5. DISCUSSION

Poultry are commonly infected with a wide variety of ***Salmonella*** serovars. Infections are generally subclinical and one serovar may be a predominant isolate in a country for several years before it is replaced by another serovar **(Wray *et al.*, 1996)**. Two serovars that have been of most concern in recent years are ***S.* Enteritidis** and ***S.* Typhimurium (Tellez *et al.*, 2001).**

Bacteriological examination is the traditional mean to obtain accurate data about the prevalence of ***Salmonella*** in the infected hosts **(Commission of The European Communities, 1992)**.

In the present study, a total 1073 samples collected from apparently healthy, diseased or dead broiler chickens were subjected to bacteriological examination for recovery of ***Salmonella*** spp. All isolates showed red colonies with black center on XLD agar media and white colonies with black center on S.S agar media. Gram stained colonies showed Gram negative and non spore forming rods **(Carsiotis *et al.*, 1984)**. Biochemically they appeared as non lactose fermenting colonies, oxidase negative; methyl red and citrate positives and most isolates produce H_2S **(Collier *et al.*, 1998)**. تكتب بقية كما بالجدول التفاعلات

The incidence of ***Salmonella*** spp. in four farms located in Damietta governorate was recorded in this work. Out of 414 different samples collected from apparent healthy chickens, 9 ***Salmonella*** spp. (2.2%) was isolated while out of 157 diseased chickens, only 15 isolates (9.6%) of ***Salmonella*** spp. was detected. From 502 dead chickens, 17 isolates (3.4%) of ***Salmonella*** spp. were found.

These results nearly agreed with **Mølbak and Neimann (2002); Kimura *et al.* (2004) and Trawińska *et al.* (2008)**. Also, **Snow *et al.* (2008)** isolated ***Salmonella*** in a rate of 10.7 % in the United Kingdom, while **van Overbeke *et***

al.* (2006) and Pieskus *et al*. (2008)** reported that the incidence of ***Salmonella in broiler farms was 29% in Lithuania, 20% in Italy and 11% in the Netherlands. **ElAmine (2007)** found that the infection rate of ***Salmonella*** in broiler chickens was 17.5 % in Germany which was comparatively high and put Germany in the upper range in comparison with other European Union members.

Strains of ***Salmonella*** are classified into serovars on the basis of extensive diversity of lipopolysaccharide (LPS) somatic antigens (O) and flagellar protein antigens (H) in accordance with the Kauffmann-White Scheme; currently approximately 2500 serovars are recognised **(Popoff *et al*., 1994 and Popoff, 2001).**

This study showed the percentage of ***Salmonella*** serotypes isolated from different chicken samples. Among the identified serovars, 13 isolates of ***S.* Enteritidis** (31.7%); 8 isolates of ***S*. Infantis** (19.5%); 6 isolates of ***S*. Kentucky** (14.6%); 3 isolates of ***S.* Chiredzi** (7.3%); 7 isolates of ***S.* Typhimurium** (17.1%) and 4 isolates of ***S.* Tsevie** (9.8%) were identified.

EFSA (2010) at European Union level reported that the most four frequently isolated ***Salmonella*** serovars in broilers were respectively in decreasing order, ***S*. Infantis** (29.2%), ***S.* Enteritidis** (13.6%), ***S*. Kentucky** (6.2%) and ***S.* Typhimurium** (4.4%).

In the present study, it could be observed that the most predominant and frequently isolated ***Salmonella*** serovars were ***S*. Enteritidis** as 13 serovars out of 41 (31.7%) were isolated from three broiler chicken flocks. This result supported by that of **Hoszowski and Wasyl (2005). Strzałkowski *et al.* (2000)** recovered ***Salmonella*** rods from dead broiler chickens where ***S.* Enteritidis** serovar showed the highest rate (90.88%). Moreover, **Marin and Lainez (2009)** recorded that ***S.* Enteritidis** was the most prevalent serotype isolated during broiler rearing (66.7%).

