# *التعرف على ميكروب السالمونيلا انتريتيديس فى بيئة الدجاج*

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# DETECTION OF SALMONELLA ENTERITIDIS IN CHICKEN ENVIRONMENT

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Dedicated to

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# LIST OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES



# LIST OF PHOTO

# 1. INTRODUCTION

Avian Salmonellosis is one of the economic problems concern to all phases of poultry industry from production to marketing **(Stinert et al., 1990 and Nagaraja et al., 1991)**.

Among the factors which interfere with the success in poultry production are diseases, one of the widely distributed is that group caused by *Salmonella* species which are responsible for major economic losses due to drop off performance parameter such as egg production, fertility and hatchability and increase early chick mortality **(Abd-El-Latif.,1995)**

Generally, the rate of growth in poultry farms is affected by genetic structure, nutritional states and environmental condition including air, drinking water, feed and litter.So good productivity of poultry require proper management, improving feeding system as well as efficient control of disease **(Rehab El-Zarka., 2003).**

The main sources of *Salmonella* contamination in poultry farm are feed, carrier birds, hatchery, litter and surrounding environment **(Kosugi et al., 1985)**

Salmonellosis in chickens can be classified into three diseases: pullorum disease caused by *Salmonella Pullorum,* fowl typhoid caused by *S. Gallinarum* and paratyphoid infections due to a diverse group of serovars related to foodborne illness in humans. *Salmonella Typhimurium* and more recently *Salmonella Enteritidis* have been the serovars most frequently isolated from cases of human food poisoning in which chicken products have been implicated. **(Oliveira et al., 2002)**.

Detection of *S. Enteritidis* in the environment of laying flocks is an indication that contaminated eggs might be produced **(Garber et al., 2003).**

*Salmonella Enteritidis* continues to be an internationally important human pathogen that causes Salmonellosis (food borne zoonotic diseases which may lead to food poisoning) outbreaks in countries in both northern and southern hemispheres **(Humphrey et al., 1996, and Jorgensen et al., 2002).**

Traditional detection methods for *salmonella* which based on culture using selective media and characterization of suspicious colonies by biochemical and serological tests are generally time consuming **.(Wallace et al.,1999).**

Bacteriological examination may yield false negative results when Salmonella Enteritidis over grown by other *Salmonella* serotypes (**Van Zijderveld et al., 1992).** Also false negative results could be obtained by bacteriological examination, when the initial number of Salmonella is low in the sample **(Fricker, 1987).**

Therefore, a rapid and sensitive primary screening methods with a proper sampling plane is required to detect *Salmonella* in the flock or in the food industry using immunological and genetic methods .Among these ,Polymerase chain reaction (PCR) which has been applied to various types of samples **(Stone et al.,1994; Bennett et al., 1998; Abouzeed et al .,2000; Whyte et al .,2002).**

As the PCR technique proved to be very sensitive, very specific and relatively rapid test as it takes less than 24hrs to obtain the result compared to culture methods which takes four days to obtain a negative result and up to seven days to get confirmed positive result **(Andrews et al., 1995).**

Another advantage that PCR is not dependent on utilization of a substrate or the expression of antigens, there by circumventing the phenotypic variations in biochemical patterns and lack of detectable antigens **( Hoorfar et al., 1999).**

**The aim of the study**

The present work was designed for :

1- Isolation and identification of *S. Enteritidis* from environment of chicken houses including litter, drinking water, feed,cloacal swabs and air of chicken houses.

2- Confirmative diagnosis with PCR technique as rapid and accurate method for detection of *S. Enteritidis*.

3- Testing of PCR sensitivity for detection of *S. Enteritidis* directly from litter samples.

# 2. REVIEW OF LITERATURE

## 1- History

*Salmonella* was discovered since more than 100 years ago by Dr. Daniel Salmon. It is related to family enterobacteriacae due to its normal inhabitant in intestinal tract of worm and cold blooded animals, man, birds and widely distributed through the environment as water, feces, soil, plant.

