5%). This obtained result was somewhat higher than that reported by **Mosupye and Holy (1999)** (3.3%) and more higher (1%) than that reported by **Jordan *et al.* (2006)**, this may be due to no paper recorded. Which 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* isolated from raw unpasteurizend milk was (15%). This result was supported by results of **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 findings were higher than that obtained by **Jayarao and Henning (2001)**who recorded an incidence of (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* form milk at all, such as **Ekici*et al.*(2004)**and**Abd El-Aal (2008)**.

The obtained data showed the incidence of *S.enteritidis* isolated from ice-cream samples was (10%). These results were higher than obtained incidence by **El-Sharef *et al.* (2005)** who recorded an incidence of (5%) from ice-cream samples. This variation may be due to the processing and manufacturing of ice-cream which affected by contamination during processing or from food handlers' contaminated hands or contaminated utensils. Our results were also higher than that of **Thomas *et al.* (1996)** who recorded a (3%) incidence in collected samples from ice-cream manufactured from pasteurized milk and this incidence of isolation may have come from post-manufacture contamination.

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 20 serum samples of patients suffering from food poisoning manifestation, 2(10%) 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 an incidence of (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 **Steven*set al.*(2006)** 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, **Smith*et al.* (2009)** who found that all isolated *S.enteritidis* from food handlers was highly sensitive to ciprofloxacin followed by enrofloxacin then chloramphenicol and low sensitive to gentamicin, trimethoprim- sulfamethoxazole but highly resistant to ampicillin.

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