A total of 8 (19.5%) of ***S*. Infantis** were isolated from two broiler flocks. Opposite results were seen with **Snow *et al*. (2008)** who reported that ***S.***

Infantis was not detected in broiler flocks. Only 6 (14.6%) ***S.* Kentucky** serovars were recovered from three chicken flocks. The United States Department of Agriculture **(USDA) (1999)** found that although ***S.* Kentucky** was not among the most common serovars isolated from human sources, approximately 50% of the isolates from chicken and turkey sources were it. Also, 7 (17.1%) serovars of ***S.* Typhimurium** was isolates from 3 chicken flocks. This result was nearly relative to that recorded by **Chiu *et al*. (2010).** Opposite result was reported by **Snow (2008)** who isolated ***S.* Typhimurium** only in a rate 0.2%.

Surveillance studies of ***Salmonella*** serotypes conducted by the Centers of Diseases for Control and Prevention **(CDC)** identified ***Salmonella*** *enterica* **Enteritidis** and **Typhimurium** as the most commonly reported serovars associated with human illness **(CDC, 2003).**

The ability of ***S.* Enteritidis** to penetrate the intestinal mucosa was found to fall rapidly with increasing age of birds and the caudal regions of intestine (ileum and ceca) were common sit of infection **(Hinton, 1990).** ما معني هذا الكلام؟ احذفيه

Moreover, newly hatched commercial chicks were raised under controlled conditions that can delay the establishment of the definitive cecal bacterial community there by increasing the susceptibility of these chickens to ***Salmonellae*** caecl colonization **(Tellez *et al*., 2001).** ما معني هذا الكلام؟ احذفيه

In the present study, a trial was conducted to evaluate the effect of locally prepared ***S.* Enteritidis** bacterin and a commercial probiotic preparation on the prevention of broiler chickens from ***S.* Enteritidis** infection.

Three hundred, day old broiler chicks were used and divided 4 groups, submitted for vaccination with locally prepared ***S.* Enteritidis** bacterin or treated with a probiotic, then experimentally infected with ***S.* Enteritidis** to evaluate the efficiency of these tools in protection.

It has been accepted worldwide that vaccination to prevent or reduce ***Salmonella*** infection in poultry is practically possible (**Barrow *et al*., 2007)**. In big poultry producing countries as Brazil, commercial vaccines are commonly used in layers as well as in broilers to control the outbreak of salmonellosis **(Paiva *et al*., 2009)**. There are many factors affecting the vaccine efficacy like challenge strain, route of administration, infective dose, age of birds and species/line of birds (**Woodwards *et al*., 2002 and Young *et al*., 2007**).

In this study, infected groups showed signs of depression, anorexia and watery diarrhae 3 days post experimental infection. The morbidity rate was the lowest in the vaccinated group (5.33%) when compared with probiotic treated (12%) and the infected non treated groups (30.67%). ***S.* Enteritidis** may produce clinical disease in chicks up to six weeks of age and occasionally in adult laying birds as affected birds showed depressed, reluctant to move and commonly have diarrhoea **(Wray *et al.*, 1996)**. Older chicks may show uneven growth and stunting and birds may be rejected at slaughter with lesions of pericarditis and septicaemia **(Lister, 1988 and O'Brien, 1988)**.

The protection rate was significantly ($P \leq 0.05$) increased in group vaccinated with locally prepared ***S.* Enteritidis** bacterin (82.61%) than infected non treated group. These results agreed with **Gast *et al*. (1993); Timms *et al*. (1994) and Feberwee *et al*. (2000)** who concluded that ***S.* Enteritidis** vaccine provided birds with high protection against ***S.* Enteritidis** challenge. **Ghosh (1989)** reported that vaccination with formalin-killed ***S*. virchow** reduced mortality from 85 to 0% in chicks when inoculate the organism intraperitoneal. Also, **Timms et al., (1990)** found reduced mortality from 100 to 50% against ***S*. Enteritidis** challenge in broiler chickens.