Since the 1940s, there has been a rapid increase in the isolation of the non-host-specific *Salmonella* serovars from humans and animals and these serovars continued to cause a significant disease losses in young poultry
**(Calnek, 1997** and **Poppe, 1999)**.

Since the mid-to-late 1980s, the incidence of Salmonellosis has been reported by public health authorities throughout the world. Despite intensive eradication efforts, this pathogen persists as an important problem for poultry and also threatens the public health **(Wierup et al. 1988; Hogue et al.1997; and Davison et al., 2003)**. Because *S. Enteritidis* is the serotype most frequently isolated from eggs, contaminated eggs are recognized as the most probable sources of transmission of *S. Enteritidis* to humans **( van de Giessen et al. 1992, and Henzler et al. 1994).**

## 2- Classification

Kauffman-white classification of the genus Salmonella is subdivided into more than two thousands serotypes (2300 serotypes) containing different combination of antigens . The identification of these serotypes depends on detection of the O (somatic) and H (flagellar) antigens by means of agglutination tests with specific antisera **(Collee et al 1989)**.

From 2300 serotypes of Salmonellae known at present only 10 to 15 are of epidemic importance, in the first place *S.Enteritidis* and *S.Typhimurium* **(Perales and Audicana, 1988).**

More recent classification for the genus *Salmonella* has been divided into 2 species which namely *S. enterica* and *S. bongori*

The species *S.enterica* is divided into 6 subspecies as follow (Salmonella subspecies) **Subspecies 1: Enterica ; Subspecies ll : Salamae ; Subspecies lll a :Arizona ; Subspecies lllb :Diarizonae ; Subspecies 1V :Houtenae and Subspecies V1 :Indica .**

Salmonella subsp enterica 1 contains most of the Salmonella that are significant animal pathogens ex. S. enterica serovar Typhi, S. enterica serovar Typhimurium, S. enterica serovar Enteritidis. Which called *S. Typhi*, *S. Typhimurium* and *S. Enteritidis* respectively. So *S. Enteritidis* (SE) is a serovar in the subspecies S.enterica 1.

## 3- Incidence of *Salmonella Enteritidis* in poultry farm:

**WHO (1989)** reported that *S. Enteritidis* and *S. Typhimurium* were more invasive than other serotypes of *Salmonella* found in eggs before the egg shell was formed .This might occur due to either ovarian infection or ascending oviduct contamination . Also, birds may become contaminated with *S. Enteritidis* via other routes of infection such as air, food, water vermin, wild birds, insects and the environment.

**Ashton (1990)** stated that *S.Enterica* was a facultative intracellular pathogen that capable of causing disease in a wide range of host's. Concerning poultry, it caused a severe systemic disease responsible for heavy economic losses to the commercial poultry industry through mortality and reduced egg production.

**Giessen et al. (1991)** recorded that the incidence of *S. Enteritidis* was 15% out of the screened poultry flocks with higher incidence in broiler flocks (94%) than in the layer flocks (47%) and concluded that examination of fecal samples yielded the best results in screening of

poultry flocks .

In Denmark, **Brown et al., (1994)** found that *S. Enteritidis PT1* was the most common type among isolates of poultry origin (57.6%) followed by *S. Enteritidis* PT4 (28.8) .

**Poppe (1994)** isolated *S. Enteritidis* from 2.7% of environmental samples of layer flocks and of 3% of broiler flocks.

**Producers (1995)** mentioned that the production of *S. Enteritidis* contaminated eggs depending on the environmental condition of the house.

**Sasipreeyajan et al. (1996)** reported that *Salmonella* could be isolated from samples of feed, drinking water, faeces and litter from all broiler and breeder flocks and 87% of the layer flocks. Also they found that out of 1488 samples examined from all flocks , *Salmonella* was recovered from samples of litter (42%), water in drinking troughs (36%), feed left over in the feed trays (28%),water in main tanks (17%) and stock feed (8%).

**Gast et al. (1998)** stated that direct contact with infected birds and indirect contact with contaminated environmental surfaces were known to be important factors in the dissemination of *S. Enteritidis* in poultry flocks.

