INTRODUCTION

Salmonella is considered to be one of the most important causative agents which infects animal population causing great mortalities and various morbidity changes and at the same time considered as one of the most common and important zoonotic diseases in the world ***(UK Ministry of Agriculture, Fisheries and Food, 1983; UK Public Health Laboratory Service, 1984 and Wall et al., 1995)***.

Salmonella infection in poultry is responsible for a variety of disease conditions such as: Pullorum disease caused by *Salmonella pullorum*, fowl typhoid caused by *Salmonella gallinarum* and paratyphoid infection caused by serotypes other than *Salmonella pullorum* and *Salmonella gallinarum* usually caused by *Salmonella typhimurium* ***(Faddoul and Fellows, 1966; Klein, 1989; Wray and Wray, 2000)***.

The clinical disease caused by salmonella is usually divided into four syndromes: gastroenteritis, enteric fever, septecaemia and asymptomatic infection or carrier state.

Avian salmonellosis is an inclusive term designating a large group of acute and chronic diseases of poultry caused by one or more member of the genus Salmonella. Poultry and poultry products are consistently identified as important source of Salmonella that cause human illness ***(Snoeyenbos and Williams, 1991 and Tietjen and Fung, 1995)***.

Salmonella is belong to the family *Enterobacteriacea* (or Enterobacteria) defined in, which has diverse natural habitats including animal intestines, as well as plant, soil and water. The major characteristics that serve to identify this family are Gram negative, rod-shaped bacilli bacteria that can grow aerobically and anaerobically, produce a catalase but not oxidase, ferment glucose not lactose, hydrogen peroxide producer, usually motile by means of peritrchous flagella and non spore-forming ***(Joshua Lederberg, 2000; Encyclopedia of Microbiology)***.

The molecular typing is considered as a very important epidemiological marker ***(Crowley et al., 2002)***. The plasmid profiling of Enterobacteria was the first geneotypic method used for strain separation. The plasmids ranging in size from 2 to 150 kb, but

frequencies and size distributions vary between serovars according to ***Shaberg et al. (1981)***.

Analysis of the whole cell protein patterns has been used extensively to the study of the differences among bacterial genera, species and strains ***(Walia et al., 1988)***. Outer membrane protein (OMP) analysis has proved to be useful technique in the characterization of Salmonella ***(Fadl et al., 2002; Ochea-Reparaz et al., 2004)***.

Attention has been directed towards development of rapid and effective identification of food poisoning microorganism genetically based; primer mediated enzymatic amplification of target DNA called polymerase chain reaction (PCR) method ***(Bej et al., 1993)*** which has been successfully applied to overcome this obstacle. PCR has been shown to be a fast and reliable means for detection of bacteria from food and can amplify a single copy one million fold of a gene or DNA segment unique to a target microbial pathogen, shows a high degree of specificity and is extremely sensitive. Polymerase chain reaction offers advantages over conventional diagnostics method ***(Pfeffer et al., 1995)***.

AIM OF THE WORK

Trails to find rapid and highly sensitive test by using molecular biology techniques to differentiate the different Salmonella isolates for accurate genotyping assay.

LITERATURE REVIEW

1. Occurrence of salmonella in poultry:

Barrosamad and Martins (1985) recovered (101) isolates from poultry farms which included 23 of *S.typhimurium*, 19 of *S.typhimurium var Copenhagen*, 26 of *S.enteritidis*, 10 of *S.berta* and 7 of *S.havana*. Also, ***Rudy, (1985)*** identified salmonella from internal organs of broilers with incidence of 38% from faeces and 22% from bedding (litter) samples. The commonest serovars were *S.typhimurium, S.gallinarum* and *S.enteritidis*. While, 53% of 2603 isolates of salmonellae belonging to 50 serovars was reported by ***Schellner, (1985)*** as *S.typhimurium*, 18.5% *S.dublin* and 7.9% *S.tennessee*.

