2. REVIEW OF LITERATURE

2.1. Incidence of *Salmonella* in poultry in world:

Bernardo and Machado (1990) recorded the incidence of ***Salmonella*** among broiler carcasses passed for humans consumption and revealed the following : (1) the rate of isolation was 31.1% from non refrigerated carcasses; (2) the incidence of ***Salmonella*** was greater in the hot season (May to September), the frequency of the principle serovars found in broiler carcasses was : ***S.* Enteritidis** (65.5%), ***S.* Agona** (11.7 %), ***S.* Sainptpaul** (6.4 %), ***S.* Newport** (6.4%), ***S.* Typhimurium** (2.9%).

Hoop and keller (1991) detected ***S.* Enteritidis** phage type (4) from 12 out of the hens, being localized in the ovaries and/or oviduct in 8 of them.

Poppe *et al.* (1991) detected the prevalence of ***Salmonella*** infection among Canadian egg producing poultry flocks. The most prevalent serovars were ***S.* Heidelberg**, ***S.* Infantis**, and ***S.* Hadar** and ***S.* Schwarzengrund**.

Barnhart *et al.* (1992) isolated 15 different serovars from the ovaries of commercial layer hens at time of slaughter. ***S.* Heidelberg** was the predominant serovar, representing 56.5% of ***Salmonella*** detected, followed by ***S.* Agona**, ***S.* Oranienburg**, ***S.* Mbandaka**, ***S.* Kentucky**, ***S.* Montevideo**, ***S.* Iondon**, ***S.* Typhimurium**,***S.* Infantis**, ***S.* Schwarzenqrund**, ***S.* Ohio**, ***S.* Cerro**, ***S.* Anatum**, and ***Salmonella*** untypeable were also found. The authors recorded that ***S.* Enteritidis** phage type 23 was isolated from only one (2.4%) of the flocks.

Cox *et al.* (1992) isolated ***Salmonella*** in an incidence of 15.2%, 5%, and 12% from egg fragments, fluff, and paper pads, respectively. The serotypes isolated were : ***S.* Berta, *S.* California, *S.* Give**, ***S.* Hadar**, ***S.* Mbandaka**, ***S.* Senftenberg** and ***S.* Typhimurium**.

Waltman *et al*. (1992) cultured 3700 pooled cecal samples from layer houses for isolation of ***S.* Enteritidis** and found that ***Salmonella*** were isolated from 2418 out of 3700 pooled caecal samples (65.4%), but only six isolates were ***S.* Enteritidis**.

Ebel *et al.* (1992) detected the prevalence and distribution of ***S.* Enteritidis** in USA, in commercial egg production flocks. Where, 23431 pooled caecal sampled were collected from a total of 406 layer houses ***Salmonellae*** and ***S.* Enteritidis** were recovered from 24% and 3% of the pooled samples, respectively. Overall, the prevalence of ***Salmonella***-positive houses was 86%.

Dreesen *et al.* (1993) estimated the frequency of ***S.* Enteritidis** and other ***Salmonella*** serovars in the caecal contents of spent laying hens at a hen-processing plat in USA. Of the 1920 pooled caecal samples (three caecal per sample) from 38 flocks representing 23 procedures, tested for the presence of ***S.* Enteritidis** and other ***Salmonella*** serovars. 359 samples (18.7%) from 37 of the flocks (97.4%) showed characteristic reactions for ***Salmonellae*** on Triple Sugar Iron agar (TSI) slants. 29 out of the 359 ***Salmonella*** positive samples (8.1%) were belonging to group D positive, all of which were found to be ***S.* Enteritidis** on further serotyping. The ***S.* Enteritidis** positive samples were from 7 out 38 flocks (18.4%). Serotyping of the 330 TSI positive group D-negative ***Salmonella*** revealed 37 different serovars and ***S.* Heidelberg** was the predominant isolates (49.1%).

Ebel *et al. (*1994) stated that the Northern region of the United States had the highest ***S.* Enteritidis** recovery, with 20% of samples submitted from plants in the region being culture positive for this serotype. ***S.* Enteritidis** was found in 10%, 15% and 60% of the samples submitted from the South East, Central and Western regions of U.S.A., respectively.

Huang *et al*. (1994) isolated fifteen isolates of ***Salmonella*** from 120 duckling had clinical signs of lethargy, anorexia, diarrhea and death. The morbidity was 19.3, 28.9% and the mortality was 9.5, 14.3 and 32.95% respectively, during the early, middle, and late stages of illness which lasted for 7 days.

Baggesen and Wegener (1995) recorded that ***S.* Typhimurium** was one of the 2 commonest ***Salmonella*** serotypes causing human Salmonellosis in Denmark. To illustrate the significance of different production animals as a source of infection, 1461 isolates were characterized by phage typing. The isolates originated from human patients and from cattle, pigs and poultry. By phage typing the isolates could be separated in to 35 different phage types. Five types (10, 12,66, 110 and 135) predominated and comprised 78.8% of the isolates. In humans, 57.3% of the isolates were phage type 12. This phage type was also dominant in pig herds and to lesser degree in cattle. Phage types No. 110, 120, 135 and constituted 86.5% of the poultry isolates while these phage types only made up 12.9% of the human isolates.

Verma and Gupto (1995) detected 243 ***Salmonella*** isolates serologically from various states during 1990-1993. The isolates belonged to 19 serovars of which ***S.* Virishow** (27.98%) was the most predominant followed by ***S.* Gallinarum** (23.04), ***S.* Typhimurium** (18.1%), ***S.* Enteritidis** (9.87%) and ***S.* Bovis-Moribificans** (5.34%). ***S.* Gallinarum** was the commonest on poultry farms.

Carraminana *et al*. (1996) studied the distribution of 133 ***Salmonella*** strains (8 from faeces samples, 12 from surface samples of Knife, 18 from cold water samples, 1 from chlorinated water samples, 58 from surface samples of broiler carcasses and 36 from broiler liver samples for their distribution in subspecies, serogroup and serotypes. 119 strains belonged to subspecies I (***S.***

enterica subsp. *enteica*) and 14 to the subspecies II (***S.*** *enterica* subsp. ***salamae***). ***Salmonella*** serogroups B and D were the most frequently isolated (14.3% and 79.9%, respectively). The sergroups C1 (3%) and C2(3%) were also found. The serotyping yielded 6 different serotypes. ***S.*** **Enteritidis** was the predominant serovar, representing 79.7% of the ***Salmonella*** detected. These data corroborated the current situation throughout the world where broiler and poultry products were the main reservoir of ***S.*** **Enteritidis** and therefore, often incriminated in human diseases. Among the 3 serovars usually recovered ***S.*** **Enteritidis.**

Johansson *et al*. (1996) examined 91 commercial layers, 61 replacement pullets and 1062 eggs. ***S.*** **Enteritidis** phage type 1 (PT1) was isolated from livers (5%), ovaries (2%) and from caeca (3%), 8% of 105 pooled egg samples were positive, of which 2 were only detected from contents and 3 from shells, indicating both oviductal and faecal contamination routes of eggs.

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.

Hafez and Stadler (1997) stated that the most frequently isolates from commercial turkey flocks were ***S.*** **Newport** (34.6%) and ***S.*** **Reading** (30.3%), followed by ***S.*** **Bredeny** (10.6%). ***S.*** **Enteritidis** phage type 8 which was detected for only a short period (5 weeks) in one flock.

Schaar *et al*. (1997) detected the prevalence of ***S.*** **Enteritidis** and ***S.*** **Typhimurium** in 34 flocks of laying chickens. After investigation by cultural methods, 47.0% of the floor flocks and 35.3% of the battery flocks were ***S.*** **Enteritidis** or ***S.*** **Typhimurium** respectively.

Hofer *et al*. (1998) identified ***Salmonella*** strains from feed stuff and poultry feeds from several regions of Brazil. The predominant serovars were ***S.* Montevideo**, ***S.* Senftenberg**, ***S.* Havana**, ***S.* Mbandaka**, ***S.* Tennessee**, ***S.* Infantis**, ***S.* Agona**, ***S.* Anatum**, ***S.* Cerro** and ***S.* Bredeney**.

Riemann *et al*. (1998) stated that during the mid 1970s through the mid 1980s, there was an increase in the number of ***S.* Enteritidis** isolates from humans in United Stated from 1.12 to 7.16 isolates per 100.000 persons per year. ***S.* Enteritidis** accounted for 28% of all ***Salmonella*** isolates for humans. Epidemiological evidence suggested that this increase may have been due to consumption of contaminated eggs.

Al-Nakhli *et al*. (1999) isolated eleven ***Salmonella*** serogroups representing 38 different ***Salmonella*** serovars and identified then glby means of antigenic analysis. The majority of the 276 isolates (26.2%) of ***Salmonella*** types were recovered from liver, heart and intestines of the broilers and layers. The most prominent ***Salmonella*** serogroups isolated were serogroup C1 (392 isolates, 37.26%), B (289 isolates, 27.47%) and D1 (269 isolates, 25.69%). However, untypable and multiple serogroups were also encountered.

Carli *et al*. (2001) examined 814 caeci of turkeys obtained during in-line processing of 28 broiler and 5 layer flocks and found that they were contaminated with four different ***Salmonella*** serovars. ***S.* Enteritidis** was only recovered from layer birds whereas; ***S.* Enteritidis** (81.5%), ***S.* Agona** (7.6%), ***S.* Thompson** (10.1%) and ***S.* Sarajane** (0.8%) were isolated from broiler birds.

Hui and Das (2001) collected samples from different tissues from dead, diseased and apparently healthy chickens. A total of 280 samples comprising 94 from intestines, 70 from liver, 43 from spleen, 51 from gall bladder and 22 from heart. The samples yielded 15 (5.32%), ***Salmonella*** strains. Also, They observed that the highest percentage (8.7%) of ***Salmonella*** was recovered from

the liver ,followed by intestine (5.32%), heart (4.55%), gall bladder (3.92%) and spleen (2.33%). Serotyping revealed 15 ***Salmonella*** strains. ***S.* Enteritidis** and ***S.* Typhimurium** constituting 12 (80%) and 3 (20%) respectively.

Matsumoto *et al.* (2001) investigated the differences in ***Salmonella*** isolation rates in environmental samples taken from several types of hen houses in Chiba and Japan, In addition, for the detailed epidemiologic survey, environmental samples, hens and rodent samples were collected from windowless houses in 3 farms. ***Salmonella*** was isolated from four (80%) of five farms with windowless hen houses. ***S.* Enteritidis** was isolated from single windowless house. In contrast, only one serotype of ***Salmonella*** was isolated from 1 (6.7%) of 15 farms with open hen houses. In ***S.* Enteritidis** contaminated windowless hen houses, the isolation rates of it were 90.9% of environments, 94.1% of hens and 86.4% of rats.

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

Guerin *et al.* (2005) found that ***S.* Typhimurium**, ***S.* Heidelberg**, ***S.* Hadar**, ***S.* Kentucky**, and ***S.* Thompson** were the most frequently isolated serovars, approximately 60% of the ***S.* Typhimurium** were isolated from cattle, whereas over 90% of the ***S.* Heidelberg**, ***S.* Hadar**, ***S.* Kentucky**, and ***S.* Thompson** were isolated from chickens. There was an increasing trend in isolates from chickens, cattle, and pig, and a decreasing trend in isolates from turkeys.

