PATHOGENESIS OF SALMONELLOSIS WITH RESPECT TO CARRIER STATES IN POULTRY AND ITS PUBLIC HEALTH IMPACT

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87-ag-645

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN PATHOLOGY

UNIVERSITY OF VETERINARY AND ANIMAL SCIENCES, LAHORE-PAKISTAN
2006
To

The Controller of Examinations,
University of Veterinary and Animal Sciences,
Lahore.

We, the Supervisory Committee, certify that the contents and form of
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IN THE NAME OF ALLAH,
THE MOST BENEFICIENT
AND MERCIFUL
ALLAH AND HIS ANGELS SEND BLESSINGS ON THE HOLY PROPHET (P.B.U.H.)
O YE THAT BELIEVE!
SEND YE BLESSINGS ON HIM
AND SALUTE HIM WITH
ALL RESPECT (Al-Quran)
DEDICATION

I dedicate this humble effort and study to

my beloved parents

who always prayed for my brilliant career
ACKNOWLEDGEMENTS

The author feels actuated from within to offer his humblest and sincerest thanks to Almighty ALLAH! The most Beneficent and Merciful, who bestowed him the ability to perceive and pursue higher ideals of life.

Heart is warm with love and thoughts have turned to Holy Prophet Muhammad (PBUH), the very special entity God has brought into our lives, whose saying learnt from cradle to grave awakened the strong desire in myself to undertake this course of studies.

Special and personal thanks and appreciation to Prof. Dr. Atta-ur-Rehman, as a Chairman, HEC and also to the Higher Education Commissioner of Pakistan Islamabad for sponsoring and funding my Ph.D. programme.

Special thanks to Prof. Dr. Mauzoor Ahmad, Vice-Chancellor, University of Veterinary and Animal Sciences, Lahore for his moral support, encouragement and ever helping behaviour and valuable guidance.

Special thanks and appreciations for Prof. Dr. Zafar Iqbal Chaudhry Chairman, Department of Pathology for his remarkable supervision and guidance during the Ph.D. programme.

I feel immense pleasure in recording my heartfelt and sincerest thanks to Prof. Abdul Rafe Shukoori, Director, School Biological Sciences, University of the Punjab, Lahore for his Co-Supervision, guidance, encouragement and ever helping behaviour.

I take pride in expressing my deep sense of obligation to Dr. Muhammad Akram Muneer, Professor of Microbiology and Dean, Faculty of Veterinary Science, University of Veterinary and Animal Sciences, Lahore for his supervision, guidance and ever encouraging attitude.

I have the honour to express my deep sense of gratitude and indebtedness to Prof. Dr. Muhammad Ather Khan, Chairman, Department of Preventive Medicine and Public Health, University of Veterinary and Animal Sciences, Lahore for his skillful guidance, learned patronage and inspiring attitude during the Ph.D. programme at U.V.A.S., Lahore.

I will be failing in my duties if I do not extend my special thanks to Prof. Dr. Haji Ahmad Hashmi, Director, Advanced Studies and Research, U.V.A.S, Lahore, for his moral support and technical guidance for preparation of this manuscript.

Special thanks to Dr. Azhar Marhoob, Associate Professor, Department of Parasitology, University of Veterinary and Animal Sciences, Lahore for his everlasting coordination, cooperation and guidance during my Ph.D. programme.

I will never forget the guidance, kindness and supportive attitude of Mr. Nasir Mehmoond Ch., Ph.D. Scholar, School Biological Sciences, University of the Punjab, Lahore for his valuable support and help during my research experiments, of Ph.D. programme.
Special complements to Dr. Muhammad Ramzan, Dr. Zafar Hayat, Dr. Rashid Munir, Dr. M. Kamran, Dr. Hafiz Ghulam Murtaza, Dr. Waseem Zehzad, Dr. Zahid Nazir, Muhammad Babur Ph.D. scholars and Dr. Jawad Nazir and Dr. Amir Ghafour Bajwa, Lecturers, Department of Microbiology, UVAS, Lahore.

This chapter will incomplete if I do not extend my heartiest thanks to Mr. Badar Munir, Muhammad Javed, Muhammad Irfan Najmi, Sajjad Ahmad and Muhammad Zargar office of the Dean, Faculty of Veterinary Sciences, UVAS, Lahore for their moral and academic support during Ph.D. study programme and finally composing this manuscript by heart and soul.

I would appreciate the role of Mr. Muhammad Saeed, Rana Muhammad Shahid, Rana Abdul Rasheed and Javed Younus, Department of Pathology, University of Veterinary and Animal Sciences, Lahore for their coordination, cooperation and ever lasting help during my Ph.D Research programme.

I wish to extend my zealous thanks to all my respected brothers especially to my elder brother Rana Muhammad Ayub Advocate and my younger sister for their ever lasting support and encouragement in my life.

It was impossible to achieve this goal without a full support of my loving wife Samra Younus Lecturer, Govt. Islamia College for women, Lahore Cantt who really sacrificed a lot of her precious time for me. I will never forget her care and concern throughout my life.

MUHAMMAD YOUNUS
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<td>showing leukocytic infiltration and inflammation</td>
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<td>showing atrophy of bursal follicles</td>
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<td>4.28</td>
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<td>showing leukocytic infiltration and necrosis of bursal</td>
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<td>4.30</td>
<td>Representative figure of lean muscle of group A</td>
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<td>experimentally infected with <em>Salmonella enteritidis</em></td>
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<td>showing muscular degeneration and necrotic areas</td>
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<td>4.31</td>
<td>Representative figure of lean muscle of group B</td>
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<td>experimentally infected with <em>Salmonella typhimurium</em></td>
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<td>showing muscular degeneration and necrotic areas</td>
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<td>4.32</td>
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INTRODUCTION
CHAPTER 1

INTRODUCTION

As the human population in Pakistan is expanding exponentially, the protein requirements can be met through broiler poultry meat which is relatively cheaper and easily available in the market. The demand of broiler meat will also increase in the coming years. An estimated average per capita and per annum consumption is 61.54 eggs and 2.34 kg poultry meat, respectively (Economic survey of Pakistan, 2001). In Pakistan, poultry industry commands an annual return of some 45 billion rupees and currently annual losses amounting to 4.5 billion rupees, mainly as a result of losses due to diseases (Economic survey of Pakistan, 2001).

The Salmonella genus of bacteria was identified in 1885 by Salmon and Smith. Salmonella is a genus of gram negative, non-sporeforming, motile by flagella and rod shaped bacteria of family entero-bacteriaceae. There are more than 2000 species of Salmonella. Salmonellosis is a convenient etiological term to describe a variety of conditions that affect humans and many animal species. In poultry it causes pullorum disease (salmonella pullorum), fowl typhoid disease (Salmonella gallinarum) and fowl paratyphoid (Salmonella enteritidis and Salmonella typhimurium) and in human it cause a wide range of diseases such as enteric/typhoid fever, food poisoning, gastro-enteritis, diarrhea and bacteremia (Antonio et al., 2000).
INTRODUCTION

Salmonellosis is the most wide spread food borne and zoonotic problem throughout the world. Its incidence has increased dramatically in developing countries over the past 50 years. The microbiologically contaminated poultry meat and eggs remains a hot topic since decades. Researchers are consistently finding the methods in producing 100% Salmonella free chicken meat.

It has been clearly demonstrated that salmonella species especially *Salmonella enteritidis* and *Salmonella typhimurium* are transferable via transovarian route to both market eggs and newly hatched chickens. Salmonellosis has been declared by WHO and FAO as one of the most common and important zoonoses since 1950 and has been included in the list of commonest zoonoses and also in the terrestrial animal health code of OIE (OIE, 2004). There is extensive literature, documenting adverse effects of Salmonellosis on human health. There is much evidence for the possible transmission of bacterial organisms from feed, poultry meat, eggs and contaminated water to humans causing typhoid fever and food poisoning (Gupta, 1999; Robert et al., 1998).

Typhoid fever and food poisoning are well recognized as a public health problem in developing countries like Pakistan. It is estimated that more than 13 million cases occurring alone in Asia including Pakistan. Mostly, the causal agents are transmitted from feed, poultry meat, eggs, and contaminated water, to humans which result in typhoid fever and food poisoning (Lutwick and Zenilman, 1997; Butta and Mansurali, 1999).

**Typhoid fever/enteric fever** is a severe infection caused mainly by *Salmonella typhi* but other *Salmonella* such as *Salmonella enteritidis* and *Salmonella*
*Salmonella typhimurium* may also cause enteric/typhoid fever showing symptoms of high fever, constipation, extreme fatigue, head ache, joint pain and peritonitis which may appear in the form of death (Williams et al., 2001).

**Food poisoning** is a general term for health problem arising from eating contaminated food and is a bacterial infection which mainly caused by *Salmonella enteritidis* and *Salmonella typhimurium* but other bacteria may also cause food poisoning showing symptoms of severe diarrhea, fever and vomiting (Williams et al., 2001).

When the organisms infect a human in sufficient numbers, severe gastroenteritis may result, lasting for 1-7 days. If the person is immuno compromised, more serious illness and possibly death may result, (Hunton, 2002). In some cases, the salmonella infection may spread from the intestines to the bloodstream and then other body sites and may cause death if not treated with antibiotics (Rzedzicki et al., 2000).

The incidence of Salmonellosis in poultry and other birds is studied and concluded that *Salmonella pullorum* and *Salmonella gallinarum* are confined only to poultry causing pullorum and fowl typhoid disease while *Salmonella enteritidis* and *Salmonella typhimurium* causes fowl paratyphoid in poultry (disease of young and adult chicks) showing signs of profuse watery diarrhea and dehydration in acute cases, laboured breathing, pasting of vent and huddling together but also transferable to human causing gastroenteritis, enteric/typhoid fever, food poisoning and diarrhea (Anjum, A.D., 1997)
The pathological changes and lesions observed by giving oral infection of *Salmonella enteritidis* and *Salmonella typhimurium* in poultry are severe enteritis accompanied by focal necrotic lesions in the mucosa of the small intestine, cheesy cecal cores, spleen and liver congested, kidneys enlarged and congested, peri-hepatitis and peri-carditis, co-agulated yolk material in the yolk sack, purulent arthritis, air-saculitis and cell death also may occur Saif (2003).

Bacteriological methods (Clinical signs/symptoms and lesions, Isolation and identification of organism) imposed by current regulations are laborious and time consuming. There is risk of false or negative results arising from the periodic nature of Salmonella excretion. In these cases, serological tests (whole blood plate test, spot agglutination test, rapid serum plate test, widal test and ELISA test) are of great value. Among them ELISA by considering sensitive and quick test has been used in the control of Salmonella infection in poultry flocks (Thoms and Levine et al., 2001).

Diagnostic polymerase chain reaction (PCR) is an extremely powerful rapid and reliable method for diagnosis microbial infections and genetic diseases, as well as for detecting microorganisms in environment and food samples. PCR testing offers the possibility to improve detection and characterization of pathogenic bacteria, since one can target species-specific DNA regions and specific traits of pathogenicity, especially genes coding for toxins, virulence factors, or major antigens. The PCR technique has several advantages over classical bacteriology with respect to detection limit, speed, and potential for automation (Hanai et al., 1997; Vaneechoutte and Van Eldere, 1997). The latter
capacity is indeed necessary for application of the test in extensive screening programs. Currently, probes and PCR methods are available for many important food-borne pathogens, including Salmonella. In order to increase the sensitivity, specificity, and speed of detection, several different DNA methods have been developed (Olsen et al., 1995; Hoorfar et al., 2000). However, due to the lack of common genes for toxins or other virulence factors, the approach for isolation of specific DNA probes has been to select randomly cloned chromosomal fragments. Furthermore, ribosomal RNA-based oligonucleotide probes have been used successfully in a single-phase hybridization assay to detect a large number of serovars of Salmonella.

In Pakistan, due to lack of reliable data base with regard to the quality of poultry products for human consumption, the consumer is considered to be a dead end host and also never comes to know what is the status of the product, he is going to enjoy. For this very reason, efforts will be made in the form of this project to determine the possible levels of bacterial loads, its transmission through contamination of the poultry meat and eggs and also by studying the pathogenesis of Salmonella enteritidis and Salmonella typhimurium with respect to carrier states in poultry and its public health impact. The export of poultry products will also be increased by controlling the diseases/infections in poultry through improving the animal and human health status and ultimately strengthening the economy of the country. Thus, this research endeavour is being made to generate data to improve the quality of poultry products and human health awareness.
Objectives of the Project

i) To ascertain the quality of poultry products with respect to salmonella infection to broiler meat available in the market for human consumption.

ii) To generate data regarding quality of poultry products with World Trade Organization (WTO) standards.

iii) To improve human health awareness in the society.

iv) To achieve human resource development in the field of good quality control.

v) To study the chain of Salmonella enteritidis and Salmonella typhimurium in feed, poultry and humans.
CHAPTER 2

REVIEW OF LITERATURE

Poultry Feed

Cox et al. (1983) collected Poultry feed (mash and pelleted) and meat and bone meal samples from commercial mills. All samples were analyzed for Enterobacteriaceae count (ENT). The genus and species of the various Enterobacteriaceae present were also determined. The average ENT for mash, pelleted, and meal samples was $10^{4.1}$, $10^{8}$, and $10^{1.8}$/g, respectively. Enterobacteriaceae were present in 100%, 60%, and 92% and Salmonella in 58%, 0%, and 92% of the mash, pelleted, and meal samples, respectively.

Nashed (1986) found that in countries with a prevailing Poultry production, salmonellosis is of growing importance. The Poultry contaminated with Salmonellae plays a significant role in the food poisoning of men and in decreasing the breeding results. The study investigated the viability of Salmonella typhimurium in feed and litter contaminated with this germ, at different temperatures. The organism remained viable at 37°C in feed up to 6 weeks, in litter for 2 weeks, at room temperature in the feed up to 71 weeks in the litter up to 78 weeks, and at 7°C in feed and litter up to 79 weeks. Recommendations are given for the control of salmonellosis by referring to the sources and possibilities of contamination.
Rouse et al. (1988) contaminated sterilized commercial Poultry feed was contaminated with either *Salmonella typhimurium* or with a strain isolated from commercial broiler carcasses. They found that after treatment with a chemical preservative resulted in the elimination of detectable *Salmonella* from heavily contaminated feed within 72 hours and from lightly contaminated feed within 24 hours. Nonsterilized feed showed similar results. Commercial feed fed to broilers for 56 days and inoculated with *Salmonella* daily for Days 35 to 56 was treated with the chemical preservative at .5 and 1.0% for the last 7 days. Treatment resulted in a reduction of the number of fecal and intestinal samples positive for *Salmonella*, demonstrating elimination of *Salmonella* in the feed by use of the feed preservative.

Shackelford (1988) reported that *Salmonellae* are everywhere in our environment. The organism seems to have adapted to changes in its environment; it has survived improvements in sanitation practices, chemical treatments, and antibacterial drugs. *Salmonellae* can be introduced into the Poultry production cycle through the hatchery, feed, broiler house, rodents, and man. Once colonized in one broiler, the organism can be shared with other broilers, either internally or externally. The Poultry transport container provides an avenue for organisms to transfer from one broiler's excrement to at least the feathers of other broilers. Much effort has been expended on research and development by equipment manufacturers and researchers to improve the microbiological quality of Poultry carcasses. Innovations such as spray scalding, automation of eviscerating process, provision of hand wash nozzles
for manual work stations, immersion chilling, and cooling of carcasses packed in plastic bags offer possibilities for reducing microbial loads on carcasses.

Veldman et al. (1995) surveyed between July 1990 and April 1991 the rate of contamination with Salmonella species of Poultry feeds and feed components used by the Dutch feed industry. Ten per cent of 360, 10 g samples of Poultry feeds were found to be contaminated. Mash feeds, mostly used for layer-breeders, were far more frequently (21 per cent) contaminated than pelleted feeds (1.4 per cent). The rate of contamination of 130 samples of fish meal was 31 per cent, of 83 samples of meat and bone meal 4 per cent, 58 samples of tapioca 2 per cent and of 15 samples of maize grits 27 per cent. Twenty-eight serotypes of Salmonellae including Salmonella typhimurium and Salmonella enteritidis were isolated.

Martin et al. (1996) reported that freshly hatched chickens show a very high susceptibility to Salmonella infections and control measures are therefore frequently focused on the period shortly after hatching. Experimental investigations using one strain against itself, differentiated by different antibiotic resistance markers, have shown that colonization with Salmonella prevents the establishment of subsequently inoculated challenge organisms in the chicken gut. The inhibition effect lasts for several days and is detectable even when a challenge dose of $10^8$ organisms is used.

Antonio et al. (2000) evaluated 3 rapid molecular typing methods for the identification of three frequent, Salmonella serovars on the basis of ease,
simplicity and reproducibility of the chosen methods. The three different serovars of Salmonella i.e. *Salmonella enteritidis*, *Salmonella typhimurium* and *Salmonella virchow* studied gene reproducible and distinguishable profiles by using PCR methods. The conserved patterns in each serovar allowed for easy differentiations from other serovars of Salmonella.

Maciorowski *et al.* (2000) highlighted that Salmonellosis is a cyclic problem in the food industry, to which animal feed has been a contributor. Current conventional methods of *Salmonella* species detection require 96 hours for detection and confirmation. With modern and just-in-time production schedules, a 96 hours hold represents a significant expense in storage and decontamination. The commercially available assay, ‘BAX’ for Screening *I. Salmonella*’ (BAX), is based on the principle of the polymerase chain reaction and may represent a significant decrease in assay time. Seven fresh feed formulations, two fresh feed ingredients, seven stored feeds and two stored feed ingredients were artificially contaminated with a primary Poultry isolate of *Salmonella typhimurium* and analysed by conventional and BAX methodology. Specific sequences of Salmonella DNA that were extracted from Poultry diets could be detected with BAX.

Shirola *et al.* (2001) in order to investigate contamination of chicken farms with Salmonella, feed and eggs sampled from 16 commercial layer farms in eastern Japan between 1993 and 1998. *Salmonella enterica* subsp. *enterica* isolates belonging to 19 serovars were obtained from the feed. Six of the 19 serotypes, including *Salmonella serovar enteritidis*, were observed in isolates
recovered from the eggs. *Salmonella serovar enteritidis* strains obtained from a feed sample and egg contents in a layer farm showed *pulsed-field gel electrophoresis* patterns that were genetically related and belonged to a single phage type, suggesting that the contamination of the farms was linked to the occurrence of *Salmonellae* in feed.

Boqvist *et al.* (2003) presented data Salmonella from animals, feedstuffs and feed mills in Sweden between 1993 and 1997. During that period, 555 isolates were recorded from animals, representing 87 serotypes. Of those, 30 serotypes were found in animals in Sweden for the first time. The majority of all isolates *from animals* were *Salmonella typhimurium* (*n* = 91), followed by *Salmonella dublin* (*n* = 82). There were 115 isolates from cattle, 21 from broilers, 56 from layers and 18 from *swine*. The majority of these isolates were from outbreaks, although some were isolated at the surveillance at slaughterhouses. The number of isolates from the feed industry was similar to that of the previous 5-year period. Most of those findings were from dust and scrapings from feed mills, in accordance with the HACCP programme in the feed control programme. It can be concluded that the occurrence of *Salmonella in animals* and in the feed production in Sweden remained considerable during 1993-97.

Lim *et al.* (2003) developed a multiplex polymerase chain reaction (PCR) assay for the identification of *Salmonella enterica serovar typhimurium*. Three sets of *primers* were designed for detecting O4, H:i, and H:1,2 antigen genes from the antigen-specific genes *rfbJ, fliC*, and *fliB*, respectively. These were
evaluated in a multiplex PCR assay by using DNAs from *Salmonella enterica serovar typhimurium*, 15 other *Salmonella* serovars, and 8 non-Salmonella enteric pathogens. Multiplex PCR proved to be capable of identifying *Salmonella enterica serovar typhimurium* specifically and differentiating it from other Salmonella serovars in addition to non-Salmonella enteric pathogens. Thus, this multiplex PCR assay can be practically applied to the identification of *Salmonella enterica serovar typhimurium*.

Heres (2004) found that Salmonella and Campylobacter are undesirable pathogens on poultry. Therefore, the effect of fermented feed on the colonization in the gastro-intestinal tract of the chicken, the introduction of both bacteria in a chicken flocks, and the transmission between chickens was studied. Broilers that were fed with fermented feed were significantly less susceptible for Salmonella and Campylobacter than chickens on a standard chicken feed. The spread of Salmonella between broiler chickens was reduced. However, the results also showed, like for other known control measures, that this feed can not absolutely guarantee the absence of Salmonella and Campylobacter. Therefore fermented feed must be seen as one of the hurdles in a so called multiple hurdle strategy. The combination of different hurdles should prevent the introduction and transmission. The effect of fermented feed on Campylobacter and Salmonella is partially caused by the presence of high concentrations of organic acids. In chickens fed with liquid feed the acidic barrier in the first part of the GI-tract was clearly improved. Besides organic acids there are other changes in the GI-tract. Changes in colonization levels of
indicator organisms, changes in levels of organic acids and an increased pH in ileum and caecum. These changes indicate a stabilised GI-flora in fermented feed fed Poultry. The research confirmed that by changes in the composition of the feed (carbohydrates, acids, or micro-organisms) the GI-health can be promoted and therefore can contribute to the control of food pathogens in farmed animals.

Jones and Richardson (2004) collected 886 samples (68 feed ingredient samples, 189 dust samples, and 629 feed samples) from 3 feed mills each of which produced between 100,000 and 400,000 tons of feed a year. Samples were collected on 3 days (Monday, Wednesday, and Friday), during 2 seasons (early spring and summer), and between 0700 and 1700 hours approximately once per hour. Samples were collected from 5 locations within each mill: ingredient receiving, at the mixer, at the pellet mill, from pellet coolers, and at load-out. Temperatures were taken of the samples obtained at the pellet mill immediately following collection. All samples were analyzed for Enterobacteriaceae counts (EC) and Salmonella. The data confirm that feed ingredients and dust can be a major source of Salmonella contamination in feed mills. There were no differences ($P < 0.05$) in the Salmonella contamination rates of samples collected in spring as compared with samples collected in summer. Salmonella contamination rates were observed to be higher in samples collected on Friday compared with samples collected on Monday or Wednesday, an effect that may be management related. Data collected at the pellet mill clearly illustrate the uneven distribution of Salmonella contamination
in feed as well as the need for control of dust around the pellet mill. Feed samples (both mash and pellets) contaminated with Salmonella contained significantly higher EC than samples not contaminated with Salmonella. Thus, EC may provide some indication of the likelihood of Salmonella contamination in feed samples.
Poultry Meat (Small and Large Intestine)

Ozari and Kotter (1986) found that certain pathogenic micro-organisms in
or on food of animal origin still constitute a particular hygienic risk. Salmonellae
are found chiefly on meat and in meat products including Poultry. Most of the
infections with Salmonellae in men are presumably caused by the consumption
of raw products of animal origin. They suggested that their stock of slaughter
animals were most often latently infected. During the slaughter and the
processing meat surfaces are contaminated with Salmonellae.

Barrell (1987) isolated of Salmonellae from humans and food products
for the period 1981-85 and attempted to investigate the relationship between
serotypes isolated from humans and those from meat products. The
predominant serotypes isolated from humans were Salmonella typhimurium,
Salmonella enteritidis and Salmonella virchow. Salmonella typhimurium was
commonly isolated from a range of meat products. S. derby was one of the
most common serotypes isolated from tripe and sausages but was relatively
uncommon in humans. Salmonellae were found in less than or equal to 0.5% of
most cooked meat products apart from tripe and udder (3.2%) and pet foods
(12.4%). Isolations from raw meats ranged from 3% for pork to 28% for Poultry.
Incidence of Salmonella infection in humans in Manchester increased between

Tokumaru et al. (1991) collected pork, beef and chicken meat samples
from slaughter houses, Poultry-processing plants and meat shops. Rates of
incidence of Salmonella spp., Campylobacter jejuni and Campylobacter coli with respect to the sample size were compared and the most probable number for these species were determined. Salmonella spp. were detected in 69 (24.1%) of 286 chicken meat samples, in three (3.2%) of 94 pork samples, and in one (1.9%) of 52 beef samples. With chicken meat, the rates of detection were: 19.9% in 25-g, 15.7% in 10-g, and 12.2% in 1-g samples. The populations in most probable numbers, that gave positive results in 31 (20.8%) of 149 samples, ranged from 30 to 10(4) per 100 g, the majority (93.5%) being between 30 and 10(3) per 100 g. Campylobacter jejuni and Campylobacter coli were detected in 106 (67.9%) of 156 chicken meat samples, in two (2.1%) of 94 pork samples, and none of 52 beef samples. The results obtained with different sample sizes of chicken were compared. Positive rates were 55.8%, 39.7%, 27.6% in 10g, 1g, and 0.1g, respectively.

Noordhuizen and Frankena (1994) reported that Salmonella enteritidis infections in Poultry appear to be of major public concern. Prevalence levels in veal calves and pigs are rather low. Because of the complex of socio-psychological, welfare, economic and public health aspects great emphasis should be put on prevention and control. This paper deals with some clinical epidemiological approaches for prevention and control of Salmonella enteritidis. Emphasis is set on multi-factorial background of infection occurrence, epidemiological methods and features of monitoring and surveillance for evaluation of measures taken during a follow-up period. Finally, it is stated that the application of Risk Assessment and Analysis principles in this problem area,
integrating the concepts previously addressed, might prove to be a valuable perspective.

Wilson *et al.* (1996) carried out a prospective survey in Northern Ireland between February and August 1994 to assess the current levels of *Salmonella* in retail chickens, and to determine whether contamination rates differed between chicken sold in supermarkets and by butchers. One hundred and forty chilled and frozen raw chicken were sampled from retail display and examined for the presence of *Salmonella* using an approved method. Contamination was commoner in chicken that were chilled, sold from smaller premises, and reared or slaughtered outside Northern Ireland. *Salmonella enteritidis* was the most commonly isolated type. The contamination rate of about 7% is considerably lower than in Great Britain and this may partially explain the lower rate of human *Salmonella* infections in Northern Ireland.

Barrow (1997) found that bacterial infections of Poultry remain of great importance world-wide in terms of economic effects and public health. They include infections caused by *Salmonella, Escherichia coli, Campylobacter* and *Pasteurella*. Through the introduction of rigid hygienic measures it is possible to breed and rear Poultry free of these pathogens. However, the cost to the industry would be prohibitive and economically disastrous. Biological measures have been introduced albeit in a relatively empirical way. Antibiotic therapy and prophylaxis is used extensively with the associated problems of development of resistance. Killed vaccines are used but are not usually very effective. Live vaccines are increasingly becoming acceptable and studies are under way to
increase our understanding of the pathogenesis of these infections so that vaccine development may become less empirical. Work with live vaccines to be used against Salmonella has shown that they may be administered orally to newly-hatched chicks. These measures are very exciting and open up opportunities for disease control for the future.

Corry and Hinton (1997) described that zoonoses are diseases, the infections of which can be transmitted between man and animals. Only a few are of importance with respect to Poultry meat and meat from cattle, sheep, horses and goats. Advances in the control of diseases such as tuberculosis, brucellosis and trichomoniasis in animals have reduced the hazards posed to workers in the meat industry and to consumers of meat. However, inspection of animals ante- and post-mortem cannot detect all infectious agents present. This applies particularly to bacteria such as Campylobacter, Salmonella, verotoxigenic and other pathogenic Escherichia coli and Yersinia. Protection of meat workers from infection depends upon taking normal hygienic precautions, which also protect the meat from contamination from the workers. Consumers are exposed to a smaller range of zoonoses than meat workers because they encounter only meat that has passed inspection.

Haapapuro et al. (1997) reported that foodborne illness remains a common and serious problem, despite efforts to improve slaughterhouse inspection and food preparation practices. A potential contributor to this problem that has heretofore escaped serious public health scrutiny is the feeding of animal excrement to livestock, a common practice in some parts of
the United States. In 1994, 18% of Poultry producers in Arkansas collectively fed more than 1,000 tons of Poultry litter to cattle, and the procedure is also common in some other geographic areas as a means of eliminating a portion of the 1.6 million tons of livestock wastes produced in the United States annually. While heat processing reliably kills bacterial pathogens, its use is limited by expense and other factors. Deep-stacking and ensiling are commonly used by farmers to process animal wastes, but the maximal temperatures achieved in stacked Poultry litter are typically in the range of 43°C to 60°C, below the inactivation temperatures of pathogenic Salmonella and Escherichia coli species, and far below the USDA's recommended cooking temperatures of 71°C to 77°C for potentially manure-tainted meat products.

Henson (1997) described the use of the Delphi method to estimate the incidence of food-borne Salmonella in the UK and the effectiveness of alternative control measures. A panel of experts of food-borne Salmonella participated in the Delphi survey, which involved five rounds of questioning which took place in the period July 1993 to January 1994. Participants were asked to give initial estimates for a number of parameters and invited to revise these estimates through progressive rounds of the survey at which the group responses were reported back. This process resulted in a reduction in the variation between the estimates given by individual experts. The final estimated annual incidence of food-borne Salmonella in the UK was 537,000, although significant variation remained between individual estimates. The foods judged to be the most important modes of transmission were Poultry and Poultry
products (50% of cases) and eggs and egg products (26% of cases). The panel was also requested to estimate the effectiveness of strategies available to reduce the incidence of food-borne Salmonella from all sources.

Mulder (1997) reported that the revolutionary industrialization of the Poultry farming in the last 30 years has made the Poultry meat available for large groups of consumers. Due to its nutritional, sensory and economical characteristics, Poultry meat is by far the most popular animal food product world-wide. Epidemiological reports, however, incriminate Poultry meat as a source for outbreaks of human food poisoning. The organisms involved are Salmonella spp., Campylobacter spp. and, to a lesser extent, Listeria monocytogenes, Escherichia coli, Staphylococcus aureus, Yersinia enterocolitica, Clostridium perfringens and Aeromonas spp. Contamination of the end-product with pathogenic microorganisms is a reflection of the contamination of the live birds and, therefore, measures to be taken by industry to avoid contamination of the consumer-ready product should start at that level.

White et al. (1997) reported that Food borne illness is a major public health concern. The largest number of food borne illness cases attributed to Poultry and Poultry products are caused by paratyphoid serotypes of Salmonella and by Campylobacter jejuni. The effective prevention of food borne disease requires an understanding that contamination can be introduced into foods at numerous points along the food chain. Since multiple entry points exist for food borne pathogens, multifaceted intervention approaches are required to successfully control contamination of Poultry during the various phases of the
growth period and processing procedure of broiler chickens. Strategies during the grow-out period (the period during which day-old chicks are raised to six- to seven-week-old broiler chickens) include sanitation, biosecurity, vaccine and drug therapy, and biological control procedures, such as those aimed at preventing colonization. There are also many critical control points identified in the processing plant which reduce contamination. These include temperature controls (washer and product), chemical interventions, water replacements and counter-flow technology in the scalding and chilling, and equipment maintenance. Transportation and food handling at retail outlets and by the consumer (i.e., storage at the proper temperature and adequate cooking) are the final critical control points in the farm-to-table continuum. It is important to apply risk reduction strategies throughout the food chain.

Fuzihara et al. (2000) found that Salmonella is the leading cause of human foodborne infections in Latin America, and Poultry meat is one of the main vehicles. Small Poultry slaughterhouses (fewer than 200 birds slaughtered per day) represent an important economic activity in certain regions. The slaughtering process in these abattoirs is manual and rudimentary, and frequently the hygienic conditions are poor. This study reports results of a detailed evaluation of the prevalence of Salmonella serotypes in carcasses, utensils, and environmental samples collected in 60 small Brazilian slaughter houses. In the second step of the study, one of these slaughterhouses was selected to monitor the dissemination of Salmonella along the slaughtering process. Forty-one percent of samples harbored Salmonella species, including
42% of carcasses, 23.1% of utensils, 71.4% of water, and 71.4% of freezers and refrigerators. Seventeen serotypes were detected. *Salmonella enteritidis* predominated (30%), followed by *Salmonella albany* (12%), *Salmonella hadar* (12%), *Salmonella indiana* (10%). All samples collected along the slaughtering process in the selected slaughterhouse were Salmonella positive. More than 30% of the samples contained more than one serotype, and 12.5% presented three serotypes. The widespread occurrence of Salmonella in small slaughterhouses reinforces the need for implementation of effective control measures.

*Naught et al.* (2000) developed a model of the transmission of Salmonella through the Poultry meat production chain, to predict the effects of intervention strategies for Salmonella control. The model first describes the situation before intervention in terms of Salmonella prevalence at flock level and some transmission parameters. After single control measures are translated into effects on these transmission parameters, the effects of sets of control measures (intervention strategies), can be calculated with the model. As research data are lacking, the model input parameters were derived from expert opinion. As an example, the effects of two intervention strategies proposed for the Dutch Poultry industry are predicted. A sensitivity analysis is performed to indicate where the most effective control measures may be expected. Additionally, the reliability of the model predictions is studied by an uncertainty analysis. The use of the model as a tool for policy makers deciding about Salmonella control strategies is discussed.
Pope and Cherry (2000) conducted two trials to evaluate the presence of Salmonella, campylobacter, and generic Escherichia coli on broilers raised on Poultry Litter Treatment (PLT) enhanced litter in comparison with those raised on untreated litter. Two Company A farms included three houses on each farm as the treated group and three houses per farm as controls. Two complete grow outs were evaluated on each farm. The Company B study included 10 farms with two paired houses per farm, one house as the treated group and one house as the control. One grow out was evaluated per farm. The pathogen sampling consisted of litter sampling and whole bird rinses on the farm and in the processing plant. Litter pH, ammonia concentration, total litter bacteria, temperatures, and humidity were also recorded. The study with Company A resulted in lower mean levels of pH, ammonia concentration, total litter bacteria, litter E. coli, and bird rinse counts for Salmonella and E. coli in houses treated with PLT. The results for Company B closely resembled those for Company A, but also included campylobacter data, which showed no difference between treated and control groups. The data indicate that PLT may be a beneficial component for on-farm pathogen reduction.

Frenzen et al. (2001) reported that food manufacturers in the United States are currently allowed to irradiate raw meat and poultry to control microbial pathogens and began marketing irradiated beef products in mid-2000. Consumers can reduce their risk of food borne illness by substituting irradiated meat and Poultry for non-irradiated products, particularly if they are more susceptible to food borne illness. The objective of this study was to identify the
individual characteristics associated with willingness to buy irradiated meat and Poultry, with a focus on five risk factors for food borne illness: unsafe food handling and consumption behavior, young and old age, and compromised immune status. However, there was no difference in consumer acceptance by any of the food borne illness risk factors. It is unclear why persons at increased risk of food borne illness were not more willing to buy irradiated products, which could reduce the hazards they faced from handling or undercooking raw meat or poultry contaminated by microbial pathogens.

Sarwari et al. (2001) reported that if raw meat and Poultry are the primary point of entry for Salmonella species into human populations, a correlation might be expected between the sero types distribution of Salmonella species isolated from animals at the time of slaughter and that of isolates found in humans. For 1990-1996, a sufficient national data were available to permit such a comparison. A mathematical model was developed to predict sero-type distributions of Salmonella isolates among humans on the basis of animal data. There was a significant mismatch between the sero-types distributions among humans predicted by the model and those actually observed that the risk of transmission to humans is equal for all food product categories and that Salmonella sero-types have an equal ability to cause human illness.

Valancony et al. (2001) noted that Salmonella enteritidis contaminated Poultry houses have to be cleaned and disinfected. The objective of this study was to determine the most appropriate points to take samples to validate the efficiency of the decontamination. 14 sites were so described and analysed in
1999 and 2000. From each site hundreds of samplings were realized for research on Salmonella, before and after cleaning and disinfection. The samplings made in cages are not reliable indicator of Salmonella contamination. After cleaning and disinfection, the neighbour hoods can remain contaminated as well as certain elements of the hens house which are difficult to access or which have been forgotten.

Aysegul and Carli (2002) developed a real time probe specific PCR to rapidly detect Salmonella inv A gene based PCR products from chicken feces and carcasses by a fluorescence resonance energy transfer essay. The sensitivity and the specificity of this system were determined as 3 colony forming units per ml and 100%, respectively. Seventy two cloacal swabs, 147 intestines and 50 carcass samples were examined. Thirteen (8.8%) and 25 (17%) of the intestinal samples were found to harbor Salmonella by bacteriology and PCR, respectively. Forty five of 50 (90%) carcass samples were Salmonella positive by both methods. Results indicate that this essay has the potential for use in routine monitoring and detection of Salmonella in infected flocks and carcasses.

Whyte et al. (2002) identified contaminated Poultry meat, as one of the principal foodborne sources of Salmonella. The development of rapid detection assays for Salmonella would enable official agencies and food industries to identify contaminated foodstuffs in a more timely manner. In this study, a survey was carried out to determine the prevalence of Salmonella in raw broiler carcasses. A total of 198 neck skin samples were obtained from within 40 flocks
at a commercial broiler slaughtering facility. The presence of Salmonella was assessed by traditional culture methods and by a Salmonella-specific polymerase chain reaction (PCR) test. Salmonella was recovered from 32 (16%) of all samples using traditional culture methods. In contrast, the PCR assay proved to be more sensitive and detected Salmonella DNA in 38 (19%) of the samples tested. The pathogen was detected in 45 (23%) of the 198 samples when culture and PCR results were combined. The sensitivity of the PCR test was also greater than culture when detecting Salmonella from within flocks (53% of flocks by PCR, 30% of flocks by culture). The combination of both tests revealed that 55% of the flocks were contaminated with Salmonella. The PCR assay proved to be a highly specific and sensitive method for detecting Salmonella.

