Molecular Studies on Salmonella Isolated From Chicks and Human

A Thesis Presented by

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To

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TO MY FATHER AND MY LOVING MOTHER: *WHO OVER THEIR LIFE PRAYING, SUPPORT AND LOVE WHICH IS MUCH GREATER THAN I CAN ACKNOWLEDGE.*

TO MY BROTHERS: *WHO ENCOURAGE ME IN MY LIFE.*

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LIST OF ABBREVIATIONS

CDC	**Centers for Disease Control and Prevention**
DIASALM	**Diagnostic Semisolid Salmonella Medium**
E.U.	**European Union**
EFSA	**European Food Safety Authority**
FSIS	**Food Safety Inspection Service**
MIPA	**Magnetic Immuno PCR Assay**
MR	**Methyle red**
MSRV	**Modifies Semisolid RV**
MSs	**Monash Science Society.**
PCR	**Polymerase chain reaction**
RV	**Rappaport-Vassiliadis**
U.S.	**United states**
U.S.D.A	**United States Department of Agriculture**
UV	**ultraviolet**
VP	**Voges-Proskaur**

INTRODUCTION

Food-borne pathogens continue to cause major public health problems worldwide. Among all the food-borne pathogens, Salmonellae is the most common cause of food-borne infectious diseases in the world **(Plym and Wierup, ٢٠٠٦).**

Salmonellosis is one of the leading causes of foodborne bacterial enteritis in many countries **(Centers for Disease Control and Prevention). (CDC, ٢٠٠٤).**

Salmonellae are some of the major causes of food poisoning in the developed world. Food products like meat, eggs and poultry are common sources of Salmonellosis. (**Gill *et al.* ١٩٨٣; Bullock *et al.* ١٩٨٩ and Nicholas *et al.* ١٩٩١).**

Poultry products are known to be significant reservoir for and the most important source of *S. Enteritidis* infection in humans. More over, with great expansion of the poultry industry, the wide spread occurrence of avian Salmonellosis has ranked it as one of the most important egg-borne bacterial diseases of poultry **(Hayes *et al.* ١٩٩٩; Davis and Berslin, ٢٠٠١; Molbak and Neimann, ٢٠٠٢).**

The most common contaminated foods associated with human Salmonellosis include poultry, beef, pork, eggs, milk, seafood and fresh products such as sprouts **(Al-Sheddy *et al.* ١٩٩٥; Sivapalasingam *et al*. ٢٠٠٤ and Bohaychuk *et al*. ٢٠٠٦).**

From ٢٥٠٠ serovars known at present, only ١٠ to ١٥ serovars are of epidemic importance. In the first place *S. Typhimurium* and *S. Enteritidis* **(Meyer, ١٩٩٩),** and *S. Enteritidis* phage type ٤ in poultry **(Barrow *et al*. ٢٠٠٣),** both serotypes **(Foley *et al.* ٢٠٠٦),** *S. Enteritidis* **(Yukiko, T. *et al.* ٢٠٠٩).**

Transmission of Salmonellosis is of particular concern because of the worldwide distribution of this bacterium and the large variety of different host species, including insects, fish, amphibians, reptiles, birds, and mammals that can harbor this organism, often without clinical signs of illness.

Salmonella infection per year in the U.S is ١,٤ million cases **(Voetsch *et al*. ٢٠٠٤ and CDC, ٢٠٠٥).**

Perhaps the most important advantage of PCR technology is its potential to cultural enrichment with specific nucleic acid sequence enrichment, there by decreasing detection time **(Wilson, ١٩٩٧).**

The aim of the present study was to:

Throw light on the most common Salmonella species circulating in human and chicks in that time and what is new in their sensitivity to antibiotics. To achieve this object, we carried the following points:

١- Isolation of Salmonella species from human and poultry.

٢- Identification of the suspected recovered isolates with the traditional methods (morphological, culture characteristics, biochemical characteristics and serological).

٣- Diagnostic Molecular confirmation of the isolates.

٤- Examination of their sensitivity to antibiotics.

REVIEW OF LITERATURE

١. Salmonellae in Human:

Steffen (١٩٨٤) said that *Salmonella Enterica* has been identified as a source of human infection, possibly related to ingestion of eggs or other poultry-related products.