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%).

Islam *et al*. (2006) studied the seroprevalence and pathology of ***Salmonella*** infections in layer chickens of Dhaka and Gazipur regions of Bangladesh and to isolate and characterize ***Salmonella*e** from layer chickens during the period from January to May 2006. They found that a total of 33 (21.02%) ***Salmonella*e** from live and dead birds were isolated. The isolation rate of ***Salmonella*e** was higher in seronegative (31.6%) group than seropositive (3.2%) group. Out of 33 ***Salmonella*** isolates, 25 were ***S.* Pullorum**, 3 were ***S.* Gallinarum** and the rest 5 were motile ***Salmonellae***.

Michael *et al*. (2006) stated that ***S.* Agona** plays an important role in Brazil as causative agent of salmonellosis in food-producing animals in particularly in pigs and poultry as well as in humans.

Shahada *et al*. (2008) reported that ***Salmonella*** was isolated from 563 (14%) samples in 179 (71%) flocks. The flock situation varied from ***Salmonella***-negative holdings (n = 9), positive-flocks from persistently infected holdings (n = 21), and holdings (n = 19) that showed fluctuations with alternating negative and positive flocks for variable time periods. Fourteen holdings (negative, n = 5 and positive, n = 9) were sampled once throughout the study period. Seasonality component was not observed, and ***Salmonellae*** were found colonizing broiler ceca in warm and cold months. Predominant serovar was ***S.* Infantis** (93.3%; n = 525).

Pieskus *et al*. (2008) reported that the incidence of ***Salmonella*** in "conventional" broiler farms was 29% in Lithuania, 20% in Italy and 11% in The Netherlands, while in Germany ***Salmonella*** was not detected. ***Salmonella*** was isolated from organic broiler flocks in Italy (18.1%) and in Netherlands

(3.7%). The authors indicated that ***S.* Enteritidis** and ***S.* Typhimurium** dominated in Lithuanian broiler flocks while ***S.* Infantis** and ***S.* Javiana** were predominant in the Netherlands. ***S.* Hadar** and ***S.* Heidelberg** seemed to be prevalent in Italy.

Jarquin *et al.* (2009) stated that poultry may carry some ***Salmonella*** serovars without any symptoms of disease and without causing any adverse effects to the health of the bird.

Akhtar *et al.* (2010) revealed that overall serovar **Entertidis** prevalence rate in 206 ***Salmonella*** positive samples was 75.24% (155). Out of 58 isolates of ***Salmonella*** recovered from human stool samples, 44 (75.86%) were ***S.* Enteritidis**. Isolation frequency of ***S.* Enteritidis** from total isolates (148/206) in poultry sources was 111/148 (75 %) which indicated the zoonotic potential of ***S.* Enteritidis**.

Betancor *et al.* (2010) stated that the national prevalence of ***S.* Enteritidis** infection was estimated to be 6.3%. ***Salmonellae*** were recovered from 58 of 620 pools made up of 20 eggs each, demonstrating a prevalence of at least 1 in every 214 eggs. Surprisingly, the majority of the isolates were not ***S.* Enteritidis**. Thirty-nine isolates were typed as ***S.* Derby**, 9 as ***S.* Gallinarum**, 8 as ***S.* Enteritidis**, and 2 as ***S.* Panama**. Despite the highest prevalence in eggs, ***S.* Derby** was not isolated from humans in the period of analysis, suggesting a low capacity to infect humans.

2.2. Incidence *Salmonella* in Egypt:

Abd-Allah (1991) revealed that the incidence of ***Salmonella*** in broiler and parent chickens 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.* Typhimurium**, ***S.* Enteritidis**, ***S.* Dublin** and ***S.* Reading.**

Ibrahim (1992) stated that the rate of ***Salmonella*** serovares from poultry feeds as a whole was 2%. It was 5.33% from feeds of poultry origin, 4% from fish meal, 2% from corn and 1.47% from concentrates. They recorded that ***S.* Typhimurium** was the predominant serovar in all poultry feed stuff. On the other hand, ***S.* Blockely** was isolated from concentrates and ***S.* Virchow** from fish meal.

Abd-Allah (1995) detected 25 isolates of ***Salmonellae*** from different domestic birds and their environmental surroundings. Serological typing of 25 ***Salmonellae*** revealed the differentiation of 6 serotypes namely: 10 isolates as ***S.* Enteritidis** (40%), 6 as ***S.* Typhimurium** (24%), 4 as ***S.* Montevideo** (16%); 3 as ***S.* Gallinarum pullorum** (12%) and one from each ***S.* California** and ***S.* Newport** (4% each).

Abdel Rahman *et al.*(2000) isolated ***Salmonellae*** species from El-Arish region of Egypt from broiler chickens in percentage of 9%.

Sleim (2003) examined 185 chicken flocks (150 broiler flocks, 16 balady flocks and 19 layer flocks), 40 fertile eggs, 43 dead-in-shell embryos, 20 duck eggs, 10 duck farms,16 rats and 55 feed samples. A total of 14 ***Salmonella*** isolates were recovered and were typed as 3 isolates of ***S.* Enteritidis**, 6 isolates of ***S.* Typhimurium**, 2 isolates of ***S.* Kentucky** and 2 isolates of ***S.* Montevideo** whereas,1 isolate was untyped.

Hanan (2005) recovered 56 isolates of ***Salmonellae***,19 were recovered from feed, 22 from poultry, 10 from cattle and 5 from human. ***S.* Montevideo**, ***S.* Agona**, ***S.* Blockley**, ***S.* Hadar** and ***S.* Virchow** were the most important serovars recovered from feed, while, ***S.* Agona**, ***S.* Arizona**, ***S.* Cerro**, ***S.* Enteritidis**, ***S.* Gallinarum pullorum** and ***S.* Typhimurium** were from poultry. Also, ***S.* Anatum**, ***S.* Enteritidis** and ***S.* Sandiego** and ***S.* Typhimurium** were

isolated from cattle. ***S.* Paratyphi A**, ***S.* Paratyphi B** and ***S.* Typhi** were recovered from samples from diseased human cases.

Abdel Rahman (2007) studied the prevalence of ***Salmonella*** among layer farms in different provinces. The results showed that there was a moderate spread of ***Salmonella*** and the serological typing of ***Salmonellae*** were. ***S.* Essen**, ***S.* Ayinde** and ***S.* Ljubljana** in table eggs, ***S.* Kentucky** in fertile eggs, ***S.* Ayinde**, ***S.* Enteritidis**, ***S.* Kentucky**, ***S.* Tennessee** and ***S.* Winston** in feed samples, ***S.* Eingedi**, ***S.* Cremieu**, ***S.* Magherafelt**, *S.* **Rechovot**, ***S.* Enteritidis**, ***S.* Ayinde** and ***S.* Kentucky** from drag swabs, ***S.* Ferruch**, ***S.* Neumuenster**, ***S.* Rechovot**, ***S.* Ljubljana, *S.* Ayinde** and ***S.* Enteritidis** from paper lining box and also ***S.* Enteritidis** and ***S.* Yaounde** from internal organs of chickens.

Radwan (2007) examined 22 table egg layer flocks, 11 samples of feed and feed ingredients and 23 rodents of various types. A total of 9 ***Salmonella*** isolates were recovered. Two isolates from laying flocks (***S.* Enteritidis** and ***S.* Typhimurium**), 2 isolates from rearing flocks (***S.* Enteritidis**), 1 isolate from feed ingredients and 4 isolates from rodents (***S.* Enteritidis**, ***S.* Typhimurium** and Untypable ***Salmonella*** isolates).

Hazem (2010) examined 150 cloacal swabs were obtained from different chicken farms and examined bacteriologically to recover the organism. The percentage of positivity for ***Salmonella*** was 12.6 %. Serotyping using available antisera revealed that 19 isolates were identified as ***Salmonella***. ***S.* Typhimurium** (5), ***S.* Heidelberg** (2), ***S.* Pullorum** (6) and ***S.* Enteritidis** (6).

2.3. Different types of vaccines as a trial for vaccination against *Salmonella* infection in poultry:

2.3.1. Bacterins:

Timms *et al*. (1990) prepared an oil adjuvant inactivated ***S.* Enteritidis** bacterin containing 10^{11} colony forming units (CFU) of the organism. That bacterin was used subcutaneously in 3 weeks or 3 and 6 weeks old specific pathogen free (SPF) chickens, then the birds were intramuscularly or intravenous challenged with virulent strain of ***S.* Enteritidis** containing $l0^{9}$ or 10^{8} CFU at 5 and 8 weeks old, respectively. The results showed that vaccine protected or intravenous with virulent strain of performed an experimentally inactivated ***S.* Enteritidi** chickens against the massive challenges at either age.

Barbour *et al*. (1993) evaluated six ***S.* Enteritidis** bacterins formulations differing in adjuvant content and whole-cell inactivation procedures in egg - laying chicken vaccinated subcutaneously at age of 3.5 and 4.5 months. The birds were orally challenged after one month from second vaccination with ***S.* Enteritidis**. Protection against shedding and cecal colonization was evaluated via cloacal and cecal swabs respectively. A bacterin containing ***S.* Enteritidis** inactivated by acetone and with modified Freund's incomplete adjuvant performed the best.

Gast *et al*. (1993) used an acetone-killed oil-emulsion bacterin made from 10^{10} cells/dose of phage type 13a ***S.* Enteritidis** to vaccinate SPF white leghorn hens. Hens were vaccinated at 23 weeks of age in one experiment and at 45 weeks of age in another. The bacterin was administered again 6 weeks later in both trials. Three weeks after the second vaccination, all hens were challenged orally with approximately 10^{9} cells of a highly invasive heterologous phage type 14b ***S.* Enteritidis**. The results in both trials, demonstrated that ***S.* Enteritidis** was isolated from fewer internal organs (spleens and livers) and pools of egg contents from vaccinated than from nonvaccinated controls. No ***S.* Enteritidis** was isolated from any ovary or oviduct samples of vaccinated hens, but ***S.* Enteritidis** was isolated from a significantly higher percentage of ovaries and oviducts of control hens. The authors concluded that immunization of chickens

with ***S.* Enteritidis** bacterins reduced but did not eliminate the frequency of ***S.* Enteritidis** isolation from the internal organs and egg contents.

Nakamura *et al*. (1994) evaluated the efficacy of ***S.* Enteritidis** formalin-inactivated oil-emulsion bacterin against ***S.* Enteritidis** challenge at a dose simulating natural infection (trial 1)10^6 or 10^3 cells/dose and at a very high dose (trial 2)10^9 cells/dose. SPF Leghorn chickens were vaccinated twice, 4 weeks apart, in both trials with the first vaccination given at 14 weeks of age in trial (1) and at 8 weeks of age in trial (2). The challenge ***S.* Enteritidis** organism was administered orally 6 weeks following the second vaccination in trial (1) and 4 weeks following second vaccination in trial (2). The efficacy of the vaccine was evaluated by counting ***S.* Enteritidis** shedding in cecal droppings. In both trials, ***S.* Enteritidis** was recovered significantly less frequently and in lower number from cecal droppings of vaccinated hens than of control hens. ***S.* Enteritidis** was recovered from fewer livers and spleens of vaccinated chickens than these control chickens.