Summer et al. (2004) stated that during the 1990s, there was radical change in regulation of meat and poultry hygiene in Australia, and Australian Standards were developed for each sector of the meat industry. Systems for industry/government co-regulation and company-employed meat inspection were introduced based on company HACCP programs approved and audited by the Controlling Authority. However, in the 5 years since regulatory changes took full effect, rates of salmonellosis have not decreased (surveillance and reporting systems have remained unchanged). Using statistics gathered by the National Enteric Pathogens Surveillance Scheme, an attempt was made to link Salmonella serovars isolated from meat and Poultry with those causing salmonellosis. Two periods were studied, 1993/1994, before regulations were
introduced, and 2000/2001, when regulations should be having an effect. For red meat, the same serovars were prominent among the top 10 isolates both before and after regulation, and there was little linkage with salmonelloses. For Poultry, frequently isolated serovars differed pre- and post-regulation, however, in both periods there was some linkage between serovars isolated from Poultry and those causing salmonelloses. The present paper illustrates difficulties faced by governments in measuring public health outcomes of changes to food hygiene regulation.

Mead (2004) reported that contamination of Poultry meat products with Salmonella and Campylobacter spp. continues to be the main microbiological problem affecting both industry and consumers. In some countries, control measures appear to have reduced flock infection with Salmonella, but other issues have arisen; these include an increase in multiple resistance to antimicrobials in certain serotypes and the recent emergence of Salmonella paratyphi B variant Java, also frequently multi-resistant. For intensive production systems, there is much information on sources and routes of Salmonella transmission and the relative risk of infection/contamination at different stages of production. Some possible reasons for control failures are discussed. In contrast, less is known about Campylobacter in these respects, and a better understanding is needed of the physiology and host-interactions of the organisms, and of their behaviour in Poultry operations. On such knowledge may depend the future development of effective controls.
Poultry Eggs

Duguid et al. (1991) recorded evidence on the extent of the part played by infected hens' eggs in currently much exaggerated. Prevention should be sought through improved catering practices and kitchen hygiene and attempts to eradicate Salmonellosis from laying flocks are likely to be in effective.

Kovarik et al. (1991) cultured three sites (Shellsarfax, shell membranes and yolk) in 760 eggs from four Poultry farms. Salmonellae were isolated from the yolk of 4 human and 4 Poultry Salmonella enteritidis isolates were phage type 8, 2 Poultry isolates were phage type 23 and the other human isolate was phage type 4.

Dorn et al. (1993) performed plasmid analysis of Salmonella enteritidis isolates from human gastroenteritis cases and from two commercial egg-producing Poultry flocks, to determine if the Poultry flocks were the source of the human infections. The plasmid profile and restriction fragment pattern (fingerprint) of five Salmonella enteritidis isolates from human cases matched those of nine isolates from internal organs of egg-laying hens in one flock which was the source of eggs consumed by the cases. Another commercial flock was epidemiologically associated as the source of eggs consumed by affected persons in four separate gastroenteritis outbreaks from which Salmonella enteritidis isolates were available. Five Salmonella enteritidis isolates from human cases in these four outbreaks had the same profile and fingerprint, and they all matched those of the 24 isolates from hens in this flock. These results
provide further documentation of egg-borne transmission of *Salmonella enteritidis* to humans.

Gast and Beard (1993) observed when it became evident that the association of human *Salmonella enteritidis* (SE) outbreaks with the consumption of contaminated Grade A eggs posed a threat to public health and to the economic viability of the egg industry, research programs were rapidly initiated to investigate the many unanswered questions about SE in eggs and chickens. Research efforts have focused on the dynamics of deposition, survival, and growth of SE in eggs, the pathogenesis of SE in chickens, strategies for detecting SE-infected flocks, opportunities for intervening to prevent infection, the sources of SE in laying flocks, options for effectively cleaning Poultry houses, and the epidemiology of SE infections of humans and chickens. This research has provided a substantially better understanding of the SE problem in Poultry, but many further questions about the basis for and the prevention of eggborne transmission of SE remain to be answered.

Pittler (1993) observed an increased occurrence in Great Britain of *Salmonella enteritidis* infections in human beings which were predominantly caused by raw egg dishes. The Commission of the EEC also started activities designed as a protection against such an obvious new dimension of Salmonella infections. Mainly prompted by British activities, the Commission of the EEC elaborated a proposal for a Zoonosis Directive of the EEC. This proposal was intended to provide for the recording of data on specific zoonoses and their pathogenic agents--i.e. a monitoring--as well as for the measures to be
introduced at Community level in the member states. The Directive was adopted in the Council of Ministers of Agriculture on 17 December 1992 (Directive 92/117/EEC), it is to be translated by a Poultry Salmonella regulation of the Federal Ministry of Food, Agriculture and Forestry. It will provide for controls by the farm manager, for official controls as well as for official measures including quarantine of the Poultry stock and killing of the affected fowl. Beginning in 1995, there will probably be reports on EEC measures in the feeding stuffs sector (microbiological controls) as well as on national hygiene measures in the fields of egg marketing, raw egg consumption in canteens and voluntary control in laying hen farms in anticipation of an EEC regulation on these stocks, which resulted in the reduction of Salmonella organisms.

Schutze et al. (1996) determined whether Poultry shell eggs are a major reservoir of Salmonella enteritidis in Arkansas. One hundred dozen commercially purchased shell eggs were cultured for the presence of Salmonella spp. After each dozen eggs were examined, the contents of the 12 eggs were separated from their shells. The contents and the shells were separately pooled and cultured. One dozen of the 100 dozen egg shells cultured were found to be externally contaminated with Salmonella heidelberg, while none of the contents of the 100 dozen eggs were found to contain Salmonella organisms. The reevaluation of previously obtained telephone follow-up data on 204 patients with Salmonella infections from 1992-1993 revealed that 30 had consumed raw eggs before their salmonellosis but only one patient was infected with Salmonella enteritidis. These data suggest that
Poultry shelf eggs are not a major cause of human illness due to *Salmonella enteritidis* in Arkansas.

Kapperud *et al.* (1998) reported the epidemiological progression of human salmonellosis in Norway is parallel to trends noted elsewhere in Europe. During the past two decades, the number of reported cases has increased steadily, with a special sharp rise in the early 1980s due to the emergence of *Salmonella enteritidis*, followed by a leveling off in recent years. However, in contrast to the situation in most other European countries, about 90% of the cases from whom a travel history is available, have acquired their infection abroad. The incidence of indigenous *Salmonella* infections as well as the prevalence of the microorganism in the domestic food chain, are both comparatively low. In 1993-94, a national case-control study of sporadic indigenous *Salmonella* infections was conducted to identify preventable risk factors and guide preventive efforts. Ninety-four case patients and 226 matched population controls were enrolled. The only factor which remained independently associated with an increased risk is the consumption of Poultry meat and eggs.

Cox *et al.* (2000) observed that the presence of *Salmonellae* in fertile broiler hatching eggs has been clearly identified as a critical control point in the *Salmonellae* contamination of broiler chickens. This paper reviews the published research studies on a) the penetration and proliferation of *Salmonellae* in hatching eggs, b) the consequences of this contamination on the contamination of the final product, and (c) the egg's defenses against
invading Salmonellae. A better understanding of the material in this review paper will assist Poultry researchers and the Poultry industry in continuing to make progress in reducing and eliminating Salmonellae from fertile hatching eggs, hatcheries, and breeder flocks.

Gast and Holt (2000) recorded egg yolk and albumen differ substantially in their abilities to support bacterial growth, the initial level and location of Salmonella enteritidis deposition are critical for determining whether proposed standards for refrigerating eggs are likely to protect public health by preventing extensive microbial multiplication. In the present study, three groups of laying hens were infected with oral doses of approximately $10^{9}$ cells of different Salmonella enteritidis strains (two were phage type 4 and one was phage type 13a) in two replicate trials. For all three Salmonella enteritidis strains, the incidence of yolk contamination (approximately 2.5% overall) was significantly greater than the incidence of albumen contamination (approximately 0.5% overall). The phage type 13a strain was less often isolated from fecal samples at 2 wk post-inoculation than were the phage type 4 strains, but no significant differences between strains were observed in the incidence of egg contamination. Most freshly laid contaminated eggs contained fewer than 1 Salmonella enteritidis cell/ml of egg yolk or albumen, and no sample contained more than 67 Salmonella enteritidis cells/ml.

Gast and Holt (2001) stated that infected laying flocks is a vital part of many efforts to control egg-associated transmission of Salmonella enteritidis to humans. The relationship between the development of a specific antibody
response in infected hens and the deposition of *Salmonella enteritidis* in eggs is important for establishing the epidemiologic relevance of serologic testing methods. In two trials, laying hens were infected with large oral doses of phage types 13a and 14b isolates of *Salmonella enteritidis*. Approximately 38% of all infected hens produced at least one contaminated egg, at an overall incidence of 5.2%, between 3 and 23 days post-inoculation. As determined by enzyme-linked immunosorbent assay with an *Salmonella enteritidis* flagellar antigen, 91.7% of inoculated hens produced specific serum antibodies. Although hens with very high antibody titers were associated with a significantly elevated frequency of egg contamination, a consistently direct relationship was not evident between the magnitude of the antibody responses of individual hens and the frequency at which they laid contaminated eggs.

Durecko et al. (2004) reported that most of the patients with salmonellosis mentioned consumption of meals containing Poultry products, primarily eggs and egg products as a source of their disease. Microbiological analysis of samples showed that in more than 88% of cases the infectious agent was identified as *Salmonella enteritidis*. This serovar is the most frequent pathogen isolated at Poultry farms. In the period of the past five years, 171 outbreaks of Salmonellosis were recorded in Slovakia. However, within the group of animal tenders at these farms, no case of Salmonellosis was confirmed. The alimentary character of Salmonellosis led us to check results of analysis of samples of food and foodstuff of animal origin examined for *Salmonella* spp. performed during the past five years. The control of results
indicated that out of these 228, 545 samples of food and foodstuff of animal origin only 0.21% were confirmed as being Salmonella positive and the average ratio of Salmonella enteritidis occurrence in samples was 0.1% per year. A higher incidence (1.43 %) was recorded only in eggs and egg products. The workers indicated that there was a change in tenacity of Salmonella enteritidis due to its increased resistance, primarily against elevated temperatures including that of pasteurization. An everyday requirement for decreasing the incidence of salmonellosis is based on strict hygienic behaviour "from stable to table". The objective of salmonellosis elimination strongly depends on amending the current legislation as to the establishment of hygienic conditions in complete food chains.

Leon-Velarde et al. (2004) reported an increase in the prevalence of Salmonella enterica serotype typhimurium. This study examined the prevalence of this microorganism in Poultry environmental samples from commercial layer flocks and pullet environments as well as the sensitivity and specificity of a PCR-based method, and multiple antibiotic resistance profile of Salmonella serogroup B isolates in relation to the serotype and phagetype reference method for the identification of Salmonella typhimurium. A total of 435 Salmonella isolates were obtained from Poultry house environmental samples tested during a 20-month period representing a prevalence of 5.5%. Of these, 313 (72%) isolates were identified as Salmonella serogroup B isolates. These isolates were tested by a PCR-based assay, and for resistance to five antibiotics: ampicillin, chloramphenicol, streptomycin, sulfonamides, and
tetracycline for the rapid identification of *Salmonella typhimurium*. Upon comparing the antibiotic resistance and PCR results with serotype and phage type data, the sensitivity and specificity for the identification of *Salmonella typhimurium* of both methods were found to be 100%, and 99.6%, respectively. Both methods can be completed within 24 hours after obtaining an isolate, while serotyping and phagetyping required more than 5 days to complete.

Richard *et al.* (2004) observed that internal contamination of eggs laid by hens infected with *Salmonella enteritidis* has been a prominent international public health issue since the mid-1980s. Considerable resources have been committed to detecting and controlling *Salmonella enteritidis* infections in commercial laying flocks. Recently, the Centers for Disease Control and Prevention also reported a significant association between eggs or egg-containing foods and *Salmonella heidelberg* infections in humans. The present study sought to determine whether several *Salmonella heidelberg* isolates obtained from egg-associated human disease outbreaks were able to colonize reproductive tissues and be deposited inside eggs laid by experimentally infected hens in a manner similar to the previously documented behavior of *Salmonella enteritidis*. In two trials, groups of laying hens were orally inoculated with large doses of four *Salmonella heidelberg* strains and an *Salmonella enteritidis* strain that consistently caused egg contamination in previous studies. All five *Salmonella* strains (of both serotypes) colonized the intestinal tracts and invaded the livers, spleens, ovaries, and oviducts of inoculated hens, with no significant differences observed between the strains for any of these.
parameters. All four *Salmonella heidelberg* strains were recovered from the interior liquid contents of eggs laid by infected hens, although at lower frequencies (between 1.1% and 4.5%) than the *Salmonella enteritidis* strain (7.0%).

Elson *et al.* (2005) recorded the epidemiological investigations of the unusual number of *Salmonella enteritidis* outbreaks associated with the use of eggs in catering premises in England and Wales during 2002. The aims of the study, performed between April and May 2003, were to establish the rate of Salmonella contamination in raw shell eggs from catering premises, investigate any correlation between the origin and type of eggs and the presence of particular serotypes or phage types (PTs) of Salmonella, and examine the use of raw shell eggs in catering premises in the United Kingdom. A total of 34,116 eggs (5,686 pooled samples of six eggs) were collected from 2,104 catering premises, most of which were eggs produced in the United Kingdom (88%). Salmonella was isolated from 17 pools (0.3%) of eggs. The Salmonella contamination rate of eggs produced in the United Kingdom appears to have decreased significantly since 1995 and 1996.
Isolation and Characterization of *Salmonella enteritidis* and *Salmonella typhimurium*

Bokanyi et al. (1990) conducted a study to determine and characterize Salmonella contamination on ready-to-cook broilers or parts in the Columbus, metropolitan area. Ten to twelve samples per store were examined, using a whole-carcass rinse method. The Salmonella cultures isolated were tested for resistance to a series of nine drugs and, subsequently, were sent to the National Veterinary Services Laboratory at Ames, for serotype identification. Cultures identified as *Salmonella* were also examined for the presence of plasmids. Salmonella was isolated from one or more samples obtained from 11 of the 12 stores, and from 43% of the 142 samples examined. The serotypes isolated most often were *Salmonella hadar*, *Salmonella heidelberg*, and *Salmonella johannesburg*. Of the 55 cultures tested for drug resistance, 32.7% were sensitive to all nine drugs. The most-common patterns of drug resistance were triple sulfa (41.8% of the cultures) and tetracycline (34.5% of the cultures). Plasmids were found in 41.7% of the 36 cultures analyzed. The results of the present study indicate that the probability is high that carcasses from retail stores will have at least a few drug-resistant Salmonella cells.

Fantasia et al. (1991) reported that *Salmonella enteritidis* accounted for 5.45% of the 118,685 Salmonella isolates from man and for 2.65% of the 3,315 Salmonella isolates from food in Italy in the eleven year period 1978 to 1988. In the years 1978-1982 no *Salmonella enteritidis* strain was isolated from eggs
and Poultry; in the years 1983-1988 the 53% of *Salmonella enteritidis* isolates from food were from eggs and Poultry. In 1989 *Salmonella enteritidis* accounted for 744 isolates from man and 22 from food of which 80% were from eggs and Poultry (partial data). In that year 18 outbreaks caused by *Salmonella enteritidis* were reported to the National Centre of Enteric Pathogens in Rome. Characteristics of 81 *Salmonella enteritidis* isolates were examined of which 27 were from sporadic cases involving humans and 40 from outbreaks in humans; 14 isolates were from food, all but one connected with the outbreaks.

Bailey (1993) completed the primary mission of the USDA (United States department of Agriculture), Agricultural Research Service, Poultry Microbiological Safety Research Unit is to develop technology preventing commensal intestinal colonization of chickens by human bacterial enteropathogens, including *Salmonella* and *Campylobacter*. The different mechanisms of colonization and the wide variety of sources of these pathogens combined with the different factors that affect host susceptibility to colonization indicate that a multifaceted research approach will be required to control *Salmonella* and *Campylobacter* during Poultry production. The working hypothesis is that only by delivering chickens free of *Salmonella* or *Campylobacter* to the processing plant can cause significant reductions in the number and levels of contamination of chickens leaving the plant with these pathogens. As the means are developed to substantially reduce or eliminate the intestinal colonization of chickens by these pathogens, the pathogen-free
chickens can be delivered to the processing plant, thereby eliminating or at least reducing the prevalence and levels of Salmonella on processed broilers.

Novak et al. (1993) recorded that culture of Postmortem material from chicks and broilers and cloacal swabs from broilers and layers yielded Salmonella from 457 of 7746 samples in 1990, the 457 isolates included 164 Salmonella enteritidis (35.9% of the isolates), 149 Salmonella typhimurium and 73 Salmonella Infantis. In 1991, there were 880 Salmonella isolates from 6879 samples; 447 Salmonella enteritidis (50.8% of the isolate), 139 Salmonella typhimurium and 110 Salmonella Infantis. In 1992 there were 715 (72.4%) Salmonella enteritidis, 173 Salmonella typhimurium and 80 Salmonella Infantis in the 988 isolates.

Brito et al. (1995) reported that day-old chicks were inoculated either via the feed or by direct oral inoculation with Salmonellae which were either invasive or non-invasive (serotypes typhimurium and kedougou, respectively). Colonization of the alimentary tract and visceral organs, determined by microbiological examination, occurred more quickly in birds inoculated orally with Salmonella serotype typhimurium compared with feed-challenged birds. By contrast, Salmonella serotype kedougou remained confined to the alimentary tract. In birds inoculated either orally or via the feed, Salmonella serotype typhimurium, but not serotype Kedougou, was identified in the lamina propria of the caecum by immunostaining. Electron microscopic examination confirmed that the organisms were within macrophages.
Cast and Benson (1996) recorded that phage type 4 *Salmonella enteritidis* strains have only recently been isolated from Poultry and humans in the United States, although this phage type predominates in many other countries. The present study assessed the ability of *Salmonella enteritidis* isolates of various phage types found in the United States (including phage type 4) to colonize the intestinal tract and invade to reach internal organs in experimentally infected chicks. Groups of 5-day-old single-comb white leghorn chicks were inoculated with a range of oral doses of three phage type 4 isolates and three isolates of other phage types. Although some significant differences were observed between individual *Salmonella enteritidis* isolates in the frequencies at which they colonized the intestinal tracts and invaded to reach the spleens of inoculated chicks, no consistent overall pattern differentiated phage type 4 isolates from isolates of other phage types.

Taunay et al. (1996) recorded from 1950 to 1990 a total of 45,862 strains (31,517 isolates from human sources, and 14,345 of non-human origin) were identified at Instituto Adolfo Lutz. No prevalence of any serovars was seen during the period 1950-66 among human source isolates. Important changing pattern was seen in 1968, when *Salmonella typhimurium* surprisingly increased becoming the prevalent serovar in the following decades. During the period of 1970-76, *Salmonella typhimurium* represented 77.7% of all serovars of human origin. Significant rise in *Salmonella agona* isolation as well as in the number of different serovars among human sources strains were seen in the late 70’ and the 80’s. More than one hundred different serovars were identified among non-
human origin strains. Among serovars isolated from human sources, 74.9%, 15.5%, and 3.7% were recovered from stool, blood, and cerebrospinal fluid cultures, respectively. This evaluation covering a long period shows the important role of the Public Health Laboratory in the surveillance of salmonellosis, one of the most frequent zoonoses in the world.

Hogue et al. (1997) observed that the isolation rate for Salmonella enterica serotype enteritidis (SE) in humans in the United States of America (USA) increased from 1,207 sporadic isolates identified in 1976 (0.6 isolates/100,000 population) to 10,201 identified in 1995 (4.0/100,000 population). The proportion of reported Salmonella isolates which were SE increased from 5% to 25% during the same time period. In 1990, 1994, and 1995, SE was the most commonly reported Salmonella serotype in the USA. Much of this increase has been associated with the consumption of contaminated shell eggs.

Henzler et al. (1998) analyzed data for 60 Poultry flocks voluntarily enrolled in the Pennsylvania Salmonella enteritidis Pilot Project and determine management and environmental risk factors associated with production of Salmonella enteritidis contaminated eggs. Sixty flocks for which at least one environmental sample (manure or egg-handling equipment) was positive for Salmonella enteritidis. For this purpose, samples of manure, egg-handling equipment, and mice were submitted for bacterial culture of Salmonella enteritidis. When Salmonella enteritidis was isolated from environmental samples, 1,000 eggs were collected from the flock every 2 weeks for 8 weeks
and submitted for bacterial culture. Eighteen flocks were found to have produced contaminated eggs. Estimated overall prevalence of contaminated eggs was 2.64/10,000 eggs produced, but flock-specific prevalence ranged from 0 to 62.5/10,000 eggs. Flocks with high levels of manure contamination were 10 times as likely to produce contaminated eggs as were flocks with low levels. However, 5 flocks with low levels of manure contamination produced contaminated eggs.

Telo et al. (1998) recorded that 80 samples of imported Poultry meat were investigated for the presence of Salmonella strains during the 1st quarter of 1997. Salmonella species were detected in 10 out of 80 Poultry meat samples (12.5) Further sero-typing of isolated strains was performed with Salmonella monovalent anti-sera. Salmonella enteritidis was the most frequent strain encountered (3 out of a sero-typed strains).

Al-Nakhli et al. (1999) described the source and prevalence of pathogenic Salmonella serovars among Poultry farms in Saudi Arabia. A total of 1,052 (4%) Salmonella isolates were recovered from 25,759 samples of Poultry (broilers, layers, broiler breeders and layer breeders) and Poultry environments (box liner, litter, drag swab, droppings, mice and feed) were examined bacteriologically between 1988 and 1997 at the Poultry Disease Laboratory at the National Agriculture and Water Research Center in Riyadh. Eleven Salmonella serogroups representing 38 different Salmonella serovars were identified by means of antigenic analysis. The majority of the 276 isolates (26.2%) of Salmonella typed, were recovered from liver, heart and intestines of
the broilers and layers. The most prominent Salmonella serogroups isolated were as follows: serogroup C1 (392 isolates, 37.26%), B (289 isolates, 27.47%) and D1 (269 isolates, 25.69%). *Salmonella enteritidis* (85 isolates, 98.8%), *Salmonella virchow* (48 isolates, 57.8%), *Salmonella paratyphi* B var. Java (41 isolates, 57.7%) and *Salmonella Infantis* (30 isolates, 20.6%) were distributed the most widely as all were encountered in Poultry and in Poultry environments.

Alexandre *et al.* (2000) described that *Salmonella enteritidis* is a frequent cause of diarrhea, and is transmitted mainly by *Salmonella enteritidis* contaminated eggs or Poultry meat. The frequency of *Salmonella enteritidis* contaminated eggs or chicken meat and the risk for acquiring this pathogen is unknown in Chile. To measure *Salmonella enteritidis* contamination in eggs Poultry meat and entrails offered in retail markets in the Metropolitan Area during two consecutive years (1998-1999). Samples were placed in sterile bags and transported to the laboratory before 4 hours at 4°C. Microbiologic detection was done using a standard procedure and an immuno-detection assay. *Salmonella enteritidis* was found in one of 1081 egg samples (0.09%). The contaminated sample was offered in a supermarket under their own commercial name. Six percent of 1154 Poultry meat samples were contaminated by *Salmonella enteritidis* and 2.3% by other *Salmonella* serotypes. Eggs and other avian products are contaminated by different *Salmonella enteritidis* phagotypes and other Salmonella serotypes, implicating a risk for the consumers.

Chang (2000) conducted to determine the presence of *Salmonella* species in raw broilers and shell eggs in Korea. In total, 135 dozen shell eggs
and 27 raw broilers were tested. Salmonella species were detected in 25.9% of raw broilers, and Salmonella serotypes isolated from raw broilers were *Salmonella enteritidis, Salmonella virchow, and Salmonella virginia*. D-values (death values) and antibiotic resistance of Salmonella isolates were also investigated. D-values of *Salmonella enteritidis, Salmonella virginia*, and *Salmonella virchow in tryptic soy broth* at 55°C were 2.36, 2.13, and 0.70 min and 0.53, 0.37, and 0.20 min at 60°C, respectively. All Salmonella isolates showed multiple antibiotic resistance patterns and were resistant to *penicillin* and *vancomycin*. One strain of *Salmonella enteritidis* showed resistance to 12 antibiotics used in this study.

Raja *et al.* (2000) examined *Salmonella typhimurium* isolates type 104 (DT 104) from feed ingredients or Poultry sources from different geographical locations in Minnesota during 1995-1997. Antibiotic susceptibility studies indicated that 15 of 50 isolates of *Salmonella typhimurium* had an antibiotic resistance pattern (ampicillin, *chloramphenicol*, streptomycin, sulphonamides and tetracycline), that is usually observed with multi* drug resistant* *Salmonella typhimurium* DT 104. Out of the 15 isolates showing the antibiotic resistance pattern, 8 isolates were phage type 104, 3 isolates were typed as phage type 104 complex and remaining 4 isolates belonged to phage types 193, 81 and 126.

Levine *et al.* (2001) conducted microbiological testing programme for ready to eat meat and Poultry products in 1800 samples. All samples were
collected at production facilities and not at retail. The cumulative 3 year Salmonella prevalence for dry and semidry fermented sausages was 1.43%.

Patrick et al. (2001) isolated and characterized member of the family Enterobacteriaceae from the gallbladder pus of a food handler. Conventional biochemical tests suggested Salmonella enterica serotype Typhi, but the isolate agglutinated with poly(O), 2O, 9O, and Vi Salmonella antisera but not with poly(H) or any individual H Salmonella antisera. 16S rRNA gene sequencing showed that there were two base differences between the isolate and Salmonella enterica serotype Montevideo, four base differences between the isolate and serotype typhi, five base differences between the isolate and Salmonella enterica serotype typhimurium, and six base differences between the isolate and Salmonella enterica serotype Dublin, indicating that the isolate was a strain of Salmonella enterica. Electron microscopy confirmed that the isolate was aflagellated. The flagellin gene sequence of the isolate was 100% identical to that of the H1-d flagellin gene of serotype Typhi. Sequencing of the rfbE gene, which encoded the CDP-tyvelose epimerase of the isolate, showed that there was a point mutation at position +694 (G→T), leading to an amino acid substitution (Gly→Cys). This may have resulted in a protein of reduced catalytic activity and hence the presence of both 2O and 9O antigens. They therefore concluded that the isolate was a variant of serotype Typhi. Besides antibiotic therapy and cholecystectomy, removal of all stones in the biliary tree was performed for eradication of the carrier state.
Schrank et al. (2001) attempted the rapid detection of Salmonella enterica. They have coupled a culture procedure with PCR amplification of the genus-specific invE/invA genes. The method was applied to different kinds of samples from the Poultry industry and evaluated by using hydrolyzed feather meal, meat meal, litter and viscera, all experimentally inoculated with a known number of Salmonella followed by cultivation in selenite–cystine broth prior to the PCR reaction. The expected 457 bp specific DNA fragment could be amplified from dilutions containing as few as 5.7 cfu, indicating that the PCR technique can be successfully coupled with culture in an enrichment broth to distinguish Salmonella species from other enteric bacteria present in samples from the Poultry industry. Tetrathionate broth proved to be a much better enrichment media compared to selenite-cystine when the presence of Salmonella was evaluated by PCR in 1-day-old chicks experimentally infected with known numbers of Salmonella. Samples included cecal tonsils and viscera, collected at 48 hours and 7 days post-infection. The PCR technique was more sensitive in detecting infected animals than the standard microbiological procedure, which detected only 47% of all PCR positive samples.

White et al. (2001) noted that Salmonella is a leading cause of food borne illness. The emergence of antimicrobial resistant Salmonella is associated with the use of antibiotics in animals raised for food, resistant bacteria can be transmitted to humans through foods, particularly those of animal origin. They identified and characterized strains of Salmonella isolated from ground meats purchased in the Washington, D.C. area. Salmonella was
isolated from samples of ground chicken, beef, turkey and pork purchased at 3 super markets. The isolates were characterized by serotyping, antimicrobacterial testing, phage typing and pulsed field gel electrophoresis and also by PCR. It was found that out of 200 meat samples, 41 samples contain Salmonella with 13 serotypes of Salmonella in which Salmonella enteritidis and Salmonella typhimurium also present. It was also observed that resistant strains of Salmonella are common in retail ground meats.

Heyndrick et al. (2002) collected data on the prevalence of Salmonella at different stages during the life cycle of 18 broiler flocks on different farms as well as during slaughter in different Poultry slaughterhouses. For the isolation of Salmonella, the highest sensitivity (93.9%) was obtained by enrichment in the semi-solid agar Diasalm agar. Ten of the 18 flocks received a Salmonella positive status with the highest shedding occurring during the first 2 weeks of rearing. Significant associations were found between the contamination level of a flock and hygiene of the broiler house, feed and water in the broiler house and both animal and non-animal material sampled in the environment. No correlation was found between contamination during the rearing period and contamination found after slaughtering. Improved hygiene management during transport of broilers and in some slaughterhouses could significantly reduce the risk of Salmonella contamination of Poultry meat.

Roy et al. (2002) isolated five hundred and sixty-nine Salmonellae out of 4745 samples from and Poultry environment in 1999 and 2000 from the Pacific northwest. These Salmonellae were identified to their exact source, and some
were serogrouped, serotyped, phage typed, and tested for antibiotic sensitivity. Food product samples tested included rinse water of spent hens and broilers and chicken ground meat. Poultry environment samples were hatchery fluff from the hatcheries where eggs of grandparent broiler breeders or parent broiler breeder eggs were hatched and drag swabs from Poultry houses. Diagnostic samples were of liver or yolk sac contents collected at necropsy from the young chicks received in the laboratory. Of these samples tested, 569 were Salmonella positive (11.99%). Ninety-two Salmonella were sero grouped with polyvalent antisera and polymerose chain reaction. It was observed that Salmonella enteritidis and Salmonella typhimurium were found in 5.15% samples along with other Salmonella serotypes.

Tibaijuka et al. (2003) undertaken a cross-sectional study to determine the presence and prevalence of Salmonellae in retail raw chicken meat and giblets (gizzard and liver) in supermarkets in Addis Ababa (Ethiopia). A total of 301 samples (244 chicken meat, 32 gizzards and 25 livers) were collected from 22 randomly selected supermarkets and examined for the presence of Salmonellae. For the isolation and identification of Salmonellae, the technique recommended by the International Organization for Standardization (ISO 6579, 1998) was used. Salmonellae were detected from 54 (17.9%) of the 301 samples examined. Chicken meat and giblet samples in 68.2% (15/22) of the supermarkets were contaminated with Salmonellae. The contamination level of Salmonella was higher in chicken giblets as compared to chicken meat, which were respectively 12.3%, 53.1% and 28.0% in chicken meat, gizzard and liver.
samples. Out of the 54 Salmonella isolates, nine different serotypes were identified: *Salmonella braenderup* (31.5%), *Salmonella anatum* (25.9%), *Salmonella saintpaul* (14.8%), *Salmonella uganda* (11.1%), *Salmonella haifa*, Salmonella group B, *Salmonella rough form* and *Salmonella typhimurium* (each 3.7%) and *Salmonella virchow* (1.8%). The high level of Salmonella contamination of chicken meat and giblets observed in the present study indicated the need in an improvement in the microbiological quality of retail chicken in Ethiopia.

Gast *et al.* (2004) found that *Salmonella enteritidis* in the environment of commercial laying hens is critical for reducing the production of contaminated eggs by infected flocks. In the present study, an inexpensive and portable electrostatic air sampling device was used to collect *Salmonella enteritidis* in rooms containing experimentally infected laying hens. After hens were orally inoculated with a phage type 13a *Salmonella enteritidis* strain and housed in individual cages, air samples were collected 3 times each week with electrostatic devices onto plates of 6 types of culture media (brilliant green agar, modified lysine iron agar, modified semisolid Rappaport-Vassiliadis agar, Rambach agar, XLD agar, and XLT4 agar). Air sampling plates were incubated at 37°C, examined visually for presumptive identification of typical *Salmonella enteritidis* colonies and then subjected to confirmatory enrichment culturing. Air samples (collected using all 6 culture media) were positive for *Salmonella enteritidis* for 3 weeks post-inoculation. Because visual determination of the presence or absence of typical *Salmonella enteritidis* colonies on air sampling
plates was not consistently confirmed by enrichment culturing, the post-enrichment results were used for comparing sampling strategies. The frequency of positive air sampling results using brilliant green agar (66.7% overall) was significantly greater than was obtained using most other media. A combination of several plating media (brilliant green agar, modified lysine iron agar, and XLT4 agar) allowed detection of airborne *Salmonella enteritidis* at an overall frequency of 83.3% over the 3 wk of sampling. When used with appropriate culture media, electrostatic collection of airborne *Salmonella enteritidis* can provide a sensitive alternative to traditional methods for detecting this pathogen in the environment of laying flocks.

Rybolt *et al.* (2004) recorded that the poultry industry is now operating, under increased regulatory pressure following the introduction of the pathogen reduction and hazard analysis critical control point (HACCP) rule in 1996. This new operation scheme has greatly increased the need for on-farm food safety risk management of food borne bacteria, such as Salmonella. Information needed to make informed food safety risk management decisions must be obtained from accurate risk assessments, which rely on the sensitivity of the isolation techniques used to identify Salmonella in the production environment. Therefore, better characterization of the Salmonella isolation and identification techniques is warranted. One new technique, immunomagnetic separation (IMS), may offer a benefit to the Poultry industry, as it has been shown to be efficacious in the isolation of Salmonella from various sample matrices, including some Poultry products. In this work, authors compared the isolation
ability of 4 Salmonella-specific protocols: IMS, tetrahionate (TT) broth, Rappaport-Vassiliadis R10 (RV) broth, and a secondary enrichment (TR) procedure. All 4 methods were compared in 4 different spiked sample matrices: Butterfield’s, Poultry litter, broiler crops, and carcass rinses. IMS was able to detect Salmonella at 3.66, 2.09, 3.06, and 3.97 log10 cfu/mL in Butterfield’s, Poultry litter, carcass rinse, and broiler crop matrices, respectively. For the broiler litter and Butterfield’s solution, there were no (P>0.05).

Takahashi et al. (2004) found that Salmonella infection is the most common cause of foodborne bacterial outbreaks and deaths in the United States. The effectiveness of Salmonella surveillance for detecting outbreaks depends on timeliness of reporting. We evaluated the public health Salmonella surveillance system in King County, Washington, during an outbreak and at baseline. They assessed the timeliness of the Salmonella surveillance system in King County using the Public Health-Seattle and King County Laboratory (County PHL) database from 1998 to 1999. They determined median days for key steps involved in the Salmonella identification and reporting process and the percentage of suspected Salmonella isolates confirmed. Time intervals for key steps during a Salmonella outbreak were compared to baseline surveillance: Of the 652 suspected Salmonella isolates sent to the County PHL from 22 clinical laboratories, 617 (94.6%) were confirmed as Salmonella. Salmonella confirmation rates improved from 1998 to 1999, and 41% of the submitting laboratories, contributing 32.4% of the isolates, had 100% confirmation rates for both years. The median total identification time during the
outbreak did not differ significantly from baseline (13 days vs 17 days). The time interval for serotyping contributed most to the total identification time.

Orji et al. (2005) carried out a survey of Salmonella contamination of Poultry droppings used as manure, retail fresh beef, fresh beef retailers’ aprons and fresh beef retail tables. In this regard a total of 120 samples of Poultry droppings collected from five Poultry farms, 96 fresh beef samples, 96 beef retailers’ aprons and 96 fresh beef retail tables were examined for the presence of Salmonella species. Different Salmonella serotypes were isolated from all the sources. The serotypes isolated from the sources included Salmonella typhimurium, Salmonella enteritidis, Salmonella gallinarum, Salmonella pullorum, Salmonella typhi and Salmonella agama. Salmonella typhi was not isolated from poultry droppings throughout the survey. They concluded that there is a need to create more environmental and personal hygiene awareness among the Nigerian population, especially among food vendors.

Parsons et al. (2005) MADE a comprehensive review of both the scientific literature and industry practices to identify and quantify all sources of contamination throughout the entire Poultry meat production chain by Salmonella spp. This information was used to develop a quantitative risk assessment (QRA) model for Salmonella in the production chain from the breeder farm to the chilled carcass. This was subsequently used as the basis on which to compare the merits of three approaches to QRA modelling in such systems. The original model used a Bayesian Network (BN). The second method was a Markov chain Monte Carlo (MCMC) approach, The third method
was a more detailed simulation model. Finally, the simulation offers greater flexibility, such as consideration of the individual carcass, but may be more complex to implement as a result and sacrifices the ability to propagate evidence.
Calculation of LD$_{50}$

Williams et al. (1980) inoculated orally, 16 *Salmonella typhimurium* strains belonging to 12 phage types varied greatly in their ability to kill 1-day-old chickens; variation was noted even between strains of the same phage type. Fourteen strains belonging to 11 food poisoning serotypes other than *Salmonella typhimurium* were practically non-lethal when examined in this manner. All of them were lethal by the intramuscular route but some were more so than others. Two were more lethal by this route than one of the *Salmonella typhimurium* strains that was highly lethal when given orally. With age, chickens rapidly became resistant to fatal infection with the food poisoning strains; given orally, a *Salmonella typhimurium* strain killed 79% of 1-day-old chickens but only 3% of 2-day-old chickens. Of 2 specific poultry pathogenic strains, one, of *Salmonella gallinarum*, was lethal by oral inoculation to chickens of all ages but the other, of *Salmonella pullorum*, was only lethal to very young ones. Some salmonella strains, such as those of *Salmonella infantis* and *Salmonella menston*, were more efficient at infecting and colonizing the alimentary tract of chickens than were the more virulent *Salmonella typhimurium* strains, the *Salmonella gallinarum* and *Salmonella pullorum* strains and a *Salmonella cholerae-suis* strain.