The protection rate of the probiotic treatment was estimated in this work and it was significantly ($P \leq 0.05$) increased in the probiotic treated group (60.87%) than infected non treated one (0%). This result coincides with **Samanta and Biswas (1995)** who detected that supplementation of probiotics to

poultry reduced the mortality rate. Similarly, **Soomro *et al*., (2002) and Takahashi *et al*., (2005)** mentioned that the mortality rate was significantly affected by the probiotic treatment in the first few days of chicken's life as this treatment have beneficial effect on the health and reduced mortalities in ***S*. Enteritidis** infected birds. Probiotics have been shown to accelerate the development of normal microflora in chicks and increased the resistance to infection by some enteric bacterial pathogens (**Madian and Wafaa, 2006; Higgins *et al*., 2007b and Vicente *et al*., 2007a and b)**.

Probiotic compounds containing *Lactobacilli* have also been widely reported to produce antibacterial compounds called bacteriocins, and the effect of bacteriocins have been hypothesized to be the mechanism by which *Lactobacilli* exert cytotoxic effects *in vivo* **(Bogovic-Matijasic *et al*., 1998 and Ocana *et al*., 1999)**.

Concerning the faecal shedding rate of ***S*. Enteritidis**, there was significant ($P \leq 0.05$) decrease in the shedding rate in the vaccinated and probiotic treated birds during the 1st, 2nd and 3rd week post challenge. Our finding is parallel to these of **Tellez *et al*. (2001**) **and Rahimi *et al*. (2007), Fulton *et al*. (2002**) **and Yokoyama (1998)** who detected reduction in shedding rate of ***S*. Enteritidis** after vaccination of broilers, duckling and piglet and calves, respectively. Also this result agrees with **Gast *et al*., (1993)** who demonstrated reductions in the rate of faecal shedding when birds were challenged with ***S*. Enteritidis** two weeks after the second subcutaneous dose of ***S*. Enteritidis** bacterin. Reduced excretion of ***Salmonella*** in faeces was also obtained when hens were vaccinated with acetone-killed ***S*. Enteritidis** bacterins mixed with Freund's incomplete adjuvant **(Barbour *et al*., 1993)**. **Nakamura *et al*.,** also showed reduced excretion in faeces and of bacterial numbers in the tissues of birds vaccinated twice and challenged at laying age **Nakamura *et al*., (1994)**. There was a considerable difference concerning the carriage of ***Salmonella*** in the cecal contents, indicating that broilers are able to clear the systemic

infections, but can remain intestinal carries **(Bjerrum *et al*., 2003)**. The intestinal carrier status is most important in control of contamination during transportation and processing of broilers, where cross contamination plays a major role **(Feberwee *et al*., 2000; Gurtler *et al*., 2004; Higgins *et al*., 2008 and Dorea *et al*., 2010)**.

Regarding the re-isolation of ***S.* Enteritidis** from different chicken's organs after experimental infection with ***S.* Enteritidis** and after booster dose of vaccination, it was found that there was significant ($P \leq 0.05$) decrease of ***S.* Enteritidis** re-isolation at the 3rd week post infection in all groups than at the 1st and 2nd week post infection, except in infected non treated group where no significant ($P \leq 0.05$) difference was found during the observation period. In Addition, we found that there was significant ($P \leq 0.05$) decrease of ***S.* Enteritidis** re-isolation in vaccinated group than probiotic treated group and the infected non treated one during three weeks post infection.

These results concur with **Gast *et al*., (1993)** who recorded that fewer number of the challenge ***S.* Enteritidis** strain was isolated from the spleen, ovaries and oviducts when compared with controls. Also, reduced bacterial number of ***S.* Enteritidis** in the bird's organs was reported after double shots of vaccination and challenging at laying age **(Nakamura *et al*., 1994)**. Moreover, **and Miyamoto *et al*., (1999); Okamura *et al*., (2007) and Young *et al*., (2007)** stated that vaccination with ***S.* Enteritidis** bacterins significantly reduced the frequency of recovery of organism from internal organs. It was demonstrated that commercially available killed ***S.* Enteritidis** bacterin played a significant role in the reduction of ***S.* Enteritidis** in layers especially when combined with improvement in the biosecurity and hygiene **(**R. Davis, pers. comm). Above mentioned findings disagree with that of **Clifton-Hadley *et al*., (2002)** who mentioned that there was no effect of vaccination on internal organs colonization after oral challenge with ***S.* Typhimurium**.