The incidence of salmonella in chicken meat and turkey meat in Egypt were studied by ***Safwat et al. (1985)***. The incidence was 9% in chicken meat and 3.4% in turkey meat. They demonstrated a variable incidence of salmonella according to the source of importation so the incidence from: France 13.4%, Israel 11.5%, Denmark 9.7%, USA 6.8%, West Germany 5%, Brazil 3.4%; serotypes were identified as *S.typhimurium*

showing the predominant serotype of isolation, *S.heidelberg, S.sandiago, S.agona, S.saintpaul, S.reading, S.ohio, S.brandenburg, S.infantis, S.virchow, S.colindale, S.munchen, S.newports, S.kentuchey, S.sofia, S.anatum, S.london, S.farchan, S.senttenberg, S.kaksony* and *S.cannis*. Five serovars from 2240 poultry carcasses examined in Accra as *S.anatum, S.birkenhead, S.typhimurium, S.poona* and *S.wippra* ***(Boachie, 1986)***.

Willinger et al. (1986) identified 19 salmonella serotypes among poultry farms, 6 serotypes were detected in feed (*S.heidelberg, S.typhimurium, S.duisburg, S.newport, S.saintpoul* and *S.drypool*), while *S.typhimurium* and *S.infantis* were present in poultry abattoirs.

The outbreak of *S.enteritidis* infection in broiler chicks in affected flock in East Angela in the UK in year 1987 was recorded by ***O'Brien, (1988)***. Up to 5% of chicks failed to grow and were culled. *S.enteritidis* phage type (4) was isolated from the heart blood. Pericarditis was subsequently seen in small number of broiler at time of slaughter.

Mcllory et al. (1989) identified *S.enteritidis* by serological and biochemical techniques in two clinically normal breeder flocks in Northern Ireland. The infected breeder flocks were slaughtered and the infection throughout the organization controlled and subsequently eradicated. The presences of salmonella in chicks were studied by ***Hahne and DeBoer, (1990)***. They examined chicken cuts and livers and found that the frequency of salmonella isolation was 54%.

Abd-Allah, (1991) revealed that the incidence of salmonella in broiler and parent chickens, in El-Fayoum governorate, was 6.4%. The isolates were belonging to group D1 (51.5%), group B (39.4%) and group C2 (9.1%). The most common serotypes were *S.gallinarum-pullorum, S.typhimurium, S.enteritidis*, *S.dublin* and *S.reading*.

After identification of 15 different serovars from the ovaries of commercial layer hens at time of slaughter. *S.heidelberg* was the most predominant serovar (56.5%), followed by *S.agona, S.soranienberg*, *S.mbandaka, S.kentucky, S.montevideo, S.london, S.typhimurium, S.infantis, S.schwarzengrund, S.ohio, S.cerro and S.anatum. S.enteritidis* phage type "23" was

recorded from only one of the flocks ***(Barnhart et al., 1992)***. In 3700 pooled caecal samples from laying hens the incidence of salmonella was 65.4%, but only 6 isolates were serotyped as *S.enteritidis* ***(Waltman et al., 1993)***. The presence of *S.enteritidis* in 2 out of 351 flocks of hens which produce hatching eggs were recovered by ***Ebel et al. (1994)***. *S.enteritidis* accounted 33.5% of human cases of salmonellosis.

A survey in large number of samples from different domestic birds and its environmental surroundings in El-Fayoum governorate was carried out by ***Abd-Allah, (1995)*** for detection of salmonella. Serological typing of the 25 salmonella isolates revealed that, 10 isolates were S.enteritidis (40%), 6 *S.typhimurium* (24%), 4 *S.montevideo* (16%), 3 *S.gallinarum pullorum* (12%) and one *S.california* and *S.newport* (4% each).

Oh and Choi, (1996) isolated 42 salmonella strains from 1577 caecal samples of chicks, the serotypes were: *S.typhimurium* (10), *S.typhimurium var Copenhagen* (5), *S.infantis* (4), *S.thompson* (3) and 20 were untypable. The most frequent isolates from commercial turkey flocks were *S.newport* (34.6%) and *S.reading* (30.3%) followed by *S.bredney* (10.6%),

S.enteritidis phage type 8 which was detected for only a short period (5 weeks) in one flock ***(Hafez and Stadler, 1997)***. The commonest isolate in poultry and for bearing animal samples was *S.enteritidis* (84.51% and 34.03% respectively) ***(Kopczewski et al., 1998)***.

A survey among 39 poultry flocks suspected of being infected with salmonella were carried out by ***Jindal et al. (1999)*** using clinical and post mortum examination. Salmonella infection accounted 5% of the total disease outbreaks 111 poultry, with overall morbidity and mortality rates of 14.22 and 12.12% respectively. Most isolates were *S.gallinarum*, while *S.enteritidis* was found in 3 flocks. The public health implications of the findings are mentioned.