Timms *et al*. (1994) made both laboratory trial in (SPF chickens) and field trial in (Rhode Island Red layers chickens) through subcutaneous vaccination of them by an inactivated oil-adjuvant ***S.* Enteritidis** PT4 bacterin at one day old and booster at 4 weeks of age. Subgroups of birds were challenged intravenous by 10^8 CFU of a virulent ***S.* Enteritidis** PT4 at 8, 12, and 16 weeks of age. The bacterin efficacy was assessed by observation of the clinical signs and mortality for 3 weeks following the challenge, by the postmortem lesions, and by the recovery of challenging organisms from organs of vaccinated and non vaccinated birds. Based on those criteria, the mean protective indices in the laboratory trial ranged from 28 to 80 and in the field trial from 38 to 70 at the various challenged ages. Those results demonstrated that the vaccine provided a protection against overwhelming challenge for up to 12 weeks post vaccination in both trails.

Mohrah and Zaki (1995) used an inactivated oil adjuvant vaccine of ***S.* Gallinarum** and ***S*. Pullorum** to vaccinate 120 chickens subcutaneously at 2 weeks of age then challenge them at 4 and 8 weeks of age with 5 ml of broth culture containing 10 lethal dose fifty (LD_{50}) live ***S*. Gallinarum** organisms. The surviving birds were tested bacteriologically 2 weeks later. In both vaccinated groups, 2.7 and 0% of the birds were positive for ***S*. Gallinarum**, while the corresponding figures for both unvaccinated groups were 34.7 and 15.7%.

Davison *et al*. (1999) evaluated the efficacy of ***S.* Enteritidis** bacterins in the field. Eleven commercial layer flocks were vaccinated with an either autogenous or a commercial ***S.* Enteritidis** bacterin. The evaluation criteria included the presence or absence of ***S.* Enteritidis** in the environment in the organs of birds and in the eggs. The results showed that all the vaccinated flocks were ***S.* Enteritidis** negative organ cultures, although 63.6% of the houses had ***S.* Enteritidis** positive environmental cultures. However, 100% of the unvaccinated flocks had birds with ***S.* Enteritidis** culture positive organs.

Miyamoto *et al*. (1999) studied the efficacy of a commercial oil-emulsion ***S.* Enteritidis** bacterin in the laying hens. White Leghorn hens were vaccinated at 38 weeks of age. Each hen in the vaccinated group was inoculated subcutaneously at the back of neck with 0.5 ml of the bacterin. The second vaccination shot was administrated 4weeks later. Two weeks later, all hens were challenged intravaginally with 10 CFU of the organism and again intravaginally challenged at 44 weeks of age with $l0^7$ CFU/dose of ***S.* Enteritidis** PT4. Significantly fewer eggs were positive for *S.* Enteritidis from the vaccinated hens (19.0%) than eggs from the nonvaccinated hens (37.0%). ***S.* Enteritidis** was recovered from liver, spleen, and cecum at significantly lower rates from vaccinated hens than from the nonvaccinated ones.

Yamane *et al*. (2000) evaluated whether the administration of ***S.* Enteritidis** bacterin affected the incidence of ***S.* Enteritidis** contamination of commercial batches of pullets flocks. Three farms with history of ***S.* Enteritidis** contamination of laid eggs were studied. The farms were contaminated with ***S.* Enteritidis** and had been demonstrated to have horizontal transmission of ***S.* Enteritidis** among flocks. Bacterin vaccinated flocks were compared with nonvaccinated control one at the same age. It was concluded that ***S.* Enteritidis** bacterin was effective in reducing the incidence of ***S.* Enteritidis** on shell eggs produced by flocks on ***S.* Enteritidis** contaminated farms.

Liu *et al*. (200l) evaluated the efficacy of vaccinating 2-weeks old SPF Leghorn chicks with a formalin-inactivated ***S.* Enteritidis** phage type 4 either orally or intramuscularly. The chicks were challenged orally with homologous ***S.* Enteritidis** after 6 weeks. The authors found that the recovery of ***S.* Enteritidis** post challenge was significantly lower in both orally and intramuscularly vaccinated than nonvaccinated challenged chickens. Moreover, the shedding during 3 days post challenge was significantly lower in the orally vaccinated group than the intramuscularly vaccinated group. In addition, the shedding rate in both groups was significantly lower than the non vaccinated control.

Woodward *et al*. (2002) compared two programs of ***Sallmonella*** vaccination in commercial layer chicken using an iron-restricted ***S.* Enteritidis** PT4 aluminum hydroxide-adjuvanted bacterin (Salenvac, Intervet). In the first program the birds were vaccinated intramuscularly at day old and at 4 weeks of age. Whereas, in the second program, the birds were vaccinated at 1 day, 4 weeks and 18 weeks of age. Vaccinated and nonvaccinated control birds were challenged intravenously with ***S.* Enteritidis** PT4 (at 8, 17, 23, 30, and 59 weeks of age for the first program, and at 23, 30, and 59 weeks of age for the second one). The results revealed that both vaccination regimes led to reduction of the

organism in both egg content and shell contamination and also the bacterin afforded protection against ***S.* Enteritidis** challenge.

Clifton-Hadley *et al*. (2002) used a commercial inactivated iron restricted ***S.* Typhimurium** and ***S.* Enteritidis** vaccine (Salenvac®T Intervet) to vaccinate chicks against ***S.* Typhimurium**. The chicks were subcutaneously vaccinated at day old and boostered at 4 weeks of age, and then challenged by a high and a low dose of ***S.* Typhimurium** given either orally or by contact with seeder birds inoculated orally with a high dose of ***S.* Typhimurium**. The results showed that the shedding of ***S.* Typhimurium** was significantly reduced in vaccinated birds compared to unvaccinated control.

Cogan and Humphrey (2003) stated a dramatic decline in infection of human with ***Sallmonella*** in the United Kingdome since 1997 following the introduction of vaccination programs to control ***S.* Enteritidis** in laying hens and meat-type chickens. The decline in ***S.* Enteritidis** infections in humans was concomitant with the introduction of vaccination; however it is likely that other factors such as improved flock biosecurity measures also played a part.

Davies and Breslin (2003) investigated the effect of introducing vaccinated commercial layers onto farms that have previously housed flocks infected with ***S.* Enteritidis**. The authors examined both fecal and environmental samples for the presence of ***S.* Enteritidis**. The results showed that the incidence of ***S.* Enteritidis** in fecal samples was reduced from 40.5% to 5.9% in the unvaccinated and vaccinated flocks respectively. Similarly, ***S.* Enteritidis** was isolated from 16.1% of environmental samples in vaccinated flocks as compared to 29.5% of such samples in nonvaccinated flocks. The authors concluded that vaccination was recommended for all commercial laying flocks to reduce the fecal shedding of ***S.* Enteritidis**, and also the elimination of ***S.* Enteritidis** from large multistage laying farms without vaccination was not

likely to be possible. Moreover, good cleaning and disinfection measures were also required.

Khan *et al*. (2003) studied the recovery of ***S.* Enteritidis** from the small intestine and cecum of chickens after vaccination with subunit vaccine. They subcutaneously vaccinated 9-week-old SPF chickens challenged then with a virulent ***S.* Enteritidis**. The results demonstrated that the number of the organisms recovered from small intestine and cecum of vaccinated chickens was significantly lower than those from the non vaccinated ones.

Okamura *et al*. (2003) examined the effects of both ***S.* Enteritidis** bacterin and experimental subunit vaccines on the cell-mediated immunity by measuring lymphocyte proliferation and the levels of interleukin 2 (IL-2) and IL-6 in the serum. The birds were subcutaneously immunized at 6 and 9 weeks of age with either a commercial killed ***S.* Enteritidis** vaccine, crude protein (CP) extract or the outer membrane protein (OMP), or were given either saline alone or saline in IFA (incomplete Freund's adjuvant). The authors reported that there were significant increases in the proliferative responses to ***S.* Enteritidis** flagella, but not to lipopolysaccharide, CP, or OMP at 1 week post immunization. The use of the killed ***S.* Enteritidis** vaccine appeared the most effective because it induced a higher flagella-stimulated lymphocyte proliferation at 1 and 2 weeks post vaccination. Significant increases in the levels of both IL-2 and IL-6 in the serum were seen at 1 week post immunization.

Davies and Breslin (**2004)** stated that the rate of contamination of the egg shells and the egg contents with ***S.* Enteritidis** was 0.1% and 0. 04% respectively, in vaccinated flocks. While, it was 0.9% and 0.3% in nonvaccinated flocks. The vaccinated flocks were vaccinated with ***S.* Enteritidis**

bacterin at 4 and 18 weeks of age. In conclusion, the vaccination had a beneficial effect on decreasing egg contamination with ***S.* Enteritidis**.

Haider *et al.* (2007) studied the efficacy of formalin killed fowl typhoid vaccine contain 2 x 10^7 CFU. The fowl typhi vaccine was produced from the locally isolated ***S.* Gallinarum**. Five commercial layer flocks (flock no. 1, 2 3, 4 & 5) were inoculated subcutaneously at 6 weeks and then at 8 weeks old. Chickens were boasted after 4 wks of vaccination. The chickens of vaccinated and non-vaccinated groups were challenged with the infective dose (2 x 10^7 CFU) of freshly prepared live ***S.* Gallinarum** bacteria and lesions were not detected in vaccinated birds at necropsy. The vaccine was proved safe and effective in terms of preventing of fowl typhoid in chickens in Bangladesh.

Okamura *et al.* (2007) studied the effect of a commercial bivalent killed ***Sallmonella*** vaccine against ***S.* Enteritidis** / ***S.* Typhimurium** to prevent egg contamination with ***S.* Enteritidis** and ***S.* Typhimurium**. Using 4 different challenge models (oral, intravaginal, intravenous and intraperitoneal). The birds were challenged 4 weeks after vaccination. In either an oral or intravaginal challenge model, the fecal shedding decreased in vaccinated hens, but egg contamination was not evaluated due to scarcity of contaminated eggs even in the unvaccinated control groups. In contrast, an intravenous and an intraperitoneal challenge resulted in the relatively high level of egg contamination in unvaccinated chickens, which significantly reduced in vaccinated chickens.

Potkonjak et al. (2007) conducted a study in broiler breeder flocks during the rearing and the production period. All tested breeder flocks were vaccinated against Sallmonella three times during rear with the inactivated commercial vaccine (Salenvac T, Intervet, Netherlands) at 6, 12 and 16 weeks of age. Serum samples and ELISA test Sera from 12 broiler breeder flocks during

the rear (at ten weeks of age), at the peak of production and in 7 broiler breeder flocks at the end of the production period, all vaccinated against ***Sallmonella***, were taken for the serology testing. In spite of the vaccination, breeder flocks were negative 4 weeks after the first vaccination, while during the production there was a strong immunological response in all tested farms, indicating that ***S.* Enteritidis** infection probably occurred during the rear. However, the level of specific antibodies showed high differences among flocks and in the same flock (data not shown). Most likely broiler breeders experience infection during the rear, and serology response cannot be attributed to vaccination only. The authors were not able to explain why the immunological response, in most of the vaccinated flocks, was poor during the rear. Some data indicated that better immune response could be achieved when inactivated vaccine is primed with live antigens. There was a substantial amount of evidence that vaccination lower the ***S.* Enteritidis** shedding when challenge with homologous strain occurs. In fact, vaccination with live vaccines provided some cross protection to some of the other ***Sallmonella*** serovars. In such circumstances, it is reasonable to apply vaccination strategy and to expect that, with good farm management, cleaning and disinfection, there will be improvement in ***Sallmonella*** spp. contamination.