Barrow et al. (1987) examined the virulence of *Salmonella typhimurium* strains for day-old chickens. The mortality following oral inoculation varied from 0 to 100 per cent. Some breeds were more susceptible than others. There was
no correlation between oral and parenteral virulence. Pathogenesis studies associated with one of the most virulent strains suggested that, after invasion, organisms multiplied in the liver and spleen and spread to other organs producing a systemic infection. The cause of death was probably a combination of anorexia and dehydration resulting from general malaise and diarrhoea. A virulent strain studied in detail spread through the body faster, persisted for a longer period and was more invasive than an avirulent strain. In the system studied invasiveness was the virulence determinant of overriding importance.

Barrow et al. (1987) reported that four strains of *Salmonella gallinarum* isolated from independent cases of fowl typhoid all possessed both an 85-kilobase and a 2.5-kilobase plasmid. Each plasmid was eliminated in turn from one of the strains by transposon labeling and curing at 42°C. Elimination of the small plasmid had no effect on the high virulence of the strain for newly hatched and 2-week-old chickens. Whereas oral inoculation of 2-week-old chickens with the parent strain produced 90% mortality with characteristic signs of fowl typhoid, inoculation of the large-plasmid-minus strain produced 0% mortality. A corresponding increase in the 50% lethal dose from log₁₀ 1.1 to greater than log₁₀ 7.3 was seen with the large-plasmid-minus strain after intramuscular inoculation. Reintroduction of the large plasmid completely restored virulence. A role for the plasmid-linked virulence genes in both invasion and growth in the reticuloendothelial system is suggested by the failure of the large-plasmid-minus strain to penetrate to the liver and spleen after oral inoculation and by its increased clearance from the reticuloendothelial system after intravenous
inoculation. These results clearly demonstrate that the large plasmid of Salmonella gallinarum contributes toward virulence in fowl typhoid of chickens.

Suzuki (1994) recorded Salmonella enteritidis is a common pathogen of all species of mammals and fowls. The recent increase in the number of outbreaks of food poisoning due to Salmonella enteritidis in man was epidemiologically analysed, and it was considered that contaminated eggs or egg products were the major source of this infection. To assist in prevention and eradication of human food poisoning many investigators have studied the pathogenicity of Salmonella enteritidis in Poultry. Gross pathological observations after natural and experimental infections with Salmonella enteritidis in Poultry revealed that this organism may cause systemic infection in chicks and laying hens accompanied by prolonged faecal shedding. Some variations in the mortality rates, clinical symptoms, faecal shedding and frequency of production of contaminated eggs were observed in the chicks and hens experimentally infected with Salmonella enteritidis isolates. Choice of bacterial strain, phage type, age of bird and inoculum size may affect the outcome of an infection. Moreover, isolation of the organisms from the ovaries, oviducts and egg contents indicates the possibility of transovarian infection of Salmonella enteritidis in chickens. Some virulence factors associated with Salmonella enteritidis are also reviewed in the present paper.

Gast and Benson (1995) studied the Phage type 4 Salmonella enteritidis has been associated with morbidity and mortality in broiler chickens in the United Kingdom. The recent isolation of this phage type from poultry in the
United States has raised concerns about whether the current regulatory approach to *Salmonella enteritidis* should be modified to consider phage type 4 differently from other phage types. The present study assessed and compared the virulence of phage type 4 *Salmonella enteritidis* isolates, *Salmonella enteritidis* isolates of other phage types, and an *Salmonella pullorum* isolate in both single-comb white leghorn and white Plymouth Rock chicks. The mean incidence of severe illness or death following oral inoculation with phage type 4 *Salmonella enteritidis* was significantly lower than the incidence associated with *Salmonella pullorum* inoculation in both lines of chicks. Nevertheless, some individual phage type 4 *Salmonella enteritidis* isolates caused severe effects at a frequency similar to that of *Salmonella pullorum* in single-comb white leghorn chicks. In general, severe morbidity or mortality following infection with *Salmonella enteritidis* isolates of all phage types tested occurred more often in single-comb white leghorn chicks than in white Plymouth Rock chicks. The mean frequency at which chicks were severely affected following inoculation with phage type 4 isolates was significantly higher than the mean for isolates of other phage types. However, in both lines of chicks, some significant differences in virulence were apparent within the set of phage type 4 strains tested. The observed virulence for chicks of recent U.S. poultry isolates of phage type 4 *Salmonella enteritidis* was similar to that of earlier isolates from various sources, including poultry isolates from the United Kingdom.

Guillot *et al.* (1995) determined the susceptibility or resistance of 9 outbred experimental or commercial Poultry lines to *Salmonella enteritidis* PT4.
Young chicks were inoculated either intramuscularly or orally just after hatching. After intramuscular challenge the lines could be divided into susceptible lines (LD 50% < or = $10^2$ Salmonella per animal), intermediate lines (LD 50% about $10^4$ Salmonella) and resistant lines (LD 50% > $10^5$ Salmonella). The results obtained after oral challenge confirmed these 3 groups for both mortality rates and the probability of the presence of Salmonellae in the spleen and liver. There was no difference between lines concerning caecal carriage.

Frances Bowe (1998) reported that *Salmonella typhimurium* infection of mice is an established model system for studying typhoid fever in humans. Using this model, they identified *Salmonella typhimurium* genes which are absolutely required to cause fatal murine infection by testing independently derived transposon insertion mutants for loss of virulence in vivo. Of the 330 mutants tested intraperitoneally and the 197 mutants tested intragastrically, 12 mutants with 50% lethal doses greater than 1,000 times that of the parental strain were identified. These attenuated mutants were characterized by in vitro assays which correlate with known virulence functions. In addition, the corresponding transposon insertions were mapped within the *Salmonella typhimurium* genome and the nucleotide sequence of the transposon-flanking DNA was obtained. *Salmonella* spp. and related bacteria were probed with flanking DNA for the presence of these genes. All 12 attenuated mutants had insertions in known genes, although the attenuating effects of only two of these were previously described. Furthermore, the proportion of attenuated mutants obtained in this study suggests that mutations
in about 4% of the *Salmonella* genome lead to 1,000-fold or greater attenuation in the mouse typhoid model of infection. Most of these genes appear to be required during the early stages of a natural infection.

Dhillon *et al.* (1999) reported that four hundred fifty day-old Hubbard broiler chicks were subdivided into 15 groups of 30 chicks each. Six groups of chicks received 0.5 ml of broth culture containing $5 \times 10^6$ colony-forming units (CFU) of *Salmonella enteritidis* phage types (PTs) 4, 8, and 23 by crop gavage. Similarly, six other groups received 0.5 ml containing $5 \times 10^8$ CFU of *Salmonella enteritidis*. One group was inoculated with 0.5 ml containing $5 \times 10^6$ CFU of *Salmonella pullorum*, and another group received 0.5 ml containing $5 \times 10^8$ CFU of *Salmonella pullorum*. A group of 30 chicks were kept as uninoculated controls. Chicks were observed daily for clinical signs and mortality. All birds were weighed at 7, 14, and 21 days post-inoculation 21 (DPI). Four chicks were randomly selected from each treatment group, euthanatized, and necropsied at 7 and 14 DPI. Gross lesions were recorded and selected tissues were collected for histopathology. The higher rates of illness and mortality were observed in chicks inoculated with $5 \times 10^6$ and $5 \times 10^8$ CFU of *Salmonella pullorum*, followed by *Salmonella enteritidis* PT4 of human origin and *Salmonella enteritidis* PT4 of chicken origin. Moderate to high mortality was observed in chicks inoculated with the higher dose of SE isolates that belonged to PT8 and one *Salmonella enteritidis* of PT23. Variable mortality was evident in groups inoculated with the lower dose of salmonella. The most consistent gross and histopathologic changes, including fibrinous pericarditis
and perihepatitis, were seen in the dead birds from various treatment groups. The lower mean body weights were present in all treatment groups compared with uninoculated controls. No illness or mortality was observed in uninoculated control groups.

Allen et al. (2001) stated that multi drug-resistant *Salmonella enterica serovar typhimurium* phage type DT104 has become a widespread cause of human and other animal infection worldwide. The severity of clinical illness in *Salmonella enterica serovar typhimurium* DT104 outbreaks has led to the suggestion that this strain possesses enhanced virulence. In the present study, in vitro and in vivo virulence-associated phenotypes of several clinical isolates of *Salmonella enterica serovar typhimurium* DT104 were examined and compared to *Salmonella enterica serovar typhimurium* ATCC 14028s. The ability of these DT104 isolates to survive within murine peritoneal macrophages, invade cultured epithelial cells, resist antimicrobial actions of reactive oxygen and nitrogen compounds, and cause lethal infection in mice were assessed. Their results failed to demonstrate that *Salmonella enterica serovar typhimurium* DT104 isolates are more virulent than *Salmonella enterica serovar typhimurium* ATCC 14028s.

Smith et al. (2002) reported that the possibility of non-culturable cells of a normally culturable bacterial pathogen may constitute a source or reservoir for infective disease was investigated. In multiple experiments and with careful attention to the statistical limitations of the assays used, *Salmonella enterica serovar typhimurium* cells rendered non-culturable by carbon and nitrogen
stress in the presence of chloramphenicol were administered orally and intraperitoneally to over 300 female BALB/c mice. Neither infection nor colonization was detected in these studies, even when active but non-culturable (ABNC) cells, as defined by the Kogure cell elongation assay, were present in the inoculum. Doses of ABNC cells exceeding the oral and intraperitoneal LD(50) values by 3.5 and 2 orders of magnitude, respectively, were administered. It was concluded that ABNC cells of the Salmonella strains used could not be considered potentially infective and that their detection in samples from material being evaluated as a potential source or reservoir of infection by the Kogure test does not specifically represent an infective hazard.
Identification of Salmonella species from small and large intestine of poultry (Carrier States)

Rigby (1980) stated that cultural monitoring was used to determine the incidence and sources of salmonellae in a 4160-bird broiler flock raised on litter in 32 pens. Twenty-five of the pens remained apparently free of salmonellae during the 49-day growing period. Salmonella johannesburg, first detected in the meat meal component of the starter ration, was recovered from the litter of seven pens and from the intestines of dead or culled chicks from two pens. Salmonella alachua was also recovered from two of these pens. Culture of swabs collected from the plastic crates used to transport this flock for processing showed that 97/112 (86.6%) were contaminated with salmonellae (15 serovars) before the birds were loaded. The crate washer at the plant did not remove salmonellae from these crates: 97/132 (73.5%) crates sampled after washing yielded salmonellae. Eleven serovar were recovered, including Salmonella johannesburg and Salmonella alachua introduced by the infected flock. Twelve of 31 chickens (38.7%) collected when the birds were unloaded at the processing plant were intestinal carriers of Salmonella johannesburg and Salmonella alachua and 29 (93.5%) were external carriers. Salmonella johannesburg, Salmonella alachua and four other serovars were isolated from the feathers of these birds. Eleven of 25 (44%)-carcasses tested from this flock yielded salmonellae. Salmonella johannesburg or Salmonella alachua, first isolated from the infected flock, were recovered from five carcasses and
Salmonella haardt and Salmonella typhimurium, first isolated from the transport crates were recovered from six carcasses.

Ruff and Wilkins (1980) taken in vitro absorption of glucose and L-methionine in the intestine of broiler chickens was measured 7, 14 and 21 days post-inoculation with sporulated oocysts of Eimeria acervulina, E. mivati, E. maxima or E. brunetti. The small intestine of each bird was divided into 8 regions of equal length and absorption was measured on 3 tissue disks of equal size from each region. The absorption rate of each substrate with each coccidial species was measured based on (1) an equal area from each region, (2) an equal weight from each region, (3) the total absorption in each region, and (4) the total potential absorption in the intestine. Comparisons of absorption rate of equal areas in each intestinal region demonstrated that infected birds at 7 days post infection absorbed significantly less substrate per unit area in the regions of maximum infection than uninfected controls. Malabsorption was less apparent when the weight of the region was used as the unit of measurement. Compensatory absorption was seen in some uninfected regions with E. acervulina. The total potential intestinal absorption at 7 days post infection was reduced with E. mivati, E. maxima and E. brunetti but not with E. acervulina. At 14 days post infection, total L-methionine and glucose absorption in some regions of the intestine was significantly increased with E. acervulina but not with E. mivati, E. maxima or E. brunetti. No absorption differences were seen at 21 days post infection with any species.
Goren (1984) recorded in two experiments newly hatched broilers were orally inoculated either with intestinal microfloras cultured under different conditions or with a suspension of intestinal homogenate and challenged with high doses (3 X 10^5 c.f.u. per chick) of *Salmonella infantis* organisms. Inocula were prepared from intestinal material of mature SPF WL hens under aerobic or strictly anaerobic conditions (less than 5 ppm oxygen), and protected against atmospheric oxygen during storage. A very significant reduction of the number of *Salmonella infantis* bacteria in caecal content was achieved one and two weeks after challenge in those groups of chicks which were inoculated with anaerobically cultured flora or with intestinal homogenate. In order to induce protection, obligate anaerobes had to be accompanied by facultative anaerobes. Amprolium or Monensin did not interfere with protection. Protection induced by intestinal microflora depends on anaerobiosis during preparation, culturing, storage, and application of the protective bacteria. Starvation on the first day of life appeared to have a negative effect on protection.

Goren (1984) studied the efficacy of spray application of intestinal microflora of the adult bird in protection of broilers against salmonella infections in 3 experiments under laboratory and field conditions. In chicks treated soon after hatching (in the hatchers at approximately 30 per cent hatch or in the chick delivery boxes in the hatchery) with crop-caecum homogenate or a mixture of aerobically and strict anaerobically cultured intestinal flora, very good results were achieved. A very obvious protective effect against a high infection dose (3 X 10^6 c.f.u. *Salmonella infantis* bacteria per chick) and complete protection
against natural infection with different salmonella types was induced. A significant improvement of growth rate was observed in broilers treated by spray in the hatchery and reared for 7 weeks under field conditions in an environment heavily contaminated with different salmonella types.

Clarke and Gyles (1987) tested a ligated intestine model in calves, pigs, and rabbits for its value as an indicator of virulence of potential vaccine strains of *Salmonella typhimurium*. A wild virulent strain (3860C), a laboratory strain LT2, and mutants of these 2 strains were evaluated. Inoculation of calf intestinal segments with strain 3860C revealed that fluid responses were greatest in the proximal portion of the small intestine and that doses greater than 10(7) organisms were required to produce fluid responses and mucosal damage. Immunoperoxidase-stained sections of intestine revealed that a large dose of Salmonella organisms was required before mucosal invasion could be detected. Aromatic (aroA), galactose epimerase (galE), and diaminopimelic acid (dap) mutants of strain 3860C all resulted in much less fluid response, mucosal invasion, and mucosal damage compared with those by the parent organism. Strain LT2 induced such weak responses that it was not possible to evaluate reductions in virulence of its mutants. In 6-week-old pigs, there was no fluid response to any strains; however, in 1-week-old pigs, there was fluid response to the wild strain and some of its mutants. In adult rabbits, fluid responses were not observed, except when the wild strain was inoculated in the proximal portion of the small intestine. The calf and 1-week-old pig models appeared to be best suited for assessment of virulence of mutant strains of *Salmonella typhimurium*.
Hinton (1988) The feed given to young broiler chickens was contaminated artificially with *Salmonella kedougou*, a serotype associated with both subclinical infections in commercially reared chickens and food poisoning in humans. No evidence was obtained to suggest that the growth promoting antibiotic avilamycin, added to the feed at the rate of either 2.5 ppm or 10 ppm, had the undesirable side-effect of favouring the colonization of the intestinal tract of the birds with *Salmonella kedougou* when they were challenged with this organism in the feed.

Kotova *et al.* (1988) demonstrated Salmonella carrier state (42.6% *Salmonella enteritidis* and 34.4% *Salmonella dublin*) in subjects after acute salmonellosis as well as in healthy persons infected with Salmonella as a result of occupational exposure to Poultry (8.8% in humans exposed to chickens and 6.1% in those exposed to ducks) and sheep (2.8%). The carrier state was accompanied by intermittent pain in the epigastrium, diminished appetite, diarrhoea etc. The etiological role of *Salmonella typhimurium* was proved beyond doubt, as well as its ability to cause salmonellosis outbursts, sporadic cases of the disease and the carrier state. When large industrial facilities specializing in Poultry processing were investigated, the Salmonella carrier state was revealed in practically healthy Poultry in 16% of chickens and 12% of ducks.

Oyofo *et al.* (1989) the effect of carbohydrates in the drinking water of broiler chickens on *Salmonella typhimurium* colonization was evaluated. Results indicate that mannose and lactose (2.5%) significantly (P less than
0.05) reduced intestinal colonization of *Salmonella typhimurium* by at least one-half, as compared with dextrose, maltose, and sucrose. Lactose and mannose also significantly reduced (P less than 0.01) the mean log10 number of *Salmonella typhimurium* in the cecal contents. Although mannose was the most effective sugar at blocking colonization, lactose may be more practical because it is effective and costs much less than mannose. Provision of carbohydrates in the drinking water had no significant effect on weight gain.

Hassan (1991) undertaken that four-day-old chickens infected orally with a spectinomycin-resistant (Spcr) mutant of a highly invasive avian *Salmonella typhimurium* strain excreted salmonellae in the feces for at least 10 weeks. When these chickens were reinfected at this time with a nalidixic acid-resistant (Nalr) mutant of the same strain, they excreted this mutant in significantly smaller numbers (P less than 0.01) than did a previously uninfected control group. The Nalr mutant had a shorter survival rate in the tissues of the immunized chickens than in tissues of the control birds. The Spcr mutant stimulated strong IgG, IgA, and IgM responses in serum, small-intestinal contents, and bile. These were detected by enzyme-linked immunosorbent assay (ELISA) against antigens of crude whole bacterial cell protein sonicate, lipopolysaccharide, flagella, and outer-membrane proteins. There was some evidence of an anamnestic response with IgA in bile following reinfection with the Salmonella. The peak response of antibody-producing cells from the spleens of infected chickens, assayed by solid-phase ELISA, occurred at 3 weeks postinoculation. A strong delayed hypersensitivity reaction, detected by
foot-pad swelling after inoculation with either whole-cell or outer-membrane proteins, was observed between 2 and 5 weeks after infection with the Spcr mutant. The data indicate that outer-membrane proteins are major immunogens for both humoral and cell-mediated arms of the immune system.

Craven (1993) determined the ability of eight strains of Salmonella and their agar subcultured variants to colonize the intestinal tract of broiler chicks. Nalidixic-acid (NAL)-resistant and streptomycin-resistant subcultured strains (Salmonella california 1989/A and Salmonella typhimurium 3366/A) that persisted in the ceca of chicks in lower numbers than their NAL-resistant parent strains (1989/O and 3366/O) were selected for additional study Salmonella typhimurium strain 3366/A was present in the ceca of chicks in lower numbers than the parent strain 3366/O when given concomitantly with the parent strain or when the two strains were given separately to different chicks. Salmonella california 1989/A strain was present in the ceca in lower numbers than the parent strain after concomitant oral or intracloacal inoculation. Strains 3366/O and 3366/A of Salmonella typhimurium differed in growth rates in BHI broth and cecal mucus. The lipopolysaccharide (LPS) profile indicated that LPS components present in Salmonella california 1989/O were missing from strain 1989/A. A mutant of 1989/O--2095/R--was also LPS- and colonization-deficient.

Duchet-Suchaux et al. (1995) quantified the carrier state of Salmonella enteritidis in chicks (i.e., persistent asymptomatic association of Salmonella enteritidis with the host). They developed an experimental carrier state model by oral inoculation of low doses of Salmonella enteritidis in chicks at different
ages. Liver, spleen, and ceca colonizations by the challenge strains were measured weekly by enumeration of *Salmonella enteritidis* colony-forming units (CFU) for 7-12 weeks. High mortality rates, incompatible with the carrier state, were observed in chicks inoculated with $10^3$ organisms of either a parental strain of *Salmonella enteritidis* at the age of one day. Both strains colonized organs similarly, allowing us to use subsequently the SmrNalr mutant strain. The selected low doses of *Salmonella enteritidis* induced no deaths in chicks inoculated at 1 or 3 weeks of age.

Gast (1994) reported a significant proportion of human *Salmonella enteritidis* outbreaks in recent years has been traced to the consumption of contaminated eggs produced by infected laying flocks. Experimental SE infections in chickens have been used to acquire a considerable amount of basic information about the interaction between SE and the avian host. After oral or parenteral inoculation of chickens, SE can colonize the intestinal tract, invade and disseminate to reach numerous internal organ sites, and elicit the production of specific antibodies in serum and egg yolks. Experimental infection of laying hens can result in the deposition of SE in the contents of eggs before oviposition, although generally in rather small numbers and at a relatively low frequency. The consequences of experimental SE infection have been shown to vary significantly with the strain and dose of the inoculum. Some SE isolates have been shown to produce various clinical effects, including decreased egg production by hens. The information provided by experimental SE infections in chickens has played an important role in the formulation of appropriate
strategies for reducing the incidence of SE in commercial laying flocks and thereby also reducing the incidence of transmission of SE to consumers via contaminated eggs.

Cooper et al. (1994) Newly hatched chicks were dosed orally with a Salmonella typhimurium wild-type strain, an Salmonella enteritidis wild-type strain, and a genetically defined Salmonella enteritidis aroA vaccine candidate, strain CVL30. The Salmonella typhimurium strain, 2391 NaIr, was virulent in newly hatched chicks and caused deaths in 7 of 20 chicks after an oral dose of 10(5) organisms. The Salmonella enteritidis wild-type strain, LA5, caused death in 1 of 25 chicks and gross pathology including pericarditis and perihepatitis in 6 of the 24 survivors after an oral dose of 10(9) organisms. Salmonella enteritidis aroA CVL30, attenuated by ca. 6.5 log10 in BALB/c mice, was nonvirulent when administered orally to chicks and did not cause morbidity. When newly hatched chicks were dosed, the pattern of invasion and colonization of the reticuloendothelial system by strain CVL30 was similar to that of its parent strain, LA5, irrespective of the dose. Oral inoculation of newly hatched chicks with < 10 organisms of Salmonella enteritidis LA5 or CVL30 was followed by multiplication in the cecal contents. Within 3 days of hatching, the pH of the cecal contents was reduced from ca. 7 to 5. Samples of gut contents were inoculated in vitro. The S. enteritidis strains multiplied in samples taken from the ileum and duodenum irrespective of age but multiplied in the cecal samples from newly hatched chicks only. Invasion from the gut by Salmonella enteritidis LA5 and CVL30 was both age and dose dependent.
Badar Craven (1994) Salmonella typhimurium strain 3333/O was used to assess the role of bacterial lipopolysaccharide (LPS) in intestinal colonization of broiler chicks by salmonellae. LPS-defective TnPhoA mutants of this strain were isolated. The sensitivities of the mutants to smooth and rough phages and LPS banding patterns in sodium dodecyl sulfate-polyacrylamide gel electrophoresis indicated a defect in the polysaccharide side chain of the LPS molecule. Colonization was determined by orally administering 10^8 cells each of the wild-type and/or the mutant strains per chick and counting the colony-forming units (CFU) from the ceca 1 to 3 weeks after gavage. CFU of chicks given the LPS-deficient strains either were not detected or were significantly lower than the CFU from chicks given the wild-type strain. The incidence of the wild-type strain in spleens was higher than incidence of the mutant strains. In vitro binding studies with LPS-deficient mutants derived in this study and from Salmonella typhimurium LT2 suggest that LPS side-chain components may shield the bacterial cell from entrapment in the chicken mucus. The LPS layer appears to enhance persistence of Salmonella in the avian intestinal tract.

Hargis (1995) Much previously published research has focused on the role of cecal and intestinal Salmonella contamination of poultry carcasses within commercial processing plants. Presently, we have evaluated the persistence of experimentally inoculated Salmonella enteritidis in the crops and ceca of commercial broiler chickens during the last week of growth (Weeks 6 to 7) and the presence of crop and cecal Salmonella in 7-wk-old broilers in a commercial processing plant. When broilers were inoculated with 1 x 10^6 cfu S. enteritidis
at 6 weeks of age by oral gavage, the incidence of crop and cecal contamination was equivalent 2 days after challenge (30%), with only 1 of 29 crops contaminated and 0 of 29 ceca contaminated at 7 days following challenge. When broilers were inoculated with $1 \times 10^8$ cfu Salmonella enteritidis at 6 weeks of age by oral gavage, 2 days after challenge the crops and ceca were observed to be 57 and 67% positive for Salmonella enteritidis, respectively. Seven days after inoculation with $1 \times 10^8$ Salmonella enteritidis, the crops and ceca were 37 and 57% positive, respectively, for the challenge organism. At a commercial broiler processing plant, 286 of 550 crops from three flocks were Salmonella-positive, whereas only 73 of 500 ceca from these flocks were contaminated. Furthermore, data from this plant indicated that the crops were far more likely to rupture than ceca (86-fold) during processing, increasing the possibility of carcass contamination with Salmonella derived from crop contents. The results of these studies suggest that the crop may serve as a source of carcass contamination with Salmonella within some processing plants.

Ramirez (1997) Previous research regarding Salmonella contamination in poultry has focused predominantly on cecal and intestinal contamination. Recently, the crop has been implicated as an important source of carcass contamination within the processing plant. In the present study, broiler chickens were orally challenged with $1 \times 10^8$ cfu Salmonella enteritidis at 6 weeks of age. At 7 weeks of age, birds were randomly divided into two groups consisting of full access to feed, or total feed withdrawal, 18 h prior to sample collection. At
the time of sample collection, crops and ceca were aseptically removed and cultured for the presence or absence of *Salmonella enteritidis* by enrichment. The incidence of *Salmonella enteritidis* positive crops was consistently higher (range: 2.8- to 7.3-fold increases) following feed withdrawal than the incidence in samples collected from full-fed broilers in four experiments. Similarly, the incidence of *Salmonella enteritidis* isolation was consistently higher (range: 1.4 to 2.1-fold increases) in ceca following feed withdrawal than in samples collected from full-fed broilers in these experiments. In a subsequent experiment, ceca and crops were aseptically collected and cultured for the presence of *Salmonella* immediately prior to or following 8 h feed withdrawal at a commercial broiler house. Similar to the laboratory experiments, the incidence of *Salmonella* isolation was significantly (*P<0.01*) greater from crops following feed withdrawal (36/100) than from samples obtained immediately prior to withdrawal (19/100). However, the incidence of *Salmonella* in the ceca was not significantly higher following feed withdrawal (31/100) than in samples obtained immediately prior to withdrawal (25/100) in this field experiment. These studies indicate that feed withdrawal increases the incidence of *Salmonella* in broiler crops prior to slaughter and provide further evidence that the crop may be an important critical control point for reducing *Salmonella* contamination of broiler carcasses.

Arthur *et al.* (1998) recorded from a collection of 2800 Tn5-TC1 transposon mutants of *Salmonella typhimurium* F98, 18 that showed reduced intestinal colonization of 3 week old chicks were identified. The sites of
transposon insertion were determined for most of the mutants and included
insertions in the lipopolysaccharide biosynthesis genes rfaK, rfaY, rfbK and rfbB,
and the genes dksA, clpB, hupA, and sipC.

Promsopone (1998) Salmonella typhimurium colonizes the intestinal tract
of poultry and causes food-borne illness in humans. Reduction of Salmonella
*typhimurium* colonization in the intestinal tract of poultry reduces potential
carcass contamination during slaughter. The purpose of this study was to
determine the effect of an avian-specific probiotic and Salmonella *typhimurium*
specific antibodies on the colonization of *Salmonella typhimurium* in broilers
and on body weights. Broiler chicks were spray-vaccinated at the hatchery with
the commercial product, Avian Pac Plus, which contains *Lactobacillus
acidophilus*, *Streptococcus faecium*, and *Salmonella typhimurium*-specific
antibodies. At placement, these chicks were administered Avian Pac plus in the
water. Six hours post-placement, chicks were orally challenged with 1.8 x 10^7
CFU of *Salmonella typhimurium*. Chicks were administered Avian Pac Plus for
two additional days post-challenge. Chicks were evaluated for *Salmonella
typhimurium* colonization and shedding every 3 to 4 days for the first 2 weeks
and every 7 days for 8 weeks. The mean cecal and colonic concentration of
*Salmonella typhimurium* from the Avian Pac Plus-treated group was
significantly lower at day 31 (P = 0.0001), day 38 (P = 0.0005), and day 43 (P =
0.0001) than the non-treated control group. These results indicated that a
combination of *Lactobacillus acidophilus*, *Streptococcus faecium*, and
*Salmonella typhimurium* specific antibodies have a beneficial effect in reducing the colonization of *Salmonella typhimurium* in market-aged broilers.

Tellez (2001) conducted an experiment on the *Salmonella enteritidis* colonizes the intestinal tract of poultry and causes food borne illness in humans. Reduction of *Salmonella enteritidis* colonization in the intestinal tract of poultry reduces potential carcass contamination during slaughter. The purpose of this study was to determine the effect of an avian-specific probiotic combined with *Salmonella enteritidis*, *Salmonella typhimurium* and *Salmonella heidelberg*-specific antibodies on the cecal colonization and organ invasion of *Salmonella enteritidis* in broiler as well as on body weights. The treatment group was defined as chicks spray-vaccinated with Avian Pac Plus at the hatchery and given Avian Pac Plus for the first 3 days after placement. An intermediate treatment was given at 10 and 14 days, 2 days prior to vaccination and 2 days post-vaccination. All birds were vaccinated with Newcastle disease vaccine, La Sota virus (one drop/eye) at 12 days of age. A final treatment was given 3 days pre-slaughter. The control group was defined as chicks not given Avian Pac Plus at any time. Six hours after oral administration of the probiotic suspension (treatment group) or water (control group) at placement, the chicks were challenged with *Salmonella enteritidis*. All chickens were orally inoculated with 0.25 ml of *Salmonella enteritidis* that contained $4 \times 10^{7}$ CFU/1.0 ml. Cecal colonization and organ invasion were evaluated for *Salmonella enteritidis* on days 0, 1, 3, 7, 10, 17, 24, 31, 38, and 41. The probiotic-treated group had a significantly lower concentration of *Salmonella enteritidis* cecal colonization at
days 3, 7, 10, 17, 24, 31, 38, and 41 when compared to the non-treated, control group (P<0.05). Similarly, there was a significant difference (P<0.05) in the isolation of *Salmonella enteritidis* from the internal organs (liver and spleen) when probiotic-treated and non-probiotic-treated groups were compared. There was no significant difference (P>0.05) in the mean body weight between the two experimental groups at each collection period. These results indicated that a combination of *Lactobacillus acidophilus*, *Streptococcus faecium*, and *Salmonella enteritidis*, *Salmonella typhimurium*, and *Salmonella Heidelberg*. Specific antibodies have a beneficial effect in reducing the colonization of *Salmonella enteritidis* in market-aged broilers.

Salvatore Scalzo (2004) determined the effect of continuous in-feed administration of *anticoccidial* agents on antimicrobial sensitivity and the level of bacterial shedding in poultry experimentally infected with *Salmonella enterica* subsp. *enterica* serotype *typhimurium* definitive type 104 (DT104) were investigated. On day 0, 1,200 1-day-old *Salmonella*-free broiler chicks were placed into 50 pens, and the pens were randomly allocated to one of five treatments: non supplemented (negative control; T1), monensin at 120 mg/kg of diet (T2), salinomycin at 60 mg/kg of diet (T3), semduramicin at 20 mg/kg of diet (T4), or semduramicin at 25 mg/kg of diet (T5). Each bird was inoculated with a well-characterized strain of serotype *typhimurium* DT104 on day 10. On day 49, the birds were euthanatized humanely. Bulk fecal samples were collected on days 13, 43, and 48 and were examined for organisms which had acquired resistance. The genetic basis of acquired resistance was determined from
representative samples of isolates. Of 784 *Salmonella*-selective plates supplemented with antimicrobial agents, only 33 showed growth. These isolates came from all treatment regimens, including the non-supplemented control. A number of phenotypic changes were observed; these included changes in motility, phage type, and agglutination properties. Supplementation of the diet with an anticoccidial drug does not appear to affect antimicrobial resistance or the level of excretion of *salmonellae*. Most of the changes observed do not seem to be related to the presence of a supplement in feed. *Salmonellae* appear to be capable of acquiring antimicrobial resistance and phenotypic changes independently of specific antimicrobial selection pressures.

Sadreyen et al. (2004) observed that *Salmonella enterica* serovar *enteritidis* carrier state in Poultry has serious consequences on food safety and public health due to the risks of food poisoning following consumption of contaminated products. An understanding of the mechanisms of persistence of Salmonella in the digestive tract of chicken can be achieved by a better knowledge of the defects in the control of infection in susceptible versus resistant animals. The gene expression of innate immune response factors including anti-microbial molecules, inflammatory and anti-infectious cytokines was studied in the caecal lymphoid tissue associated with the carrier state. Expression levels of these genes were assessed by real-time PCR and were compared in two inbred lines of chickens differing in resistance to the carrier state following oral inoculation of *Salmonella enterica* serovar *enteritidis* at one week of age. A high baseline level of defensin gene expression was recorded in
young animals from the susceptible line. In contrast, a significantly low expression of interferon-gamma (IFN-gamma) gene was observed in these susceptible infected animals in comparison to resistant ones and healthy counterparts.

Van Immerseel (2004) found that in recent years, a dramatic increase in incidence of the dextro-rotatory tartrate-positive variant (dT+) of Salmonella enterica subspecies enterica serovar Paratyphi B has been observed in poultry and poultry products. In the present study the interactions of this bacterium with the host were studied in vivo and in vitro in an attempt to explain the preferential association of this serotype with poultry. The ability of this organism to invade and multiply in chicken intestinal epithelial cells and the intracellular behaviour in chicken macrophages was studied in vitro using chicken cell lines. In vivo challenge experiments in specific pathogen-free chickens were carried out to determine the level of colonization of caeca and internal organs early after experimental infection. An in vivo trial with commercial broiler chickens, using a seeder model, was performed to determine whether Salmonella paratyphi B dT+ could persist and spread in broilers until slaughter. Salmonella paratyphi B dT+ invaded and multiplied in the chicken epithelial cell line and survived in a chicken macrophage cell line. The strain used colonized caeca and internal organs of chickens to a high extent 1 week after infection with a low-dose inoculum. Moreover, the strain was efficiently transmitted within a group of broilers and persisted until slaughter. It was concluded that Salmonella
*Salmonella* *paratyphi* B dT+ was well adapted to poultry and therefore it is suggested that specific control measures against this serotype should be considered.

Jennifer et al. (2004) reported *Salmonella enterica* serovar typhimurium causes self-limiting gastroenteritis in humans and a typhoid-like disease in mice that serves as a model for typhoid infections in humans. A critical step in the *Salmonella* pathogenesis is the invasion of enterocytes and M cells of the small intestine via expression of a type III secretion system, encoded on *Salmonella* pathogenicity island 1 (SPI-1), that secretes effector proteins into host cells, leading to engulfment of the bacteria within large membrane ruffles. The in vitro regulation of invasion genes has been the subject of much scientific investigation. Transcription of the *hilA* gene, which encodes an OmpR/ToxR-type transcriptional activator of downstream invasion genes, is increased during growth under high-osmolarity and low-oxygen conditions, which presumably mimic the environment found within the small intestine. Several negative regulators of invasion gene expression have been identified, including HilE, Hha, and Lon protease. Mutations within the respective genes increase the expression of *hilA* when the bacteria are grown under environmental conditions that are not favorable for *hilA* expression and invasion. In this study, the intracellular expression of invasion genes was examined, after bacterial invasion of HEp-2 epithelial cells, using *Salmonella* strains containing plasmid-encoded short-half-life green fluorescent protein reporters of *hilA*, *hilD*, *hilC*, or *sicA* expression. Interestingly, the expression of SPI-1 genes was down-regulated after invasion, and this was important for the intracellular survival of
the bacteria. In addition, the effects of mutations in genes encoding negative regulators of invasion on intracellular *hilA* expression were examined. Our results indicate that Lon protease is important for down-regulation of *hilA* expression and intracellular survival after the invasion of epithelial cells.