**Chambers et al . (1998)** examined 635 broiler chickens to determine the total prevalence of *Salmonella* in broiler farms in Canada .They found that over all prevalence of contamination was low (4.3%). Prevalence was higher in broiler sampled in Quebec (5.8%) than in those sampled in Ontario (2.2%). Also they could isolate *S. hadar* and *S. heidelberg*; while no *S. Enteritidis* was isolated.

**Al-Nakhli et al. (1999)** described the sources and prevalence of pathogenic *Salmonella* serovars among poultry farms in Saudi Arabia between 1988 and 1997. They recovered a total of 1052 (4%) *Salmonella* isolates out of 25759 samples of poultry (broiler, layer,

broiler breeder and layer breeders) and poultry environments (box liner, litter, drags swab, dropping, mice and feed).

**Carli et al. (2001)** investigated the presence of *Salmonella* in 28 broiler and 5 layer flocks in turkey. They isolated *Salmonella* from 11 out of 28 (39.3%) of the broiler flocks and From 3 out of 5 (60%) of the layers. *S. Enteritidis* was the Only serotype isolated from layers where as it was isolated from broiler birds at (81.5%) with other serotypes .

**Knape et al. (2002)** isolated *Salmonella spp*. from 72% of the environmental samples collected from hen houses.

**Kinde et al. (2004)** examined a total 133 commercial egg laying farms in California, and found that the overall *S. Enteritidis* prevalence was 10.5%.

**Gast et al. (2004)** said that *S. Enteritidis* in the environment of commercial laying hens was critical for reducing the production of contaminated eggs by infected flocks.

**Liljebjelke et al. (2005)** studied the ecology of *Salmonella* within an integrated commercial broiler production system and found that 15 serotypes were identified and found that the most common serotypes isolated are *S.Typhimurium* (55%) and *S.Enteritidis*(9.7%). They suggested that vertical transmission of these serotypes occurred in this poultry production system.

**Moore et al. (2006)** stated that heat stress and short-term withdrawal of feed and water have been associated with an increase in *Salmonella* isolation from flocks.

**Al-Zenky et al. (2007)** examined 2882 environmental samples (litter, feed, water and air) to determine the prevalence of *Salmonella* spp. in poultry farms. They found that the overall prevalence of *Salmonella* spp. was 5.4% and *S.Enteritidis* was the most prevalent serotype.

**Lee et al. (2007)** investigated an integrated broiler chicken operation

(hatchery, broiler farm and chicken slaughter house) for presence of Salmonella and found that the recovered Salmonella serotypes from the broiler farm were *S. Enteritidis*, *S. senftenberg*, *S. gallinarum* and *S. blockly*.

**Snow et al. (2007)** monitored *Salmonella* infection in 454 commercial layer flock holdings in England. They found that the most common serovar identified was *S. Enteritidis* at a prevalence of 5.8%.

**Esteban et al. (2008)** examined 60 flocks of free-range chicken for presence of *Salmonella*. They found that 2.9% of the flocks were positive for *S. Enteritidis*. They concluded that the free-range rearing might have an advantageous effect on diminishing *Salmonella.*

## 3-1- Incidence of Salmonella Enteritidis in litter:

**Hacking et al . (1978)** isolated *Salmonellae* from 6 out of 35 new wood shaving samples and 44 out of 267 used litter samples. These results indicated that broiler chicken flocks were infected with diverse Salmonellae introduced in wood shaving and residual contamination from the preceding flock.

**Morgan and Susan (1980)** studied the occurrence of *Salmonella* during rearing of broilers and found that no *Salmonella* species were isolated from environmental samples (water trough, litter and feed) before occupation ,once the house occupied, *Salmonellae* were recovered from litter within 24 hours after chick has been placed.

**Baker et al. (1980)** stated that once cecal tonsil colonization of *Salmonella* was established, the bacterium was consistently shed in the feces.

**Bhatia and McNabb (1980**) examined bacteriologically the litter of 15 different poultry farm flocks and found that fresh straw litter was contaminated with *Salmonella* and might be a source of flock infection. They concluded that the litter at 3 and 6 weeks could be used as an indicator of flock infection.