Radwan (2007) reported that vaccination with ***S.* Enteritidis** vaccines, regardless the type of vaccine (killed or live), conferred good protection against fecal shedding and colonization of the challenge ***S.* Enteritidis** strain in internal organs which would resulted in reducing pollution of the poultry environment, then reducing the incidence of human infection.

2.3.2. Live vaccine:

Barrow *et al.* (1990) intramuscularly gave 9R ***S.* Gallinarum** vaccine twice two weeks apart, and an experimental live ***S.* Enteritidis** (aromatic) aroA mutant vaccine, was given twice two weeks apart both orally and

intramuscularly, in 24-week-old commercial hens. Two weeks following the final vaccination, the chickens were orally challenged with virulent ***S.* Enteritidis** PT4. The 9R vaccine significantly reduced isolations of challenge ***S.* Enteritidis** from liver, spleen, ovary, and from both eggs shell and eggs content.

Cooper *et al*. (1990) tested ***S.* Enteritidis** aroA mutant as a vaccine. SPF Leghorn chicks were orally vaccinated with $l0^9$ CFU at 1 day of age, 10^7 CFU at 1 and 14 days of age, or $l0^5$ CFU at 1 and 7 days followed by 10^9 CFU at 14 and 21 days of age. The chicks were orally challenged after 2 weeks of the final vaccination with a virulent ***S.* Enteritidis** PT4. All vaccinated groups had reduced fecal shedding of challenge organisms compared to nonvaccinated ones. The chicks that given four doses had a significant reduction in challenge organisms in liver, spleen, and feces compared to nonvaccinated ones. The oral vaccination with ***S.* Enteritidis** aroA vaccine provided a significant protection from a chicken-virulent strain of ***S.* Enteritidis**.

Cooper *et al*. (1992) orally vaccinated SPF white leghorn chicks with either $l0^9$ CFU/dose at one day of age or $l0^5$ CFU/dose at 1, 7, 14, and 21 days of age with two ***S.* Enteritidis** PT4 aroA mutants live attenuated vaccines. The chickens were either intravenously or orally challenged between 8 and 9 weeks of age with 10^8 CFU of virulent ***S.* Enteritidis**. There was significant reduction in the intestinal shedding of challenge ***S.* Enteritidis** and the recovery of challenge organism from spleens, livers, and feces of vaccinated birds with either vaccine than from nonvaccinated birds.

Cooper *et al*. (1993) evaluated a live ***S.* Enteritidis** aroA vaccine in two trials, in the first trial, chickens were vaccinated at one day of age with $l0^9$ CFU of the ***S.* Enteritidis** aroA vaccine, and then housed at 3 weeks of age with nonvaccinated chickens that had been orally infected with 10^8 CFU of ***S.* Enteritidis** PT4. Over six weeks, non of the vaccinated chickens shed ***S.***

Enteritidis PT4 heavily, far fewer of them shed at all, and they shed for a shorter time than did nonvaccinated control chickens similarly exposed to infected chickens. In another trial, oral vaccination at 1 and 14 days of age produced solid cecal protection, and oral booster dosing at 16 and 18 weeks made cecal protection complete. So, the oral boosting before lay increased intestinal mucosal immunity, but that parenteral boosting might be necessary for protection of the ovary.

Meyer *et al*. (1993) used live vaccines against ***S.* Dublin**, ***S.* Typhimurium** and ***S.* Cholerasuis**, under the collective intensive condition. The incidence of Salmonllosis was reduced from 107 outbreaks in 1980 to 150 in 1985, a fall of 85%. All the 3 live vaccine were found safe and effective if used appropriately. Use of ***S.* Typhimurium** live vaccine was also claimed to eliminate ***S.* Enteritidis** from egg producing farms. Those results were confirmed in 2 trials against experimental oral ***S.* Enteritidis** infection. Live vaccines also produced protective result against ***S.* Typhimurium** in turkey poults and goslings.

Cooper *et al*. (1994) examined different programmes of vaccination with a live attenuated oral ***S.* Enteritidis** aroA vaccine. Day-old chicks were orally vaccinated with that vaccine then were challenged intravenously with virulent ***S.* Enteritidis** at 8 weeks of age and one group of them was given an intramuscular booster dose at 16 weeks of age then both groups were intravenously challenged at 23 weeks of age with virulent ***S.* Enteritidis**. vaccinated day-old chicks were orally challenged at 8 weeks of age with virulent ***S.* Enteritidis**. Day-old chicks were orally vaccinated with the vaccine and were challenged the following day with virulent ***S.* Enteritidis**. The results showed that the oral live attenuated vaccine appeared to induce sufficient mucosal and systemic immune responses to reduce intestinal shedding, invasion from the gut, and colonization of internal organs from field challenge.

Hassan and curtiss *(1997)* evaluated the efficacy of an virulent live ***S.* Typhimurium** in prevention of colonization and invasion of chickens by homologous and heterologous ***Salmonella*** serotypes. Chickens were vaccinated orally at 2 and 4 weeks of age then assessed for protection against oral challenged with ***S.* Typhimurium** and ***S.* Enteritidis** at 3, 6, 9, and 12 months of age. A comparison of ***Salmonella*** isolation from vaccinated and non vaccinated layers after challenge with ***S.* Typhimurium** and ***S.* Enteritidis** showed that ***S.* Typhimurium** induced excellent protection against intestinal, visceral, reproductive tract, and egg colonization invasion, and/or contamination by ***Salmonella***. Protection lasted for 11 months after vaccination, when the experiment was terminated. ***S.* Typhimurium** and ***S.* Enteritidis** strains were isolated from the yolk, albumen, and shells of eggs laid by non vaccinated challenged chickens. ***S.* Typhimurium** caused pathological lesions in non vaccinated chickens, whereas vaccinated and nonvaccinated ***S.* Enteritidis** challenged chickens showed no pathological lesion in visceral and reproductive organs. Vaccination with ***S.* Typhimurium** prevented transmission of ***S.* Typhimurium** or ***S.* Enteritidis** into eggs laid by vaccinated layers with no effect on egg production. That was believed to the first published confirmation that vaccination live virulent ***Salmonella*** can induce long-term protection against ***Salmonella*** infection in layers.

Barbezange *et al*. (2000) compared the safety characteristics of three commercially available live ***Salmonella*** vaccine strains (vac T, Zoosaloral, and chi3985) in relation to their persistence in individual birds but also within a flock and in the environment. In a first experiment, the digestive and systematic distributions in chickens were followed for 10 days in individually reared chickens that were orally inoculated at 1 day of age. strains chi3985 quickly disappeared from the digestive tract but remained in liver until the end of the experiment, whereas strains vac T, Zoosaloral colonized the liver as well as the

gut for 10 days. In the second trial, the behavior of vaccine strains was studied in groups of 20 chickens during 10 weeks after single oral administration to individual birds. Strain vacT remained in the environment of inoculated birds for 4-5 weeks. Six weeks after the inoculation, vacT was not recovered from internal organs such as liver and spleen, and vacT disappeared from digestive tract between the 6 and 10 weeks. Comparatively, both Zoosaloral, and chi3985 vaccine strains persisted longer in the environment (8 weeks at least). Of the vaccine strains, chi3985 showed the greatest colonization of both systemic and digestive organs.

Cerquetti and Gherardi (2000a) orally administrated a live temperature-sensitive mutant of ***S.* Enteritidis** vaccine to chicks at 1, 2, 3, and 7 days of age and were challenged at 14 or 21 days of age with wild type of ***Salmonella*** of different serotypes. The results showed that the immunization of newly hatched chickens with attenuated live ***S.* Enteritidis** vaccine reduced ***Salmonella*** shedding, cecal colonization and internal organ invasion.

Cerquetti and Gherardi (2000b) used a live temperature-sensitive mutant of ***S.* Enteritidis** vaccine orally, intraperitoneally or by combination of two routes to chicks at one day of age and again at 2 weeks of age. The chicks were orally challenged at 4 weeks of age with virulent ***S.* Enteritidis**. It was found that vaccination reduced the number of chickens shedding challenge ***S.* Enteritidis**, and chickens that vaccinated first orally had the fewest shedders. The vaccination reduced the number of the organism in cecal contents and also the duration of colonization was reduced in chickens immunized first orally. The vaccination reduced spleen colonization, especially in birds vaccinated intraperitoneally first, then orally. Interestingly, oral rout of vaccination boostered by oral or intraperitoneal one effectively reduced ***S.* Enteritidis** shedding and colonization of cecum and spleen.

Springer *et al*. (2000) investigated the safety and efficacy of a new live ***S.* Enteritidis** vaccine strain for chicken. No adverse effects were observed in 1-day-old chicks after oral administration of the vaccine. Laying performance and egg weight were not affected by vaccination. The vaccine strain of ***Salmonella*** could not be isolated from egg and internal organs of slaughtered chickens even when they were vaccinated with a triple dose. The vaccine induced cell mediated immunity and development of complement-fixing antibodies in mice. Challenge trials demonstrated that the vaccine could prevent or significantly reduce adverse effects of ***S.* Enteritidis** infection.

Feberwee *et al*. (200la) stated that 80 commercial layer flocks were subcutaneously vaccinated at 6 weeks of age and again at 14 to 16 weeks of age with a live attenuated ***S.* Gallinarum** 9R strain vaccine. The vaccinated flocks were compared with 1854 nonvaccinated flocks hatched in the same period. The nonvaccinated control flocks were examined for ***S.* Enteritidis** at 72 weeks of age by serological monitoring. The vaccinated flocks were monitored at 4 weeks of age by routine bacteriology of pooled cecal droppings and also at 10, 16, 24, 32, 40, 48, 56, 64 & 72 weeks of age by routine serology for ***S.* Enteritidis**. Only one of the 80 vaccinated flocks was verified as ***S.* Enteritidis** positive during the trial, while 214 (11.5%) of the control flocks became serologically positive. There was no evidence of spread of the vaccine organism into eggs. The vaccine contributed in the reduction of ***S.* Enteritidis** infections in commercial layer flocks.

Feberwee *et al*. (200lb) stated that 4 different rearing farms and 1 layer farm that vaccinated with a live attenuated ***S.* Gallinarum** 9R strain vaccine and were housed adjacent to or at the same house with non vaccinated flocks. Showed no evidence of the fecal spread of ***S.* Gallinarum** 9R vaccine.

van Immerseel *et al*. (2002) evaluated the effect of vaccination with ***S.* Enteritidis** aroA mutant on early cellular responses in cecal lamina propria of newly-hatched chickens. Day-old chicks were vaccinated with ***S.* Enteritidis** aroA mutant vaccine. Then they challenged one day later with a virulent ***S.* Enteritidis** strain. The authors found that vaccinated chicks had a much lower number of challenged organisms in organs and cecal contents during the first days post challenge than controls. Immune cells infiltrated cecal lamina propria within 12 to 24 hours following vaccination and those cells might contribute to inhibit subsequent colonization by a virulent ***Salmonella*** strain.