Bärbel Stecher *et al.* (2005) *Salmonella enterica* subspecies 1 serovar *typhimurium* is a common cause of bacterial enterocolitis. Mice are generally protected from *Salmonella serovar typhimurium* colonization and enterocolitis by their resident intestinal microflora. This phenomenon is called "colonization resistance" (CR). Two murine *Salmonella* serovar *typhimurium* infection models are based on the neutralization of CR: (i) in specific-pathogen-free mice pretreated with streptomycin (StrSPF mice) antibiotics disrupt the intestinal microflora; and (ii) germfree (GF) mice are raised without any intestinal microflora, but their intestines show distinct physiologic and immunologic characteristics. It has been unclear whether the same pathogenetic mechanisms trigger *Salmonella* serovar *typhimurium* colitis in GF and StrSPF mice. In this study, we compared the two colitis models. In both of the models *Salmonella* serovar Typhimurium efficiently colonized the large intestine and triggered cecum and colon inflammation starting 8 hours post-infection. The type III secretion system encoded in *Salmonella* pathogenicity island 1 was essential in both disease models. Thus, *Salmonella* serovar *typhimurium* colitis is triggered by similar pathogenetic mechanisms in StrSPF and GF mice. This is remarkable considering the distinct physiological properties of the GF mouse gut. One obvious difference was more pronounced damage and reduced
regenerative response of the cecal epithelium in GF mice. Overall, StrSPF mice and GF mice provide similar but not identical models for Salmonella serovar typhimurium colitis.
Histopathology

Girard-Santosuosso et al. (1998) observed genetic resistance of four chicken lines against Salmonella enteritidis (SE) phage type 4 (PT4) systemic colonization was investigated. Thirteen-week-old chickens were intravenously inoculated with 10^6 SE colony-forming units, and the levels of SE colonization were determined at various time intervals after inoculation in liver, spleen, genital organs, and ceca. The course of SE infection showed a rapid contamination of liver, spleen, and genital organs, whereas the ceca were infected later. A significant (P < 0.001) effect of the chicken line on levels of SE was detected on day 3 post-inoculation (PI) in liver and ceca, on day 10 PI in ceca, and on day 15 PI in spleen. Because an early control of systemic Salmonella infection by the lty/Nramp1 gene has been demonstrated in mice, we aimed to study the early resistance of chickens to SE. As a consequence, we then focused our study on the between- and within-line variabilities of SE levels on day 3 PI. According to the SE levels in liver on day 3 PI, the chicken lines could be classified as susceptible (Y11 and L2) or resistant (PA12 and B13). This early variability was explored in resistant B13 and susceptible L2 lines. Differences between these two lines were confirmed in liver but not in ceca. A large within-line variability was observed in all organs of these two lines. The genetic origin of this variability will have to be determined as a prerequisite to an eventual selection.
Gast and Beard (1989) assessed the effects of age at exposure on the persistence of Salmonella in various tissues of chicks in two experiments. Broiler chicks, housed on wire floors in isolation cabinets, were orally inoculated with Salmonella typhimurium at various ages (1 to 8 days after hatching). The post-inoculation mortality of chicks declined significantly (P<0.05) as the age at inoculation increased. In one experiment the effect of age at inoculation was investigated on the persistence of Salmonella typhimurium in the cecum. Salmonellae persisted for 7 weeks after inoculation in 81.3% of the chicks inoculated at 1 day of age and in 62.5% of the chicks inoculated at 8 days of age. The mean number of cecal Salmonellae at 7 weeks post-inoculation was also greater for chicks inoculated on day 1 than for those inoculated on day 8. The second experiment examined the effect of age at inoculation on the adherence of Salmonella typhimurium to and penetration through the cecal epithelium. The ceca of chicks inoculated at 1 day of age were colonized by significantly more adhering Salmonella at 2 days post-inoculation than those of chicks inoculated at 3, 5, or 7 days of age, but age did not affect the recovery of Salmonella typhimurium from livers or spleens.

Gorham et al. (1994) examined infected orally one-day-old and 7-day-old specific-pathogen-free chickens were orally infected with a field isolate of Salmonella enteritidis phage type 13A. Chickens were sequentially euthanatized at various intervals until 42 days of age, and selected tissues were collected for microscopic evaluation. Eleven of 53 chickens (21%) infected at 1 day of age and 2 of 28 chickens (7%) infected at 7 days of age died. Gross and
microscopic lesions were more frequent and severe in chickens infected at 1 day of age and in chickens that died (almost all of these were 1-day-infected as well). Lesions were characterized by mild to severe fibrinous pericarditis, airsacculitis, perihepatitis, peritonitis, cecal cores, and enlarged, firm yolk sacs. Gross the microscopic lesions were present from post-inoculation day 2 until termination of this study.

Desmidt et al. (1997) inoculated white leghorn specific-pathogen-free chickens orally with Salmonella enteritidis phage type 4 at the age of one day (group 1) and four weeks (group 2). From 3 h until 4 weeks post inoculation (pi), birds were sacrificed. Gross lesions were recorded and different sites of the intestine and visceral organs were collected for bacteriological and histopathological examination. Clinical disease and mortality were only observed in group 1. Mortality was 8%. The birds were depressed, had diarrhoea and an indurated yolk sac. Infection of the liver and the heart was present within 12 h pi in both groups. The percentage of infected organs was very high and similar in both groups during the first week pi. Thereafter, the isolation rate of Salmonella was declining faster in group 2. The crop, the proventriculus, the lower intestinal tract and the bursa of Fabricius were the predilective sites of isolation in both groups. Most prevalent lesions were serous typhlitis, omphalitis and polyserositis. Histopathology revealed inflammation in the intestines and visceral organs. In some birds granulomatous nodules were present in the caeca. Antibodies were detected from 18 and 5 days pi in group 1 and 2, respectively. Granulomatous nodules were typical of infection with this
strain of *Salmonella enteritidis* phage type 4. These granulomatous nodules together with the retained yolk sac possibly are a source of Salmonella organisms that may account for intermittent faecal shedding by carrier birds.

*Kinde et al. (2000)* examined two strains of 27-wk-old commercial laying chickens (strain A, brown egg laying type and strain B, white egg laying type) were inoculated either orally (PO) or intravenously (IV) with a field isolate of *Salmonella enteritidis* phage type 4. Chickens were sequentially necropsied at regular intervals throughout the 17 weeks observation period. Gross and microscopic lesions were most evident between 1 and 14 days post-inoculation (DPI). Gross lesions consisted of enlarged livers with white foci, enlarged and mottled white spleens, fibrinous exudate in the peritoneum, and atretic, misshapen ovarian follicles. Microscopic lesions included multifocal coagulative necrosis of hepatocytes and inflammation, fibrinous exudation in vascular sinuses of the spleen, and fibrinosuppurative inflammation of the peritoneum and ovarian follicles. The proportion of reproductive organ infections (ovary and oviduct) in the IV group, 83% (20/24, P = 0.007; 50% and 33% for strains A and strain B birds, respectively), was higher than that of the PO group, 46% (11/24; 29% and 17% for strains A and B, respectively), for the first 16 days of observation post-inoculation. Overall the percentage of culture-positive birds did not differ significantly (P>0.05) between birds with and without lesions, but isolation of *Salmonella enteritidis* tended to be more frequent when lesions were evident. This experiment also demonstrated that brown-egg-laying-type chickens were more susceptible than white-egg-laying-type chickens to
Salmonella enteritidis phage type 4 isolated from California based on gross and microscopic lesions and bacteriologic findings.

Buhr et al. (2001) challenged broilers with $10^9$ml *Salmonella typhimurium* and then were provided with 0 to 15 glucose during feed with diet on litter. After feed with diet broilers were processed and their crops were aseptically removed and weighed. It was found that, inhibition of the growth of *Salmonella typhimurium* in the crops of broilers provided that cocktail supplemented with 7.5% glucose was generally associated with increased growth and lactic acid bacteria and decreased crop.

Dhillon et al. (2001) studied two hundred sixty one-day-old specific pathogen-free (SPF), Single Comb White Leghorn chicks to determine pathology caused by *Salmonella enteritidis* isolated from a Poultry environment. The chicks were subdivided into 10 equal groups of 26 chicks each. Eight groups of chicks were inoculated individually with 0.5 ml of brain heart broth culture of *Salmonella enteritidis*. One group of 26 chicks were inoculated with 0.5 ml *Salmonella pullorum* per bird. Another group of 26 chicks were kept as an uninoculated control group. All the chicks were observed daily for clinical signs and mortality. *Salmonella* was reisolated from different organs at 7, 14, 21, and 28 post-inoculation (DPI). All of the chicks were weighed individually at each interval. Two chicks at random from each group were taken and necropsied at each DPI for gross pathology. Selected tissues were examined for histopathological changes at 7 and 14 DPI. Dead chicks were examined for gross and histopathological lesions. Mortality rates were 30.7 and 7.6% in the
groups inoculated with *Salmonella pullorum*, *Salmonella enteritidis*, PT5A, and
*Salmonella enteritidis*, respectively. No mortality or clinical sign were observed
in other treatment groups or in uninoculated control groups. Cecal pouches are
found to be the ideal organ. Mean body weights were reduced to 1.8 to 12.6% in
inoculated groups compared with the uninoculated control group. The
consistent gross and histopathological lesions were of peritonitis, perihepatitis,
yolk sac infection, and enteritis. Sub-clinical *Salmonella* infection identified in
this study resulted in reduced body weights of inoculated birds compared with
uninoculated controls.

Roy et al. (2001) recorded the pathogenicity of one isolate of *Salmonella
typhimurium*, four isolates of *Salmonella heidelberg*, three isolates of
*Salmonella kentucky*, two isolates of *Salmonella montevideo*, one isolate of
*Salmonella hadar*, and two isolates of *Salmonella enteritidis* (SE), one
belonging to phage type PT13a and the other to PT34, was investigated in
specific-pathogen-free chicks. Three hundred eighty-four chicks were separated
into 16 equal groups of 24 chicks. Thirteen groups were inoculated individually
with 0.5 ml of broth culture containing $1 \times 10^7$ colony-forming units (CFU) of
either *Salmonella typhimurium* (one source), *Salmonella heidelberg* (four
sources), *Salmonella montevideo* (two sources), *Salmonella hadar* (one
source), *Salmonella kentucky* (three sources), *Salmonella enteritidis* PT 13a
(one source) or *Salmonella enteritidis* PT 34 (one source) by crop gavage. Two
groups of 24 chicks were inoculated in the same way with $1 \times 10^7(7)$ CFU of
*Salmonella enteritidis* PT4 and *Salmonella pullorum*. Another group of 24
chicks was kept as an uninoculated control group. The chicks were observed daily for clinical signs and mortality. Isolation of Salmonella was done from different organs at 7 and 28 days post-inoculation (DPI). All the chicks were weighed individually at 7, 14, 21, and 28 DPI. Two chicks chosen at random from each group were euthanatized and necropsied at 7 and 14 DPI and all the remaining live chickens, at 28 DPI. Selected tissues were taken for histopathology at 7 and 14 DPI. Dead chicks were examined for gross lesions and tissues were collected for histopathology. Chicks inoculated with Salmonella pullorum had the highest mortality (66.66%), followed by Salmonella typhimurium (33.33%). Chicks inoculated with Salmonella heidelberg and Salmonella enteritidis PT4 had 12.5% mortality and 8.3% mortality, respectively, with Salmonella enteritidis PT 13a. Ceca were 100% positive for Salmonellae at acute or chronic infection compared with other organs. Mean body weight reduction ranged from 0.67% (inoculated with Salmonella kentucky) to 33.23% (inoculated with Salmonella typhimurium) in the inoculated groups at different weeks compared with uninoculated controls. Gross and microscopic lesions included peritonitis, perihepatitis, yolk sac infection, typhilitis, pneumonia, and enteritis in some groups, especially those inoculated with Salmonella typhimurium, Salmonella heidelberg, Salmonella enteritidis PT4 and Salmonella pullorum.

Bolder et al. (2002) reported that Salmonella infections originating from Poultry are one of the major causes of food-borne disease. For the control of Salmonella in Poultry a multi-factorial approach is more likely to be effective,
and the genetic resistance of Poultry breeds to Salmonella infections may be a valuable contribution. Experimental *Salmonella enteritidis* infections were examined in three different broiler outbred lines: the FC line, which had been selected for feed conversion efficiency; the R line, which had been selected for growth rate; and the C line, a commercially available line. The FC line had the highest mortality rate after intramuscular inoculation with $5 \times 10^6$ colony forming units (CFU) of *Salmonella enteritidis* at 2 weeks of age (40% versus 21 and 20% in the other lines). However, at slaughter age, the number of birds carrying Salmonella in caecal contents, and the concentration of Salmonella in the caecal contents, was lowest in the FC line. The FC and R lines were compared by inoculation with doses ranging from $10^2$ to $10^7$ CFU *Salmonella enteritidis*. At sublethal doses ($10^5$ CFU or less), the FC line carried significantly less Salmonella in caecal contents and the rate of systemic infection was lower. The start of shedding was also delayed compared with the R line. At doses of $10^6$ CFU *Salmonella enteritidis* or higher, there were no differences in Salmonella carriage between the lines, and the FC line showed higher mortality. In conclusion, resistance to mortality and resistance to the carriage of *Salmonella enteritidis* do not necessarily coincide within lines, as the FC line showed high mortality but low carriage, both in survivors of high infection doses and in all birds at lower infection doses.

Saif (2003) observed the pathological changes by giving oral infection of *Salmonella enteritidis* and *Salmonella typhimurium* in Poultry and found severe enteritis accompanied by focal necrotic lesions in the mucosa of the small
intestine, cheesy cecal cores, spleen and liver congested, kidneys enlarged and congested, peri-hepatitis and peri-carditis, coagulated yolk material in the yolk sack, purulent arthritis, air-saculitis. Inflation of the epithelium and lamina propria of the colon and ceca and cell death.

Beat et al. (2004) found that Salmonella enterica remains one of the most important food-borne pathogens of humans and is often acquired through consumption of infected Poultry meat or eggs. Control of Salmonella infections in chicken is therefore an important public health issue. Infection with Salmonella enterica serovar typhimurium results in a persistent enteric infection without clinical disease in chickens of more than 3 days of age, and represents a source for contamination of carcass at slaughter and entry into the human food chain. Data presented indicate a profound effect of age at initial exposure on the persistence of infection and a lesser effect on the development of effective immunity to re-challenge. The percentage of birds positive for Salmonella was high until 8-9 weeks of age, regardless of the age at which the birds were infected (1, 3 or 6 weeks). The birds infected at 3 and 6 weeks of age produced a more rapid and higher antibody response than those infected at 1 week of age, but in all cases infection persisted for a considerable period despite the presence of high antibody levels. Following a re-challenge infection with Salmonella typhimurium, all three previously-infected groups had fewer bacteria in the gut, spleen and liver compared with age-matched birds receiving a parallel primary infection. However, the birds primary infected at 3 and 6 weeks of age cleared infection more rapidly than those infected at a younger
age. Interestingly, older-primed birds had higher specific T lymphocyte proliferative responses and specific circulating levels of IgY antibody at time of re-challenge. Although birds initially infected at 1 week of age and those that were previously uninfected produced a stronger antibody response following re-challenge, they were slower to clear Salmonella from the gut than the older-primed groups which expressed a stronger T lymphocyte response. The data presented indicate that clearance of Salmonella from the gut is age-dependent and we propose that this relates to the increased competence of the enteric T cell response. The findings that Salmonella persists beyond 8-9 weeks, irrespective of age at exposure, has implications for the broiler sector and indicates the need to remain Salmonella free throughout the rearing period. Moreover, the re-challenge data demonstrates that infection at a young age is less effective in producing protective immunity than in older chickens. This feature of the development of protective immunity needs to be considered when developing vaccines for the broiler sector of the Poultry industry. Moreover, the rechallenge data demonstrates that infection at young age.

Lima-Filho et al. (2004) experimentally exposed plasmid free non pathogenic Escherichia coli (EMO) or not (control) 10 days before challenge with Salmonella typhimurium (10^5) colony forming units (CFU)/mouse. Survival after challenge was higher (P < 0.05) in the experimental group (16%) than in the control animals (0%). Histopathological examination of the colon and ileum mucosa of the experimental group showed less extensive lesions such as edema, cell inflammatory infiltration and hyperemia. The epithelial cells of the
mucosal surface and the production of the mucous layer were also better preserved in the experimental group. The population levels of Salmonella typhimurium in the feces were initially 10-fold lower (P < 0.05) in the experimental groups. However, 3 days after challenge both experimental and control groups showed similar population levels ranging from $10^8$ to $10^9$ CFU/g of feces. The intestinal contents of total and anti-Salmonella typhimurium sIgA were higher in the experimental groups 10 days after inoculation of E.coli EMO strain. Translocation of Salmonella typhimurium to the spleen was 10-fold lower (P < 0.05) in the experimental group only on day 3 after infection. This was not related to an increase in the bacterial blood clearance of the animals, as shown by experimental venous challenge with E. coli B41. In conclusion, treatment of mice with E. coli EMO strain promoted a relative protection against experimental infection with Salmonella typhimurium. This protection was not due to the reduction of the population of pathogens in the intestine but was probably related to stimulation of the immune response.

Van Immerseel et al. (2004) recorded that Poultry are very likely to become infected with Salmonella in the early period, due to environmental contamination. The purpose of their study was to evaluate the effect of infection dose on the risk of persistent infection in laying hens. In this study, young layer chicks were orally infected with a low $10^2$ at 1 day posthatch) or a high dose $10^9$ cfu at one week posthatch of Salmonella enteritidis. The pattern and duration of fecal shedding was studied for 18 wk. All chickens shed Salmonella early after infection and shed Salmonella intermittently during the whole study.
period. There were more positive birds in the high-dose group than in the low-dose group in the first weeks following Salmonella exposure. From 10 weeks post-infection onwards, however, birds that were orally infected with the low dose of Salmonella enteritidis shed more Salmonella than the birds that received the high dose. At 18 wk of age, there was no difference in cecal colonization between the treatment groups. It can be concluded that infection of newly hatched chicks with a low dose of Salmonella enteritidis can lead to persistent infection until onset of lay, hereby excreting Salmonella bacteria intermittently.

Isaacs et al. (2005) detected an outbreak due to Salmonella enteritidis (SE) phage type 30 (PT30), a rare strain, in Canada. The ensuing investigation involved Canadian and American public health and food regulatory agencies and an academic research laboratory. Enhanced laboratory surveillance, including phage typing and pulsed-field gel electrophoresis, was used to identify cases. Case questionnaires were administered to collect information about food and environmental exposures. A case-control study with 16 matched case-control pairs was conducted to test the hypothesis of an association between raw whole almond consumption and infection. Almond samples were collected from case homes, retail outlets, and the implicated processor, and environmental samples were collected from processing equipment and associated farms for microbiological testing. One hundred sixty-eight laboratory-confirmed cases of SE PT30 infection (157 in Canada, 11 in the United States) were identified between October 2000 and July 2001. The case-control study
identified raw whole almonds as the source of infection (odds ratio, 21.1; 95% confidence interval, 3.6 to infinity). SE PT30 was detected in raw whole natural almonds collected from home, retail, distribution, and warehouse sources and from environmental swabs of processing equipment and associated farmers' orchards. The frequent and prolonged recovery of this specific organism from a large agricultural area was an unexpected finding and may indicate significant diffuse contamination on these farms. Identification of almonds as the source of a food borne outbreak is a previously undocumented finding, leading to a North American recall of this product and a review of current industry practices.
Typhoid Fever

Vallenas et al. (1985) made comparative study in 118 pediatric patients, (2 to 13 years old) with suspected typhoid fever. Only 47% of children 2 to 6 years, as compared with 89% in children 7 to 13 years. At least one was positive for Salmonella typhi in 43 patients. Bone marrow cultures were positive in 84% of the confirmed cases, a sensitivity significantly greater than for duodenal contents (42%), blood (44%) and stool (65%) cultures. Higher recovery rates for blood cultures were found during the first week of illness than later. Bone marrow cultures remain the most effective method for the recovery of Salmonella typhi. Stool cultures appear to be more effective in children than in adults.

 Milkovic et al. (1990) observed that in the 4 year period there was a considerable increase in both human and Poultry infections with Salmonella enteritidis. Of the 299 – 627 Poultry organ samples examined annually, 48.6% were infected in 1986, 60% in 1987, 20.4% in 1988 and 33.1% in 1989, compared with 0.6% of 849 in 1985. Of the 26118 in 3345/human fecal samples examined annually, 41.4% were infected in 1986, 63.6% in 1987, 41.4% in 1988 and 44.6% in 1989, compared with 7.1% in 1985. The main sources of the human infections are Poultry products.

 Luby et al. (1998) conducted a study to evaluate risk factors for developing typhoid fever in a setting where the disease is endemic in Karachi, Pakistan. They enrolled 100 cases with blood culture-confirmed Salmonella
typhi between July and October 1994 and 200 age-matched neighbour hood controls. Cases had a median age of 5.8 years. In a conditional logistic regression model, eating ice cream (Odds ratio [OR] = 2.3; 95% confidence interval [CI] 1.2-4.2, attributable risk [AR] = 36%), eating food from a roadside cabin during the summer months (OR = 4.6, 95% CI 1.6-13.0; AR = 18%), taking antimicrobials in the 2 weeks preceding the onset of symptoms (OR = 5.7, 95% CI 2.3-13.9, AR = 21%), and drinking water at the work-site (OR = 44.0, 95% CI 2.8-680, AR = 8%) were all independently associated with typhoid fever. There was no difference in the microbiological water quality of home drinking water between cases and controls. Typhoid fever in Karachi resulted from high-dose exposures from multiple sources with individual susceptibility increased by young age and prior antimicrobial use. Improving commercial food hygiene and decreasing unnecessary antimicrobial use would be expected to decrease the burden of typhoid fever.

Bhatta et al. (1999) evaluated the diagnostic sensitivity and specificity of two dot-enzyme linked immunoassays, assessing IgG and IgM antibodies against the outer membrane protein of Salmonella typhi and the widal test in comparison with blood culture in a consecutive group of children with suspected typhoid fever of 97 children on clinical grounds. An alternative diagnosis was made in 26, 27%. It was observed that there is a regional difference in the genomic structure and plasticity of the Salmonella typhi.

Kraus et al. (1999) reported that enteric fever is a systemic illness caused by Salmonella infection, with Salmonella typhi, Salmonella paratyphi
and *Salmonella enteritidis* being the most common serotypes. Humans are the only reservoir for *Salmonella typhi* and its predilection for the ileum is due to the fact that organisms enter the body by translocation across specialized Peyer's patch epithelium and then proliferate in the mucosal macrophages. Although rare in developed countries, enteric fever should be considered in any patient with recent travel to endemic areas and in the context of illness thought to be related to contaminated foods.

*Rzedzicki et al.* (2000) recorded that there has recently been observed a growing increase of *Salmonella* infections in humans. Most of these infections are caused by *Salmonella enteritidis*. An important factor in preventing human Salmonellosis is interrupting the infection chain which originates in Poultry. Bacteriological methods imposed by current regulations are laborious and time consuming. In these cases, serological tests are of great value. Among the many serological methods, the Elisa has been increasingly used in the control of *Salmonella* infections in poultry flocks because it is a sensitive and quick test. Antibiotic therapy is considered an important factor that can influence bacterial variability. Antibiotic therapy may promote the spread of resistant-strains.

Bishop *et al.* (2001) reported that Salmonellosis is the major cause of illness in the USA. A total of 441,863 *Salmonella* isolates were reported. Annual isolation rates decreased from 19 to 13/100,000 persons, however, trends varied by serotype. *Salmonella* infections continue to be an important-cause of illness especially among infants. Recent declines in food associated serotype may reflect changes in the meat, Poultry and eggs.
Qasim Khan (2001) reported that although typhoid fever almost has disappeared from the developed countries but is still very much prevalent in the developing countries including Pakistan. According to WHO estimates worldwide incidence of typhoid fever is 365/100,000 with 16-20 million cases occurring worldwide annually. The incidence of typhoid fever in developing countries is 540 / 100,000 and 7.7 million cases in the Asia alone, with a total of 600,000 death cases due to typhoid fever in developing countries per year. Although resistance to the conventional antityphoid drugs like chloramphenicol; ampicillin and cotrimoxazole started in 1962 but globally reported in 1972. In Pakistan, Salmonella typhi remained uniformly sensitive to these drugs till 1987, when First MDR (multi dug resistant) Salmonella typhi were isolated. The resistance increased from 10% in 1987 to 60% in 1990 and then continuous reports from different centres regarding resistance varied from 10-70%.

Waqar et al. (2002) a descriptive study in 75 patients, admitted with suspected typhoid fever. Out of 75, 47 were male and 28 were female patients. Maximum incidents was seen in 5-15 years age group (73%). Forty (53%) patients had duration of fever of 7-14 days. The fever was mostly high grade (67%) with a history of gastro intestinal complaints like diarrhea, vomiting, abdominal pain etc. (This study has been done during 2000-2001 in Sheikh Zaid hospital Lahore).

Massi et al. (2003) developed a rapid diagnosis method for Salmonella typhi infection in blood specimens from patients with typhoid fever. Primers were designed from the flagellin gene sequence, which would give an
amplification product of 367 base pairs. In this study, the specificity of the assay, with no amplification, was seen for the other Salmonella strains with the flagellin gene, and not for non-Salmonella bacteria. For the sensitivity test, the protocol described allowed the detection of two to three copies of the Salmonella typhi genome, as determined by serial dilution of genomic DNA from Salmonella typhi. With the PCR technique, genomic DNA of Salmonella typhi was detected in 46 of 73 blood samples collected from patients with clinically suspected typhoid fever who had fever within 3 days of admission to the General Hospital, Makassar, South Sulawesi, Indonesia, and who had had no prior antibiotic treatment. The PCR results (63% positive cases) were compared with those of blood culture (13.7% positive cases) and the widal test (35.6% positive cases), using the same samples from each of the 73 patients admitted to the General Hospital in Makassar. The time taken for PCR analysis of each sample was less than 12 hours, compared with 3 to 5 days for blood or clot culture. The PCR with one pair of primers can be used as a novel, rapid diagnostic method for typhoid fever, particularly when results of standard culture assays are negative.
Food Poisoning

Eiguer et al. (1990) reported outbreaks of food-borne diseases due to *Salmonella enteritidis* which occurred in Argentina between 1986 and 1988. In 39 registered episodes 210 strains were isolated from human feces (28 outbreaks) and 59 from food (23 outbreaks). More than 2,500 people in different provinces were affected, the chief characteristics of the clinical picture being the gravity of the symptoms (high temperature, vomiting, diarrhea and severe dehydration). The main source of infection was related to raw eggs, eaten in the form of home-made mayonnaise. It is considered necessary to carry out an effective control of Poultry products, as well as a permanent surveillance of salmonellosis.

Palmer et al. (1990) reported an outbreak of *Salmonella typhimurium* food poisoning due to contaminated chicken pieces affecting at least 196 delegates at a medical conference. Twelve per cent (12%) of the cases reported suffering paraesthesiae. Nine reported serious complications of infection which included rectal prolapse and perianal abscess and severe diarrhoea.

Giessen et al. (1992) reported that following an incident of food poisoning involving 100 persons, the eggs were responsible and were traced to 10 farms through an immuno essay two farms were positive for Salmonella by detecting anti bodies to group D Salmonella in egg yolk and eggs. *Salmonella*
*Enteritidis* was isolated from faeces from 3 of the 4 flocks on these farms. All flocks came from the same breeding stock.

Muramatsu and Nishizawa (1992) recorded in an outbreak of municipal water-associated food poisoning by *Salmonella enteritidis* occurred in Takatoh Town, Nagano prefecture during September 4 to 19, 1989. The major symptoms observed in the 680 patients consisted of diarrhea (70.9%), abdominal pain (51.2%), fever (44.6%), headache (27.9%), nausea (5.9%) and vomiting (5.7%). In the outbreak, *Salmonella enteritidis* was the only suspected etiological agent isolated from both patients and municipal water supply. In other respects, the isolates conformed to the general characteristics of *Salmonella*. The outbreak above seems to be the most large-scale among those of *Salmonella* found poisoning associated with drinking water in Japan.

Ortega-Benito and Langridge (1992) recorded a large outbreak, in July 1989, of food poisoning (68 cases) occurring at a private club in Teddington (London). Initial enquiries indicated that the peak of the outbreak occurred between 20th and 26th July. An epidemiological investigation (using self-completed questionnaires) was set up to determine the probable source of infection. Two groups among those exposed were selected: club staff (129), and cricket teams playing in a club tournament (105). Response rates were 89% and 64%, respectively. Overall 50% (89) amongst the affected had gastrointestinal symptoms, including two hospital admissions. A highly significant association was found between illness and eating sandwiches containing mayonnaise. Microbiological investigations found *Salmonella*
*Salmonella typhimurium* DT4 in 36 of 68 faecal samples taken. The probable source was identified as a flock of one of the egg suppliers.

Aseffa *et al.* (1994) noted a sudden outbreak of food poisoning occurring between December 31, 1991 and January 4, 1992 among students of the Gondar College of Medical Sciences. Out of 344 students, 79 (23%) had manifest disease. *Salmonella* was isolated from the stool of six students and three food handlers. The mean incubation period for the excretors was 48 hours. Main symptoms of those evaluated by physician were mild diarrhoea (86%) and abdominal cramps (71.4%). The only meal shared by all was a breakfast of undercooked eggs served after 14 hours of storage at room temperature.

Murase *et al.* (1996) did an epidemiological analysis of *Salmonella enteritidis* from a food poisoning out break, using pulsed-field gel electrophoresis (PFGE) of fragments of chromosomal DNA of isolates. *Salmonella enteritidis* isolates obtained from 19 patients had identical PFGE patterns. Therefore, a strain giving the same pattern was considered to be the causative agent of this outbreak. In addition, four isolates that had different PFGE patterns were obtained from three patients, suggesting that the observed variations in PFGE patterns might occur as the result of some point mutations of chromosomal DNA during growth or from the existence of several *Salmonella enteritidis* strains from various sources. Subsequent PFGE analysis of continuously subcultured strains supported the former possibility.
Humbert and Salvat (1997) reported that while Salmonellas can cause disease problems among Poultry, they remain essentially a concern for public health, as a cause of outbreaks of food poisoning. The principal site of multiplication of these bacteria is the digestive tract, particularly the caecum, which may result in widespread contamination of the environment. The pathogenicity of Salmonellae depends on the invasive properties and the ability of the bacteria to survive and multiply within cells, particularly macrophages. These properties are the source of vertical transmission which, in the case of survival of the embryo, can result in contamination of a flock or, in the case of embryonic mortality, can result in an explosion of contaminated eggs. Salmonella infection can be diagnosed by isolating the bacteria and/or serological testing of the flock. However, the most effective means of reducing food poisoning remains adequate cooking of food and maintenance of the cold chain.

Kusunoki et al. (1997) recorded since 1989, outbreaks of Salmonella, enteritidis food poisoning have dramatically increased in Tokyo, and a total of 31 outbreaks has been reported in 1989. Twenty-one of these 31 outbreaks were caused by Salmonella enteritidis PT34, but 8 outbreaks were caused by Salmonella enteritidis PT4. After 1990 instead of SE PT34, food poisoning due to PT4, which was a very common phage type in the UK, has increased in Tokyo. Between 1989 and 1995, there were 144 food poisoning outbreaks caused by Salmonella enteritidis, and 64 of these outbreaks were by due to Salmonella enteritidis PT4, which was one of the main phage types in Tokyo. A
total of 56 (87.5%) of 64 outbreaks was found to have been caused by these types of *Salmonella enteritidis*. Several kinds of egg-related foods were suspected as the vehicles of transmission among 24 outbreaks. Especially, in 5 outbreaks, *Salmonella enteritidis* strains were isolated both from patients and suspected food which were cooked with egg. This strongly suggests that these foods may be the potential source of infection in *Salmonella enteritidis* outbreaks.

Nastasi *et al.* (1997) performed polymerase chain reaction (PCR) on 243 strains of *Salmonella enterica* serotype *enteritidis* isolated during the year 1980–1994 from 58 food borne out breaks occurring in different regions of Italy. The majority of the out breaks were attributed to phage type 4, followed by PT1 (seven out breaks); the latter was identified in 1993 in Italy in epidemic strains of *enteritidis*. In eight cases more that one phage was recognized from a single event. Nine PCR ribo types (PCR-RTS) were detected. PCR ribo typing proved to be effective and reliable tool for sub-typing isolates of *enteritidis* belonging to most frequent phage types. The technique of PCR is the best one suited for reference laboratories.

Zhang-Barber *et al.* (1999) stated that *Salmonella* species are facultative intracellular pathogens causing localized or systemic infections, in addition to a chronic asymptomatic carrier state. They are of worldwide economic and public health significance. In Poultry, which represent important sources of cheap protein throughout the world, fowl typhoid and pullorum disease continue to cause economic losses in those parts of the world where the Poultry industries
are continuing to intensify and where open sided housing is common. A number of serotypes that cause human gastro-enteritis are also increasing. The costs or impracticality of improvements in hygiene and management together with the increasing problems of antibiotic resistance suggest that vaccination in Poultry will become more attractive as an adjunct to existing control measures.

Godoy et al. (2000) reported a clinico-epidemiological and microbiological investigation conducted into an outbreak of gastrointestinal infection due to Salmonella enteritidis, which is due to most likely food vehicle. An historic study was conducted out among persons exposed to menus at a school canteen. Data were gathered on age, sex, foods consumed and clinical symptoms. School premises and menus were inspected, food samples obtained (spaghetti and meat balls), and stool samples taken from 30 affected subjects and 8 food handlers. Isolated strains were studied using pulsed-field electrophoresis. Attack rates were computed, and the odds ratio adjusted for the remaining foodstuffs (ORa) used to calculate the independent contribution made by the respective foods to risk of infection. Study coverage was 75.7% (140/185). The overall attack rate was 72.1% (101/140), with 12.9% of those affected requiring hospitalization. Salmonella enteritidis was isolated in stool cultures from 28 affected subjects, and in 2 blood and 6 stool cultures from food handlers. Moreover, Salmonella enteritidis was also isolated in the food samples. On pulsed-field electrophoresis, the strains registered the same electrophoresis pattern. They concluded this outbreak serves to underscore the gravity of Salmonella species food poisoning, the danger of using inadequately
cooked eggs, and the importance of interviewing food handlers to ensure proper classification as patients or carriers. Pasturized egg products should be used for eating purpose.

Mead (2000) reported that in newly hatched chicks, the rapid establishment of an adult-type intestinal microflora, via the oral route, produces almost immediate resistance to colonization by any food poisoning Salmonellae that gain access to the rearing environment. Exploitation of the 'competitive exclusion' (CE) effect is now an accepted part of the overall strategy by which Poultry-associated Salmonellas are being controlled in some countries.

Thorns (2000) recorded in many countries of the world, bacterial food-borne zoonotic infections are the most common cause of human intestinal disease. Salmonella and Campylobacter account for over 90% of all reported cases of bacteria-related food poisoning world-wide. Poultry and Poultry products have been incriminated in the majority of traceable food borne illnesses caused by these bacteria, although all domestic livestock are reservoirs of infection. In contrast to the enzootic nature of most Salmonella and Campylobacter infections, Salmonella enteritidis caused a pandemic in both Poultry and humans during the latter half of the 20th Century. Salmonella typhimurium and Campylobacter appear to be more ubiquitous in the environment. The dissemination of Salmonella Enteritidis along the food chain is fairly well understood, and control programmes have been developed to target key areas of Poultry meat and egg production. Recent evidence indicates that these control programmes have been associated with an overall reduction
of Salmonella enteritidis along the food chain. Future control strategies need to consider variations in the epidemiologies of food-borne zoonotic infections, and apply a quantitative risk analysis approach to ensure that the most cost-effective programmes are developed.

Dohtsu et al. (2001) experienced a hospital outbreak of Salmonella food poisoning after ingestion of omelet which was the hospital evening meal on August 8, 1999. Total number of patients was sixty-two (Male 25; female 37) and the mean age was 52.1 years old. Salmonella enteritidis was isolated from the stool in 59 cases. Clinical symptoms of the patients were composed of watery diarrhea (100%), fever (88.7%), abdominal pain (82.3%), nausea (45.2%) and vomiting (25.8%). The laboratory data revealed leukocytosis (15/47 = 31.9%), increased (44/46 = 95.7%), elevated creatinine (1/37 = 2.7%) and hypokalemia (5/42 = 11.9%). MICs of 20 strains isolated in the laboratory almost coincided with each other indicating that the source of bacteria was probably the same. It was suspect that the Salmonella food poisoning was due to infected eggs. The partially cooked omelet would permit the growth of a sufficient inoculum to cause disease. To prevent food poisoning, the food should be cooked well (at 75°C, for more than 1 minute) and should not have omelets during the hot summer season.

Kupek et al. (2001) described the epidemic profile of out breaks of food infection caused by Salmonella enteritidis, 96 out breaks were observed and 79 were investigated, involving 7,802 people with signs and symptoms consistent with the infection. Most frequent symptoms were diarrhea (92%), abdominal
pain (73%) fever 70%, vomiting 49% and nausea 45%. The out breaks most frequently occurred at home (60%) but the largest infections resulted from industrial kitchens (78%). The age group between 20 and 30 years was most affected. No relationships between the number of out breaks and outside temperature or humidity were found. The attack rate should significant difference between men and women. Better attention to storage of food products at home and in industrial kitchens is needed to control this common gastrointestinal illness.