The 231 strains of *S.typhimurium* phage type DT104 of animal origin isolated from geese, turkeys, poultry and porcine ***(Szmolleny et al., 2000)***. While, *Salmonella enteric* subsp. *enteric* serovar *enteritidis* recovered from laying fowls. *S.enteritidis, S.agona, S.thompson* and *S.sarajane* were isolated from broiler birds. Isolation of *S.thompson* and *S.agona* are reported for the first time in turkey, while isolation of *S.sarajane* from chickens is the first report in the world ***(Carli et al., 2001)***. *Salmonella enteric*

serotypes *Derby, mobandaka, Montevideo, Livingstone* and *sanftenberg* were among the most 10 prevalent serotypes isolated from farm animals in England and Wales ***(Liebana et al., 2001b)***.

The prevalence of Salmonella serovars among Danish turkeys between 1995 and 2000 were detected by ***Pedersen et al. (2002)***, the most five prevalent serotypes which accounted for 58.5% of the isolates were *S.heidelberg, S.agona, S.derby, S.muenster* and *S.anatum*. In addition, a few rough isolates and isolates belonging to the antigenically incomplete formulae 6, 7: a and 9, 12:b were found.

Salmonella are widespread in humans and animals worldwide. In industrialized countries, non-typhoid Salmonellae are an important cause of bacterial gastroenteritis. In the Netherlands, the estimated incidence of salmonellosis is three cases per 1000 inhabitants per year ***(Van den Brandhof et al., 2003)***.

The incidence of salmonella in Spanish poultry products was examined by ***Capita et al.,(2003)***. Samples included chicken carcasses, chicken parts and processed chicken products. *Salmonella enteritidis, Salmonella poona*, *Salmonella paratypi* B

and *Salmonella Worthington* were isolated in 34.3%, 11.4%, 2.8% and 1.4% respectively.*S.typhimurium, S.heidelberg, S.hadar, S.kentucky* and *S.thompson* were the most frequently isolated serovars. Over 90% of the *S.heidelberg, S.hadar, S.kentucky* and *S.thompson* were isolated from chickens. *S.enteritidis* was rarely isolated. There was an increasing trend in isolates from chickens, cattle and pigs, and a decreasing trend in isolates from turkeys ***(Guerin et al., 2005)***.

Tsai and Hsiang, (2005) recovered 10 serotypes of salmonella from cloacal swabs obtained from 100 duck farms in Taiwan, the serotypes were *S.potsdam* (31.9% of isolates), *S.dusseldorf* (18.7%), *S.indiana* (14.3%), *S.typhimurium* (7.7%), *S.hadar* (5.5%), *S.newport* (4.4%), *S.derby* (4.4%), *S.montevideo* (2.2%), *S.schwarzengrund* (2.2%) and *S.asinnine* (1.1%).The *S.enterica subsp. enterica serovar agona* plays an important role in Brazil as causative agent of salmonellosis in food-producing animals in pigs and poultry as well as in humans ***(Michael et al., 2006)***.

El-Zeedy et al. (2007) examined a total of 620 egg samples from different poultry species (chickens, ducks and ostriches) and 1615 poultry samples

(chicken, ducks, pigeons, quails, turkeys and ostriches) for salmonella infection in Egypt. Twelve salmonella isolates were obtained from egg samples and 67 isolates from poultry samples. Salmonella isolates were serotyped into *S.enteritidis, S.typhimurium, S.rubislaw, S.infantis, S.montevideo, S.cerro, S.virginia, S.agona, S.poona, S.derby, S.kentucky* and *S.sandiago.*

2. Antibiogram studies among salmonellae:

The sensitivity of 222 *S.typhimurium* strains, 250 *S.dublin*, 188 *S.choleraesuis*, 61 *S.enteritidis* and 73 *S.gallinarum-pullorum* to chloramphenicol, streptomycin, ampicillin, neomycin, colistin, nitrofurantoin and sulfathiazole were determined by ***Hoszowski and Truszczynski, (1980)***. All of 99 salmonella strains of animal origin belonging to 17 serotypes were of variable resistance, between one and ten of antibacterial used. The highest resistance rate was found with polymyxin B (95%), followed by bacitracin (83%), erythromycin (51.5%), penicillin (47.5%), viomycin (33.3%), oxytetracycline (26.3%), only one was resistant to chloramphenicol and one to ampicillin ***(Brahma et al., 1982)***. So, the resistance of salmonella strains was most frequent to streptomycin and tetracycline ***(Pohl et al., 1991)***.