Gantois *et al*. (2006) reported on the effect of oral vaccination with live ***S.* Enteritidis** vaccine, live ***S.* Typhimurium** vaccine and with both vaccines on colonization of the reproductive tract and internal egg contamination of laying hens with ***S.* Enteritidis**. Three groups of 30 laying hens were vaccinated at 1 day, 6 weeks and 16 weeks of age with either one of the vaccine strains, or a combination of both vaccine strains, while a fourth group was left unvaccinated. At 24 weeks of age, the birds were intravenously challenged with *c* PT4. Both vaccines either separately or combined reduced the isolation of challenge ***S.* Enteritidis** from oviducts and from internal eggs content than from the nonvaccinated hens. The vaccination of laying hens with these live vaccines could be considered as a valuable tool in controlling internal egg contamination and reduction of ***Salmonella*** colonization in the reproductive tract.

Bohez *et al*. (2008) evaluated the effect of pre-treatment with a homologous live ***Salmonella*** hilA mutant strain on the long-term colonization and transmission of ***S.* Enteritidis** in broilers. For this purpose, three treatment groups of newly hatched broilers were created. Each group consisted of 4 pens with 25 birds per pen. The first and second group were orally inoculated with a ***S.* Enteritidis** hilA mutant strain (Nalr), whereas the third group was not. In the second and third group, 20% of the birds were challenged 1 day later with a ***S.***

Enteritidis wild type strain (Strepr). The ***S.* Enteritidis** hilA mutant strain showed no residual virulence in the chicken host and was largely cleared from the chickens at 6 weeks of age. A significant long-term inhibition of faecal shedding and caecal and internal organ colonization of the wild type ***S.* Enteritidis** strain was observed in the birds pre-treated with the hilA mutant strain. Although pre-treatment with a hilA mutant strain could not fully prevent the spread of ***S.* Enteritidis** amongst the broilers, a significant reduction of transmission was observed in comparison to the non-pre-treated groups. The observed colonization-inhibition (CI) indicates that administration of live attenuated hilA mutant ***Salmonella*** strains to newly hatched chicks might, in combination with other protective control measures, contribute to the control of ***Salmonella*** infections in broilers**.**

2.3.3. Live vaccines plus bacterin:

Methner *et al*. (1994) found that immunization of broiler parent chickens with ***S.* Typhimurium** live or inactivated vaccines (orally and/or parentally) resulted in a considerable antibody response. Maternally produced antibodies were transferred via egg to progeny, but the transferred antibodies did not increase considerably the resistance of day old chicks to oral ***S.* Typhimurium** challenge. The results of the oral challenge of the parent birds showed protective effect induce by the oral immunization with the ***Salmonella*** live vaccine strain. That protection was expressed by a reduced number of ***Sallmonella*** bacteria in caeci and internal organs.

Nassar *et al*. (1994) investigated the efficacy of the live 9R ***S.* Gallinarum** vaccine given subcutaneously at 8 weeks of age in a combined program with ***S.* Enteritidis** bacterin given subcutaneously at 18 weeks and again at 22 weeks, compared to separate use of either vaccine alone in commercial layer chickens. The hens were orally challenged at 24 ,27 and 30

weeks of age with ***S.* Enteritidis** PT4 intramuscularly. Throughout the experiment, isolation of the challenged organism from egg shells and contents of nonvaccinated layer was higher than those of any vaccinated layers. Isolation of the challenged organism from cloaca1 swabs was less in the groups given a combination of live and killed vaccine or killed vaccine only than the controls or the group given live vaccine only. The results showed that combined live and killed vaccination program, and in association with improved hygiene, good biosecurity, and rodent control would provide an excellent comprehensive program for control of ***S.* Enteritidis** in layers.

Schaller (1996) reported that the live attenuated ***S.* Typhimurium** vaccine induced high homologous immunity against ***S.* Typhimurium** and partial heterologous immunity against ***S.* Enteritidis**, reduced horizontal and vertical transmission, eggs shell contamination and induced antibodies in the eggs. The inactivated ***S.* Enteritidis** bacterin gave high homologous immunity to ***S.* Enteritidis**, induced systemic as well as local immunity in the intestine, reduced colonization of organs including ovary, reduced vertical transmission, reduced eggs shell contamination, reduced shedding period in case of ***S.* Enteritidis** infection and induced high levels of ***S.* Enteritidis** antibody in the eggs. The live vaccine provided some protection against ***S.* Enteritidis**, which then was strengthened through the use of the killed ***S.* Enteritidis** bacterin. Interestingly, the vaccination could provide a means of preventing spread of ***S.* Enteritidis** by keeping infection frequency and egg transmission at lower levels than was possible without vaccination, and that the reduced infection pressure should ease eradication of ***S.* Enteritidis** at a poultry production site.

Feberwee *et al*. (2000) compared between the efficacy of living and inactivated ***S.* Enteritidis** vaccine in reducing the reinfection with ***S.* Enteritidis**. The study was carried out over a 2 years course and was included flocks in 3 groups. Group A, which comprised of 15 flocks were vaccinated with

an attenuated live ***S.* Typhimurium** vaccine in drinking water at 1 day of age and, at 7 weeks of age and again subcutaneously with ***S.* Enteritidis** bacterin at 16 weeks of age. Group B, which comprised of 49 flocks was intramuscularly vaccinated with ***S.* Enteritidis** bacterin at 12 and at 16 weeks of age. Group C, which comprised of 608 nonvaccinated flocks were kept as a control group. The authors found that although in most cases the proportion of ***S.* Enteritidis** infected flocks was lower for the vaccinated group, differences were not statistically significant because the number of vaccinatd flocks was very small. the authors indicated that vaccination against ***S.* Enteritidis** contributed in reduction of ***S.* Enteritidis** reinfection in broiler breeder flocks.

Babu *et al*. (2003) evaluated the impact of live and killed ***Sallmonella*** vaccines on cell-mediated immunity (CMI) in 18 and 32 weeks-old White Leghorn chickens. Overall, oral vaccination with live attenuated ***Salmonella*** vaccine was more effective than the killed vaccine, in increasing the proliferative response in both age group birds. Those results indicated that the live vaccine may be a more valuable tool in controlling the ***S.* Enteritidis** infection.

Babu *et al*. (2004) evaluated the impact of using of live and killed vaccines on ***S.* Enteritidis** clearance in young chicken. SPF Leghorn chickens at 2 weeks and at 4 weeks of age were vaccinated with either a live attenuated ***S.* Typhimurium** or ***S.* Enteritidis** bacterin. The chickens were orally challenged with 10^{10} CFU/ bird of ***S.* Enteritidis** PT4 at 6 weeks of age. At 7 weeks of age, the birds were euthanized for evaluation of immunological responses and ***S.* Enteritidis** clearance. ***S.* Enteritidis** shedding was reduced in chickens given live vaccine compared to control or those given bacterin. The live vaccine potentiated cell-mediated immunity, but the killed one suppressed it. In contrast,

the killed vaccine induced the highest ***S.* Enteritidis** specific antibody response. The author indicated that the vaccination with attenuated live ***Sallmonella*** could be an effective method for controlling ***S.* Enteritidis** in young chicken.

Bailey *et al*. (2007) evaluated the intestinal humoral immune response to a live vaccine used on hatchings with and without maternal antibody and related this response to challenge with a blend of two antibiotic-resistant ***Salmonella*** marker strains. Forty week of age ISA Brown (Institute de Selection Animale, France) breeders from a ***Salmonella***-free flock were vaccinated twice at a three weeks interval with commercially-prepared autogenous trivalent bacterin, serogroups B, C and D1 or ***S.* Enteritidis** bacterin. Half of the progeny from these treatments (hatched from eggs laid 3 weeks after second bacterin dose) were given a live ***S.* Typhimurium** mutant vaccine by coarse spray on arrival in the brooding premises. On days 3, 13 and 34, intestinal immunoglobulins (Ig) A and G were sampled and measured on enzyme-linked immunosorbent assay plates coated with ***S.* Enteritidis** (SELPS) or ***S.* Typhimurium (**STLPS) Lipopolys-accharide. On the same days, a second group of birds was challenged with a blend of antibiotic-resistant ***S.* Enteritidis** and ***S.* Typhimurium**. Cecal, liver, heart, and spleen samples obtained 7 days post-challenge were cultured and colonies were enumerated. Maternal IgG that observed up to 13 days had no effect on subsequent Live ***S.* Typhimurium** stimulated antibody production. No protective effect of maternal antibody was demonstrated, except when combined with Live ***S.* Typhimurium** given to the progeny. Killed vaccines delivered to the breeders combined with a live vaccine delivered to the progeny resulted in reduced invasiveness after challenge, as shown by a reduction in liver-heart-spleen ***Salmonella*** counts. One dose of Live ***S.* Typhimurium** enhanced intestinal IgG [Optical Densities (OD) >0.576] up to 34 days when measured on STLPS, but only to 13 days when measured on SELPS, with titers decreasing with age. Increased IgA was observed only at 13 days. Three and 13 but not 34

days bacterial counts were decreased by the live ***S.* Typhimurium** vaccine treatment, for both cecal (1.05 and 1.09 log) and liver-heart-spleen (0.32 10 and 0.06 log) samples, indicating that a second dose might be necessary for prolonged protection. The 10 protective effect of the live vaccine, but not of maternal IgG, leads us to hypothesize that protection might be due to stimulation of cell-mediated intestinal immunity and/or a competitive exclusion effect of the Live ***S.* Typhimurium** vaccine. Reduction but not elimination of ***Salmonella*** colonization by vaccination highlights the importance of vaccines as complementary tools and not substitutes of integral biosecurity programs to control ***Sallmonella*** in poultry.

Pakpinyo *et al*. (2008) evaluated the efficacy of ***S*. Typhimurium** live vaccine and ***S.* Enteritidis** inactivated one against ***S.* Enteritidis** in layer. The chickens were into 4 groups, 30 birds in each group as follows: Group 1: ***S*. Typhimurium**, 2 doses at 1 and 14 days old and ***S.* Enteritidis,** 1 dose at 12 weeks old and challenge with ***S.* Enteritidis** at 16 weeks of age. Group 2: Non vaccination but challenge with ***S.* Enteritidis** at 16 weeks old. Group 3: ***S.* Enteritidis,** 2 doses at 1 and 14 days old and ***S.* Enteritidis**,1 dose at 12 weeks old and non challenge. Group 4: Non vaccination and non challenge. The results of that study indicated that ***S*. Typhimurium** live vaccine and ***S.* Enteritidis** inactivated were effective in reducing the ***S.* Enteritidis** colonization hence re-isolation. The vaccinated group also showed better feed conversion rate (FCR) (6.45) compared with non vaccinated group (9.37).

2.4. Probiotics:

Impey and Mead (1989) explained the mechanism of action of competitive exclusion (CE) flora in part by a bacteriostatic effect on ***Salmonella*** in the caeca.