Thorns (2001) observed that in most of the countries of the world, bacterial food borne zoonotic infections are the most common cause of human intestinal disease. Salmonella and Campylobacter account for over 90% of all reported cases of bacteria related food poisoning world wide. Poultry and Poultry products have been incriminated in the majority of traceable food borne illness caused by these bacteria. Salmonella enteritidis caused a pandemic in both Poultry and humans during the latter half of the 20 century. Salmonella typhimurium and Campylo bacter appear to be more ubiquitous in the environment, cloning a greater variety of hosts and environmental niches. The dissemination of Salmonella enteritidis along the food chain is fairly well understood and control programmes have been developed to target key areas of Poultry meat and egg production.

Hau-Yangtsen (2002) found that Salmonella enterica serovars typhimurium, typhi and enteritidis are serious food pathogens which may cause human disease and animal infections. In an attempt to elucidate the
clonal relationship in each of these species, found the most disseminated and re-circulating strains in food poisoning cases and to discern the possible transmission of these strains from different origins and areas, are have used phage typing, anti biograms and molecular typing methods, such as plasmid profiles, pulsed field gel electrophoresis (PFGE) and random amplified polymorphic DNA (RAPD) to identify sub types of these Salmonella strains. The results showed that in Salmonella typhimurium and typhi strains, considerable genetic diversity are found while in Salmonella enteritidis, high genetic similarity was observed. Also possibly, the most disseminated and re-circulating strains of Salmonella typhimurium and Salmonella enteritidis might be the most prevalent strains and transmission of strains between different areas and origins might be possible.

Pan and Lin (2002) developed the polymerase chain reaction and the multiplex polymerase chain reaction for detection of Salmonella and for identification of the serotype enteritidis. Three sets of primers were selected from different genomic sequences amplifying a 429 bp fragment specific for the genus Salmonella within a randomly cloned sequence, including a 250 bp fragment within the spv gene, and a 310 bp fragment within the sefA gene, specific for Salmonella enteritidis. The polymerase chain reaction and the multiplex polymerase chain reaction were used for detecting Salmonella enteritidis isolated from stool samples during outbreaks of food borne gastroenteritis between 1992 and 1998 in Taiwan. The sefA gene was detected in all 27 strains of Salmonella enteritidis by this polymerase chain reaction
method. Multiplex polymerase chain reaction could detect 3 genes in all strains, but could not detect the spv gene in 2 strains. The sensitivity of the polymerase chain reaction and the multiplex polymerase chain reaction were $10^4$ and $10^5$ cells/ml, respectively. In double polymerase chain reaction, the sensitivity increased to 100 cells/ml. These data indicate that the specificity and sensitivity of the polymerase chain reaction and the multiplex polymerase chain reaction make them potentially valuable tools for diagnosis of *Salmonella enteritidis* infection and that they may be used for the identification of *Salmonella enteritidis* responsible for sporadic enteritis cases.

Parry et al. (2002) found that domestic kitchen food handling risk factors for sporadic Salmonella food poisoning are largely unknown. They compared food consumption and food handling practices, opportunities for cross contamination and refrigerator temperature control, in 99 households in South East Wales in 1997-78 with a case of Salmonella food poisoning, and control households matched for electoral ward. On univariate analyses, cases were significantly more likely than control respondents to have purchased free-range eggs in the preceding week, and more likely than control households to have handled frozen whole chicken in the previous week, and to handle raw chicken portions at least weekly. In multivariate analysis, only consumption of raw eggs and handling free-range eggs were significant risk factors, independent of the age structure of the family and of the season.

Sarna et al. (2002) investigated an outbreak of gastroenteritis amongst attendees of a local community dinner in a Perth suburb. Of the 98 people
interviewed (response rate 98%). 53 reported gastrointestinal symptoms (attack rate 54%). Faecal cultures from 11 cases, 2 food preparers, 1 waitress and leftover mock ice-cream dessert grew *Salmonella typhimurium* PT135. Of the 3 food handlers, one was asymptomatic, another gave an unclear history of onset of illness and the waitress claimed illness onset 9 days after the dinner. Eggs used to make the mock ice-cream dessert were supplied directly from the producer who used inappropriate shell cleaning methods. The method of preparation of the dessert encouraged contamination. The cause of this outbreak was almost certainly the ice-cream dessert with contamination most likely resulting either from the eggs used to make the dessert or one or both of the food preparers, coupled with inadequate cooking of the dessert. The pasteurized egg products or egg pulp should be used in the preparation of uncooked or minimally cooked dishes.

Parry *et al.* (2004) believed that food hygiene precautions in domestic kitchens are an important strategy in efforts to reduce the incidence of sporadic food poisoning, but recent research has shown that people who have suffered food poisoning handle the same types of foods and adopt similar food hygiene precautions in their kitchens to the rest of the population. This suggests the need to examine other factors. A case-control study of sporadic Salmonella food poisoning was conducted to investigate several domestic kitchen risk factors. Measures of perception of risk, knowledge, and control associated with food poisoning in case and control respondents are reported here. It was found that perceived personal risk from food poisoning in the home was less than
perceived risk to other people. In contrast, ratings of personal knowledge about food poisoning and personal control over food poisoning in the home were seen to be greater than other people's knowledge and control. There were no differences between the cases and the controls in their ratings of knowledge about food poisoning or their control over food poisoning.
FLOW CHART OF EXPERIMENT NO.1

POLYMERASE CHAIN REACTION (PCR):

Samples of poultry feed, small and large intestine and eggs

Processing of samples for DNA extraction

Optimization of DNA extraction protocol

Confirmation of DNA by agarose gel electrophoresis

PCR analysis
   (Optimization of PCR reaction condition)

PCR Amplified product analysis on agarose gel

If no required size DNA band on gel
   Sample does not contained pathogen
      No. of negative samples were recorded
         Data analysis

If required size DNA band on gel
   Sample contained pathogen
      No. of positive samples were recorded
         Data analysis
CHAPTER 3

MATERIALS AND METHODS

A series of five experiments were conducted to study and explore the project as mentioned below:

EXPERIMENT NO. 1

Identification of Salmonella species from poultry feed, eggs and meat prevalent in Lahore area through polymerase chain reaction (PCR).

COLLECTION OF SAMPLES

A total of 400 samples of poultry feed, intestines and eggs were collected randomly through systematic random sampling method for the identification of Salmonella species (Salmonella enteritidis and Salmonella typhimurium) from Lahore area and prepared for polymerase chain reaction (PCR) (Radstorm et al., 2003, Sachse and Frey, 2003).

Systematic Random Sampling Method:

The 20 bags from each poultry farm were tagged serially and selected through systematic random sampling method i.e. 5th, 10th, 15th, 20th bag of each type feed were selected from all the five areas of Lahore city. Similarly one poultry shop from each of the five areas having eggs and both of the desi and broiler birds for marketing were selected. The broilers of one cage and desi birds of an other cage were tagged with serial numbers from 1-50. Then every fifth bird
was picked/selected from desi and broiler cages separately. The following serial numbered birds were removed, slaughtered and their small and large intestines were separately collected in sterilized small plastic bottles containing normal saline solution: 5th, 10th, 15th, 20th, 25th, 30th, 35th, 40th, 45th and 50th. The birds each of broiler and desi were thus randomly selected through systematic random sampling method. Similarly 25 eggs from the baskets were tagged and every fifth egg of layer, desi, double yolk and broken eggs were selected through systematic random sampling method.

i) Poultry Feed:

One hundred feed samples of broilers and layers (3 kinds of feed used in layers i.e. starter, grower and finisher), whereas, 2 kinds used in case of broilers (i.e. starter and finisher) were collected randomly through systematic random sampling method from different poultry farms present in Lahore Area (i.e. Wahga Boarder Road, Thokar Niaz Baig Road, Bedian Road, Chungi Amar Saddu and Ferozepur Road, Lahore).

ii) Poultry Meat (Small and Large Intestines):

Two hundred samples of Poultry Intestines (100 samples of small intestines; 50 from Local Poultry Breeds (Desi) and 50 from broiler chickens) and (100 samples of Large Intestines; 50 from local Poultry breeds (Desi) and 50 from broiler chickens) were collected randomly through systematic random sampling method from different commercial poultry market points of Lahore area (i.e. Station Area, Shadman Area, Thokar Niaz Baig area, Chungi Amar Saddu area and Ferozepur Road Ichara area Lahore).
iii) Poultry Eggs (Albumin and Yolk):

One Hundred samples of eggs were collected randomly from different Poultry market points of Lahore area (i.e. Station Area, Shadman Area, Thokar Niaz Baig area and Chungi Amar Saddu area and Ferozepur Road Ichara area, Lahore) in the following order:

1. Layer eggs = 25
2. Desi eggs = 25
3. Double Yolk eggs = 25

The albumin and yolk of eggs were checked/investigated for the presence of Salmonella enteritidis and Salmonella typhimurium.

All above mentioned samples were collected in sterilized plastic bottle/urine containers and brought immediately to the school of biological sciences, University of Punjab, Lahore for further processing. The samples were analyzed for the identification of Salmonella enteritidis and Salmonella typhimurium which were considered most probable causative agents for typhoid fever and food poisoning in humans, by using polymerase chain reaction (PCR) (Wang and Yeh 2002, Sachse and Frey, 2003)

POLYMERASE CHAIN REACTION (PCR)

A. Chemicals and Regents Used

1. Buffered Peptone Water 225 ml (prepared 1000 ml of stock solution by dissolving 10gm of Enzymatic digest of animal tissue/experimental sample (1%), 5gm Nacl (0.5%), 9gm Na₂HPO₄,
12H₂O (09%) and 1.5gm KH₂PO₄ (0.15%) in 1000 ml of double distilled water by heating and autoclaving it and then took 225 ml from stock solution as required concentration.

2. TE Buffer 300 ul (1mM EDTA, 10mM Tris-HCl PH 8.0):

3. PCR Buffer 1X (50 mM KCl, 20 mM Tris-HCl, pH 8.4) (One time concentration with reference to stock solution)

4. dNTPs 0.4 mM (Milli molar)

5. MgCl₂ 1.5 mM (Milli molar)

6. Taq. Polymerase 2.5 U (units used for enzymatic activity)

7. Forward Primer 100 p mol/ul (pico mole/ul)

8. Reverse Primar 100 p mol/ul (pico mole/ul)

9. DNA (supernatant) 5ul (microliter)

10. dH₂O Variable

a. Processing of Samples (Rad Storm et al, 2003; Sachse and Frey, 2003)

   1. Added 25gm of the sample to be investigated to 225 ml of buffered peptone water using 1-L culture flask and homogenize the sample by vigorous stirring.

   2. Incubated the pre-enrichment sample at 37°C for 16-20 hours without shaking.

b. Standardization of DNA Extraction Protocol

DNA Extraction was carried out by the method as described by Wang and Yeh, (2002) and Sachse and Frey, (2003).
Materials and Methods

1. Transferred 1 ml microbial pre-enrichment sample into a clean 1 ml micro centrifuge tube. Spinned the tube in a micro centrifuge for 5 min. at 10,000 gm and at 4°C.

2. Discarded the supernatant carefully.

3. Re-suspended the pellet in 300 ul of TE buffer (Trisacetate buffer), for more efficient removal of PCR inhibitors.

4. Incubated the micro centrifuge tube for 15 min. in a shaker water bath at 100°C. After incubation, chilled it immediately on ice.

5. Centrifuged the tube for 5 min. at 13,000 gm at 4°C. Transferred the supernatant carefully into a fresh eppendorf.

6. Used 5-ul aliquot of the supernatant as template DNA in the PCR.

7. Stored the supernatant at -20°C. (Wang and Yeh, 2002; Sachse and Frey, 2003)

c. Confirmation of DNA

The presence of DNA was confirmed by 0.7% Agarose gel electrophoresis by following procedure:

1. The DNA sample 5ul (1ug/ul) was mixed with 2 ul of gel loading dye and loaded in a gel containing 0.7% agarose.

2. The electrophoresis was carried out at a constant voltage of 90 volts for 1 hour.

3. The total genomic DNA was visible on ultra-violet (uv) gel documentation system and was photographed. The required genomic DNA fragment was visible at 23 Kb position (Fig 1.1).
B. PCR AMPLIFICATION

a. Primer sets used

The Primer sets were used as described by Wang and Yeh, (2002) and Sachse and Frey, (2003) and commercially prepared and presented in (table-1.1).

b. Reaction Mixture:

The PCR was performed in a 50ul reaction mixture (table 1.2 and 1.3) for Salmonella enteritidis and Salmonella typhimurium containing 1 x PCR Buffer, 0.4 nM dNTPs, 1.5 mM MgCl₂, 2.5 u Taq Ploymerase, 100 p mol/ul of each primer, 5 ul of DNA extracted and variable distilled water.

c. Amplification Condition

The PCR condition was optimized by considering different melting temperature (Tₘ) values (45°C, 50°C and 55°C) and optimized Tₘ value was find out to be 50°C and the optimized cycling condition for DNA samples was denaturation at 94°C for 5 minutes and 30 cycles which involved annealing (binding) at 50°C for 30 seconds and extension at 72°C for 30 seconds prior to final extension at 72°C for 7 minutes. The PCR product was amplified by using thermocycler (Applied Biosystem Model # 2720). The PCR product was then analyzed by 2.2% agarose gel electrophoresis (Fig-1.2).

C. DETECTION OF PCR AMPLIFIED TARGET DNA

Material and Equipments

- An electrophoresis chamber and power supply
- Gel casting trays
Materials and Methods

- Sample Comb
- Ethidium Bromide
- Gel Loading Dye
- Micropipettes and tips to load dye samples
- Electrophoresis buffer, Tris-acetate – EDTA (TAE)
- Ultra Violet (UV) Transluminator
- Polaroid Camera

To detect the amplified product of PCR; the following steps were adopted:

a. Preparation of Gel
   - To make 2.2% agarose gel for PCR product analysis, 100 ml (1X) TAE buffer was taken and added 2.2 gm agarose (Prepared 1000 ml (50X TAE) buffer (stock solution) by adding 242 gm trisbase + 57.1 ml acetic acid + 100 ml (0.5 molar) EDTA.
   - The mixture was heated in a microwave oven on high setting for 1.5 minutes or until mixture began to boil.
   - A folded paper towel was used to hold the neck of the flask/beaker, the gel mixture was swirled well to dissolve the agarose.
   - The molten gel solution was removed carefully from microwave using a folded paper towel to hold the neck of the flask/beaker.
   - The agarose was allowed to cool for 10-15 minutes at room temperature before the gel was poured.

b. Preparation of Gel Tray and Pouring of Gel
   - The gel tray was prepared by sealing ends with tape
Materials and Methods

- The comb was placed in gel tray about 1 inch from one end of the tray and positioned the comb vertically such that the teeth were about 0.1 to 0.2 mm above the surface of the tray.

- Before pouring, ethidium bromide was added to the gel at this point to a concentration of 0.5 μg/ml to facilitate visualization of DNA after electrophoresis.

- The gel solution was poured into tray to a depth of about 5 mm.

- The gel was allowed to solidify about 20-30 minutes at room temperature.

C. Preparation DNA Samples

- The DNA samples were prepared during the process of gel solidification.

- A small piece of Para film was cut and placed on bench near gel.

- 8 ul of 6 x loading dye was spotted at Para film for each sample to be loaded on gel, to increase the sample density and to provide a visible marker to monitor the process of electrophoresis.

- 50 ul sample was drawn into a pipette tip and was pipleted up and down at a spot of loading dye to mix.

D. Loading the Samples

- The tape was removed from the ends of gel chamber.

- The gel was placed in horizontal electrophoresis chamber with the wells near the negative electrode.
The gel chamber was filled with sufficient 1 x TAE Buffer in such a way that the level of liquid just covered the gel.

On the data sheet the samples details were noted.

*Gene ruit*™ DNA ladder Mix (Fermentas inc. USA) prepared ladder was loaded in one well and the numbered dye samples were loaded in others wells.

### E. Running the Gel

- The lead and power load were placed on the *apparatus*.
- The electrophoresis unit was connected to power supply.
- The power supply (90 Volts) was turned on.
- The *proper* operation was confirmed by checking for gas production (bubbles) at electrodes (*electrolysis of water*).
- The migration of DNA towards the *anode* was noted.
- The gel was run until the DNA loading dye was *approximately* ¾ the way across the gel.

### F. Photography

- *The power* was turned off.
- The gel was removed from the gel chamber.
- The gel was placed in an ultra-violet (UV) transilluminator to visualize the DNA.
- The UV transilluminator was turned on and *looked* at the gel.
- To confirm the presence of DNA *and* to estimate the size it was compared with DAN ladder *and* was photographed with a Polaroid camera.
Fig. 1.1 Confirmation of Total Genomic DNA Extracted From Experimental Samples.

M = Marker DNA (Gene ruler) = 10 Kb Ladder
TSE = Total Genomic DNA for *Salmonella enteritidis*
TST = Total Genomic DNA for *Salmonella typhimurium*
**PCR primer sequences and expected product size**

**Primers used for salmonella enteritidis (Set-1)**

<table>
<thead>
<tr>
<th>Primers Sequences</th>
<th>Expected Size</th>
</tr>
</thead>
<tbody>
<tr>
<td><code>5'-AGTGCCATACTITTAATGAC-3'</code> (Forward primer)</td>
<td>316 bp</td>
</tr>
<tr>
<td><code>5'-ACTATGTCGATACGGTGGG-3'</code> (Reverse primer)</td>
<td></td>
</tr>
</tbody>
</table>

**Primers used for salmonella typhimurium (Set-2)**

<table>
<thead>
<tr>
<th>Primers Sequences</th>
<th>Expected Size</th>
</tr>
</thead>
<tbody>
<tr>
<td><code>5'-GTGAAATTATCGCCACGTCCGGGCAA-3'</code> (Forward primer)</td>
<td>284 bp</td>
</tr>
<tr>
<td><code>5'-TCATCGCACCAGTCAAAGGAACC-3</code> (Reverse primer)</td>
<td></td>
</tr>
</tbody>
</table>

Note: “The primers of (set-1) were same as described by Wang & Yeh, 2002”

“The primers of (set-2) were same as described by Sachse & Frey, 2003”
Fig. 1.2  PCR amplification condition

30 Cycles

\[ \begin{array}{c|c|c|c|c|c|c|c}
94^\circ C & 94^\circ C & 72^\circ C & 72^\circ C \\
5:00 \text{ min} & 0:30 \text{ min} & 0:30 \text{ min} & 7:00 \text{ min} \\
\hline
50^\circ C & \ & \ & \ \\
0:30 \text{ min} & \ & \ & \ \\
\hline
4^\circ C & \ & \ & \ \\
\alpha & \ & \ & \ \\
\end{array} \]
<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Stock Concentration</th>
<th>Required Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR buffer</td>
<td>10 X</td>
<td>1 X</td>
</tr>
<tr>
<td>dNTPs</td>
<td>2.5 mM</td>
<td>0.4 mM</td>
</tr>
<tr>
<td>MgCl(_2)</td>
<td>25 mM</td>
<td>1.5 mM</td>
</tr>
<tr>
<td>Taq. Polymerase</td>
<td>5 U/µl</td>
<td>2.5 U</td>
</tr>
<tr>
<td>Forward Primer</td>
<td>50 p mol/µl</td>
<td>100 p mol/µl</td>
</tr>
<tr>
<td>Reverse Primer</td>
<td>50 p mol/µl</td>
<td>100 p mol/µl</td>
</tr>
<tr>
<td>DNA (supernatant)</td>
<td>5 µl</td>
<td>5 µl</td>
</tr>
<tr>
<td>dH(_2)O</td>
<td>Variable.</td>
<td>Variable.</td>
</tr>
</tbody>
</table>

**Note:** Chemicals used for 3 reactions.
Table-1.3: **Chemicals used for PCR condition optimization of Salmonella typhimurium.**

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Stock Concentration</th>
<th>Required Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR buffer</td>
<td>10 X</td>
<td>1 X</td>
</tr>
<tr>
<td>dNTPs</td>
<td>2.5 mM</td>
<td>0.4 mM</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>25 mM</td>
<td>1.5 mM</td>
</tr>
<tr>
<td>Taq. Polymerase</td>
<td>5 U/μl</td>
<td>2.5 U</td>
</tr>
<tr>
<td>Forward Primer</td>
<td>50 pmol/μl</td>
<td>100 pmol/μl</td>
</tr>
<tr>
<td>Reverse Primer</td>
<td>50 pmol/μl</td>
<td>100 pmol/μl</td>
</tr>
<tr>
<td>DNA (supernatant)</td>
<td>5 μl</td>
<td>5 μl</td>
</tr>
<tr>
<td>dH₂O</td>
<td>Variable</td>
<td>Variable</td>
</tr>
</tbody>
</table>

**Note:** Chemicals used for 3 reactions.
Fig. 1.4 Optimization of PCR condition for *Salmonella typhimurium*

M | 1 | 2 | 3
---|---|---|---
500 bp |  |  |  
284 bp |  |  |

*M = DNA marker upper band 500 bp.*

1 = Reaction condition applied as given in table 3 and $T_m$ adjusted at 45 °C.
2 = Reaction condition applied as given in table 3 and $T_m$ adjusted at 50 °C.
3 = Reaction condition applied as given in table 3 and $T_m$ adjusted at 55 °C.

**Key:** Experiment repeated 2 times for each condition.
FLOW CHART OF EXPERIMENT NO. 2

SCHEME USED FOR THE ISOLATION AND CHARACTERIZATION OF
SALMONELLA ENTERITIDIS AND SALMONELLA TYPHIMURIUM

Samples (Poultry feed, intestines and eggs)

Enrichment broth at 37 °C for 48 hours (Selenite broth)

XLD and Brilliant green agar at 37 °C for 24 hours

Suspected Colonies

T.S.I

Confirmation by

Biochemical tests

Serological tests
EXPERIMENT NO. 2

Isolation and Serological characterization of *Salmonella enteritidis* and *Salmonella typhimurium* was conducted by the following procedure (OIE, 2000).

2.1 Collection of Samples

The samples of the material (poultry feed, intestines and eggs) which have been identified as highly positive for *Salmonella enteritidis* and *Salmonella typhimurium* by PCR in the school of biological sciences, University of the Punjab, Lahore were selected for the isolation of Salmonella species. These samples were pooled and kept in sterilized plastic bottles/urine containers and brought to the department of Microbiology/Pathology, University of Veterinary and Animal Sciences, Lahore for further processing.

2.2 Sterilization of glassware

The glassware was thoroughly washed, cleaned, dried and wrapped with paper or plugged with cotton wool, where necessary and sterilized in hot air oven at temperature 180 °C for 60 minutes.

2.3 Preparation of Culture Media

The following bacteriological media were prepared according to the instructions as described by manufacturers.

2.3.1 Routine Media

The bacteriological media like MacConkey agar, Brilliant green agar and nutrient agar etc. were rehydrated and PH adjusted, where necessary and sterilized according to the instruction of manufacturers. The media plates and
tubes were incubated at 37°C for 24 hours for ascertaining their sterility and stored in the refrigerator.

2.3.2 Enrichment Media/Selenite broth

Selenite broth was rehydrated in accordance with the manufacturers instructions, heated to boiling point and distributed in 10 ml quantity per tube. The broth was freshly prepared before use and this media was not autoclaved.

2.3.3 Selective Media

Bismith sulphate agar was used as selective media for primary isolation of Salmonella. The media was rehydrated as per manufacturer's instructions and heated to boiling point and poured in plates. This media was not autoclaved. The plates were incubated at 37°C for 24 hours for ascertaining their sterility and stored in refrigerator.

2.3.4 Sugar Media

These media were prepared as follows.

A 10% stock solution of each sugar was prepared and sterilized by lyophilization and peptone water was prepared by dissolving 10 gm of peptone and 5 gm of sodium chloride in 1000 ml of distilled water.

The phenol red was used as an indicator and 0.2% stock solution was prepared. In routine 5 ml of this solution was added to each 100 ml of peptone water. The PH of peptone water was adjusted at 7.2. Then the medium was dispensed in 5 ml quantity in each tube, inverted Durhams fermentation tubes were inserted to each test tube and were stopped with cotton wool. These tubes were then sterilized in autoclave at 121°C for 15 minutes. After cooling, each
tubes was added 0.25 cc of 10% solution of sugar. The tubes were incubated for 48 hours for testing their sterility. The tubes showing changes in colour were discarded.

2.4 Isolation Procedure

The isolation was carried out as described by Quinn et al. 2002.

2.4.1 Enrichment of Culture

Duplicate sets of freshly prepared selenite broth tubes (10 ml each) were inoculated with 3 gms of the sample and subsequently incubated aerobically at 37°C for 48 hours. Turbidity of the tubes showed the enhanced growth of Salmonella.

2.4.2 Culturing on Selective Media

The samples from the tubes showing turbidity were subcultured on selective media (brilliant green agar and xylose lysine deoxycolate (XLD) agar). The plates were incubated aerobically at 37°C for 24 hours. After 24 hours the colonies were observed for identification and further processing.

2.5 Identification of organisms (OIE, 2000)

2.5.1 Culture Characteristics

a) Morphological examination

b) Colony examination

c) Motility examination

da) Morphological examination

The colonies from pure growth suspected for Salmonella were used for making a smear on a clean microscopic slide, stains with Grams method of
staining and examined under oil immersion lens. This gave a clear view of staining reactions and morphology of the isolate.

b) Colony examination

Suspected colonies of the isolates streaked on brilliant green plates and incubated at

37 °C for 24 hours were observed for color, consistency, size and appearance of colonies (Circular and pink colonies).

c) Motility examination

A loopful of growth from overnight culture in nutrient broth was placed on cover slip, which was inverted over a cavity glass slide and examined under the microscope with high power.

The organisms constantly changing their position in the microscopic field were considered to be exhibiting true motility.

2.5.2 Biochemical tests

Following biochemical reactions were performed as described by Cappuccino and Sherman (2004)

a) Triple sugar iron test

b) Urease test

c) Indole test

d) Methyl red test (MR test)

e) Voges proskauer test (VP test)

f) Citrate utilization test

g) Sugar fermentation test
a) **Triple sugar iron test**

The lactose negative colonies were inoculated on triple sugar iron test (TSI) agar. This medium contains three sugars, glucose, lactose, sucrose, an indicator and ferrous sulphate. This medium was used for studying the ability of organisms to ferment any or all of these three carbohydrates and to produce hydrogen sulphide gas. Triple sugar iron agar medium was tubed in such a way that it gave a deep butt and a short slant.

Single colony of each isolate was picked up with the help of straight inoculating wire and stabbed in the butt and streaked gently over the surface of the slant. Inoculated tubes were incubated at 37°C for 24 hours. Interpretation of results on TSI agar was carried out as follows.

<table>
<thead>
<tr>
<th></th>
<th>Acidic butt (yellow)</th>
<th>Alkaline slant (red)</th>
<th>Glucose fermented</th>
<th>Lactose not fermented</th>
<th>Sucrose not fermented</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Acid through out the medium (butt and slant yellow)</td>
<td></td>
<td>Glucose fermented, Lactose fermented, Sucrose fermented</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Alkaline butt and slant</td>
<td></td>
<td>Glucose not fermented, lactose fermented, Sucrose not fermented</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Blackening in butt</td>
<td></td>
<td>H2S production</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Breaking of medium colour</td>
<td></td>
<td>Gas produced</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The isolates giving acidic butt and alkaline slants with or without gas production and with H2S production, were carried over to urea broth in order to determine their urease activities.
b) Urease test

The isolates confirming the triple sugar iron agar reaction were inoculated in to urea broth and incubated at 37 °C for 48 hours. The observations were recorded at an internal of 8, 12, 24 and 48 hours. Red colouration of medium (due to production of ammonia/indicated a urease positive reaction and no change in colour (yellow) were considered as negative reaction.

The isolates giving negative reaction were selected as possible salmonella and transferred to nutrient agar slants for further study.

c) Indole test

The peptone water was inoculated with the culture and incubated at 37 °C for 24 hours. Then 1 ml of Kovac’s reagent was added in 5 ml of culture. Red color ring at top of the tube indicated positive results while no coloration indicated negative results.

d) Methyle red test (MR test)

The MRVP medium was inoculated with the culture and incubated at 37 °C for 48 hours. At the end of incubation period five drops of methyle red reagent was added to each tube and the reaction was noticed immediately. A bright red colour was taken as positive and yellow as negative reaction.

e) Voges Prokauer Test (VP test)

MR-VP broth was inoculated with culture of the isolates and incubated at 37 °C for 48 hours. After incubation 3ml each of alcoholic alphanephol (5%) and potassium hydroxide (40%) was added to the medium. A positive reaction was indicated by an eosin red colour which developed within five minutes.
f) Citrate Utilization Test

Kosers citrate medium was inoculated with a loopful of growth and incubated at 37°C for 96 hours. A positive reaction was indicated by turbidity in the medium.

g) Sugar fermentation test

Besides three sugars of TSI medium, mannitol, sorbitol, inositol, Dulcitol, arabinose and Adonitol were used for studying the fermentation activities of salmonella isolates. A duplicate set of each sugar tube was inoculated with a loopful of isolate incubated at 37°C for 48 hours. Acid production was indicated by a change in the colour of the indicator from red to yellow and gas production was observed by trapping of air bubbles in Durhams tube. Negative tubes were further incubated for 24 hours.

2.6 Serological identification of the organisms (OIE, 2000)

Rapid slide agglutination test was applied for the detection of somatic ‘O’ and flagellar ‘H’ antigens of Salmonella isolates. Poly valent group specific and monovalent antiserum (Denka Seiken Co-Ltd, Tokyo, Japan manufacturer) were used for serological characterization.

2.6.1 Preparation of Antigen

i) Somatic antigen

ii) Flagellar antigen

1) Preparation of somatic antigen

i) Salmonella culture from the stock culture was inoculated in to a blood agar base (BAB) plate. Incubate overnight at 37°C aerobically.
ii) A smooth colony was selected to carry out a slide agglutination test for ensuring that the required somatic antigen was present.

iii) Using a sterile loop, nutrient agar slope was inoculated in a universal bottle from the selected colony.

iv) Culture was incubated for 12 hours at 37 °C aerobically.

v) Using a Pasteur pipette, the culture was washed off, preferably inside a safety cabinet, with approximately 2ml of absolute alcohol and transferred into a sterile universal bottle.

vi) Antigen was left for 4-6 hours at room temperature to enable the alcohol to kill the bacteria and detach flagella.

vii) Centrifugation of the material was done at 1000xg for 5 minutes. Supernatant was poured off and 5 ml of phenol saline was added in sediment to make the antigen.

viii) Standard titration was carried out with known serum to ensure that the antigen is positive for the required factor.

ix) Stored in a refrigerator at 4 °C until required.

2) Preparation of flagellar antigen

i) Salmonella stock culture was inoculated into a blood agarbase plate (BAB) plate, and culture was incubated overnight at 37 °C aerobically.

ii) Reculturing was performed in semisolid agar (about 0.3%) in a Craigie's tube, to induce optimum expression of the appropriate flagellar antigen. The antiserum corresponding to the phase to be suppressed was added to the agar.
iii) Slide agglutination test used to check that the Salmonella was in the required phase. Then a loop of culture was inoculated into 20 ml of nutrient broth and incubated for 18 hours at 37 °C aerobically for optimum growth.

iv) 250μl of 40% formaldehyde was added into the antigen suspension and leave overnight.

v) Antigen was tested by spot agglutination test (SAT) using the appropriate typing serum.

2.6.2 Spot Agglutination test with group specific polyvalent antiserum and monovalent antiserum

Material
1. Standard salmonella antisera (polyvalent and monovalent antiserum)
2. Antigen
3. Glass slides
4. Loop
5. Sterilized dropper.

Technique/Test procedure

i. Serological identification of salmonella by spot agglutination test required a 24 hours fresh culture of the isolates on nutrient agar slants. A drop of normal saline was placed on a clean microscope slide and a loopful of growth from Nutrient agar slant was emulsified with this drop. This acted as an antigen and in it added a drop of polyvalent group specific antiserum 'O' mixed thoroughly and observation was recorded.
The clumping of organisms occurring within a minute was taken as a positive reaction.

ii. Identification of species within groups was carried out by checking agglutination reaction with antiserum against phase I and phase II of flagellar antigen by using craigie tube method (Quinn et al 2002).

iii. The sero group was determined on the basis of positive agglutination reaction with polyvalent group specific antisera.

iv. For further categorization of salmonella isolate with each serogroup, the agglutination test was carried out with mono specific antiserum against somatic "O" antigen.

**Interpretation**

<table>
<thead>
<tr>
<th></th>
<th>Clean agglutination with sediment bottom</th>
</tr>
</thead>
<tbody>
<tr>
<td>++</td>
<td>Clean agglutination with some sediment</td>
</tr>
<tr>
<td>+</td>
<td>Clean agglutination with slight sediment</td>
</tr>
<tr>
<td>±</td>
<td>Agglutination just floating without sediment</td>
</tr>
<tr>
<td>-</td>
<td>Only cloudiness without agglutination</td>
</tr>
<tr>
<td></td>
<td>Clean fluid</td>
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FLOW CHART OF EXPERIMENT NO.3

DETERMINATION OF LD50 OF SALMONELLA ENTERITIDIS AND SALMONELLA TYPHIMURIUM IN BROILER CHICKS

Day old chicks
100 (in each case)

Infected groups (1-9) containing 10 birds each
\[10^{-1}-10^{-9}\] of culture @ 1ml/bird
\rightarrow
Mortality and morbidity analysis for next 7 days period
\rightarrow
Data analysis for calculation of LD50 by Reed and Muench method

uninfected group (10th) containing 10 birds each

Non inoculated (only normal saline injected) @ 1ml/bird
\rightarrow
Mortality and morbidity analysis for next 7 days period
\rightarrow
Data analysis for calculation of LD50 by Reed and Muench method
EXPERIMENT No.3

Determination of LD<sub>50</sub> of *Salmonella enteritidis* and *Salmonella typhimurium* in Broiler Chicks

a. Calculation of LD<sub>50</sub> of *Salmonella enteritidis* in broiler chicks

A total number of 100, day-old chicks were purchased from commercial hatchery; Big Bird, Pvt. Ltd, Lahore and shifted in the experimental rooms of Department of Pathology, UVAS, Lahore. These birds were provided clean water and fresh feed. The birds were divided into 10 groups (each comprising of 10 birds). Amongst them, 9 groups were given infection and 10<sup>th</sup> group was kept uninfected as control. The loopful of purified culture of *Salmonella enteritidis* was inoculated in (10ml) broth media and incubated at 37°C, aerobically, overnight in order to obtain the uniform turbidity. In order to prepare 10 fold dilution of the culture 18ml of normal saline (sterilized) was taken in 10 tubes and marked as 1-10. 2 ml of the overnight culture was added in first tube and mixed thoroughly by the help of pippet. Then from 1<sup>st</sup> tube 2ml of the material was transferred to 2<sup>nd</sup> tube and mixed thoroughly. The same procedure was adopted to make a 10 fold dilution upto 9<sup>th</sup> tube. In the 10<sup>th</sup> tube no bacterial culture was added and kept as negative control. On the 7<sup>th</sup> day of age infection was given orally by bacterial culture of *Salmonella enteritidis* @ 1 ml /bird (10<sup>1</sup> to 10<sup>9</sup> , respectively). The LD<sub>50</sub> serologically characterized culture of *Salmonella enteritidis* was calculated as described by Reed and Muench method (David et al., 1998).
Nine groups of the birds were inoculated orally with each dilution i.e. $10^{-1}$, $10^{-2}$ ---- $10^{9}$ of the culture @ 1ml/bird, while $10^{th}$ group was inoculated with normal saline @ 1ml/bird and kept as negative control. The birds were examined for a period of next 7 days for any morbidity and mortality and data collected was used to calculate LD$_{50}$ of the birds (David et al., 1998).

b. Calculation of LD$_{50}$ of *Salmonella typhimurium* in broiler chicks

A total number of 100, day-old chicks were purchased from commercial hatchery; Big Bird, Pvt. Ltd, Lahore and shifted in the experimental rooms of Department of Pathology, UVAS, Lahore. These birds were provided clean water and fresh feed. The birds were divided in to 10 groups (each comprising of 10 birds). Amongst them, 9 groups were given infection and $10^{th}$ group was kept uninfected as control. The loopful of purified culture of *Salmonella typhimurium* was inoculated in (10ml) broth media and incubated at 37°C, aerobically, overnight in order to obtain the uniform turbidity. In order to prepare 10 fold dilution of the culture 18ml of normal saline (sterilized) was taken in 10 tubes and marked as 1-10. 2 ml of the overnight culture was added in first tube and mixed thoroughly by the help of pippet. Then from 1st tube 2ml of the material was transferred to 2nd tube and mixed thoroughly. The same procedure was adopted to make a 10 fold dilution upto 9th tube. In the 10th tube no bacterial culture was added and kept as negative control. On the 7th day of age infection was given orally by bacterial culture of *Salmonella typhimurium* @ 1 ml / bird ($10^{-1}$ to $10^{9}$, respectively). The LD$_{50}$ serologically characterized culture of *Salmonella typhimurium* was calculated as described by Reed and Muench method (David et al., 1998).
Nine groups of the birds were inoculated orally with each dilution i.e. $10^{-1}$, $10^{-2}$ ---- $10^{-9}$ of the culture @ 1ml/bird, while 10th group was inoculated with normal saline @ 1ml/bird and kept as negative control. The birds were examined for a period of next 7 days for any morbidity and mortality and data collected was used to calculate LD 50 of the birds (David et al., 1998).
FLOW CHART OF EXPERIMENT NO.4 (A)

POLYMERASE CHAIN REACTION (PCR)

Samples of small and large intestine of broiler chickens

Processing of samples for DNA extraction

Optimization of DNA extraction protocol

Confirmation of DNA by agarose gel electrophoresis

PCR analysis
  (Optimization of PCR reaction condition)

PCR Amplified product analysis on agarose gel

If no required size DNA band on gel
  Sample does not contain pathogen
  No. of negative samples were recorded
  Data analysis

If required size DNA band on gel
  Sample contained pathogen
  No. of positive samples were recorded
  Data analysis
FLOW CHART OF EXPERIMENT NO.4 (B)

HISTOPATHOLOGY OF DIFFERENT ORGS OF BROILER CHICKENS EXPERIMENTALLY INFECTED WITH SALMONELLA ENTERITIDIS AND SALMONELLA TYPHIMURIUM

Tissue samples
↓
Fixation
↓
Dehydration
↓
Cleaning
↓
Impregnation with paraffin wax
↓
Sectioning
↓
Staining
↓
Mounting (DPX)
↓
Microscopic examination
EXPERIMENT NO.4

Histopathology of broiler chickens experimentally infected with *Salmonella enteritides* and *Salmonella typhimurium* to see the pathogenesis at various phases of disease

Experimental Chicks

A total number of 300 day-old broiler chicks, were obtained from commercial hatchery Big bird (Pvt.) Lahore. The chicks were reared in the sheds of Department of pathology, University of Veterinary and Animal Sciences, Lahore, under standard managerial conditions. The birds were fed with commercial poultry feed (Big feed) (Pvt.) Lahore.