Cicek and Kovarik, (1994) studied the resistance of 293 *S.typhimurium* and 260 *S.enteritidis* to chloramphenicol, neomycin, tetracycline, strepto-mycin, colistin, ampicillin, kanamycin and sulfafurazole. About 90% of the isolates were sensitive to the all antibacterial used, 51 (7.4%) of the *S.typhimurium* and 3 (1.2%) of the *S.enteritidis* showed resistance, 32 (10.9%) of *S.typhimurium* were resistant to sulfafurazole, 33 (11.3%) to streptomycin, 13 (4.4%) to tetracycline, 5 (1.7%) to chloramphenicol, 3 (1.0%) to ampicillin and 1 (0.3%) to colistin.

Urumova et al. (1998) examined 58 *S.typhimurium*, 12 *S.bangi* and 15 *S.enteritidis* strains isolated from broilers for resistance to ampicillin, carbencillin, streptomycin, gentamycin, chloramphenicol, kanamycin, tetracycline, erythro-mycin, nalidixic acid, oxolinic acid, flumequine and enrofloxacin. The isolates showed high percentage of resistance (64-90%) to ampicillin, carbencillin, tetracycline and erythromycin, about 20% resistance to nalidixic acid and 10% to streptomycin and kanamycin and about 5% to gentamycin and chloramphenicol. None of the strains were resistant to oxolinic acid, flumequine and enrofloxacin.

Peresi et al. (1999) found that 13.79% out of 160 salmonella isolated from chicken carcasses were resistant to the examined antimicrobial agents (sulfonamides, fosfomycin, tetracycline, cefuroxime, gentamycin, cefalofin and cefoxitin).While, the resistance of salmonella to enrofloxacin and commonly used antimicrobial agents were examined by ***Pirro et al. (1999)***. The strains were isolated randomly from intestinal contents of chickens at slaughter and their susceptibility was quantitatively determined. Resistance to enrofloxacin was detected in 9 isolates (0.45%), all belonging to *S.hadar*. *S.typhimurium* phage types DT104 possess resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline (ACSSUT) resistance. Several laboratories have shown that the antibiotic resistance gene of DT104 are chromosomally encoded and involved integrons, this gene conferring the ACSSUT-resistant phenotype have been cloned and sequenced. The resistance rates were 7.2% for neomycin, 9.5% for trimethoprim-sulfadiazine, 25.3% for ampicillin and 62.6% for streptomycin ***(Connie et al., 1999)***.

The study of the antibacterial susceptibility test of 5 salmonella isolates from poultry Showed that, all

strains were sensitive to nalidixic acid and rifampicin ***(Chachra et al., 2000)***. While, the salmonella isolated from turkeys were sensitive to enrofloxacin (92%), norfloxacin (84%), neomycin (82%) and amoxicillin (74%) ***(Koncicki et al., 2000)***. Also, all strains isolated from chickens (*S.enteritidis, S.anatum* and *S.enterica subsp. enterica serovar* 3, 10: e,h) were resistant to penicillin G ***(Bauerfeind et al., 2001)***.

Lee et al. (2003) performed antibiotic susceptibility test against 258 isolates of *S.gallinarum* isolated from Korea using 12 antimicrobial agents. All isolates from 1995 appeared to be susceptible to all of the antimicrobial agents tested except for tetracycline and oxytetracycline. While the vast majority of isolates from 2001 showed the reduced susceptibility to ampicillin (13%), gentamycin (43.4%), kanamycin (69.6%), enrofloxacin (6.5%), ciprofloxacin (10.9%), norfloxacin (52.5%) and ofloxacin (82.6%). The prevalence of the completely resistant isolates to one or more drugs rapidly increased from 0% in 1995 to 93.5% in 2001.The most strains of *S.typhimurium* were resistant to ampicillin, chloramphenicol, streptomycin, sulfonamids and tetracycline ***(Botteldoorn et al., 2004)***.