Schneitz *et al.* (1992) recorded that the rapid transfer of the normal flora from the hen to the chick is impossible in modern mass production systems and the development of microflora is therefore, considerably delayed.

Ha *et al.* (1994)detected the possible mode of action of CE flora by production of VFA by the bacteria, competition for receptor sites in the intestinal tract and competition between pathogens and native microflora for nutrients may also play a role.

Eric Line *et al*. (**1998)** concluded that ***Saccharomyces* Boulardii** in poultry feed might become a useful tool in controlling ***Salmonella*** colonization of chickens.

Mead (2000) stated that CE is a prophylactic measure that aims to increase the resistance of young chicks to ***Salmonella*** or other infections by fastening the development of normal gut microflora. The first method of application was to incorporate the preparation in the drinking water. Uptake of the drinking water in the first 24h after hatch however, is very variable and the viability of the anaerobic strains in the water may be reduced. Moreover there is a delay between hatch and placement in rearing houses.

Schneitz and Mead (**2000)** demonstrated that CE agents are considered to exert their effect by one or more of four general principle actions, namely the creation of a restrictive physiological environment, the competition for bacterial receptor sites, the elaboration of antibiotic like substances and/or the depletion of essential substrates.

Seo *et al.* (2000a) recorded that CE flora could be used after curative antibiotic therapy to eliminate an existing ***Salmonella*** infection and, in this case, the CE flora restores the microflora. The use of normal avian gut flora (NAGF) as part of a CE regimen may provide 1 day old chicks with a good defense

against ***S.* Enteritidis** colonization and may prevent the establishment of a persistent ***S.* Enteritidis** infection. Once a persistent infection is established, judicious use of antibiotics like enrofloxacin in combination with a CE culture reconstituted with intestinal flora may be effective in eliminating these infections from the flock.

Ayed *et al*. (2004) studied the effects of incorporating additives into the diets of growing broilers over a period of 50 days. Four groups of 100 birds each were divided into four treatments with 4 replication (25 x 4). Birds were 1 day old at the beginning of the experiment. Each group received one of 4 diets: A) maize + soybean; B) maize + soybean +avilamycin; C) maize + soybean + activis; and D) maize + soybean + avilamycin + activis. Body weight (kg), food conversion index (g food intake/ g weight gain), daily gain (g/d/bird), dry and organic matter digestibilities (%), nitrogen retention (%), carcass yield (%), abdominal fat%, and gizzard weight (g) were determined by growth period for the four diets. Broilers receiving diet D had the lowest ($p<0.05$) daily gain (42.6) and the highest ($p<0.05$) feed conversion index (2.43) among all groups. The heaviest gizzard (42.2) and the lowest ($p<0.05$) abdominal fat% (1.84) were observed for the C and B diets, respectively. Organic matter (73.61) and dry matter (67.45) digestibilities and nitrogen retention (60.93) were the highest for the C diet. It appeared that activis might replace avilamycin in broiler diets. These two additives should however not be given together to chicks.

Madian and Wafaa (2006) indicated that addition of prebiotic (1g/kg diet), symbiotic (o.25 g/kg feed) and probiotic(1ml/L drinking water) were significantly($p<0.05$) increased the final body weight (BW) by 17.8%, 12.9% and 9.9%, respectively when compared with that of control, whereas that of broiler received virginiamycin (1gm/kg basal diet) was numerically higher than those of control but statistically insignificant. Feed additives had a marked and significant impacts on body weight gains (BWG) and feed efficiency in the

manner of improvements versus to control during the entire 42 days experimental period. The total coliform and aerobes counts in the small intestine and caeca of birds which received prebiotic and synbiotic at 6 weeks of age were significantly ($p<0.05$) lower than the that of the control birds. . The result showed that inclusion of tested additives except virginiamycin yielded a significant enhancement in the immune responses included by a significant increase in the total antibody titer to sheep red blood cells (SRBC), hypergammaglobulinaemia in serum protein electrophoresis and less pronounced histological alternations in immunogenic organs (bursa of fabricus, thymus and spleen). So that prebiotic, synbitics and probotic can improve the overall performance, enhance the immune response and contribute to on-farm pathogen reductive to antibiotic growth promoters in the broiler chicken diets**.**

Wafaa *et al*. (2006) evaluated the effect of combined competitive exclusion culture with mannan-oligosaccharides, and ciprofloxacin on ***S.* Enteritidis** colonization in broiler chickens. Indicated that infected-untreated chicks had a reduced mean body weight and feed efficiency than those in the uninfected-untreated group, whereas these parameters were significantly better in groups supplemented with dietary CE plus MOS (0.25 gm/kg feed) and MOS alone (1gm/kg diet) than those in untreated and those in ciprofloxacin(10gm/kg. b. wt.) medication groups. ***S.* Enteritidis** challenge-untreated birds showed heavy caecal colonization and high percentage of ***S.* Enteritidis** positive samples from liver and spleen when compared with unchanged-untreated control group that was negative in all samples. A significant ($p<0.05$) reduction in the number of ***S.* Enteritidis** caecal colonization and in the percentage of culture positive birds were seen in chicks supplemented with dietary CE plus MONS mixture, MONS and in those medicated with ciprofloxacin at the 7.14, 21.28 days post challenge when compared with challenged-untreated of birds. Immunologically, compared with depressed immunological response to sheep

red blood cells (SRED) was observed in ***S.* Enteritidis** infected-untreated broilers as the primary antibody titers were diminished at the different sampling intervals, while CE plus MOS and MOS treated birds experienced ability to good response to SRED and showed also high level of gammaglobulins in electrophoretic patterns of serum proteins than those treated with ciprofloxacin. CE plus MOS and MOS treatments overcame the adverse effect of ***S.* Enteritidis** on liver and Kidneys functions and improve them.

Higgins *et al*. (2007a) evaluated the ability of a commercially available lactic acid bacteria-based probiotic culture (LAB) to reduce ***S.* Enteritidis** or ***S.* Typhimurium** in day-of-hatch broiler chicks. In these experiments, chicks were challenged with ***S.* Enteritidis** or ***S.* Typhimurium** and treated with LAB an hour postchallenge. Following treatment, cecal tonsils and ceca were aseptically collected for ***S.* Enteritidis** or ***S.* Typhimurium** enrichment or ***S.* Enteritidis** enumeration, respectively. In experiments 1 to 3, LAB significantly reduced the incidence of ***S.* Enteritidis** (60 to 70% reduction) or ***S.* Typhimurium** (89 to 95% reduction) recovered from the cecal tonsils of day-old broiler chicks 24 hours following treatment as compared with controls ($P < 0.05$). Additionally, administration of LAB caused a >2.9 $\log_{10}$ reduction of total cecal ***S.* Enteritidis** recovered 24 h following treatment as compared with controls ($P < 0.05$). In experiments 4 to 7, upon sample enrichment LAB significantly reduced the recovery of ***S.* Typhimurium** from the cecal tonsils at 24 h, but not 6 or 12 h post treatment ($P < 0.05$). However, in experiments 6 and 7, when total cecal ***S.* Enteritidis** recovery was enumerated, a significant treatment-associated reduction was observed 12 h post treatment, although in cecal tonsil samples there was no difference in ***S.* Enteritidis** incidence at 12 h ($P < 0.05$). In those studies, LAB treatment significantly reduced recovery of ***Salmonella*** in day-of-hatch broilers.

Higgins *et al*. (2007b) evaluated the effect of probiotic treatment in broiler chicks on intestinal macrophage numbers and phagocytosis of ***S.* Enteritidis** by abdominal exudate cells (AEC). Day-of-hatch chicks were challenged with ***S.* Enteritidis** and then treated with the probiotic culture 1 h later. Three other treatment groups were not treated (negative control), challenged only, or treated with probiotic only. In all experiments, probiotic treatment on the day of hatch reduced ($P < 0.05$) cecal ***S.* Enteritidis** recovery as compared with the control treatment. In experiments 1 and 2, immunohistochemistry was used to evaluate the presence of macrophages in the ileum and cecum of 7 to 10 chicks per group at 24 h post treatment. In experiment 1, the number of macrophages observed per 10,000 microm in the ileum of ***S.* Enteritidis** -challenged chicks was higher ($P < 0.05$) than that of nonchallenged chicks. In the cecum, there were more ($P < 0.05$) macrophages per 10,000 microm in chicks receiving probiotic treatment without challenge than in negative control chicks. However, in experiment 2 we found no differences among treatments in the numbers of macrophages for both the ileum and cecum. Experiments 3 and 4 were performed to evaluate the ability of AEC from chicks to phagocytose ***S.* Enteritidis** in vitro. Abdominal exudate cells were isolated from the abdominal cavity, maintained in tissue culture plates overnight, and then assayed for phagocytic activity by incubating with ***S.* Enteritidis**. In experiment 3, more ($P < 0.05$) ***S.* Enteritidis** was recovered from AEC derived from probiotic-treated chicks than in any other treatment. However, in experiment 4, all treatments resulted in similar levels of AEC, and phagocytosis of ***S.* Enteritidis** was at low levels in all groups. Although not conclusive, the modest differences detected in experiments 1 and 3, and the fact that those differences were not repeatedly detectable, suggest that these macrophage-related changes were not solely responsible for the reductions of ***S.* Enteritidis** following probiotic treatment.

Vicente *et al*. (2007) evaluated the ability of 2 probiotic cultures (P1 and P2) to reduce environmental ***Salmonella*** in commercial turkey flocks 2 weeks prior to processing with or without the use of a commercial organic acid (OA). ***Salmonella***-positive flocks were identified 3 to 4 weeks before processing by using standard assembled drag swabs. Two weeks after treatment, drag swabs were used again for ***Salmonella*** recovery. In the trial, 22 ***Salmonella***-positive houses were selected to evaluate 6 treatments: control, OA, P1, P2, OA + P1, and OA + P2. Two weeks after treatment, the recovery of ***Salmonella*** was significantly reduced (P <0.05) in houses in which P1 and P2 cultures were administered in combination with the OA product. The results suggested that the administration of selected probiotic candidate bacteria in combination with OA may reduce environmental ***Salmonella*** in turkey houses, and also this practice could help to reduce the risk of ***Salmonella*** cross-contamination in the processing plant.

Revolledo *et al*. (2009) evaluated the effects of broiler chicks treatment with a competitive exclusion (CE) product, an experimental dietary probiotic, and the abiotic β-glucan on cecal colonization, organ invasion, and serum and intestinal IgG and IgA levels to ***Salmonella*** challenge. Four groups of day-old chicks were treated by orally with an appropriate dose of a commercial CE product. Three groups received daily doses of probiotic, β-glucan, or both for 6 days. Three other groups were fed daily from day-old onwards with probiotic, β-glucan, or both. Subgroups of 30 chicks from each group were challenged on day 1, 9, 16, or 23 with 10^7 cfu/ mL of ***S.* Typhimurium** and killed 7 day later. Control groups were maintained untreated and remained unchallenged (negative control), or were challenged with ***S.* Typhimurium**. Cecum, liver, and spleen samples were examined for the presence of ***Salmonella***, whereas serum and intestinal fluid samples were assayed for total antibody (IgG and IgA) concentrations. In comparison with other treatments, those involving CE product

and β-glucan, with or without probiotic during the first week, resulted in a superior inhibition of cecal colonization and organ invasion by ***Salmonella*** and also offered a higher level of protection ($P < 0.05$). During the second week, treatments containing experimental dietary probiotic and β-glucan, with or without CE product, resulted in an inhibition of liver invasion ($P < 0.05$). The IgA levels were significantly higher ($P < 0.05$) in intestinal fluid compared with serum, whereas IgG had low levels. The results in the first and third week indicated that combination treatments involving CE product, probiotic, and β-glucan were a more effective control of ***Salmonella*** colonization than the corresponding individual preparations.