Mashhoor *et al.* (١٩٨٧) examined rectal swabs from ٧ farms workers who suffered from gastrointestinal disturbances and revealed that only one of them (١٢ years old boy) had *S. Gallinarum-Pullorum.*

Davis and Wray (١٩٩٦) reported that the production of contaminated eggs by infected laying hens has been frequently identified as the principal source of human *S. Enteritidis* infection.

Craven and Williams (١٩٩٧) found that the poultry products are the major source of infection with Salmonella in many developed countries from poultry source. Most contamination result from the release and spread of the intestinal contents of healthy chickens that are colonized with Salmonellae.

Meyer (١٩٩٩) concluded that foodborne infection of intoxications of men including Salmonellosis increased as a whole in the last years. From ٢٥٠٠ serovars known at present, only ١٠ to ١٥ are of epidemic importance. In the first place *S. Typhimurium* and *S. Enteritidis*. Mainly Salmonella-contaminated foodstuffs from animal are source of infection in men. In this connection raw eggs and raw egg-containing food, as well as raw meat and meat products are of prime importance.

Recomm (٢٠٠١) concluded that an infectious dose of Salmonella is small, probably from ١٥ to ٢٠ cells. Typically, non-typhoidal produces a self-limiting febrile gastrointestinal illness that is indistinguishable from that caused by other bacterial enteric pathogens. Dehydration is the principal clinical concern. The incubation period (the time between ingestion of bacteria and the onset of illness) varies from ٦ to ٧٢ hours.

Bouvet *et al.* (٢٠٠٢) reported that is Salmonella is one of the major sources of infection in humans in France**.**

Barrow *et al.* (٢٠٠٣) found that the incidence of human Salmonellosis has increased greatly over the past ٢٠ years and this can mostly be attributed to epidemics of *S. Enteritidis* phage type ٤ in poultry in numerous countries**.**

Berghold and Kornschober (٢٠٠٤) said that the incidence of Salmonellosis in Austria was ١٠٣ cases per ١٠٠,٠٠٠ citizens, followed by Campylobacteriosis with ٤٦,٥ cases per ١٠٠,٠٠٠ citizens.

Crump *et al.* (٢٠٠٤) said that typhoid fever caused by *S. Typhi*, remains a significant public health problem in underdeveloped and developing countries. The incidence of typhoid fever is ٢١ million cases worldwide with more than ٢٠٠,٠٠٠ deaths annually mainly among children.

Guerin *et al.* (٢٠٠٤) cited that in Sweden usually one to four sporadic Salmonellosis cases are reported annually.

Matsui *et al.* (٢٠٠٤) concluded that outbreaks are reported frequently, implicating different kinds of food contaminated with Salmonella**.**

Rasschaert *et al.* (٢٠٠٥) said that Salmonella species is an important food-borne pathogen responsible for disease in animals and humans.

Voetsch *et al.* (٢٠٠٤) estimated Salmonella infections per year in the United States (U.S.) to be ١,٤ million cases.

CDC (٢٠٠٥) estimated that ١,٤ million cases occur annually. Approximately ٦٠٠ deaths are caused by infections in the U.S. every year, accounting for ٣١٪ of all food-related deaths.

Foley *et al.* (٢٠٠٦) cited that the annual cost of medical treatment for Salmonellosis, in addition to loss of productivity, imposes a significant financial burden on many countries. At present more than ٢,٥٠٠ serotypes of Salmonella are known. Serotypes *S. Enteritidis* and *S. Typhimurium* accounted for the majority of cases of human Salmonellosis in Ireland in ٢٠٠٦.

Dominguez *et al.* (٢٠٠٧) found that Salmonella causes two types of diseases in humans: enteric fever (typhoid), resulting from bacterial invasion of the blood stream and Acute gastroenteritis, which is restricted to intestinal tract. Typhoid fever is endemic in many developing nations and non-typhoidal Salmonellosis is a major food-borne disease worldwide.

Yukiko Toyota-Hanatani *et al.* (٢٠٠٩) said that *S. Enteritidis* is the most serious human health risk factor associated with chicken eggs.