<table>
<thead>
<tr>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected with <em>Salmonella enteritides</em></td>
<td>Infected with <em>Salmonella typhimurium</em></td>
<td>Un-Infected/control</td>
</tr>
<tr>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

The birds were divided in equal 3 groups A, B, and C each comprising of 100 birds and were kept up to (91 days) 3 months. *Salmonella enteritides* and *Salmonella typhimurium* inoculum was prepared after calculation of LD50 according to Reed and Munch method (David et al., 1998) in broiler chicks.

Inoculation of Chicks

The inoculation of broiler chicks of experimental groups was done through oral route at the age of one week (7 days) and waited till the appearance of disease. *Salmonella enteritides* and *Salmonella typhimurium* inoculum was given at the dose rate of LD50 i.e. $10^{3.58}$/ml and $10^3$/ml per bird, respectively.

Two parameters in this experiment at different phases of disease were studied as below:
i. **Identification of Salmonella species at different phases of disease in broiler chickens through PCR.**

Five birds per group were randomly selected (i.e. all the birds were tagged serially from 1-100 and the birds from number 1-5 every week through purposive random sampling method) on 6th day of age (pre inoculation) followed by five birds per week per group (post inoculation) on 14th, 21st, 28th, 35th, 42nd, 49th, 56th, 63rd, 70th, 77th, 84th, 91st, days of age and samples of small and large intestine from all 5 birds were collected in sterilized plastic bottles and brought to the school of biological sciences, University of Punjab, Lahore for the identification of *Salmonella enteritidis* and *Salmonella typhimurium* by polymerase chain reaction (PCR) (Wang and Yeh, 2002; Sachse and Frey, 2003). Same procedure of PCR was adopted as mentioned in experiment No. 1 at page No. 116 to 129.

ii. **Histo-pathological studies of different organs of broiler chickens at different phases of disease**

The organs such as Bursa of abricious, small intestine, large intestine, lung, liver, spleen, kidney and lean muscles from all 5 birds of all groups (experimental and control groups) on 6th days (pre inoculation) and then (post inoculation) on 14th, 21st, 28th, 35th, 42nd, 49th, 56th, 63rd, 70th, 77th, 84th, 91st, days of age were collected and preserved in 10% buffered formalin Solution, then fixed tissue was processed in ascending grades of alcohol, cleared in xylene, embedded on parafine wax, sections of 3-6 microns thick were cut and stained with hematoxyline and Eosin stain (Bancroft and Gamble, 2002) for
histopathological studies in the laboratory, Department of Pathology, University of Veterinary and Animal Sciences, Lahore.

The histopathological examination of these tissues of all the groups on each sample was performed by the following order.

i. **FIXATION:**
Tissues were fixed in 10% buffered formalin:
Buffered formalin was prepared by the following method.

- 40% formalin: 100 ml
- Distilled water: 900 ml

Sodium Phosphate: 6.5 gm  
Dibasic (anhydrous)  
Sodium phosphate: 4.0 gm  
Monobasic

ii. **DEHYDRATION:**
After fixation, the tissues were placed in the ascending order of alcohol for dehydration as under in the schedule.

- 50% Alcohol 5 hours
- 70% Alcohol 5 hours
- 95% Alcohol 5 hours
- 100% Alcohol 5 hours

iii. **CLEARING:**
Pure xylene was used as a clearing agent in the following order.

- Pure xylene 3 hours
- Pure xylene 3 hours
iv. **IMPREGNATION WITH PARAFFIN WAX:**

After clearing, the tissue sample was placed in paraffin wax in the following order.

- Paraffin wax at temperature. 50-58 °C for 12 hours
- Paraffin wax at temperature. 56-58 °C for 12 hours

v. **SECTIONING:**

The prepared paraffin wax containing processed tissue section was cut in small sections of 3-5μ thick in size by tissue sectioner. After slight warming in hot water bath at temperature 40° C, tissue sample were then placed on clean glass slides covered with a thin layer of egg yolk.

vi) **STAINING:**

**Haematoxyline and eosin staining**

Dipped the prepared slides in the following solution in the following order:

<table>
<thead>
<tr>
<th>Solution</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure xylene</td>
<td>5 minutes</td>
</tr>
<tr>
<td>Pure xylene</td>
<td>5 minutes</td>
</tr>
<tr>
<td>100% Alcohol</td>
<td>3 minutes</td>
</tr>
<tr>
<td>90% Alcohol</td>
<td>3 minutes</td>
</tr>
<tr>
<td>80% Alcohol</td>
<td>3 minutes</td>
</tr>
<tr>
<td>70% alcohol</td>
<td>3 minutes</td>
</tr>
<tr>
<td>Distilled water</td>
<td>3 dips</td>
</tr>
<tr>
<td>Haematoxyline stain</td>
<td>3-5 minutes</td>
</tr>
<tr>
<td>Washed with tap water</td>
<td>1-2 minutes</td>
</tr>
<tr>
<td>Distilled water</td>
<td>3 dips</td>
</tr>
<tr>
<td>Ammonia water</td>
<td>1-2 dips</td>
</tr>
<tr>
<td>Eosin stain</td>
<td>5-10 minutes</td>
</tr>
</tbody>
</table>
Washed with tap water 1-2 minutes
70% Acid alcohol 1-2 minutes
80% Alcohol 3 minutes
90% alcohol 3 minutes
100% Alcohol 3 minutes
Xylool 3 minutes
Xylool 3 minutes

vii) MOUNTING:

Placed a drop of mounting material (DPX) on the stained tissue section, applied a cover slip on it with pressure and studied the stained slides under different magnifications of the microscope. The prominent lesions were photographed.
FLOW CHART OF EXPERIMENT NO. 5

POLYMERASE CHAIN REACTION (PCR)

Samples of suspected typhoid fever and food poisoning patients (stool & blood)

Processing of samples for DNA extraction

Optimization of DNA extraction protocol

Confirmation of DNA by agarose gel electrophoresis

PCR analysis
(Optimization of PCR reaction condition)

PCR Amplified product analysis on agarose gel

If no required size DNA band on gel
Sample does not contain pathogen
No. of negative samples were recorded
Data analysis

If required size DNA band on gel
Sample contained pathogen
No. of positive samples were recorded
Data analysis
EXPERIMENT No. 5

Public Health Impact of Salmonellosis through Consuming the Meat and Eggs of the carrier’s birds

Collection of Samples

A total of 400 samples of humans stool and blood were collected (200 samples of human stool and 200 samples of human blood) of 200 patients suspected of suffering from typhoid fever and food poisoning from different hospitals i.e. Myo-Hospital, Services Hospital, General Hospital, Jinnah Hospital in the Lahore area. One hundred samples from each of the four hospitals were collected i.e., 25 patients suspected of typhoid fever and 25 patients suspected of food poisoning. From each patient two samples i.e., one of stool and one of blood was collected simultaneously at each visit from the hospital laboratories, wards, out-doors and emergencies of each Hospital were collected in small plastic bottles and EDTA coated vacutainer tubes by using disposable syringes respectively. The history and data of last one week was also collected from infected human patients of suspected typhoid fever and food poisoning about eating poultry meat and eggs to ascertain the source of infection. In this regard a questionnaire was used for every patient to elicit their possible causative factors of the disease. Information was collected about their age, sex, occupation, family status, living conditions, addiction, history of contact, consumption of poultry meat, eggs and contaminated water and geographical distribution. These samples were analysed for the identification of Salmonella species by using
Polymerase chain reaction (PCR) test at School of Biological Sciences, University of the Punjab, Lahore (Wang and Yeh, 2002; Sachse and Frey, 2003).

APPLICATION OF PCR

The same procedure was adopted for the application of PCR test on the stool and blood samples of suspected typhoid fever and food poisoning patients as mentioned in experiment no. 1page no.116 to 129.

METHOD OF DATA COLLECTION

The data was collected by semi structural interview through questionnaire to determine the patients socio-economic condition i.e. housing, income, geographical, eating of poultry meat and eggs. The interview schedule and the questionnaire were pre-tested and necessary changes were made.

QUESTIONNAIRE

The questionnaire was designed for the particular study in order to collect the standard and uniform information from each patient.

1. The patients and their level of awareness about the disease and its spread.
2. Such study includes a large number of variables such as *Salmonella enteritidis, Salmonella typhimurium*. Food and food products, Poultry meat (charga, tikka and kababs) Eggs (half boiled and half fry) Bakery goods (poultry bi products)
3. The questionnaire was translated into local language at a time of interview to eliminate interviewer bias as far as possible.
4. The interviewer was the same for all the patients.
5. The advantages of the interview method are that the question can be explained to the respondent, who need not to be educated and the respondent's interest can be judged.
6. The options like no or don't know were given.
7. The respondents were given the exact instructions about the questions asked in the questionnaire.

6. The questionnaires were pre-tested (test for questionnaire that can be understood clearly so that there is no confusion).

DATA ANALYSIS

The data obtained was statistically analysed according to Wayne and Daniel (1995); Roger et al. (2003)
RESULTS
CHAPTER 4

RESULTS

Experiment No. 1

1. Poultry Feed

One hundred layer feed samples i.e., starter, grower and finisher and broiler feed samples i.e., starter and finisher were collected randomly through systematic random sampling method from different feed mills/Poultry farms present in and around Lahore area were analysed for the identification of Salmonella enteritidis and Salmonella typhimurium by polymerase chain reaction (PCR) (Wang and Yeh 2002, Sachse and Frey 2003). It was observed that out of 20 samples of feed of layer (starter), 4 samples were found positive for Salmonella enteritidis and 3 were positive for Salmonella typhimurium. Out of 20 samples of feed of layer (grower), the 3 samples were found positive for Salmonella enteritidis and 2 samples were positive for Salmonella typhimurium and out of 20 samples of feed of layer (finisher), 2 were positive for Salmonella enteritidis and 2 were positive for Salmonella typhimurium.

Thus, percentage of positive cases in the feed of layers (starter, grower and finisher) for Salmonella enteritidis were 20 %, 15 % and 10 %, respectively and for Salmonella typhimurium were 15 %, 10 % and 10 %, respectively. Similarly out of 20 samples of feed of broilers (starter), 3 were positive for Salmonella enteritidis and 2 were positive for Salmonella typhimurium and out of
20 samples of feed of broiler (finisher), 2 were positive for *Salmonella enteritidis* and 2 were positive for *Salmonella typhimurium*.

Thus, percentage of positive cases in the feed of broilers (starter and finisher) for *Salmonella enteritidis* was 15% and 10%, respectively and percentage of positive cases in the feed of broilers (starter and finisher) for *Salmonella typhimurium* was 10% and 10%, respectively (Table No.1.4)

2. **Poultry Meat (Small and Large Intestines)**

Two hundred samples of small and large intestines of Desi (local poultry breed) and Broilers were collected randomly from different Poultry farms present in Lahore area and analysed for the identification of *Salmonella enteritidis* and *Salmonella typhimurium* by polymerase chain reaction (PCR) (Wang and Yeh 2002, Sachse and Frey 2003). It was observed that out of 50 samples of small intestines of Desi chickens, the number of positive samples for *Salmonella enteritidis* were 8 and for *Salmonella typhimurium* were 7, respectively and out of 50 samples of large intestines of Desi chickens, the number of positive samples for *Salmonella enteritidis* were 6 and for *Salmonella typhimurium* 5.

Thus, percentage of both *Salmonella enteritidis* and Salmonella typhimurium in 50 samples of small intestines of Desi chickens was 2%.

Similarly, percentage of *Salmonella enteritidis* in 50 samples of large intestines of Desi birds was 16% and for *Salmonella typhimurium* was 14%.

Out of 50 samples of Small intestines of broilers, the number of *Salmonella enteritidis* were 4% and for *Salmonella typhimurium* were 2% and out
of 50 samples of large intestines of broilers, the *Salmonella enteritidis* was 18% and *Salmonella typhimurium* was 16%.

Thus, percentage of positive samples for *Salmonella enteritidis* in 50 samples of small intestines of broilers was 4% and *Salmonella typhimurium* was 2%. Similarly, the percentage of *Salmonella enteritidis* in 50 samples of large intestines was 18% and *Salmonella typhimurium* was 16% (Table No. 1.5).

3. **Poultry Eggs**

One hundred samples of eggs (25 layer, 25 Desi, 25 double yolk and 25 broken eggs) were collected randomly from different commercial poultry market points in Lahore area and analysed for the identification of *Salmonella enteritidis* and *Salmonella typhimurium* by PCR (Wang and Yeh 2002, Sachse and Frey 2003). During the analysis of poultry eggs (albumin & yolk) by PCR, it was observed that.

Out of 25 samples each of layer eggs, the number of *Salmonella enteritidis* in albumin were 4 and 3 in yolk and number of *Salmonella typhimurium* found in albumin were 3 and 2 in yolk.

Out of 25 samples of Desi eggs, the number of *Salmonella enteritidis* found were 2 in albumin and 1 in yolk and number of *Salmonella typhimurium* found positive were 1 in albumin and 1 in yolk.

Out of 25 samples of double yolk eggs, the number of *Salmonella enteritidis* found were 4 samples in albumin and 3 in yolk and number of *Salmonella typhimurium* found were 3 in albumin and 2 in yolk.
Out of 25 samples of broken eggs, the number of *Salmonella enteritidis* found were 4 in albumin and 3 in yolk and number of *Salmonella typhimurium* found were 3 in albumin and 1 in yolk.

Thus, percentage of positive samples of *Salmonella enteritidis* in albumin and yolk of layer eggs was 16 % in albumin and 12 % in yolk and percentage of *Salmonella typhimurium* were 12 % in albumin and 8 % in yolk.

The percentage of positive samples of *Salmonella enteritidis* in albumin and yolk of Desi eggs was 8 % in albumin and 4 % in yolk and percentage of *Salmonella typhimurium* was 4 % in albumin and 4 % in yolk.

The percentage of positive samples of *Salmonella enteritidis* in albumin and yolk of double yolk eggs was 16 % in albumin and 12 % in yolk and percentage of *Salmonella typhimurium* was 12 % in albumin and 8 % in yolk.

The percentage of positive samples of *Salmonella enteritidis* in albumin and yolk of broken eggs was 16 % in albumin and 12 % in yolk and percentage of *Salmonella typhimurium* was 12 % in albumin and 4 % in yolk (Table No. 1.6 and 1.7).
Fig 1.5: PCR analysis of poultry feed samples for *Salmonella enteritidis* and *Salmonella typhimurium*

M = DNA marker.
7, 8, 9 and 10 = *Salmonella enteritidis* experimental poultry feed samples.
1, 2, 3 and 4 = *Salmonella typhimurium* experimental poultry feed samples.
+ = Positive controls.
- = Negative control.
Fig 1.6: PCR analysis of poultry feed samples for *Salmonella enteritidis* and *Salmonella typhimurium*

M = DNA marker.
5 = *Salmonella enteritidis* in experimental poultry feed samples.
6 = *Salmonella typhimurium* in experimental poultry feed samples.
+ = Positive controls.
− = Negative control.
Table No. 1.4: PCR analysis of Poultry feed Samples for *Salmonella enteritidis* and *Salmonella typhimurium*

<table>
<thead>
<tr>
<th>Layer</th>
<th>Broiler</th>
<th>Layer</th>
<th>Broiler</th>
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<th>Layer</th>
<th>Broiler</th>
<th>Layer</th>
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<td>S</td>
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</table>

\( P > 0.05 \) (See appendix-I)

**Formula:**

\[
\% = \frac{\text{Number of positive samples}}{\text{Total No of samples in each group}} \times 100
\]

**Key:**

SE = *Salmonella enteritidis*

ST = *Salmonella typhimurium*

S = Starter

G = Grower

F = Finisher
Fig 1.7: PCR analysis of poultry meat samples (small and large intestines) for *Salmonella enteritidis* and *Salmonella typhimurium*

M :: DNA marker.

1, 2, 3 and 4 :: *Salmonella enteritidis* in experimental poultry meat samples of small and large intestine of local (Desi) and Broiler chickens.

5, 6, 7 and 8 :: *Salmonella typhimurium* in experimental poultry meat samples of small and large intestine of local (Desi) and Broiler chickens.

+ = Positive controls,
- = Negative control.
Table No. 1.5: PCR analysis of Poultry Meat (Small & Large Intestine)  
Samples for *Salmonella enteritidis* and *Salmonella typhimurium*

<table>
<thead>
<tr>
<th>No and type of samples collected</th>
<th>No of positive samples for SE</th>
<th>%age of positive samples for SE</th>
<th>No of positive samples for ST</th>
<th>%age of positive samples for ST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desi * Broiler</td>
<td>S.I</td>
<td>1</td>
<td>S.I</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>L.I</td>
<td>8</td>
<td>L.I</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>S.I</td>
<td>2</td>
<td>S.I</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>L.I</td>
<td>9</td>
<td>L.I</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>S.I</td>
<td>16</td>
<td>S.I</td>
<td>50</td>
</tr>
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<td>L.I</td>
<td>18</td>
<td>S.I</td>
<td>50</td>
</tr>
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<td></td>
<td>S.I</td>
<td>1</td>
<td>L.I</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>L.I</td>
<td>7</td>
<td>L.I</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>S.I</td>
<td>8</td>
<td>L.I</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>L.I</td>
<td>14</td>
<td>L.I</td>
<td>50</td>
</tr>
</tbody>
</table>

*P <0.05 (See appendix-II)*

Key:  
SE = *Salmonella enteritidis*  
ST = *Salmonella typhimurium*  
SI = Small Intestine  
LI = Large Intestine  
Desi= Local Poultry breed
Fig 1.8: PCR analysis of poultry eggs samples for *Salmonella enteritidis*

1-8 = *Salmonella enteritidis* in experimental poultry eggs of layer, Desi, double yolk and broken eggs.

+ = Positive controls.

- = Negative control.
Fig 1.9: PCR analysis of poultry eggs samples for *Salmonella typhimurium*

M  1  2  3  4  5  6  7  8  -

M     DNA marker.

1-8 = *Salmonella typhimurium* in experimental poultry eggs of layer, desi, double yolk and broken eggs.

+ = Positive controls.

- = Negative control.
### Table-1.6: PCR analysis of Poultry Egg Samples for *Salmonella enteritidis*

<table>
<thead>
<tr>
<th>No &amp; type of egg samples collected</th>
<th>No of positive samples for <em>Salmonella enteritidis</em></th>
<th>Percentage of positive samples for <em>Salmonella enteritidis (%)</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>LE</td>
<td>DE</td>
<td>DYE</td>
</tr>
<tr>
<td>---------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

P >0.05 (See appendix-III)

**Key:**
- **SE** = *Salmonella enteritidis*
- **LE** = Layer Eggs
- **DE** = Desi Eggs
- **DYE** = Double Yolk Eggs
- **BE** = Broken eggs
- **A** = Albumin
- **Y** = Yolk

**Note:** Same numbers of eggs have been tested through PCR for *Salmonella enteritidis* and *Salmonella typhimurium*
Table No. 1.7: PCR analysis of Poultry Egg Samples for *Salmonella typhimurium*

<table>
<thead>
<tr>
<th>No &amp; type of egg samples collected</th>
<th>No of positive samples for Salmonella typhimurium</th>
<th>Percentage of positive samples for ST (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LE</td>
<td>DE</td>
<td>DYE</td>
</tr>
<tr>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>P &gt;0.05 (See appendix-III)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Key:**

ST = *Salmonella typhimurium*
LE = Layer Eggs
DE = Desi Eggs
DYE = Double Yolk Eggs
BE = Broken eggs
A = Albumin
Y = Yolk

**Note:** Same numbers of eggs have been tested through PCR for *Salmonella enteritidis* and *Salmonella typhimurium*.
EXPERIMENT NO.2

The samples (Poultry feed, Intestines and eggs) which were found positive by PCR in the School of Biological Sciences, University of Punjab, Lahore showed visible turbidity after 48 hours of inoculation in the selenite broth tubes. The culture was transferred to brilliant green agar and XLD agar and after 24 hours of incubation the inoculated brilliant green agar plates showed red colonies while XLD medium showed red colonies with black center. All the colonies were circular and smooth in appearance.

MORPHOLOGICAL EXAMINATION

Microscopic examination of the smears, prepared from young cultures on solid medium, revealed the presence of gram negative short rods of varying size, occurring singly or in short chains.

MOTILITY EXAMINATION

Motility test, carried out on overnight broth cultures showed that all the cultures were actively motile.

BIOCHEMICAL TESTS

TSI AGAR

On TSI agar all the culture isolates produced a change in the colour of the butt from red to yellow indicating glucose fermentation, whereas the slants, remained red indicating non-fermentation of lactose and sucrose. Hydrogen sulphide was also produced by the isolates which was indicated by blackening in the butt. Gas was produced by all isolates.
UREASE TEST

None of the culture produced a change in the colour of urea broth indicating absence of urease activity.

CITRATE TEST

Turbidity in the Koser's citrate medium was produced by all isolates which showed positive citrate utilization test.

MRVP TEST

All the isolated cultures gave positive methyl red test and a negative VP test.

INDOLE TEST

After the addition of Kovac's reagent in 24 hours broth cultures of the isolates. There was no appearance of red color at the top of medium which indicated that isolates were indole negative.

CARBOHYDRATE FERMENTATION

Sorbitol, maltose, dulcitol, Mannitol, and arabinose were fermented by the cultures along with production of acid and gas. None of the isolates fermented Adonitol.

SEROTYPING

The five suspected colonies from the cultures were selected to prepare the somatic and flagellar antigen for their serotyping. In order to detect the serogroup of the cultures by agglutination test. All the prepared somatic antigens were treated with B, C, D and E group specific antisera. Two of the isolates showed a positive reaction with group B and three with group D antisera.
The two isolates of group B gave a positive agglutination reaction with monospecific antisera against 1, 4 and 12 somatic "O" antigen. The three isolates of group D gave a positive agglutination reaction with monospecific antisera against 1, 9 and 12 somatic "O" antigen.

The three isolates of D group when checked for the presence of flagellar antigen "H" gave a positive reaction with G and M antisera in phase I and 1, 7 antisera in phase II and hence confirmed as Salmonella enteritidis.

The two isolates of B group when checked for the presence of flagellar "H" antigen gave a positive reaction with i antisera in phase I and 1, 2 antisera in phase II and hence confirmed as Salmonella typhimurium.
### Table-2.1: Biochemical tests of the isolates

<table>
<thead>
<tr>
<th>Tests</th>
<th>Motility</th>
<th>T.S.I</th>
<th>H₂S production</th>
<th>Urease test</th>
<th>Indol test</th>
<th>M.R test</th>
<th>V.P test</th>
<th>Citrate utilization test</th>
<th>Sugar fermentation test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Results</td>
<td>+ve</td>
<td>Redisant, yellow butt with gas production</td>
<td>+ve</td>
<td>-ve</td>
<td>ve</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td>Sorbitol +ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Maltose +ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Dulcitoi +ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mannitoi +ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Arabino +ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Adonitoi -ve</td>
</tr>
</tbody>
</table>

### Table-2.2: Serological tests of the isolates

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Sero-group</th>
<th>Somatic antigen “O”</th>
<th>Flagellar Antigen “H”</th>
</tr>
</thead>
<tbody>
<tr>
<td>2, 4, 5 (S.E)</td>
<td>D</td>
<td>1, 9, 12</td>
<td>Phase-I: g, m</td>
</tr>
<tr>
<td>1, 3 (S.T)</td>
<td>B</td>
<td>1, 4, 12</td>
<td>Phase-I: l</td>
</tr>
</tbody>
</table>

S.E = *Salmonella enteritidis*

S.T = *Salmonella typhimurium*
EXPERIMENT NO.3

Table-3.1: Calculation of LD50 of *Salmonella enteritidis*

<table>
<thead>
<tr>
<th>Bacterial dilution</th>
<th>No. of birds</th>
<th>Accumulated No</th>
<th>Proportion</th>
<th>%age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dead</td>
<td>Live</td>
<td>Dead</td>
<td>Live</td>
</tr>
<tr>
<td>10^-1</td>
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<td>31</td>
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</tr>
<tr>
<td>10^-2</td>
<td>10</td>
<td>0</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>10^-3</td>
<td>8</td>
<td>2</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>10^-4</td>
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<td>9</td>
</tr>
<tr>
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<tr>
<td>10^-7</td>
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<td>0</td>
<td>39</td>
</tr>
<tr>
<td>10^-8</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>49</td>
</tr>
<tr>
<td>10^-9</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>59</td>
</tr>
</tbody>
</table>

* P < 0.05

Proportionate distance = Percentage infected at Dilution next above 50% -50

Percentage infected at Dilution next above 50% infected at dilution next below 50%

= 85-50 = 35 = .58
85-25 = 60

LD50 = 10\(^{3.58}\)/ml
Table-3.2: Calculation of LD50 of *Salmonella typhimurium*

<table>
<thead>
<tr>
<th>Bacterial dilution</th>
<th>No. of birds</th>
<th>Accumulated No</th>
<th>Proportion</th>
<th>%age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dead</td>
<td>Live</td>
<td>Dead</td>
<td>Live</td>
</tr>
<tr>
<td>$10^{-1}$</td>
<td>10</td>
<td>0</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>$10^{-2}$</td>
<td>10</td>
<td>0</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>$10^{-3}$</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>$10^{-5}$</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>$10^{-6}$</td>
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<td>10</td>
<td>0</td>
<td>35</td>
</tr>
<tr>
<td>$10^{-7}$</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>45</td>
</tr>
<tr>
<td>$10^{-8}$</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>55</td>
</tr>
<tr>
<td>$10^{-9}$</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>65</td>
</tr>
</tbody>
</table>

* $P < 0.05$

LD50 = $10^{-3}$/ml
EXPERIMENT NO.4

Clinical signs/Antemortem findings

1. Antemortem findings were recorded throughout the experiment and observed that feed intake of all the infected groups were decreased and their growth to some extent retarded as compared to control group.

2. The weight gains by survivors of the experimental *Salmonella enteritidis* and *Salmonella typhimurium* was lower than those of the control group.

3. Clinical observations among the infected chicks were profuse watery diarrhea, dehydration, laboured breathing, pasting of vent, slight depression and increased thirst, poor growth. Conjunctivitis and arthritis were also noted.

Mortality

Mortality was not observed in any of the infected groups until 5th day post infection. On the 7th day post infection, 12 chicks died from group-A (infected with *Salmonella enteritidis*) and 23 from group-B (infected with *Salmonella typhimurium*). On 14th days post infection, 5 chicks died from group-A and 8 from group-B and on 21st days post infection, 1 chick died from group-A and 2 from group-B. On 28th days post infection, no chick died from group-A and 1 died from group-B.

In group A (experimentally infected with *Salmonella enteritidis*) overall mortality was 18% (18 died out of 100). In group B (experimentally infected with *Salmonella typhimurium*) mortality was 34% (34 died out of 100). No mortality was observed in control group-C during all the experiment.
The mortality was higher in group B (experimentally infected with *Salmonella typhimurium*) 34% as compared to group-A (experimentally infected with *Salmonella enteritidis*) 18%.

**Salmonella Species Identifications at different Phases of disease through PCR**

Five birds per group were randomly selected on 6th days of age (pre inoculation) followed by five birds per week per group 14th, 21st, 28th, 35th, 42nd, 49th, 56th, 63rd, 70th, 77th, 84th, 91st days of age post inoculation. Samples of small and large intestine from all birds were collected in sterilized plastic bottles and brought to the School of Biological Sciences, University of Punjab, Lahore for the identification of *Salmonella enteritidis* and *Salmonella typhimurium* by polymerase chain reaction (PCR) (Wang and Yeh, 2002; Sachse and Frey, 2003). The group-wise detail was as under:

**Group-A: (Experimentally infected with *Salmonella enteritidis*)**

It was observed that all the samples of small and large intestine which were collected on 6th days of age (pre inoculation) from group A were found negative for *Salmonella enteritidis*. All the samples of small intestine which were collected post inoculation on 14 to 91 days of age were found negative for *Salmonella enteritidis* and all the samples of large intestine post inoculation on 14 to 63 days of age were found positive for *Salmonella enteritidis* while, on 70 and 77 days of age, 4 out of 5 samples of large intestine were found positive and 1 was found negative for *Salmonella enteritidis*. On 84 and 91 days of age 3 out of 5 samples of large intestine were found positive and 2 were found negative for *Salmonella enteritidis* (table 4.1). It was observed that all the dead birds from...
group A (18) (experimentally infected with Salmonella enteritidis) were found positive for Salmonella enteritidis in case of large intestine but negative in case of small intestine through PCR. The overall percentage of positive cases of small and large intestine from group A for Salmonella enteritidis were found 0% and 86.74%, respectively (Table-4.1).

Group-B: (Experimentally infected with Salmonella typhimurium)

It was observed that all the samples of small and large intestine which were collected on 6th days of age (pre inoculation) from group B were found negative for Salmonella typhimurium and all the samples of small intestine which were collected (post inoculation) on 14 to 91 days of age from group B were found negative for Salmonella typhimurium. In case of large intestine all the samples post inoculation on 14 to 91 days of age were found positive. It was observed that all the dead birds from group B (34) (experimentally infected with Salmonella typhimurium) was positive for Salmonella typhimurium in case of large intestine but negative in case of small intestine through PCR (Table-4.2). The overall percentage of positive cases of small and large intestine from group B for Salmonella typhimurium were found 0% and 94.94%, respectively (Table-4.2).

Group-C: (un-infected as control group)

None of the samples of small and large intestines from group C (control group) were found positive for Salmonella enteritidis and Salmonella typhimurium (Table-4.3).
Fig-4.1: PCR analysis of samples of small intestines of broiler chickens experimentally infected with *Salmonella enteritidis*

M  =  DNA Marker (Ladder)
1-5  =  Samples of small intestines of broiler chickens experimentally infected with *Salmonella enteritidis* showing negative results
+  =  Positive Control
Fig-4.2: PCR analysis of samples of large intestines of broiler chickens experimentally infected with *Salmonella enteritidis*

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>M</th>
<th>4</th>
<th>5</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>卜</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

500 bp → 316 bp

\[ M \quad = \quad \text{DNA Marker (Ladder)} \]
\[ 1-5 \quad = \quad \text{Samples of large intestines of broiler chickens experimentally infected with } Salmonella enteritidis \]
\[ + \quad = \quad \text{Positive Control} \]
\[ - \quad = \quad \text{Negative Control} \]
Fig-4.3: PCR analysis of samples of small intestines of broiler chickens experimentally infected with *Salmonella typhimurium*.

![PCR gel image]

- **M** = DNA Marker (Ladder)
- **1-2 & 3-5** = Samples of small intestines of broiler chickens experimentally infected with *Salmonella typhimurium* showing negative results
- **+** = Positive Control
**Fig-4.4: PCR analysis of samples of large intestines of broiler chickens experimentally infected with Salmonella typhimurium**

<table>
<thead>
<tr>
<th></th>
<th>DNA Marker (Ladder)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>Samples of large intestines of broiler chickens experimentally infected with <em>Salmonella typhimurium</em></td>
</tr>
<tr>
<td>1-5</td>
<td>Positive Control</td>
</tr>
<tr>
<td>+</td>
<td>Negative Control</td>
</tr>
</tbody>
</table>

![Image of PCR gel](attachment:image.png)
Fig-4.5 PCR analysis of samples of small intestines of healthy broiler chickens (control group) for *Salmonella enteritidis*

- **M** = DNA Marker (Ladder)
- **1-5** = Samples of small intestines of healthy broiler chickens (control group) by PCR for *Salmonella enteritidis* (showing negative results)
- **+** = Positive Control
Fig-4.6: PCR analysis of samples of large intestines of healthy broiler chickens (control group) for *Salmonella enteritidis*

M = DNA Marker (Ladder)
1-5 = Samples of large intestines of healthy broiler chickens (control group) by PCR for *Salmonella enteritidis* (showing negative results)
+
= Positive Control
Fig-4.7: PCR analysis of samples of small intestines of healthy broiler chickens (control group) for *Salmonella typhimurium*

M = DNA Marker (Ladder)
1-5 = Samples of small intestines of healthy broiler chickens (control group) by PCR for *Salmonella typhimurium* (showing negative results)
+ = Positive Control
Fig-4.8: PCR analysis of samples of large intestines of healthy broiler chickens (control group) for *Salmonella typhimurium*

1 2 M 3 4 5 +

500 bp → 284 bp

\[ M = \text{DNA Marker (Ladder)} \]
\[ 1-5 = \text{Samples of large intestines of healthy broiler chickens (control group) by PCR for} \]
\[ \text{Salmonella typhimurium} \text{ (showing negative results)} \]

+ = Positive Control
Postmortem findings/gross lesions

The post mortem examination of all the birds (experimental and control) was performed at the age of 6th days (pre inoculation) and then (post inoculation) on 14, 21, 28, 35, 42, 56, 63, 70, 77, 85 and 91 days of age.

No gross lesions were found on 6th days of age (pre inoculation) in all the organs, and post inoculation on 7th days of age, the organ wise detail of gross lesions were as under:

Liver:

The main necropsy findings were enlarged and creamy white liver with pinpoint haemorrhages and small necrotic foci present on the liver surface in groups A and B on 14 to 28 days of age. No gross lesions were recorded in liver after 28 days of age in group A and B. No lesions could be observed in group C.

Lungs:

In most of the cases lungs were normal in colour, shape, size and consistency but in some cases congesting and necrotic foci were present in group A and B. No lesions could be observed in group C.

Spleen:

The main necropsy findings were splenomegaly with pinpoint haemorrhages and necrotic foci scattered all over the surface in group A and B. No lesions could be observed in group C.

Kidneys:

Swollen kidneys, discolouration and friable consistency in groups A and B. No lesions could be observed in group C.
Results

Small intestine:

The main necropsy findings were haemorrhagic and necrotic enteritis. Nodules were also present in small intestine in group A and B. No lesions could be observed in group C.

Large intestine:

The main necropsy findings were haemorrhagic and necrotic enteritis, caecal cores, lymphilitis (inflammation of caecal) in group A and B. No lesions could be observed in group C.

Bursa of fabricious:

The main necropsy findings were haemorrhages and necrosis in group A and B. No lesions could be observed in group C.

Lean muscles:

The main necropsy findings were pin point haemorrhages and necrosis all over the surface of lean muscles in group A and B. No lesions could be observed in group C.

Note: The above mentioned postmortem findings/gross lesions observed in lungs, liver, spleen and kidneys only upto the age of 28 days while in case of bursa of fabracious, lean muscles and small intestine only upto the age of 21 days in groups A and B but in case of large intestines gross lesions were observed upto the age of 91 days in group A and B. No lesion was recorded in group C (control group) in all the organs in all ages.
Histopathological changes

The Histopathological examination of organs of all the birds from all the groups (experimental and control) was performed at the age of 6th days (pre inoculation) and then after (post inoculation) on 14, 21, 28, 35, 42, 58, 63, 70, 77, 85 and 91 days of age. No microscopic changes were observed in all the organs on 6th days of age (pre inoculation) and the main Microscopic changes which were recorded on (post inoculation) as described below:

Liver:

The principal lesions in the liver at the age of 14 to 28 days in groups A and B were leukocytic infiltration, necrosis and haemorrhage (Fig-4.9 and 4.10). No lesions were recorded in liver after 28 days of age in groups A and B. No lesions were found in group C (Fig-4.11). In some cases mottling and enlargement of liver, parenchymous degeneration, fatty dystrophy and congestion were also noted in groups A and B.

Lungs:

The principal lesions of the lungs at the age of 14 to 28 days in groups A and B were extravascular congestion in the form of haemorrhagic areas, necrosis and cellular infiltration of leukocytes (Fig- 4.12 and 4.13). No lesions were also recorded in lungs after 28 days of age in groups A and B. No lesions were found in group C (Fig-4.14).