Regarding the effect of the probiotic on reducing the colonization rate in the internal organs, **Tellez *et al*., (2001); Wafaa *et al.*, (2006); Wilkie (2006) and Rahimi *et al*., (2007)** demonstrated that using of probiotic could be of great benefit in reducing intestinal and internal organ colonization of ***S*. Enteritidis** in broiler chickens. It was detected that probiotic could reduce the colonization of opportunistic microorganisms in the bird's gastrointestinal tract by competition for receptor sites, stimulation of the immune system, and production of some active antimicrobial substances **(Rolfe, 2000)**.

Gradual and significant ($P \leq 0.05$) increase in the weekly body weight gain was observed in the vaccinated and probiotic treated broiler chickens than the only infected birds. Moreover, the feed conversion ration was the best in the treated group, while it was the worst in non treated infected birds. The effect of ***Salmonella*** vaccination on body weight in chickens showed that vaccination of chickens produced highly significant increase in the body weight **(Mohrah and Zaki, 1995)**.

Most of the published data concerning the effect of probiotics on the birds performance either in the presence or absent of enteric infections revealed that these compounds were effective in improving the growth of birds **(Mohan *et al*., 1996; Jin *et al*., 1998; Zulkifli *et al*., 2000; Kalvathy *et al*., 2003; Madian and Wafaa, 2006; Opalinski *et al*., 2007 and Midilli *et al*., 2009)**. **Cavit, (2003) and Ayed *et al*., (2004)** demonstrated that supplementation with probiotics improved the feed conversion ratio of the host. **Gracia *et al*., (2004)** found that supplementation with a probiotic containing ***Enterococcus facium*** increased the growth of broilers and improved the conversion rate. It was postulated that the probiotics induce better bird's performance may be though stimulating appetite **(Nahashon *et al*., 1992)**, improving microbioal balance **(Fuller, 1989)**, producing digestive enzymes **(Saarela *et al*., 2000)**, synthesizing vitamins (**Coates and Fuller, 1977)**, stimulating lactic acid **(Bailey, 1987)**, decreasing pH and releasing bacteriocins (**Rolfe, 2000)**.

In this work, after vaccination with the locally prepared ***S.* Enteritidis** bacterin or treatment with probiotic, the titre of antibodies against ***S.* Enteritidis** was measured using the MA test. After the 1st dose of the bacterin and treatment with the probiotic, the geometric mean titre (GMT) of antibodied increased to reach 65 in vaccinated group and 60.6 in probiotic group. After booster dose of the vaccine (before challenge), the GMT increased to reach 98 and 74 in vaccinated and probiotic treated birds, respectively. One week after ***S.* Enteritidis** experimental infection, the GMT increased to reach 60.6, 211.1 and 113.1 in the infected non-treated, vaccinated and probiotic treated groups, respectively. Also, Two weeks after infection, the titre increased to 130, 226.2 and 197 in infected non-treated, vaccinated and probiotic treated groups, respectively. At the 3rd week post ***S.* Enteritidis** challenge, the antibodies titre increased in the infected non treated chickens and vaccinated ones up to 139.3 and 242.5, respectively, but it declined in the probiotic treated birds to 171.

High serum IgG titres have been detected in laying hens after experimental oral inoculation with ***S.* Enteritidis (Barrow and Lovell, 1991 and Olabisi and Peter, 2008)**.

From these results it appeared that ***S.* Enteritidis** vaccine induced high level of immune response and that agrees with those previously mentioned by **Hahn (2000) and Springer *et al.*, (2000)**. They mentioned that ***S.* Enteritidis** vaccination induced protection from organism infection. It was detected that killed ***S.* Enteritidis** bacterins induced high levels of circulating specific IgG against un-specified protein antigens **(Barbour *et al.*, 1993 and Gast *et al.*, 1993)**.

The role of the probiotic in enhancing the immune response of the host was studied by **Toms and Powvie, (2001) and Koenen *et al.*, (2004)**. As well, **Revolledo *et al.*, (2009)** mentioned that the probiotic was effective in controlling of ***Salmonella*** colonization and enhancing the immune response.