2.5. Agglutination Tests:

Cooper *et al*. (1990) reported that the microantiglobulin test and the ELISA were more sensitive than the other tests and detected some infected birds that were negative by the rapid slide and tube agglutination tests, and also showed high titres in some birds from which ***Salmonella*** species could not be isolated post mortem. Sera obtained from two flocks which had a history of natural ***S.* Enteritidis** infection were evaluated by all the tests; evidence of infection was found with the microantiglobulin and ELISA tests but not with the other tests.

Gast and Beard (1990) reported that the antibody response of laying hens to experimental ***S.* Enteritidis** infection was evaluated by micro-agglutination, tube agglutination and rapid whole blood plate agglutination assays. All three tests effectively identified most exposed hens as sero-positive.

Kim *et al*. (1991) tested an ELISA for its ability to detect antibodies against ***S.* Enteritidis** in chickens. Various features of the ELISA were evaluated and optimized. The outer membrane protein antigens selected by use

of protein immunoblotting method made the assay specific and sensitive. Result of the ELISA were compared with those of conventional serum plate and microagglutination tests. The ELISA was more sensitive and specific in detection of ***S.* Enteritidis** infection than the other 2 conventional tests.

Minga (1992) evaluated an ELISA for the serodiagnosis of fowl typhoid and paratyphoid due to ***S.* Enteritidis** in chickens. The hot phenol: water lipopolysaccharide (LPS) extract of ***Salmonella*** was used as the antigen. Chicken serum, eggs and discs impregnated with chicken blood were tested for the presence of antibodies against ***Salmonella*** factor'O'9 antigen. The substrate and chromogen used were hydrogen peroxide and orthophenylenediamine respectively. Serological results from the experimentally and naturally infected chickens showed close agreement between the conventional Serum Tube Agglutination Test (SAT) and serum ELISA while serum ELISA results were in close agreement with the egg and disc ELISA results. It was noted that ELISA was highly sensitive, convenient and versatile. It was concluded that ELISA, especially disc ELISA, thought to replace SAT for seroscreening chickens against ***S.* Gallinarum** and other ***Salmonella*** Group D infections.

Thorns *et al*. (1994) developed and evaluated a latex particle agglutination test to specifically identify cultured ***S.* Enteritidis** organisms. The test was based on the use of two monoclonal antibody-coated latex reagents, one of which detected the recently discovered SEF14 fimbriae expressed predominantly by ***S.* Enteritidis** and ***S.* Dublin** organisms, while the second reagent detects the H'p' antigen of ***S.* Dublin** flagella. In a series of field trials, 141 out of 142 strains of ***S.* Enteritidis** from eighteen phage types were correctly identified by the latex test. A further 175 ***Salmonella*** isolates representing 35 serotypes were tested and only two false-positives (***S.* Dublin**) in the latex test were recorded. This is the first rapid serotype specific test for ***S.***

Enteritidis to be developed, and highlights the potential advantage of fimbrial antigens as novel diagnostic antigens of the future.

Soumet (1999) developed an immunoconcentration-PCR assay was developed for the rapid and specific detection of ***Salmonella***. That assay was compared with a conventional bacteriological method for the detection of ***Salmonella*** from environmental swabs of poultry houses. The investigated 120 samples were pre-enriched in phosphate buffered peptone water and ***Salmonella*** was separated by an immunoconcentration process using an automated system prior to PCR. The specificity of the assay was high as no false-positives were found. The sensitivity of the assay was 70%. The correlation between the ICS-PCR assay and the bacteriological method was 84%.

Charlton (2000) reported that the microagglutination test (MAT) was used to test wild turkeys trapped within or imported into California (exclusive of birds from Kansas) from 1986 to 1996 for exposure to ***S.* Pullorum** and ***S.* Typhimurium**.

Feberwee *et al.* (2001a) said that double ELISA, whole blood agglutination test and rapid plate agglutination test under field conditions were performed for the diagnosis of salmonellosis. Animal and birds with actively excreting ***Salmonella*** might be serological negative similar considerations might also apply, bacteriological culture method and negative fecal culture might not necessary indicate that an animals or birds were not infected. Serological positive samples might be due to recent infection.

Berthelot *et al.* (2003) stated that ***S.* Enteritidis** was able to colonize the gastrointestinal tract and generally produces a chronic asymptomatic carrier state in poultry, except in very young birds. They monitored the serum and gut antibody responses of these four lines to ***S.* Enteritidis** for 9 weeks post inoculation by agglutination test and ELISA. The serum IgM and IgG

antibody responses were high and the serum IgA antibody responses low. In contrast, the intestinal secretions contained mostly IgA antibodies. The serum IgM antibody values of the four chicken lines were similar. These results suggest that most antibody responses were related to cecal colonization by ***S.* Enteritidis** and other than the antibody levels were involved in the control of this colonization.

Sadek (2005) concluded that agglutination based serological tests were dependable and reliable and could be used to monitor current infection and ***Salmonella*** carrier status of chicken flocks.

Islam *et al*. (2006) studied the seroprevalence and pathology of ***Salmonella*** infections in layer chickens of Dhaka and Gazipur regions of Bangladesh to isolate and characterize ***Salmonellae*** from layer chickens during the period from January to May 2006. The used methods were serum plate agglutination (SPA) test; necropsy and histopathology; cultural, morphological and biochemical tests. They found that, a total of 33 (21.02%) ***Salmonellae*** from live and dead birds were isolated. The isolation rate of ***Salmonellae*** was higher in seronegative (31.6%) group than seropositive (3.2%) group. Out of 33 ***Salmonella*** isolates, 25 were ***S. pullorum***, 3 were ***S.* Gallinarum** and the rest 5 were motile ***Salmonellae***.

Akter *et al*. (2007) investigated a total of 225 Star cross 579 brown chickens were studied with rapid serum plate agglutination test. Livers of 200 dead birds were subjected to isolation and identification of ***Salmonellae***. In vitro antibiotic sensitivity test of isolated ***Salmonellae*** was performed with commercial sensitivity discs. The overall seroprevalence was recorded 23.11%. The prevalence was varied from age to age. The highest rate was 28% in above 20 weeks of age.

2.6. Enzyme-Linked Immunosorbent Assay (ELISA):

Hassan *et al*. (1990) developed an indirect ELISA to detect ***S.* Typhimurium** antibodies in chicken sera, using whole bacterial cell protein, flagellar protein or lipopolysaccharide as antigens. In experimental infections high concentrations of ***S.* Typhimurium**-specific IgG persisted after the faecal excretion of ***S.* Typhimurium** had ceased, whereas the specific IgM response was transitory. Some uninfected chickens placed in contact with experimentally infected birds developed high IgG titres in the absence of detectable faecal excretion. Other ***S.* Typhimurium** strains, which varied in their invasive abilities, also induced high titres of IgG. The ELISA allowed chickens infected experimentally with ***S.* Typhimurium** to be differentiated from chickens infected with 10 other serotypes, including ***S.* Enteritidis**. The use of whole blood in place of serum in the ELISA reduced the titres slightly. The storage of serum dried on to filter paper strips for four weeks produced little change in ELISA antibody titre, and the treatment of such strips with phenol or chloroform vapour had little or no effect on the antibody titre.

Nicholas (1992) investigated serologically and bacteriologically four laying flocks of chickens in Britain, each with a history of ***S.* Typhimurium** infection. ELISA and rapid slide agglutination test (RST) stained ***S.* Pullorum** were used. On site A no ***Salmonellae*** were recovered from birds in the house chosen for serological examination. Of these birds approximately 20% had antibodies to ***S.* Typhimurium** in ELISA which used either a lipopolysaccharide (LPS) or heat-extract (HE) antigen from ***S.* Typhimurium**. ***S.* Typhimurium** was recovered from birds in one other of the four houses on the same site; these birds were not tested serologically. On site B, ***S.* Typhimurium** was isolated from 8% of the birds examined. Of the total tested serologically, a third to half were seropositive by ***S.* Typhimurium** ELISA using the LPS and HE antigen respectively. A small proportion of birds was

seropositive by ***S.* Enteritidis** ELISA and RST. No ***Salmonellae*** were isolated from the other two sites although about 10% of birds tested on site C were seropositive in ***S.* Typhimurium** ELISA. Cross reactions were seen between ***S.* Typhimurium** antigens in the ELISA and experimentally prepared antiserum to ***S.* Enteritidis**. The ***S.* Enteritidis** ELISA was generally more specific although cross-reacting antibodies were detected in sera from birds on sites A and B.

van Zijderveld (1993) compared the results of bacteriological examination of faecal samples taken at 1580 visits from 545 flocks with those of a ***S.* Enteritidis** ELISA applied on 24 serum samples per visit per flock. Two flocks were found positive for ***S.* Enteritidis** by bacteriological examination; both flocks were also detected by ELISA. Ten flocks, bacteriologically negative for ***S.* Enteritidis** were found positive by ELISA. ***S.* Enteritidis** was isolated from three of these flocks by repeated and extensive bacteriological examination for verification. Verification was not possible in the fourth ELISA positive flock. ***S.* Enteritidis** infections were likely in three other flocks because of the farm histories. On the basis of the results of this study it was decided to use this ELISA, , as screening technique in the Dutch ***S.* Enteritidis** programme instead of bacteriological examination of faecal samples. The ELISA is regarded as a flock test; an extensive, confirmatory bacteriological investigation for ***S.* Enteritidis** was carried out in ELISA positive flocks to decide whether the flocks are truly infected.

Thorns *et al*. (1996) proved that the SEF14 fimbrial antigen SEF14-DAS ELISA was successfully discriminated between chickens experimentally infected with ***S.* Enteritidis** and those infected with *S.* Panama or ***S.* Typhimurium**, although the SEF14 responses in adult birds infected with ***S.* Enteritidis** were detectable but low. In contrast, ELISAs used to detect antibodies to lipopolysaccharide (LPS) and flagella were unable to discriminate

between the infected groups of chicks and adult birds infected with different ***Salmonella*** serotypes.

Tan *et al.* (1997) evaluated a competitive (c-ELISA) for detection of ***Salmonella*** spp. in chicken organs and feces. The c-ELISA used a monoclonal antibody (MAb), specific for a genus-specific epitope of the outer core oligosaccharide of ***Salmonella*e**. ***Salmonella*** lipopolysaccharide (LPS) in samples competed with ***Salmonella*** LPS coated on microtitre plates, for binding to the MAb. Competition reduced binding of the MAb to the LPS on the plate and of the secondary antibody to the MAb hence reducing the chromogenic signal. Stable coating and minimal false positive were achieved by conjugating LPS to poly-L-lysine. The c-ELISA was compared with motility enrichment culture using modified semisolid Rappaport Vassiliadis (MSRV) medium, which detected less than 10^2 CFU/g, and did not allow migration of non-***Salmonella*** species. The c-ELISA detected 10^6 CFU of enriched culture or 10^2-10^3 CFU of ***Salmonella***/g of faeces. Its limit of detection was thus higher than that of MSRV culture and it had a sensitivity of 92.9% and a specificity of 96.7%.