Spleen:

The principal lesions of the spleens were mild haemorrhage, necrosis with leukocytic infiltration at the age of 14 to 28 days in groups A and B (Fig-4.15
and 4.16). No lesions were recorded in spleen after 28 days of age in groups A and B. No lesions were found in group C (Fig-4.17).

**Kidneys:**

The principal lesions of the kidneys were marked tubular necrosis with glomerular degeneration and mild leukocytic infiltration at the age of 14 to 28 in groups A and B (Fig-4.18 and 4.19). No lesions were recorded in kidneys after 28 days of age in groups A and B. No lesions were found in group C (Fig-4.20).

**Small intestine:**

The principal lesions of the small intestine were focal necrotic lesions in the mucosa of small intestine, cheesy cecal cores, inflammation of epithelium, superficial ulceration on mucosal linings necrosis at the age of 14 to 21 days, (Fig-4.21 and 4.22). No lesions were recorded in control group C (Fig-4.23).

**Large intestine:**

The principal lesions of the large intestine were leukocytic infiltration, inflammation of epithelium and necrosis at the age of 14 to 91 days (Fig-4.24 and 4.25). No lesions were recorded in control group C (Fig-4.26).

**Bursa of fabricious:**

The principal lesions of Bursa were necrosis, degeneration of bursal follicles and mild leukocytic infiltration at the age of 14 to 21 in groups A and B (Fig-4.27 and 4.28). No lesions were recorded in kidneys after 21 days of age in groups A and B. No lesions were found in group C (Fig-4.29).
Lean muscles:

The principal lesions of lean muscle were muscular degeneration and necrotic areas at the age of 14 to 21 in groups A and B (Fig-4.30 and 4.31). No lesions were recorded in kidneys after 21 days of age in groups A and B. No lesions were found in group C (Fig-4.32).
Figure No. 4.9: Representative figure of liver of group A experimentally infected with *Salmonella enteritidis* showing leukocytic infiltration and necrosis
Figure No. 4.10: Representative figure of liver of group B experimentally infected with Salmonella typhimurium showing leukocytic infiltration, necrosis and haemorrhages.
Figure No. 4.11: Representative figure of liver of group C (control) showing no significant histopathological lesions.
Figure No. 4.12: Representative figure of lung of group A experimentally infected with *Salmonella enteritidis* showing leukocytic infiltration, mild necrosis, vascular congestion and haemorrhages.
Figure No. 4.13: Representative figure of lung of *group B* experimentally infected with *Salmonella typhimurium* showing vascular congestion, necrosis and haemorrhages.
Figure No. 4.14: Representative figure of lung of group C (control) showing no significant histopathological changes
Figure No. 4.15: Representative figure of spleen of group A experimentally infected with *Salmonella enteritidis* showing mild leukocytic infiltration and necrosis.
Figure No. 4.16: Representative figure of spleen of group B experimentally infected with Salmonella typhimurium showing mild leukocytic infiltration, necrosis and congestion.
Figure No. 4.17: Representative figure of spleen of group C (control) showing no significant histopathological lesions.
Figure No. 4.18: Representative figure of kidney of group A experimentally infected with *Salmonella enteritidis* showing mild tubular necrosis with glomerular degeneration
Figure No. 4.19: Representative figure of kidney of group B experimentally infected with *Salmonella typhimurium* showing tubular necrosis, leukocytic infiltration with haemorrhages.
Figure No. 4.20: Representative figure of kidney of group C (control) showing no significant histopathological lesions.
Figure No. 4.21: Representative figure of small intestine of group A experimentally infected with *Salmonella enteritidis* showing degeneration of mucosa with inflammatory cells.
Figure No. 4.22: Representative figure of small intestine of group B experimentally infected with Salmonella typhimurium showing necrosis, inflammation, superficial ulceration on mucosal linings of intestine.
Figure No. 4.23: Representative figure of small intestine of group C (control) showing no significant histopathological lesions.
Figure No. 4.24: Representative figure of large intestine of group A experimentally infected with *Salmonella enteritidis* showing leukocytic infiltration with necrosis and inflammation.
Figure No. 4.25: Representative figure of large intestine of group B experimentally infected with Salmonella typhimurium showing leukocytic infiltration and inflammation
Figure No. 4.26: Representative figure of large intestine of group C (control) showing no significant histopathological lesions.
Figure No. 4.27: Representative figure of bursa fabricious of group A experimentally infected with *Salmonella enteritidis* showing atrophy of bursal follicles
Figure No. 4.28: Representative figure of bursa fabricious of group B experimentally infected with Salmonella typhimurium showing leukocytic infiltration and necrosis of bursal follicles.
Figure No. 4.29: Representative figure of bursa fabricious of group C (control) showing no significant histopathological lesions.
Figure No. 4.30: Representative figure of lean muscle of group A experimentally infected with *Salmonella enteritidis* showing muscular degeneration and necrotic areas.
Figure No. 4.31: Representative figure of lean muscle of group B experimentally infected with Salmonella typhimurium showing muscular degeneration and necrotic areas
Figure No. 4.32: Representative figure of lean muscle of group C (control) showing no significant histopathological lesions.
Table 4.1: PCR analysis of small and large intestines of broiler chickens in 1-13 weeks old age experimentally infected with *Salmonella enteritidis* (Group-A).

<table>
<thead>
<tr>
<th>Age of birds in weeks</th>
<th>Slaughtering and sample collection age (in days)</th>
<th>Total No. of birds in the group</th>
<th>No. of birds died due to infection and tested for S.E</th>
<th>No. of birds slaughtered randomly per week basis and tested for S.E</th>
<th><em>Salmonella enteritidis</em> inoculation @ $10^{3.58}$/ml</th>
<th>Total no. of birds positive for <em>Salmonella enteritidis</em></th>
<th>Percentage of positive cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>6</td>
<td>100</td>
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<td>0</td>
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<tr>
<td>2</td>
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</tr>
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<td>21</td>
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<td>0</td>
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<td>5</td>
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<td>5+5=10</td>
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<td>-</td>
<td>5</td>
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<td>Total</td>
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P < 0.05 (See appendix-IV)

Key
* Pre inoculated birds
S.E= Salmonella enteritidis
Table 4.2: PCR analysis of small and large intestines of broiler chickens in 1-13 weeks old age experimentally infected with *Salmonella typhimurium* (Group-B).

<table>
<thead>
<tr>
<th>Age of birds in weeks</th>
<th>Slaughtering and sample collection age (in days)</th>
<th>Total No. of birds in the group</th>
<th>No. of birds died due to infection and tested for S.T</th>
<th>No. of birds slaughtered randomly per week basis and tested for S.T</th>
<th><em>Salmonella typhimurium</em> isolated pre* and post inoculation @ 10^3/ml</th>
<th>Total no. of birds positive for <em>Salmonella typhimurium</em></th>
<th>Percentage of positive cases</th>
</tr>
</thead>
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<td></td>
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<td>1*</td>
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<tr>
<td>Total</td>
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P < 0.05 (See appendix-IV)

key
* Pre inoculated birds
S.T= *Salmonella typhimurium*
Table 4.3: PCR analysis of small and large intestines of broiler chickens in 1-13 weeks old age of control group (group-C) for *Salmonella enteritidis* and *Salmonella typhimurium*.

<table>
<thead>
<tr>
<th>Age of birds in weeks</th>
<th>Slaughtering and sample collection age (in days)</th>
<th>Total No. of birds in the group</th>
<th>No. of birds died due to infection</th>
<th>No. of birds slaughtered randomly per week basis</th>
<th>Isolation of <em>Salmonella enteritidis</em> and <em>Salmonella typhimurium</em></th>
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</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td>Total no. of birds positive for <em>Salmonella enteritidis</em> and <em>Salmonella typhimurium</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Small intestine</td>
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</tr>
<tr>
<td>2</td>
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<td>21</td>
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<tr>
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</tr>
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</tr>
<tr>
<td>12</td>
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<td>5</td>
<td>0</td>
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<tr>
<td>Total</td>
<td>-</td>
<td>35</td>
<td>-</td>
<td>65</td>
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</table>

Key:
S.E = *Salmonella enteritidis*
S.T = *Salmonella typhimurium*
Experiment No. 5

Public health impact of Salmonellosis through consuming the meat and eggs of carrier birds.

1. PCR analysis of human stool and blood samples of suspected typhoid fever patients.

A total of 200 samples of human stool and blood of all ages and sex of 100 patients of suspected typhoid fever were collected randomly from 4 different hospitals of Lahore city. One hundred samples from each of the 4 hospitals were collected i.e. 25 patients suspected of typhoid fever and 25 suspected of food poisoning patients. From each patients two samples i.e. one of stool and one of blood was collected simultaneously at each visit from the laboratories, wards, out doors and emergencies of each hospital in a small plastic bottles and EDTA coated vacutainer tubes and brought to the school of biological sciences, university of Punjab Lahore. These samples were examined/analysed for the identification of salmonella species by PCR test.

The detail of the results per hospital is as under (Table 5.1)

25 samples of stool and 25 of blood of typhoid fever from General Hospital Lahore were collected and analysed by PCR and it was found that out of 25 samples of stool, 4 samples were found positive for Salmonella enteritidis and out of 25 of blood samples, 2 samples were found positive for Salmonella enteritidis.
Thus, percentage of *Salmonella enteritidis* out of 25 samples of stool was found 16% and out of 25 samples of blood was found 8%. Similarly, no of positive samples for *Salmonella typhimurium* out of 25 stool samples was found 2 and out of 25 blood samples was found zero.

Thus, percentage of *Salmonella typhimurium* out of 25 samples of stool was found 8% and percentage of *Salmonella typhimurium* out of 25 samples of blood was found zero. The positive samples for *Salmonella enteritidis* out of 25 samples of stool collected from Jinnah Hospital Lahore were 3 and out of 25 samples of blood were 1 and thus percentage of *Salmonella enteritidis* out of stool was found 12% and out of blood was found 4%.

Similarly, the positive samples for *Salmonella typhimurium* out of 25 stool samples were found 3 and out of blood were found 1 and thus percentage of *Salmonella typhimurium* out of stool sample was found 12% and out of blood *Salmonella typhimurium* was found 4%.

The positive samples for *Salmonella enteritidis* collected from Mayo Hospital out of 25 stool samples were found 4 and out of 25 blood samples were found 2 and thus percentage out of 25 samples for *Salmonella enteritidis* was 16% and out of 25 samples of blood for *Salmonella enteritidis* was 8%.

Similarly the positive samples for *Salmonella typhimurium* out of 25 stool were found 3 and out of 25 samples of blood positive for *Salmonella typhimurium* were found 1 and thus, percentage of *Salmonella typhimurium* out of stool samples was found 12% and out of 25 blood samples was found 4%.
The positive sample for *Salmonella enteritidis* out of 25 stool samples which were collected from Services Hospital Lahore were 3 and out of 25 of blood samples were found 1 and thus, percentage of *Salmonella enteritidis* out of 25 samples of stool was found 12% and out of 25 samples of blood were found 4%.

Similarly, no. of positive samples for *Salmonella typhimurium* out of 25 samples of stool were found 2 and out of 25 samples of blood were found zero (0). Thus, percentage of positive samples for *Salmonella typhimurium* out of 25 stool samples was 8% and percentage out of 25 samples of blood for Salmonella typhimurium was zero (0).

2. **PCR analysis of human stool and blood samples of suspected food poisoning patients**

A total of 200 samples of human stool and blood of all ages and sex of 100 patients of suspected food poisoning were collected randomly from 4 different hospitals of Lahore city. One hundred samples from each of the 4 hospitals were collected i.e. 25 patients suspected of typhoid fever and 25 suspected of food poisoning patients. From each patients two samples i.e. one of stool and one of blood was collected simultaneously at each visit from the laboratories, wards, out doors and emergencies of each hospital in a small plastic bottles and EDTA coated vacutainer tubes and brought to the school of biological sciences, university of Punjab Lahore. These samples were, examined/analysed for the identification of salmonella species by PCR test.
The detail of the results per hospital is as under (Table 5.2)

25 samples of stool and 25 of blood of food poisoning from General Hospital Lahore were collected and analysed by PCR and it was found that out of 25 samples of stool, 4 samples were found positive for *Salmonella enteritidis* and out of 25 of blood samples, 2 samples were found positive for *Salmonella enteritidis*.

Thus, percentage of *Salmonella enteritidis* out of 25 samples of stool was found 16% and out of 25 samples of blood was found 8%. Similarly, no of positive samples for *Salmonella typhimurium* out of 25 stool samples was found 2 and out of 25 blood samples was found 1%.

Thus, percentage of *Salmonella typhimurium* out of 25 samples of stool was found 8% and percentage of *Salmonella typhimurium* out of 25 samples of blood was found 4. The no. of positive samples for *Salmonella enteritidis* out of 25 samples of stool collected from Jinnah Hospital Lahore were 4 and out of 25 samples of blood were 2 and thus, percentage of *Salmonella enteritidis* out of stool was found 16% and out of blood was found 8%.

Similarly, the positive samples for *Salmonella typhimurium* out of 25 stool samples were found 4 and out of blood were found 2 and thus, percentage of *Salmonella typhimurium* out of stool sample was found 16% and out of blood *Salmonella typhimurium* was found 4%.
The no. of positive samples for *Salmonella enteritidis* collected from Mayo Hospital out of 25 stool samples were found 3 and out of 25 blood samples were found 2 and thus, percentage out of 25 samples for *Salmonella enteritidis* was 12% and out of 25 samples of blood for *Salmonella enteritidis* was 8%.

Similarly the positive samples for *Salmonella typhimurium* out of 25 stool were found 3 and out of 25 samples of blood positive for *Salmonella typhimurium* were found 2 and thus, percentage of *Salmonella typhimurium* out of stool samples was found 12% and out of 25 blood samples was found 8% (Mayo Hospital Lahore).

The no. of positive samples for *Salmonella enteritidis* out of 25 stool samples which were collected from Services Hospital Lahore were 3 and out of 25 of blood samples were found 1 and thus, percentage of *Salmonella enteritidis* out of 25 samples of stool was found 12% and out of 25 samples of blood were found 4%.

Similarly, no. of positive samples for *Salmonella typhimurium* out of 25 samples of stool were found 2 and out of 25 samples of blood were found 1 and thus, percentage of positive samples for *Salmonella typhimurium* out of 25 stool samples was 8% and percentage out of 25 samples of blood for *Salmonella typhimurium* was 4 in Services Hospital Lahore of food poisoning cases.
Fig- 5.1: PCR analysis of human stool and blood samples of suspected typhoid fever patients

M 1 2 3 4 + + -

500 bp 316 bp 284 bp

M = Marker DNA.

1 and 2 = *Salmonella enteritidis* in samples of human stool and blood of typhoid fever patients.

3 and 4 = *Salmonella typhimurium* in samples of human stool and blood of typhoid fever patients.

1 - Positive control for *Salmonella enteritidis*.

3 - Positive control for *Salmonella typhimurium*.

2 - Negative control.
Fig. 5.2: PCR analysis of human stool and blood of suspected food poisoning patients

- + 4 3 2 1 M

284 bp — 500 bp 316 bp

M = Marker DNA.

1 and 2 = *Salmonella enteritidis* in samples of human stool and blood of food poisoning patients.

3 and 4 = *Salmonella typhimurium* in samples of human stool and blood of food poisoning patients.

+ = Positive control for *Salmonella enteritidis*.

* = Positive control for *Salmonella typhimurium*.

- = Negative control.
Table 5.1: PCR analysis of Human Stool and Blood Samples of Suspected Typhoid Fever Patients

<table>
<thead>
<tr>
<th>Name of Hospital</th>
<th>No. and type of samples</th>
<th>No. of positive samples of S.E</th>
<th>% age of positive samples of S.E</th>
<th>No. of positive samples of S.T</th>
<th>% age of positive samples of S.T</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stool</td>
<td>Blood</td>
<td>Stool</td>
<td>Blood</td>
<td>Stool</td>
</tr>
<tr>
<td>General Hospital Lahore</td>
<td>25</td>
<td>25</td>
<td>4</td>
<td>2</td>
<td>16a</td>
</tr>
<tr>
<td>Jinnah Hospital Lahore</td>
<td>25</td>
<td>25</td>
<td>3</td>
<td>1</td>
<td>12a</td>
</tr>
<tr>
<td>Mayo Hospital Lahore</td>
<td>25</td>
<td>25</td>
<td>4</td>
<td>2</td>
<td>16a</td>
</tr>
<tr>
<td>Services Hospital Lahore</td>
<td>25</td>
<td>25</td>
<td>3</td>
<td>1</td>
<td>12a</td>
</tr>
<tr>
<td>Average total</td>
<td>25</td>
<td>25</td>
<td>3.5</td>
<td>1.5</td>
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</table>

P < 0.05 in ab, ac, bd and cd (see appendix-V)

Key:
SE = Salmonella enteritidis
ST = Salmonella typhimurium
Table-5.2: PCR analysis of Human Stool and Blood Samples of Suspected food poisoning Patients

<table>
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<th>Name of Hospital</th>
<th>No. and type of samples</th>
<th>No. of positive samples of S.E</th>
<th>% age of positive samples of S.E</th>
<th>No of positive samples of S.T</th>
<th>%age of positive samples of S.T</th>
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</thead>
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<tr>
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<td>Blood</td>
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</tr>
<tr>
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<td>25</td>
<td>4</td>
<td>2</td>
<td>16a</td>
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<tr>
<td>Jinnah Hospital Lahore</td>
<td>25</td>
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<td>4</td>
<td>2</td>
<td>16a</td>
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<tr>
<td>Mayo Hospital Lahore</td>
<td>25</td>
<td>25</td>
<td>3</td>
<td>2</td>
<td>12a</td>
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<tr>
<td>Services Hospital Lahore</td>
<td>25</td>
<td>25</td>
<td>3</td>
<td>1</td>
<td>12a</td>
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<tr>
<td>Average total</td>
<td>25</td>
<td>25</td>
<td>3.5</td>
<td>1.7</td>
<td>14</td>
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</table>

P < 0.05 in ab, ac, bc, bd and Hospitals (see appendix –VI)

Key:
SE= Salmonella enteritidis
ST= Salmonella typhimurium
CHAPTER 5

DISCUSSIONS

During recent years poultry industry has expanded very rapidly in Pakistan and Salmonellosis has emerged as one of the most serious problems having adverse effects on poultry as well as on human health through consuming their products. This has highlighted the need for taking extensive control measures to save the industry as well as humans. The present research project was therefore designed to study “the pathogenesis of Salmonellosis with respect to carrier state in poultry and its public health impact” for which a series of experiments were undertaken. The 1st of its series was “identification of Salmonella species in poultry feed, intestines and eggs prevalent in Lahore area by polymerase chain reaction (PCR) test.

Identification of Salmonella Species from Poultry feed, meat and eggs prevalent in Lahore area through PCR

Poultry Feed

In this experiment one hundred feed samples from different poultry farms were collected. The samples were also collected from various poultry farms located in Wahga Boarder area, Thoker Niaz Beg, Bedian Road area and Chungi Amar Saddu area Ferozpure Road Lahore. The feed samples of layer category were starter, grower, finisher and from broilers category starter and finisher. Each
category was comprising of 20 random samples selected through systematic random sampling method. These samples were subjected to (PCR) test. The percentage of positivity rate for *Salmonella enteritidis* in the poultry feed was 20%, 15%, 10%, 15%, 10% for layer starter, layer grower, layer finisher, broiler starter and broiler finisher, respectively. The percentage of positivity from the same feed samples for *Salmonella typhimurium* for layer starter, layer grower, layer finisher, broiler starter and broiler finisher was 15%, 10%, 10%, 10%, and 10% respectively. The PCR is considered an easy, simple and reproducible test among the rapid molecular typing methods. Antonio *et al.*, 2000 have reported that *Salmonella enteritidis, Salmonella typhimurium* and *Salmonella Vircho* could easily be distinguished through the gene profile by using PCR method allowing an easy differentiation from other sero-vars of *Salmonella*. Salmonellosis is a cyclic problem in the food industry for which animal feed has been a single major contributor to a significant extent. PCR method takes about 4-6 hours for the detection and confirmation of *Salmonella* species. Where as, the conventional methods required 96 hours with significant expenses. *Salmonellae* are *omni* present in the environment. The organism seems to have adopted to the environmental changes and could survive even with the improvement of sanitation practices, chemical treatments and antimicrobial drugs. *Salmonellae* can be introduced into the poultry units through the hatchery, feed, broiler house, rodents, scavengers workers, and visiting personnel. Once colonized in one broiler farm the organism is transmissible to other broilers either directly or indirectly, in side or out side of the poultry units in the poultry
production cycle. The poultry transport containers is an other source of transmission to provide an avenue or exit for the transfer of Salmonellae from one broiler units through the feathers and other contaminated material to other broiler. Innovations such as sprays, automation of administrating process, provision of hand wash nosels for manual workers, chilling and cooling of carcasses packed in plastic bags offer possibilities for reducing Salmonella load on broiler carcasses (Shackel Ford 1988).

The results of this study are quite agreeable to Veldman and Co-workers (1995) in which the 10% of 360 feed samples were contaminated with Salmonella species. Mash feed was contaminated at the rate of 21% Salmonella enteritidis and Salmonella typhimurium and other 26 sero-types were isolated. The authors project was focusing only for Salmonella enteritidis and Salmonella typhimurium, therefore results were reported only said sero-types and not for the others.

Martin and Co-authors (1996) reported that freshly hatched chickens showed a very high susceptibility to Salmonella infections and control measures were therefore frequently focused on the period shortly after hatching. Poultry production and Salmonellosis are mutually exclusive and are gaining importance because of contaminating poultry with Salmonella and its significant role in food poisoning in human beings (Nashed 1986). Further investigation revealed that the feed and litter contamination with Salmonella typhimurium remained alive and viable at 37°C in the feed for up to 6 weeks and is viable at room temperature for up to 71 weeks. The samples collected in this study were based on study as
reported by (Nashed 1986). A very high percentage of Salmonella organisms has been reported in mash, pelleted and meal samples at a percentage of 58, 0 and 92 respectively.

The positivity rate of 58% and 92% of the mash feed and meal samples could be due to the fact that the author (Cox and others 1983) just isolated any sero-vars of Salmonella in contrast to the experiment where in the author only isolated Salmonella enteritidis and Salmonella typhimurium. It is interesting to note that pelleted feed sample have zero % positivity of Salmonella because of the advanced processing techniques of the feed. This is an encouraging aspect that the pelleted feed in stead of mash feed should be used in order to avoid the Salmonella infection as a commercial poultry feed. Further more the extra ordinary high rates of 92% positivity of Salmonella as reported by Cox and his fellows 1983, because the samples were derived from meat and bone meal. The contamination of sterilized commercial poultry feed with Salmonella typhimurium from commercial broiler carcasses had been reported by (Rouse and co-authors, 1988). They found that treatment with chemical preservative resulted in the elimination of detectable Salmonella from heavy contaminated feed with in 72 hours and lightly contaminated feed with in 24 hours. Another aspect of the experiment by the same authors revealed that the commercial feed inoculated with Salmonella organisms was fed daily from day 35 to 56. It was treated with the chemical preservative at 0.5% and 1.0% for the last 7 days. The treatment resulted in a reduction in the numbers of fecal and intestinal samples for
Salmonellae demonstrating elimination of Salmonellae in the feed by use of the feed preservatives.

An interesting study was conducted by Shirota and Co-workers (2001) in which, the contamination of eggs through contaminated feed with Salmonella has been justified. The study strengthens the hypothesis of the author. For this purpose in their study samples of feed and eggs were collected from 16 commercial layer farms in Easter Japan from 1993-96. Salmonella enteritidis was isolated from a feed sample and egg contents in a layer farm through pulsed field Gel electrophoresis patterns, that was genetically related and belonged to a single phage type suggesting that the contamination of the farms were linked to the occurrence of Salmonellae in feed.

The study results of author are quite similar to Boqvist and Co-workers (2003) in which, 555 samples were collected from animal feed stuff and feed mills in Sweden between 1993 and 1997. The most prevalent isolate from feed mills was Salmonella typhimurium (N=91) followed by Salmonella Dublin (N=82). Similar No. of isolates were from animals during out breaks surveillance and at slaughter houses. Most of these findings were from dust and scrapings from feed mills in accordance with the HACCP programme in the feed control programme. It was concluded that occurrence of Salmonella in animals and in the feed were not only associated but remained considerable during 1993-96 in Sweden.
The data of 886 feed ingredients samples, dust samples and feed samples confirmed that feed ingredients and dust could be a major source of Salmonella contamination in feed mills. There was no significant difference between layers feed and broiler feed as far as identification of Salmonella enteritidis and Salmonella typhimurium was concerned (P > 0.05). However, the positivity percentage ranged between 10-20% from different feed samples which was biologically significant and in agreement to other studies (Shackel Ford, 1988, Veldman et al, 1995 and do not in agreement with Cox and falls, 1983) (Appendix-I).

Poultry Meat (small and large intestine)

Raw poultry meat is the primary point of entry and a single sole factor of Salmonellosis in human populations (Sarwari and Co-authors, 2001). The other important risk factor of Salmonellosis with respect to food borne illness in human beings due to Salmonella species were Contaminated meat, unsafe food handling, consumption behaviour and cooking practices, young and old age, compromised immune status.

For this purpose, 50 samples each of small and large intestine from desi and broiler chicks were collected from four different sites (poultry markets) of Lahore area from where most of the customers bought poultry meat and other products for the identification of Salmonella enteritidis and Salmonella typhimurium. These shops in the poultry market sold fresh poultry meat where the hygienic practices were not observed. During destining and meat processing,
the hands were not washed in the inter slaughtering interval but just hand sponging with dirty and contaminated clothes. This process communicated the existing and prior contamination to the freshly slaughtered broilers and was considered as a major risk factor for the dissemination of Salmonella to human beings. In order to understand, the indirect transmission from poultry to human beings as a risk factor, a project was designed in which 50 intestinal samples each from broiler and Desi chicks were collected from different poultry market points of Lahore area.

The intestines were packed in clean and sterilized plastic bottles which were transported in a cooler ice box to the School of Biological Sciences, University of the Punjab, Lahore for the detection of Salmonella enteritidis and Salmonella typhimurium through PCR. The positivity percentage rate of Salmonella enteritidis for small and large intestine in Desi birds was 2 and 16% respectively. Where as for broilers in small and large intestine it was 4 and 18% respectively. The positivity of Salmonella typhimurium in small and large intestine of Desi birds was 2 and 14% where as in broilers it was 4 and 16% in the small and large intestine respectively.

The results are in close agreement with Barrel (1987) who isolated the Salmonella from humans and food products from 1981 – 1985 and attempted to investigate the relationship between serotypes isolated from human and meat samples of poultry. The contaminated serotypes of humans were Salmonella enteritidis and Salmonella typhimurium and Salmonella Virchow. The Salmonella typhimurium was commonly isolated from range of meat products. Salmonellas
were found from raw poultry meat which was 28% of poultry in Manchester (England) where the Salmonella infection in human increased from 1983 – 1984 (Barrell 1987). The results are also on similar lines with an other study conducted by Tokumaru (1991) in which the Salmonella species were detected in 1969 (24.1% of 286 chicken meat samples). The catchment population gave positive results in 1931 (20.8% of 149 samples of chicken meat. The results obtained in different weights of sample sized, the detection rate was 19.9% in the sample size of 25 gm of meat, 15.7% in 10 gm of meat sample 12.2% in 1 gm meat samples.

It is worth mentioning that the contamination rate was 7% is considerably lower, almost half, as compared to the study under the authors domain i.e, 14-18% of different sero-types. Wilson and Co-workers (1996) carried out a prospective epidemiological survey in Northern Ireland between February and August 1994 to assess the current level of Salmonella in retail chickens to determine whether contamination rate difference from chicken sold in supermarket and by butchers. For this purpose a total of 140 chilled and frozen raw chicken samples from retail display were examined for the presence of Salmonella. Contamination was consistent in chicken that was chilled and sold from small premises and reared or slaughtered outside Northern Ireland. Salmonella enteritidis was the most common isolate and low contamination rate of 7% might partially explain the lower rate of human Salmonella infection in Northern Ireland (Wilson et.al, 1996). Although, the meat cooking practices in Pakistan i.e., boiling and frying of meat, vegetables and other dishes are different
from the Western style, which is also a preventive style from Salmonellosis yet the public demands of rigid hygienic measures is arising in order to breed and rear poultry free of these pathogens. Although the cost would enhance but the measures are exciting for the future disease control (Barrow, 1997). The zoonosis from poultry meat are only a few were consumers rather than the meat purpose. Protection of meat purpose form infection depends upon taking normal precautions which also protect meat from contamination from the workers (Corry and Hinton, 1997).

The food borne illness is a major public health concern in which paratyphoid sero type of Salmonella of food born illnesses are attributed to poultry and poultry products (White et.al, 1997). The effective prevention of food born diseases requires an understanding that contamination could be introduced in the food at numerous points along the food chain. There are also many critical control points identified in the processing plants which reduces contamination include temperature control, chemical intervention, water replacement and control low technology, chiller and equipment maintenance. Transportation and food handling at retail out lets and by the consumers with proper storage temperature and adequate cooking for the final critical control points in the farm to table continuum. It is important to apply risk reduction strategy through out the food chain (White et.al, 1997). Salmonella enteritidis infection in poultry appeared to be major public concern (Noordhuizen and Frankena, 1994). Haapapuro and Co-workers (1997) reported that food borne illness remained a common and serious problem despite efforts to improve slaughter house inspection and food
preparation practices. The reported single potential contributor in the chain of infectious process is the feeding of poultry excrement to livestock, a common practice in some parts of United States. In 1994, 18% of poultry producer in USA collectively fed more than one thousand tone poultry litter to cattle. The heat processing reliably could kill bacterial pathogens including Salmonella but its use is limited due to expense and other factors.

The in activation temperature of 71°C to 77°C has been recommended for potentially manure-tainted meat products. The control and preventive of poultry meat is important because they are the best transmitter of Salmonella in the UK and according to a report the food judged to be most important vehicle, where 50% cases were due to poultry and poultry products, where as 26% cases of Salmonellosis in human beings were due to eggs and egg products. So revolutionary Industrialization of poultry farming have made the poultry meat available for large groups of consumer for the last three decades. Due to its nutritional, sensory and economical considerations, poultry meat is bifar the most popular animal food product world wide. Epidemiological reports however incriminate poultry meat as a source far out breaks of human typhoid fever and food poisoning. Through the Salmonella species contamination of the end products with pathogenic micro organisms is through using the contaminated feed to the broiler and layer industry and therefore measured are needed to take care to avoid contamination of poultry meat of the consumers (Mulder, 1997).

The concept has also been supported by Fuzihara and his colleagues, (2000) where in they founded that the Salmonella was the leading cause of
human food borne infections in Latin U.S.A and poultry meat is one of the main vehicle. Small poultry entrepreneurs who slaughter and sell less than 200 birds to present an important economic role in the spread of Salmonellosis. Mostly the hygienic conditions were poor. The study reported that the high prevalence of Salmonella sero types were found in carcasses, utensils and environmental samples. Out of 60 samples 41% of small entrepreneur samples harbored Salmonella species including 42% of carcasses, 23.1% of utensils, 71.4% of water and 71.4% of freezers and refrigerators. Seventeen serotypes were attracted and Salmonella enteritidis was the most predominating i.e. 30%. The wide spread occurrence of Salmonella in small slaughter houses re-inforced the need for implementation of effective control measures Sarwari and Co-workers, (2001) supported the authors hypothesis that raw meat and poultry are primary point of entry for Salmonella species in to the human consumers. Co-relation might be expected between the serotypes distribution of Salmonella species isolated form animals at the time of slaughter and that type isolate found in humans. Both the studies were in line because all the sero-vars isolated from poultry were the isolates founded in humans through PCR (Sarwari et.al, 2001).

Valacony and his Colleague, (2001) noted that Salmonella enteritidis contaminated poultry houses should be cleaned and disinfected cyclically. Aysegul and Carli, (2002) developed real time probe specific PCR to rapidly detect Salmonella from chicken, feces and carcasses by fluorescence resolution energy transfer assay for 72 cloacal swabs, 147 intestines and 50 carcasses. Samples were examined by this assay and as a result 13 (8.8%) and 25(17%) of
the intestinal samples were found harbor Salmonella by Bacteriology and PCR respectively. A total of 45 out of 50 (90%) carcasses samples were Salmonella positive. The results are in close harmony to the authors findings. Contaminated poultry meat was identified as one of the principal food borne sources of Salmonella. The presence of Salmonella was assessed by traditional culture methods and by Salmonella PCR test. Salmonella was recovered from 32 (16%) of all the samples using traditional culture methods. In contrast, the PCR assay proved to be more sensitive and detected Salmonella DNA in 38 (19%) of the samples tested. The Pathogen was detected in 45 (23%) of the 198 samples when culture and PCR results were combined. The sensitivity of the PCR test was also greater than culture when detecting Salmonella from with in the flock (53% of flock) by PCR verves (30% of flock) by culture. The combination of both tests revealed that 55% of the flock were contaminated with Salmonella. The PCR assay proved to be the highly specific and sensitive method for detecting Salmonella (White et. al, 2002).

There was a significant difference ($P< 0.05$) between the positivity percentage of Salmonella enteritidis and Salmonella typhimurium as far as identification of Salmonellae from desi (local breed) and broiler meat was concerned (Appendix-2)

**Poultry Eggs**

Bacterial Contamination of eggs in general and Salmonella contamination in particular has remained a prominent international public health issue in every
commercial broiler growing country. Considerable resources were being utilized in detecting and controlling *Salmonella enteritidis*, *Salmonella typhimurium* and other *Salmonellae* infections in commercial layers flocks. Recently control and prevention Department of the Center of Disease Control reported a significant association between egg products and Salmonella infections in humans (Richard *et al.*, 2004).

Keeping in view the importance of egg and egg products, the layer eggs, desi (local breed) eggs, double yolk eggs and broken eggs were collected from commercial poultry market points of Lahore area for the detection of *Salmonella enteritidis* and *Salmonella typhimurium* from albumin and yolk of each egg. It was carried out in the School of Biological Sciences, University of the Punjab, Lahore. It was found that 16%, 8%, 16% and 16% egg albumin was found positive for *Salmonella enteritidis* in layer egg albumin, desi (local breed) egg albumin, double yolk albumin and broken egg albumin respectively. In each case 25 egg albumin were collected and tested for the detection of Salmonella. Similarly the egg yolk from layers, desi (local breed) double yolk and broken eggs was taken and positivity rate for *Salmonella enteritidis* was found 12%, 4%, 12% and 12% respectively. The positively rate for *Salmonella typhimurium* in both albumin and yolk was relatively less in both albumin and yolk of layers, desi double yolk and broken eggs (Table No 1.6 and 1.7). Statistically there there was no significant difference (*P* > 0.05) but the prevalence of *Salmonella enteritidis* and *Salmonella typhimurium* from different eggs ranged between 4-16% and 4-12% respectively which was biologically significant. The contamination rates due to Salmonella in
the albumin and yolk of eggs are relatively high in the authors study as compared to Elson and Co-workers (2005), Leon Vilarde and his colleagues, (2004), Durecko and his fellows, (2004), Gast and Holt, (2001), which might be due to the fact that feed was highly contaminated and may be the high rate in egg albumin and yolk might be attributed to the single and precipitating factor of contaminated feed.

Gast and Holt, (2000) found that egg yolk and albumen differ substantially in their abilities to support bacterial growth, the initial level and location of Salmonella enteritidis deposition are critical for determining whether proposed standards for refrigerating eggs are likely to protect public health by preventing extensive microbial multiplication. In the present study, three groups of laying hens were infected with oral doses of approximately $10^9$ cells of different Salmonella enteritidis strains (two were phage type 4 and one was phage type 13a) in two replicate trials. For all three Salmonella enteritidis strains, the incidence of yolk contamination (approximately 2.5% overall) was significantly greater than the incidence of albumen contamination (approximately 0.5% overall). The phage type 13a strain was less often isolated from fecal samples at 2 wk post-inoculation than were the phage type 4 strains, but no significant differences between strains were observed in the incidence of egg contamination. Most freshly laid contaminated eggs contained fewer than 1 Salmonella enteritidis cell/ml of egg yolk or albumen, and no sample contained more than 67 S. enteritidis cells/ml.
Leon-Velarde and Co-workers (2004) produced a very useful data mentioning that there was an increase in the prevalence of Salmonella enterica serotype typhimurium as compared to others. This study examined the prevalence of these micro organisms in poultry environments as well as the sensitivity and specificity of a PCR-based method and multiple antibiotic resistance profile of Salmonella sero-group B isolates for the identification of Salmonella typhimurium. A total of 435 Salmonella isolates obtained from poultry house environmental samples tested during a 20-month period representing a prevalence of 5.5%. Among these, 313 (72%) isolates were identified as Salmonella sero group B isolates. These isolates were tested by a PCR-based assay and for resistance to five antibiotics for the rapid identification of Salmonella typhimurium upon comparing the antibiotic resistance and PCR results with serotype and phage type data, the sensitivity and specificity for the identification of Salmonella a typhimurium of both methods were found to be 100% and 99.6% respectively. Both methods could be completed with in 24 hours after obtaining an isolate, while serotyping and phage typing required more than 5 days to complete. Suzuki, (1994) recorded that Salmonella enteritidis was a common pathogen of all species of fowls. The recent increase in the number of out breaks of food poisoning in humans was due to Salmonella enteritidis and was epidemiologically associated with eggs and egg products infected with the Salmonellae organisms.