For more evaluation of the humoral immune response of chickens to vaccination or probiotic treatment and then ***S.* Enteritidis** infection, an ELISA test was applied. It was documented that ELISA is considered to be an appropriate method for detecting previous infection or vaccination and also for detection of infected chickens which shed ***Salmonella*** intermittently **(Hassan *et al.*, 1990)**. **Gast (1997**) recorded that detection of previous exposure to ***S.* Enteritidis** could be occur through measuring of serum IgG as it is the very sensitive method.

In our experiment, blank control group showed no significant ($P \leq 0.05$) difference in the mean optical density values during the period of experiment. In ***S.* Enteritidis** infected non-treated control group, there was no significance ($P \leq 0.05$) difference in the mean optical density values till 20 days of age (before experimental infection) but the mean values increased significantly ($P \leq 0.05$) at 27days of age (1.781) then decreased at 34 and 41 days of age to reach 1.457 and 1.274, respectively. The marked increase in the level of anti- ***S.* Enteritidis** IgG antibodies as measured by high optical density values was in agreement with the findings of **Gast and Beard (1990); Barrow and Lovell (1991) and Olabisi and Peter (2008)** who reported that when laying hens orally infected with ***S.* Enteritidis**, high serum IgG titres were produced by most birds week post infection.

In group vaccinated with locally prepared ***S.* Enteritidis** bacterin, the optical density mean values were gradually and significantly ($P \leq 0.05$) increased from 0.234 prior vaccination to 1.614 before booster dose (10 days old) and to 2.543 after booster dose (before experimental infection at 20 days old) hen there was no significant ($P \leq 0.05$) increase in these values at 27, 34 and 41 days of age. These results are parallel with **Barbour *et al.*, (1993); Gast *et al.*, (1993); Okamura *et al.*, (2003); Davies and Breslin (2004) and Pakpinyo *et al.*, (2008)** as they recorded that ***S.* Enteritidis** vaccination induce high level of

immune response in addition to high protection from infection with ***S.* Enteritidis.**

The optical density mean values were gradually and significantly ($P \leq 0.05$) increased from 0.234 to 0.561 at day and 10 days old chickens, respectively then to 0.953 before experimental infection (20 days old). Furthermore, there as an increase in these values at 27, 34 and 41 days of age in probiotic treated group. These results revealed that probiotic play a role in increasing the immune response of the birds to infection. These findings are in agreement with those of many preceding studies. In one of those studies, **Wafaa *et al*., (2006)** found that broiler chickens treated with a probiotic showed an increase in the titre of the serum antibodies after inoculation with chicken's red blood cells and this titre was measured by haemagglutination inhibition test. **Lee *et al*., (2007)** described that probiotic containing *Pediococcus acidilactici* enhanced the serum antibody response. In addition, **Rowghani *et al*., (2007) and Alkhalf *et al*., (2010)** reported that broiler chickens fed on a diet supplemented with probiotic showed significant increase in the Newcastle diseased virus antibody titres than control group. On the other hand, **Okamoto *et al*., (2007)** demonstrated that probiotic treatment had few beneficial effects for chicks, particularly during the first days of life.

The positive effect of feeding diet containing probiotic on the immune response indicates the enhancement of the formulating bacteria on the acquired immune response exerted by T and B lymphocytes. The direct effect of the probiotic might be related to stimulating the lymphatic tissue **(Kabir *et al*., 2004)**, whereas the indirect effect may occur via changing the microbial population of the lumen of gastrointestinal tract. **Shoeib *et al*., (1997)** reported that the bursa of probiotic-treated chickens showed an increase in the number of follicles with high plasma cell reaction in the medulla. **Christensen *et al*., (2002)** suggested that the effect of the probiotic containing bacteria was due to stimulation of the secretion of cytokines mediated by immune system cells.

Also, it was detected that vaccinated group showed higher optical density mean values than probiotic treated group before and after ***S.* Enteritidis** challenge till the end of experiment which clarified that the locally prepared ***S.* Enteritidis** bacterin was more effective than probiotic in enhancing the immune response. **Priyantha (2009)** reported that vaccination was only alternative tool to control salmonellosis in chicken and other precaution like bio-security, good management practices must be taken in consideration at first. ملهاش لازمة هنا