Rajashekara *et al.* (1998) evaluated rSEF14 fimbrial antigen of ***S.* Enteritidis** for specific detection of ***S.* Enteritidis**-infected birds in latex agglutination test and ELISA. rSEF14 antigen was highly specific in identifying birds infected with ***S.* Enteritidis**. The sera from birds infected with closely related serogroup-D ***Salmonella*** and other avian pathogens did not react with rSEF14 antigen. The rSEF14 antigen identified antibodies in serum of 88% of birds during the first 2 weeks of infection, and 100% of the birds subsequently. The ***S.* Enteritidis**-specific antibodies were detected in egg yolk as early as 6 days post-infection in rSEF14- ELISA. The results suggest that rSEF14-based assays could be used as screening tests for detection of ***S.* Enteritidis** antibodies and would overcome the cross reactions observed with existing serological tests.

Solano *et al. (*2000) the results showed that the indirect ELISA, based on lipopolysaccharide (LPS), O-polysaccharide (PS) or membrane sediment (SD) antigens, enable the identification of a greater number of infected birds and discriminate field antibody responses from vaccinal ones better than the commercial IDEXX test. The indirect ELISA that used a O-polysaccharide rich fraction (PS) proved to be the most specific and sensitive test, suggesting that this indirect ELISA could be used to confirm IDEXX results, especially when the differentiation between vaccinated and infected poultry was required.

Yamane *et al.* (2000) established ELISA for field monitoring/profiling purposes for ***S.* Enteritidis** infection of poultry flocks. Serotyping rabbit sera, commercially obtained, specific for ***Salmonella*** identification sera to O2, O4, O7, O8, S. Vi, S. Hm, and O9, showed negative ELISA (E)-values (< 0.2) on ELISA, except the O9 identification serum (E-value > 0.5). Similar negative E-value results were obtained for antisera to ***Echerichia* coli**. Field serum samples originating from ***S.* Enteritidis**-isolated flocks yielded similar positive ratios on both ELISAs including the present coated deflagellated ***S.* Enteritidis** antigen and a commercially obtained flagellated SE antigen and that of rapid plate aggregation with a pullorum antigen (PD-RPA). About 100 days after the first monitoring, no ***S.* Enteritidis** isolation in the same flock was observed resulting in a carrier state of ***S.* Enteritidis** infection. Although both the monitoring results with commercially obtained ELISA and PD-RPA showed lower positive or negative ratios, the present ELISA showed a higher positive ratio than that of the first monitoring. The present ELISA was suggested to be a suitable method to do accurate profiling on the carrier state of infection.

Seo *et al.* (2002b) reported that chickens infected with ***S.* Enteritidis** showed strong secretory immunoglobulin response against flagella in both bile and crop. The optical density values of ELISA tests in positive bile and crop

were 1.17 and 0.38, respectively, and were significantly different from those of negative samples. Those results indicate that the crop may function as another site for mucosal immunity, and the ***S.* Enteritidis** flagella-based ELISA of crop samples could provide a useful screening test of ***S.* Enteritidis** exposure in chickens.

Goodridge *et al.* (2003) developed a rapid (8 h) most probable number (MPN)- ELISA for the detection and enumeration of ***S.* Typhimurium** in wastewater. The specific objectives were to (i) characterize poly- and monoclonal ***S.* Typhimurium**-specific antibodies in order to select the most specific and sensitive antibody for ***S.* Typhimurium** detection, and (ii) validate the MPN assay through a correlation between the 8 h MPN-ELISA and the traditional 48 h ***S.* Typhimurium** MPN method in poultry scald water. Poultry scald water samples were spiked with 10 and 50 CFU/ml of ***S.* Typhimurium**. The traditional MPN method used a 48 h enrichment period followed by an analysis, while the MPN-ELISA used a 5 h enrichment period followed by a 3 h ELISA analysis. No differences ($P < 0.05$) were found between the traditional MPN and the MPN-ELISA, indicating the promise of the MPN-ELISA for the rapid detection and enumeration of ***S.* Typhimurium** within an 8 h shift. This abbreviated assay will permit increased product sampling and more rapid movement of food between production and processing, resulting in reduced spoilage and quality losses.

Hong *et al*. (2003) recorded that ***Salmonella*** *invA* genes were used to design probes in PCR ELISA, as an alternative to conventional bacteriological methodology, for the rapid detection of ***S.* Enteritidis** from poultry samples. ELISA increased the sensitivity of the conventional PCR method by 100- to 1,000-fold.

Luciana *et al*. (2004) showed that both electrochemical ELISA and PCR detected ***Salmonella*** in meat products contaminated with a low number of the microorganisms (1 to 10 ***Salmonellae***/25 g) after only 5h of incubation of preenrichment broth and they were just as effective as the standard culture methods.

Jouy *et al*. (2005) compared the bacteriological examination with a serological method ELISA. This ELISA was first evaluated by the use of artificially infected hens. During these experimental infection studies, several groups of hens were inoculated with ***S.* Enteritidis**, ***S.* Typhimurium**, different vaccines and different ***Salmonella*** serovars to calculate the experimental parameters of our ELISA. Then, in a field study, 43 flocks were followed monthly using two bacteriological samples (environmental swab and pool of faeces) and 20 serological samples (sera or yolks). Twenty-seven flocks without ***S.* Enteritidis** or ***S.* Typhimurium** gave a negative serological response throughout their surveillance. Among the 10 various serovars different from ***S.* Enteritidis** and ***S.* Typhimurium** isolated in this study, ***S.* Heidelberg**, ***S.* Agona** and **S. Hadar** gave seropositive results in seven flocks. Consequently, this ELISA was not specific of ***S.* Enteritidis** and ***S.* Typhimurium** as it detected serovars sharing or not common antigens with ***S.* Enteritidis** and ***S.* Typhimurium**. Seropositive results were also obtained each month for two flocks where no ***Salmonella*** could be isolated. Finally, in seven flocks, it was to be found infected with ***S.* Enteritidis** or ***S.* Typhimurium**, the positive ELISA results appeared later than the bacteriological detection. Therefore, for the detection of chicken flocks recently infected with ***S.* Enteritidis** or ***S.* Typhimurium**, bacteriological examination currently used in France seemed to be more appropriate than this ELISA.

Haider *et al*. (2007) used rapid serum plate agglutination test and ELISA were carried out for the detection of antibody response against ***S.* Gallinarum**

vaccinated and non-vaccinated birds. Positive results of the rapid plate agglutination and the ELISA were 81% and 77% in laboratory trial, and 76.4% and 73.8% in field trial, respectively.

Potkonjak *et al*. (2007) conducted the serology control in several broiler breeder and commercial layer flocks, using ELISA test. The goal was to determine ***Salmonella*** status in selected poultry flocks and to emphasise its potential to serve as a source of infection for poultry and poultry products. In spite of the vaccination, breeder flocks were negative 4 weeks after the first vaccination, while during the production there was a strong immunological response in all tested farms. Layer flocks tested at the end of the rearing period showed low level of antibody titre that is considered negative. On contrary, during the production layer flocks tested positive. The results showed that it would be very helpful to perform serology testing especially at the end of the rear and after the peak of production in breeder and layer flocks. Nevertheless applying serology monitoring it was possible to estimate the seriousness of the problem and ELISA test should be taken into consideration in the future. Bacteriology means of ***Salmonella*** detection is still the golden standard because of the antibiotic resistance evaluation and because of the possibilities to characterize strains at the molecular level. All three aspects of ***Salmonella*** control are now days unavoidable and have to be considered as a comprehensive and useful tools to detect and recognize contaminants in poultry industry.

Kumar *et al*. (2008) development of monoclonal antibody based sandwich ELISA for rapid detection of ***Salmonella enterica*** serovar **Typhi** (***S.* Typhi**) from food and water samples and optimization of enrichment procedures for use with the developed sELISA to increase the detection sensitivity of the assay. The sELISA had the detection limit of 10^4-10^5 CFU of **S. Typhi**. Of the various broths used with sELISA, BPW was found to yield maximum ELISA values. Enrichment-ELISA, when tested in artificially inoculated food samples,

generally, could detect 10^2 **CFU *S.* Typhi** CFU /ml within 10 h from various food rinses (meat, vegetable) and milk samples. After overnight enrichment in BPW, as less as 2 bacteria per 10 ml of milk, meat rinse, and chicken rinse could be detected. Only one of the field samples (water) gave false positive result by Enrichment-ELISA. In comparison to culture, the Enrichment-ELISA is a rapid, sensitive, and specific method for detection of ***S.* Typhi** from food or water samples. This method may be used as rapid screening procedure for environmental monitoring during outbreak situation.

Pakpinyo *et al*. (2008) used ELISA for evaluated efficacy of ***S.* Typhimurium** live vaccine and ***S.* Enteritidis** inactivated vaccine against *S.* Enteritidis in layer. The chickens were into 4 groups, 30 birds in each Group as follows: Group 1: ***S.* Typhimurium** 2 doses at 1 and 14 days old and ***S.* Enteritidis** 1 dose at 12 weeks old and challenge with ***S.* Enteritidis** at 16 weeks of age. Group 2: Non vaccination but challenge with ***S.* Enteritidis** at 16 weeks old. Group 3: ***S.* Typhimurium** 2 doses at 1 and 14 days old and ***S.* Enteritidis** 1 dose at 12 weeks old and non challenge. Group 4: Non vaccination and non challenge. At 1 day old, 2, 6 and 12 weeks old, birds were negative reactors of ELISA determination and free of ***Salmonella*** spp. At 16 weeks old, the vaccinated birds were found the positive reactors of ELISA and free of ***Salmonella*** spp., whereas 3 out of 26 birds of non-vaccinated birds (group 4) showed positive reactors of ELISA determination, but ***Salmonella*** spp. was not isolated from cloacal samples. At 17 weeks old (1 week post challenge) and 21 weeks old (5 weeks post challenge), almost birds of groups 1 – 4 were found the positive reactors of ELISA. Surprisingly, at 17 and 21 weeks old of group 4 were found the positive ELISA reactors for 1 – 2 birds, without ***Salmonella*** spp. reisolation. These were possible due to the high sensitivity of the ***S.* Enteritidis** / ***S.* Typhimurium** ELISA test kits, which can detect ***S.* Enteritidis**, ***S.* Typhimurium** and other invasive group B and D ***Salmonella*** spp.

Betancor *et al*. (2010) conducted a nationwide survey over 2 years that included the analysis of sera from 5,751 birds and 12,400 eggs. Serological evidence of infection with ***Salmonella*** group O:9 was found in 24.4% of the birds. All positive sera were retested with a flagellum-based ELISA, and based on these results, Surprisingly, the majority of the isolates were not ***S.* Enteritidis**.