The European Food Safety Authority (EFSA) Journal (٢٠١٠) reported that in ٢٠٠٨, a total of ١٣١,٤٦٨ confirmed cases of human Salmonellosis (notification rate ٢٦,٤ per ١٠٠,٠٠٠ populations) were reported from ٢٧ countries. The total number of reported human Salmonellosis cases in the E.U. (European Union) has decreased steadily by several thousand cases annually since ٢٠٠٤, from ١٩٥,٩٤٧ cases to ١٣٣,٢٥٨ cases in ٢٠٠٨. The reporting of confirmed human Salmonellosis cases in ٢٠٠٨ represents a ١٣,٥٪ decrease from ٢٠٠٧ in MSs (Monash Science Society).

٢. Salmonellae in Poultry:

Seuna and Nurmi (١٩٧٩) found that *S. Enteritidis* infects the ovaries, and the organism can be isolated from eggs, so the eggs have long been recognized as potential source of infection in humans.

Lister (١٩٨٨) reported that the presence of *S. Enteritidis* infection in laying birds does not appear to have a significant adverse effect on fertility, hatchability or egg production but the initial infection of ovarian tissue with *S. Enteritidis* could lead to contamination of eggs.

Gast and Beard (١٩٨٩) concluded that horizontal dissemination of *S. Enteritidis* in poultry flocks can occur by diverse route. Newly hatched chicks are highly susceptible to infection by Salmonella, so horizontal transmission is especially likely to occur shortly after hatching.

Baskerville (١٩٩٢) said that the oral route of infection may be important for spreading for *S. Enteritidi*s. In poultry house infection can be spread by airborne droplets or dust particles carrying *S. Enteritidis*. Moreover, there was a higher rate of infection in the ovary and oviduct in laying hens through airborne infection.

Nakamura (١٩٩٤) found that *S. Enteritidis* infection in chickens is a worldwide problem. *S. Enteritidis* spread by horizontal transmission in poultry houses, where drinking water contaminated with *S. Enteritidis* might play an important role.

Davis and Wray (١٩٩٦) said that the production of contaminated eggs by infected lying is the principal source of human *S. Enteritidis* infection.

Telo *et al.* (١٩٩٩) detected *S. Enteritidis* in ١ out of ٧٩ egg pooled samples. The strain was isolated only from the egg-shell, but not from the liquid part. The isolated strain was serotyped as group C.

Chang (٢٠٠٠) tested ١٣٥ dozen shell eggs and ٢٧ raw broilers. Non of the egg yolk was found to contain organism. spp were detected in ٢٥,٩ % of raw broilers and serotypes isolated from raw broilers were *S. Enteritidis, S. Virchow* and *S. Virginia*.

Kusunoki *et al.* (٢٠٠٠) found that *S. Enterica*, a major cause of foodborne infection, is prevalent in many types of food. In Japan, poultry meat is associated with a number of food poisoning and is considered the most important target for isolation**.**

Hegazy (٢٠٠٢) examined ١٣٨ broiler flocks and isolated ٣٧ isolates. Salmonellas were serotyped as follow: ٢٣ isolates were *S. Enteritidis* (٦٥,١٦ %), while *S. Typhimurium*, *S. Kentucky* and Salmonella group E had ٢ isolates for each (٥,٤١ %) and ٨ isolates untyped (٢١,٦١ %).

Abd EL-Hamid *et al.* (٢٠٠٤) and Murugkar *et al.* (٢٠٠٥) said that among all animal species, Salmonella were most frequently reported from poultry and poultry products**.**

Bandekar *et al.* (٢٠٠٤) and Saroj *et al.* (٢٠٠٦) cited that Salmonella spp, have been isolated from minimally processed foods, fish and poultry meat from India**.**

European Food Safety Authority (٢٠٠٧) reported that a recent study on the prevalence of Salmonellosis in broiler flocks in Europe revealed that positive flocks ranged from ٢٠ -٧٠٪ in different countries.

Stephan Huehn *et al.* (٢٠٠٩) cited that Salmonella spp, are major zoonotic food-borne pathogens which cause outbreaks and sporadic cases of gastroenteritis in humans worldwide.

٣. Zoonotic importance of Salmonellae:

Cherry *et al.* (١٩٧٢) concluded that Salmonella finds its way into the river water, coastal and estuarine sediments through fecal contamination. Aquatic environments are the major reservoirs of Salmonella and aid its transmission between the hosts.