Schutze and Co-workers (1996) reported that poultry egg shells were a major reservoirs of Salmonella enteritidis in the USA. For this purpose 1200
commercially purchased egg shells cultured were found to be contaminated, externally contaminated with Salmonella hidel, while none of the contents of the 100 dozen eggs were found to be positive for Salmonella organisms. Retrospective study through telephone follow up on 204 patients from 1992-1994 revealed that 30 patients of Salmonellosis had consumed raw eggs and only one patient was infected with Salmonella enteritidis. This suggested that poultry egg shells was not major contributor of human illness due to Salmonella enteritidis in USA. Similarly Kapperud and Co-authors (1998) reported that the epidemiological progression of human Salmonellosis in Norway was parallel to trends noted else where in Europe. During the past two decades, number of reported cases increased steadily, particularly Salmonella enteritidis. However, in contrast to the situation in most other European countries, about 90% of the cases had acquired their infection from abroad. The incidence of indigenous Salmonella infections as well as the prevalence of the micro organism in the domestic food chain were both comparatively low. During a national case control study of indigenous Salmonella infections in 1993-94 case patient and 226 matched population controls were enrolled. The only factor which remained associated with increased size was the consumption of poultry meat and eggs. It was a general consensus that infected laying flocks was a vital control point in the efforts to control egg associated transmission of Salmonella enteritidis to human (Gast and Holl, 2001). Durecko and Co-workers (2004) explained that most of the patients with Salmonellosis mentioned consumption of meals
containing poultry products, primarily eggs and egg products as a source of disease. The results of the author are in agreement.

Microbiological analysis of samples showed that in more than 88% of cases the infectious agent was identified as Salmonella enteritidis. This sero-var was the most frequent pathogen isolated at poultry farms. In the period of the past five years, 171 out breaks of Salmonellosis were recorded in Slovakia. The control results indicated that out of 228 and 545 samples of food and food stuff of animal origin only 0.21% were confirmed as being Salmonella positive and the average ratio of Salmonella enteritidis occurrence in samples was 0.1% per year. A higher incidence (1.43%) was recorded only in eggs and egg products. The Salmonellosis elimination totally depended on the establishment of hygienic conditions in the food chains. Covarik and his colleagues (1991) cultured three sites (shell surface, shell membranes and yolk) in 760 eggs from four poultry farms. Salmonellae were isolated from the yolk of four human and 4 poultry Salmonella enteritidis isolates were phage type B, 2 poultry isolates were phage type 23 and the other human isolate was phage type 4. Pittler (1993) observed an increase occurrence in Great Britain of Salmonella enteritidis infections in human beings which were predominantly caused by raw egg dishes. The commission of the EEC also started activities designed as a protection against such new dimension of Salmonella infections. The results are in conformity to the author's findings.

The British Commission of EEC elaborated a proposal for a zoonosis Directive of the EEC. The directive was adopted in the Council of Ministries of
Agriculture and translated by Ministry of Food, Agriculture and Forestry. It would provide official control measures and quarantine measurers of the poultry stock and killing of the affected fowl and after adopting these measures in case of eggs, the reduction of Salmonella organisms resulted. Duguid and Associates (1991) recorded evidence on the extent of the part played by infected hens eggs in currently much exaggerated. Prevention should be sought through improved catering practices and kitchen hygiene and attempts to eradicate Salmonellosis from laying flocks were likely to be in effective.
Isolation and characterization of *Salmonella enteritidis* and *Salmonella typhimurium*

The poultry feed, poultry meat and poultry egg samples which were positive through PCR were subjected to isolation and characterization of *Salmonella enteritidis* and *Salmonella typhimurium*. Following 48 hrs pre-enrichment in selenite broth, the culture was transferred on brilliant green and xylose lysine deoxycolate agar media (XLD). The morphology, colony characteristics and biochemical behaviour of the isolate were studied. The isolates were also serotyped on the basis of their somatic antigen (O) and flagellar antigen (H). The isolated Salmonellae showed red colonies on brilliant green agar where as the colonies were red coloured with black centers on XLD media. The microscopic examination of the bacteria revealed gram negative reaction. The small rods of varying size occurring singly or in the form of short chains were seen under the ordinary microscope. The isolated bacteria were actively motile. The bacteria showed characteristics, biochemical reactions on triple sugar iron (TSI) medium with red slants, yellow butt and revealed H₂S production. The methyle red test and citrate utilization tests were positive where as the urease, indol and voges proskaur tests were negative. All the bacteria fermented sorbitol, maltose, dulcitol, mannitol, arabinose. However, the bacteria were incapable of fermenting adonitol.

The antigenic structure of the isolated Salmonellae was determined using Kaufman white technique. The sero groups were defined by the somatic
antigens. Two of the isolated sero-types showed positive reaction with group B where as 3 with group D antisera. The two isolates of group B gave a positive agglutination reaction with monospecific antisera against-1, 4 and 12 somatic ‘O’ antigen. The three isolates of D gave a positive agglutination with monospecific antisera against 1, 9 and 12 somatic ‘O’ antigen. The 3 isolates of D group when checked for the presence of flagellar antigen ‘H’ gave a positive agglutination reaction with G and M antisera in phase 1 and 1,7 antisera in phase II and hence confirmed as Salmonella enteritidis. The two isolates of B group when checked for the presence of flagellar “H” antigen gave a positive agglutination reaction with i antisera in phase I and 1, 2 antisera in phase II and hence confirmed as Salmonella typhimurium. The study of the author is not agreeable with Bokanyi and Co-workers (1990) who conducted study to determine and characterize Salmonella contamination on ready to cook broiler in the Columbus, Matropolitan area, the sero-types isolated were Salmonella hadar, Salmonella heidelberg and Salmonella Johannesburg.

The study is agreeable with Salf and Co-workers (2003) who reported that out of 10 serotypes of Salmonella most of which reported to the center of disease control and prevention, the Salmonella enteritidis and Salmonella typhimurium were the most common serotypes. They also mentioned that the distribution of Salmonella serotypes from poultry sources varies geographically and changes with time. Fantasia and colleagues (1991), Novak and Co-workers (1993), Gast and Benson (1996), Taunay and Co-authors (1996), Henzler and Co-workers (1998), T elo and Co-workers (1998), Al-Nakhli and his fellows (1999). They all
reported the isolation and characterization of Salmonella enteritidis and Salmonella typhimurium along with other species of Salmonella from different poultry houses, poultry sources and environments from different parts of the World.

Alexandre and Co-worker (2000), Chang (2000), Raja and Co-authors (2000), Levine and Co-workers (2001), Schrank and Co-authors (2001), Roy and his Colleagues (2002), Tibajuk and Co-workers (2003), Orji and Co-workers (2005). They all also brought about isolation and characterization of Salmonella species from different poultry sources from different parts of the world and found that Salmonella enteritidis and Salmonella typhimurium were the most prevalent contaminant of the poultry products. However, other species of Salmonella along with Salmonella enteritidis and Salmonella typhimurium that were reported by the said scientists could not found by the author. The possible reason for this disagreement was that the Salmonella serotypes distribution depended upon the geographical, socio-cultural and seasonal patterns and other reason is that the study of the author was only confined to the isolation and characterization of Salmonella enteritidis and Salmonella typhimurium in this experiment.
CALCULATION OF LD50

The lethal dose (LD50) meaning during an experiment 50% of the experimental animal die and 50% survive after parenteral or oral administration of the occulum. This is a good measure to estimate the virulence and pathogenicity of the pathogen (Saif 2003). For this purpose, two hundred day old chicks were purchased from commercial hatchery, one hundred for the estimation of LD50 of Salmonella enteritidis and one hundred for the estimation of LD50 of Salmonella typhimurium. The LD50 of Salmonella enteritidis and Salmonella typhimurium was determined in the broiler chicks by using Reed and Muench Method (David et al, 1998). A 10 fold serial dilutions of these Salmonella were prepared in sterilized glass tubes containing normal saline. The birds were divided in to 10 equal groups. Each comprising of 10 birds, in both the cases i.e. Salmonella enteritidis and Salmonella typhimurium. Each bird from groups 1 to 9 was given @ 1ml per bird of the dilution. The birds were examined for next seven days for any morbidity and mortality. The mortality rate was tabulated and LD50 was calculated. The LD50 value was estimated separately for Salmonella enteritidis and Salmonella typhimurium were $10^{3.58}$/ml and $10^{3}$/ml respectively. It is evident from the results that isolate of Salmonella typhimurium was more virulent than the Salmonella enteritidis. The study is in agreement with Smith and Co-workers, (2002) and Allen and Co authors, (2001) who determined the virulence of Salmonella enteritidis and Salmonella typhimurium in the same order respectively.
Williams and Co workers (1980) inoculated orally, 16 Salmonella typhimurium strains belonging to 12 phage types varied greatly in their ability to kill 1-day-old chickens; variation was noted even between strains of the same phage type. Fourteen strains belonging to 11 food poisoning serotypes other than S. typhimurium were practically non-lethal when examined in this manner. All of them were lethal by the intramuscular route but some were more so than others. Two were more lethal by this route than one of the Salmonella typhimurium strains that was highly lethal when given orally. With age, chickens rapidly become resistant to fatal infection with the food poisoning strains; given orally, a S. typhimurium strain killed 79% of 1-day-old chickens but only 3% of 2-day-old chickens. Of 2 specific poultry pathogenic strains, one, of Salmonella gallinarum, was lethal by oral inoculation to chickens of all ages but the other, of Salmonella pullorum, was only lethal to very young ones. Some salmonella strains, such as those of Salmonella infantis and Salmonella menston, were more efficient at infecting and colonizing the alimentary tract of chickens than were the more virulent Salmonella typhimurium strains, the Salmonella gallinarum and Salmonella pullorum strains and a Salmonella cholerae-suis strain.

Barrow and associates, (1987) estimated the virulence of Salmonella typhimurium strains for day-old chickens and observed that the mortality following oral inoculation depended upon breeds and found that some breeds were more susceptible than others. However, there was no correlation between oral and parenteral estimation as far as virulence was concerned. Pathogenesis
studies associated with one of the most virulent strains suggested that, after invasion, organisms multiplied in the liver and spleen and spread to other organs producing a systemic infection. The cause of death was probably a combination of anorexia and dehydration resulting from general malaise and diarrhoea. A virulent strain studied in depth spreaded through the body faster, persisted for a longer period and was more invasive than an avirulent strain. In the studied system invasiveness was the virulence of overriding importance.

Similarly Gast and Benson (1995) found that phage type 4 Salmonella enteritidis was associated with a significant morbidity and mortality in broiler chickens in the United Kingdom. The recent isolation of this phage type from poultry in the United States raised concerns about whether the current regulatory approach to Salmonella enteritidis should be modified to consider phage type 4 differently from other phage types. The present study assessed and compared the virulence of phage type 4, i.e. Salmonella enteritidis isolates, Salmonella enteritidis isolates of other phage types, and a Salmonella pullorum isolate in both single-comb white leghorn and white Plymouth Rock chicks. The mean incidence of severe illness or death following oral inoculation with phage type 4 Salmonella enteritidis was significantly lower than the incidence associated with S. pullorum inoculation in both lines of chicks. Nevertheless, some individual phage type 4 Salmonella enteritidis isolates caused severe effects at a frequency similar to that of Salmonella pullorum in single-comb white leghorn chicks. In general, severe morbidity or mortality following infection with Salmonella enteritidis isolates of all phage types tested occurred more often in
single-comb white leghorn chicks than in white Plymouth Rock chicks. The mean frequency at which chicks were severely affected following inoculation with phage type 4 isolate was significantly higher than the mean for isolates of other phage types. However, in both lines of chicks, some significant differences in virulence were apparent within the set of phage type 4 strains tested. The observed virulence for chicks of recent U.S. poultry isolates of phage type 4 Salmonella enteritidis was similar to that of earlier isolates from various sources, including poultry isolates from the United Kingdom.

The study is in close agreement to Suzuki (1994) where he stated that the virulence factors associated with Salmonella enteritidis depended upon the choice of bacterial strain phage type, age of bird and size of inoculum. He further found that virulence in terms of LD50 responsible for variation in mortality rate clinical symptoms, fecal shedding and frequency of contaminated eggs. However, the study differed from the findings of Guillot and Co-workers (1995). The strains of the Salmonellae differed widely in their ability to cause disease or death in poultry. Several investigators reported a significant difference in mortality between groups of chicks orally inoculated with isolated representing various sero types. Hence lethality for the chicks could vary with in the Salmonella sero types.

Identification of Salmonella species (from small and large intestine of poultry) at different phases of disease through PCR

A series of experiments were carried out in order to find “Pathogenesis of Salmonellosis with respect to carrier states in poultry and its public health
impact”. For this propose in the first phase, the identification, isolation and characterization of Salmonella enteritidis and Salmonella typhimurium was attempted. It was followed by the estimation of LD50 in continuation to that the experiment under study to ascertain the nature of the carrier states in poultry meat (small and large intestine).

For this purpose a total of three hundred day old broiler chicks were reared for a period of 13 weeks (age of birds was also 13 weeks) and were divided in three groups i.e., A, B and C. Each group comprising of 100 birds. Group A was experimentally infected with Salmonella enteritidis and Group B was experimentally infected with Salmonella typhimurium. The Salmonella enteritidis was inoculated at the dose rate of $10^{3.56}$ organisms/ml and Salmonella typhimurium was inoculated at the dose rate of $10^3$ organisms/ml at the age of 7 days respectively and Group C was kept as uninfected control. Five birds were randomly selected from each group per week and slaughtered and necropsied for the collection of samples from small intestine and large intestine were subjected to PCR test for the detection of Salmonella enteritidis or Salmonella typhimurium. The 1st sample was taken on 6th day i.e. (pre inoculated birds). The samples were taken at the end of each week from 5 birds as well as from the birds died due to Salmonella enteritidis and Salmonella typhimurium infection. Twelve, Five and One birds were found dead during 2nd, 3rd and 4th week of the study due to Salmonella enteritidis. The organisms were recovered from small intestine only during the first week of post inoculation and none of the organisms could be recovered uptill the 13th weeks of age i.e. termination of the experiment.
The samples taken from large intestine (ceca) 100% of the 5 birds were found positive until the eight week of age and then declined to 60% till the last day of experiment (table 4.1). As an average, 86.74% of the birds were maintaining the organism of the Salmonella enteritidis in the large intestine during the entire experimental period in contrast to the small intestine in which 0% were found positive.

A total of 23, 8, 2 and 1 birds were found dead during 2\textsuperscript{nd}, 3\textsuperscript{rd}, 4\textsuperscript{th} and 5\textsuperscript{th} week of the study due to Salmonella typhimurium. No organisms could be recovered from small intestine until the 13 weeks of age i.e. termination of the experiment. The samples taken from large intestine (ceca) 100% of the 5 birds were found positive until the last day of experiment (13 weeks of age) (table 4.2). As an average 94.94% of the birds were maintaining the organism of the Salmonella typhimurium in the large intestine during the entire experimental period in contrast to the small intestine in which 0% were found positive. There was a significant difference in the sera positivity of Salmonella enteritidis and Salmonella typhimurium in large intestine of poultry (P < 0.05) (Appendix-IV).

Five number of birds were selected randomly from control group C also per week basis On 6\textsuperscript{th} day (pre-inoculation) and then post-inoculation on 7\textsuperscript{th} to 91 days and tested through PCR for the presence of Salmonella enteritidis and Salmonella typhimurium organisms but none of the samples of small and large intestine of control group was found positive for Salmonella enteritidis and Salmonella typhimurium (table-4.3). Salif (2003) observed that Colonization of Salmonella sero types in various organs and gastro intestinal tract associated
with the age of the birds. Arthur and Co-workers, (1998) identified that the intestinal colonization is reduced from 3 weeks old chicks. The sites of transposon insertion were determined for most of the mutants.

Hassan and Co-workers, (1991) found that multiplication of Salmonellae is prohibited in small intestine of broiler chickens by the presence of IgG, IgA and IgM. The results of the author coincide with Hassan and Co-workers and are further supported with the involvement of other determinants lack of like oxygen supply and osmolarity difference is small intestine as compared to large intestine.

Barbel and Co-workers (2005) conducted one of the comparative studies and it was concluded that Salmonella serovar typhimurium efficiently colonized in the large intestine triggered cecum and colon inflammation starting 8 hours post infection. Similarly, Jennifer and Jones (2004) conducted a research experiment on salmonella pathogenicity that the high osmolarity and low oxygen conditions presumably mimic the environment for the growth of Salmonella with in the small intestine and that it secrete effector proteins into the cells leading to engulfment of bacteria with in the large membrane ruffled. The hypothesis is further strengthen with the Hargis and Co workers, (1995) who conducted a study at a commercial broiler processing plant and found that 286 out of 850 crops from 3 flocks were Salmonella positive where as only 73 of 500 ceca from these flocks were contaminated. Further more, data from this plant indicated.

Gast (1994) stated that a significant proportion of human Salmonella enteritidis (SE) outbreaks in recent years was traced due to the consumption of contaminated eggs produced by infected laying flocks. Experimental Salmonella
enteritidis infections in chickens was used to acquire a considerable amount of basic information about the interaction between Salmonella enteritidis and the avian host. After oral or parenteral inoculation of chickens, Salmonella enteritidis could colonize the intestinal tract, invade and disseminate to reach numerous internal organ sites, and elicit the production of specific antibodies in serum and egg yolk. Experimental infection of laying hens could result in the deposition of Salmonella enteritidis in the contents of eggs before oviposition, although generally in rather small numbers and at a relatively low frequency. The consequences of experimental SE infection were shown to vary significantly with the strain and dose of the inoculum. Some Salmonella enteritidis isolates have been shown to produce various clinical effects, including decreased egg production by layers. The information provided by experimental Salmonella enteritidis infections in chickens had played an important role in the formulation of appropriate strategies for reducing the incidence of Salmonella enteritidis in commercial layer flocks and thereby also reducing the incidence of transmission of Salmonella enteritidis to consumers via contaminated eggs.

Hinton (1988) recorded that the feed given to young broiler chickens was contaminated artificially with Salmonella kedougou, a serotype associated with both subclinical infections in commercially reared chickens and food poisoning in humans. No evidence was obtained to suggest that the growth promoting antibiotic avilamycin, added to the feed at the rate of either 2.5 ppm or 10 ppm, had the undesirable side-effect of favouring the colonization of the intestinal tract of the birds with Salmonella kedougou when they were challenged with this
organism in the feed. Cooper and his colleagues (1994) found that the Salmonella enteritidis strains multiplied in samples taken from the ileum and duodenum irrespective of age but multiplied in the cecal samples from newly hatched chicks only. Invasion from the gut by Salmonella enteritidis LA5 and CVL30 was both age and dose dependent.

Kotova and others (1988) demonstrated Salmonella carrier state (42.6% Salmonella enteritidis and 34.4% S. dublin) in subjects after acute salmonellosis as well as in healthy persons infected with Salmonellae as a result of occupational exposure to Poultry (8.8% in humans exposed to chickens and 6.1% in those exposed to ducks) and sheep (2.8%). The carrier state was accompanied by intermittent pain in the epigastrum, diminished appetite, diarrhoea etc. The etiological role of Salmonella typhimurium was proved beyond doubt, as well as its ability to cause salmonellosis outbursts, sporadic cases of the disease and the carrier state. When large industrial facilities specializing in Poultry processing were investigated, the Salmonella carrier state was revealed in practically healthy Poultry in 16% of chickens and 12% of ducks. The studies of Hinton (1988), Cooper and colleagues (1994) and Kotova and fallows (1988), are in agreement to the authors study.

The authors results are further supported by Duchet-Suchaux and Co-authors (1995) who quantified the carrier state of Salmonella enteritidis in chicks (i.e., persistent asymptomatic association of Salmonella enteritidis with the host). They developed an experimental carrier state model by oral inoculation of low doses of Salmonella enteritidis in chicks at different ages. Liver, spleen, and ceca
Colonizations by the challenge strains were measured weekly by enumeration of Salmonella enteritidis colony-forming units (CFU) for 7-12 weeks. High mortality rates, incompatible with the carrier state, were observed in chicks inoculated with $10^2$ organisms of either a parental strain of Salmonella enteritidis at the age of one day. Both strains colonized organs similarly, allowing us to use subsequently the SmrNafr mutant strain. The selected low doses of Salmonella enteritidis induced no deaths in chicks inoculated at 1 or 3 weeks of age.

Similarly, Sadeyen and Co-workers (2004) observed that Salmonella enterica serovar enteritidis carrier state in Poultry has serious consequences on food safety and public health due to the risks of food poisoning following consumption of contaminated products. An understanding of the mechanisms of persistence of Salmonella in the digestive tract of chicken can be achieved by a better knowledge of the defects in the control of infection in susceptible versus resistant animals. The gene expression of innate immune response factors including anti-microbial molecules, inflammatory and anti-infectious cytokines was studied in the caecal lymphoid tissue associated with the carrier state. Expression levels of these genes were assessed by real-time PCR and were compared in two inbred lines of chickens differing in resistance to the carrier state following oral inoculation of Salmonella enterica serovar enteritidis at one week of age. A high baseline level of defensin gene expression was recorded in young animals from the susceptible line. In contrast, a significantly low expression of interferon-gamma (IFN-gamma) gene was observed in these
susceptible infected animals in comparison to resistant ones and healthy counterparts. The results are agreeable.

The authors findings are in conformity with Gast and Beard (1989) who assessed the effects of age at exposure on the persistence of Salmonella in various tissues of chicks in two experiments. Broiler chicks, housed on wire floors in isolation cabinets, were orally inoculated with Salmonella typhimurium at various ages (1 to 8 days after hatching). The post-inoculation mortality of chicks declined significantly as the age at inoculation increased. In one experiment the effect of age at inoculation was investigated on the persistence of Salmonella typhimurium in the cecum. Salmonellae persisted for 7 weeks after inoculation in 81.3% of the chicks inoculated at 1 day of age and in 82.5% of the chicks inoculated at 8 days of age. The mean number of cecal Salmonellae at 7 weeks post-inoculation was also greater for chicks inoculated on day 1 than for those inoculated on day 8. The second experiment examined the effect of age at inoculation on the adherence of Salmonella typhimurium to and penetration through the cecal epithelium. The ceca of chicks inoculated at 1 day of age were colonized by significantly more adhering Salmonella at 2 days post-inoculation than those of chicks inoculated at 3, 5, or 7 days of age, but age did not affect the recovery of Salmonella typhimurium from livers or spleens.

Similarly, Dhillon and Co-workers (2001) estimated that mortality rates were 30.7 and 7.6% in the groups inoculated with Salmonella pullorum, Salmonella enteritidis, PT5A, and Salmonella enteritidis, respectively. No mortality or clinical sign were observed in other treatment groups or in
uninoculated control groups. Cecal pouches are found to be the ideal organ. Mean body weights were reduced to 1.8 to 12.6% in inoculated groups compared with the uninoculated control group. The consistent gross and histopathological lesions were of peritonitis, perihepatitis, yolk sac infection, and enteritis. Subclinical Salmonella infection identified in this study resulted in reduced body weights of inoculated birds compared with uninoculated controls.

It is further supported by Roy and others (2001) who found that ceca was 100% positive for Salmonella enteritidis at acute or chronic infection compared with other organs. Mean body weight reduction ranged from 0.67% (inoculated with Salmonella kentucky) to 33.23% (inoculated with Salmonella typhimurium) in the inoculated groups at different weeks compared with uninoculated controls.
HISTOPATHOLOGY

In continuation to the previous experiment the present study was undertaken to ascertain the histopathological changes for the better understanding of the effects of carrier states in terms of maintenance and cellular damage to the different organs. It was followed by maintenance and further histopathological changes to the stage of shedding of the organism through the feces to the environment. For this purpose a total of three hundred day old broiler chicks were reared for a period of 13 weeks (age of birds was also 13 weeks) and were divided in three groups A, B and C. Each group comprising of 100 birds. Group A was experimentally infected with Salmonella enteritidis and Group B was experimentally infected with Salmonella typhimurium. The Salmonella enteritidis was inoculated at the dose rate of $10^{3.58}$ organisms/ml and Salmonella typhimurium was inoculated at the dose rate of $10^{3}$ organisms/ml at the age of 7 days respectively and Group C was kept as uninfected as control.

The organs such as Bursa fabricious, small intestine, large intestine, lung, liver, spleen, kidney and lean muscles from all 5 birds randomly selected from all groups (experimental and control) on 6th days (before inoculation) and then (after inoculation) on 14th, 21st, 28th, 35th, 42nd, 49th, 56th, 63rd, 70th, 77th, 84th, 91st, days of age were collected and preserved in 10% buffered formaline solution, then fixed tissue was processed in ascending grades of alcohol, cleared in xylene, embedded on paraffine wax, section of 3-6 microns thick was cut and stained with hematoxyline and Eosin stain (Bancroft and Gamble, 2002) for histopathological
studies in the laboratory of Pathology Department, University of Veterinary and Animal Sciences, Lahore. The following observations were recorded:

**Clinical findings**

1. Antemortem findings were recorded through out the experiment and observed that feed intake of all the infected groups were decreased and their growth to some extent retarded as compared to control group.

2. The weight gains by survivors of the experimental *Salmonella enteritidis* and *Salmonella typhimurium* was lower than those of the control group.

3. Clinical observations among the infected chicks were profuse watery diarrhea, dehydration, laboured breathing, pasting of vent, slight depression and increased thirst, poor growth. Conjunctivitis and arthritis were also noted.

**Mortality**

Mortality was not observed in any of the infected groups until 6th day of post infection. On the 7th day of post infection, 12 chicks were died from group-A (infected with *Salmonella enteritidis*) and 23, from group B (infected with *Salmonella typhimurium*). On 14th days of post infections, 5 chicks were died from group-A and 8 from group-B and on 21st days of post infections, 1 chick was died from group-A and 2 from group-B. On 26th days of post infections, no chicks was died from group-A and 1 was died from group-B.
In group A (experimentally infected with *Salmonella enteritidis*) mortality was 18% (18 died out of 100). In group B (experimentally infected with *Salmonella typhimurium*) mortality was 34% (34 died out of 100) and no mortality was observed in control group-C. It was noted that mortality was higher in group B (experimentally infected with *Salmonella typhimurium*) 34% as compared to group-A (experimentally infected with *Salmonella enteritidis*) 18%.

Postmortem findings/gross lesions. The post mortem examination of all the birds (experimental and control) was performed at the age of 6th days (pre inoculation) and then (post inoculation) on 14, 21, 28, 35, 42, 56, 63, 70, 77, 85 and 91 days of age. It was observed that no gross lesions and necropsy findings were found on 6th days of age (pre inoculation) in all the organs, and after (post inoculation on 7th days of age, the organ wise detail of gross lesions as under:

**Liver**

The main necropsy findings were enlarged and creamy white liver with pin point haemorrhages and small necrotic foci present on the liver surface in groups A and B on 14 to 28 days of age. No gross lesions were recorded in livered after 28 days of age in group A and B. No lesions could be observed in group C.

**Lungs**

In most of the cases lungs were normal in colour, shape, size and consistency but in some cases congesting and necrotic foci were present in
group A and B on 14 to 28 days of days of age. No gross lesions were recorded in lungs after 28 days of age in group A and B. No lesions could be observed in group C.

**Spleen**

The main necropsy findings were splenomegaly with pin point haemorrhages and necrotic foci scattered all over the surface of spleen in group A and B on 14 to 28 days of age. No gross lesions were recorded in spleen after 28 days of age in group A and B. No lesions could be observed in group C.

**Kidneys**

Swollen kidneys, discoloration and friable consistency in groups A and B on 14 to 28 days of age. No gross lesions were recorded in kidneys after 28 days of age in group A and B. No lesions could be observed in group C.

**Small intestine**

The main necropsy findings were haemorrhagic and necrotic enteritis. Nodules were also present in small intestine in group A and B on 14 to 28 days of age. No gross lesions were recorded in small intestine after 28 days of age in group A and B. No lesions could be observed in group C.
Large intestine

The main necropsy findings were haemorrhagic and necrotic enteritis, caecal cores, lymphilitis (inflammation of caecal) on 14 to 91 days of age. The gross lesions were recorded in large intestine up to 91 days of age in group A and B. No lesions could be observed in group C.

Bursa of fabricious

The main necropsy findings of bursa fabricious were haemorrhages and necrosis in group A and B on 14 to 21 days of age. No gross lesions were recorded in bursa of fabricious after 21 days of age in group A and B. No lesions could be observed in group C.

Lean muscles

The main necropsy findings were pin point haemorrhages and necrosis all over the surface of lean muscles in group A and B on 14 to 21 days of age. No gross lesions were recorded in Lean muscles after 21 days of age in group A and B. No lesions could be observed in group C.
Histopathological changes

The Histopathological examination of organs of all the birds from all the groups (experimental and control) was performed at the age of 6th days (pre inoculation) and then after (post inoculation) on 14, 21, 28, 35, 42, 56, 63, 70, 77, 85 and 91 days of age. No microscopic changes were observed in all the organs on 6th days of age (pre inoculation) and the main Microscopic changes which were recorded after (post inoculation) as described below: The histo-pathology of different organs of broiler chickens i.e liver, lung, spleen, kidney, small intestine, large intestine, bursa of fabracious and lean muscles at different phases of disease was also conducted for the better understanding of pathogenesis due to salmonellosis.

Liver

The principal lesions in the liver at the age of 14 to 28 days in groups A and B were leukocytic infiltration, necrosis and haemorrhages (Fig-4.9 and 4.10). No lesions were recorded in liver after 28 days of age in groups A and B. No lesions were recorded in group C (Fig-4.11).

Lungs

The principal lesions of the lungs at the age of 14 to 28 days in groups A and B were leukocytic infiltration, mild necrosis, vascular congestion and haemorrhages (Fig- 4.12 and 4.13). No lesions were recorded in lungs after 28 days of age in groups A and B. No lesions were recorded in group C (Fig-4.14).
Spleen

The principal lesions of the spleen were mild leukocytic infiltration, necrosis and congestion at the age of 14 to 28 days in groups A and B (Fig-4.15 and 4.16). No lesions were recorded in spleen after 28 days of age in groups A and B. No lesions were found in group C (Fig-4.17).

Kidneys

The principal lesions of the kidneys were marked tubular necrosis with glomerular degeneration and leukocytic infiltration and haemorrhages at the age of 14 to 28 in groups A and B (Fig-4.18 and 4.19). No lesions were recorded in kidneys after 28 days of age in groups A and B. No lesions were found in group C (Fig-4.20).

Small intestine

The principal lesions of the small intestine were degeneration of mucosa with inflammatory cells, necrosis, inflammation, superficial ulceration on mucosal lining of intestine at the age of 14 to 21 days (Fig-4.21 and 4.22) No lesions were recorded in small intestine after 21 days of age in group A and B. No lesions were recorded in control group C (Fig-4.23).

Large intestine

The principal lesions of the large intestine were leukocytic infiltration with necrosis and inflammation at the age of 14 to 91 days (Fig-4.24 and 4.25). The
lesions were recorded up to 91 days of age in group A and B. No lesions were recorded in control group C (Fig-4.28).

**Bursa of fabricious**

The principal lesions of Bursa were atrophy & necrosis of bursal follicles and leukocytic infiltration at the age of 14 to 21 in groups A and B (Fig-4.27 and 4.28). No lesions were recorded in Bursa of fabricious after 21 days of age in groups A and B. No lesions were found in group C (Fig-4.29).

**Lean muscles**

The principal lesions of lean muscle were muscular degeneration and necrotic areas at the age of 14 to 21 days in groups A and B (Fig-4.30 and 4.31). No lesions were recorded in lean muscles after 21 days of age in groups A and B. No lesions were found in group C (Fig-4.32).

Gast and Beard (1989) assessed the effects of age at exposure on the persistence of Salmonella in various tissues of chicks in two experiments. Broiler chicks, housed on wire floors in isolation cabinets, were orally inoculated with *Salmonella typhimurium* at various ages (1 to 8 days after hatching). The post-inoculation mortality of chicks declined significantly (P<0.05) as the age at inoculation increased. In one experiment the effect of age at inoculation was investigated on the persistence of *Salmonella typhimurium* in the cecum. *Salmonellae* persisted for 7 weeks after inoculation in 81.3% of the chicks inoculated at 1 day of age and in 62.5% of the chicks inoculated at 8 days of age.
The mean number of cecal Salmonellae at 7 weeks post-inoculation was also greater for chicks inoculated on day 1 than for those inoculated on day 8. The second experiment examined the effect of age at inoculation on the adherence of *Salmonella typhimurium* to and penetration through the cecal epithelium. The ceca of chicks inoculated at 1 day of age were colonized by significantly more adhering *Salmonella* at 2 days post-inoculation than those of chicks inoculated at 3, 5, or 7 days of age, but age did not affect the recovery of *Salmonella typhimurium* from livers or spleens.

Similarly Gorham and Co-authors (1994) examined infected orally one-day-old and 7-day-old specific-pathogen-free chickens were orally infected with a field isolate of *Salmonella enteritidis* phage type 13A. Chickens were sequentially euthanatized at various intervals until 42 days of age, and selected tissues were collected for microscopic evaluation. Eleven of 53 chickens (21%) infected at 1 day of age and 2 of 28 chickens (7%) infected at 7 days of age died. Gross and microscopic lesions were more frequent and severe in chickens infected at 1 day of age and in chickens that died (almost all of these were 1-day-infected as well). Lesions were characterized by mild to severe fibrinous pericarditis, airsacculitis, perihepatitis, peritonitis, cecal cores, and enlarged, firm yolk sacs. Gross the microscopic lesions were present from post-inoculation day 2 until termination of this study.

In continuation of the experimentation Desmidt and Co-workers (1997) inoculated white leghorn specific-pathogen-free chickens orally with *Salmonella enteritidis* phage type 4 at the age of one day (group 1) and four weeks (group
2). From 3 h until 4 weeks post inoculation (pi), birds were sacrificed. Gross lesions were recorded and different sites of the intestine and visceral organs were collected for bacteriological and histopathological examination. Clinical disease and mortality were only observed in group 1. Mortality was 8%. The birds were depressed, had diarrhoea and an indurated yolk sac. Infection of the liver and the heart was present within 12 h pi in both groups. The percentage of infected organs was very high and similar in both groups during the first week pi. Thereafter, the isolation rate of Salmonella was declining faster in group 2. The crop, the proventriculus, the lower intestinal tract and the bursa of Fabricius were the predilective sites of isolation in both groups. Most prevalent lesions were serous typhilitis, omphalitis and polyserositis. Histopathology revealed inflammation in the intestines and visceral organs. In some birds granulomatous nodules were present in the caeca. Antibodies were detected from 18 and 5 days pi in group 1 and 2, respectively. Granulomatous nodules were typical of infection with this strain of Salmonella enteritidis phage type 4. These granulomatous nodules together with the retained yolk sac possibly are a source of Salmonella organisms that may account for intermittent faecal shedding by carrier birds.

In an other observation Kinde and Co-workers (2000) examined two strains of 27-wk-old commercial laying chickens (strain A, brown egg laying type and strain B, white egg laying type) were inoculated either orally (PO) or intravenously (IV) with a field isolate of Salmonella enteritidis phage type 4. Chickens were sequentially necropsied at regular intervals throughout the 17 weeks observation period. Gross and microscopic lesions were most evident
between 1 and 14 days post-inoculation (DPI). Gross lesions consisted of
enlarged livers with white foci, enlarged and mottled white spleens, fibrinous
exudate in the peritoneum, and atretic, misshapen ovarian follicles. Microscopic
lesions included multifocal coagulative necrosis of hepatocytes and inflammation,
fibrinous exudation in vascular sinuses of the spleen, and fibrinocellular
inflammation of the peritoneum and ovarian follicles. The proportion of
reproductive organ infections (ovary and oviduct) in the IV group, 83% (20/24, P
= 0.007; 50% and 33% for strains A and strain B birds, respectively), was higher
than that of the PO group, 46% (11/24; 29% and 17% for strains A and B,
respectively), for the first 16 days of observation post-inoculation. Overall the
percentage of culture-positive birds did not differ significantly (P > 0.05) between
birds with and without lesions, but isolation of Salmonella enteritidis tended to be
more frequent when lesions were evident. This experiment also demonstrated
that brown-egg-laying-type chickens were more susceptible than white-egg-
laying-type chickens to Salmonella enteritidis phage type 4 isolated from
California based on gross and microscopic lesions and bacteriologic findings.

On the other hand Dhillon and Co-workers (2001) studied two hundred
sixty one-day-old specific pathogen-free (SPF), Single Comb White Leghorn
chicks to determine pathology caused by Salmonella enteritidis isolated from a
Poultry environment. The chicks were subdivided into 10 equal groups of 26
chicks each. Eight groups of chicks were inoculated individually with 0.5 ml of
brain heart broth culture of Salmonella enteritidis. One group of 26 chicks were
inoculated with 0.5 ml Salmonella pullorum per bird. Another group of 26 chicks
were kept as an uninoculated control group. All the chicks were observed daily for clinical signs and mortality. Salmonella was reisolated from different organs at 7, 14, 21, and 28 post-inoculation (DPI). All of the chicks were weighed individually at each interval. Two chicks at random from each group were taken and necropsied at each DPI for gross pathology. Selected tissues were examined for histopathological changes at 7 and 14 DPI. Dead chicks were examined for gross and histopathological lesions. Mortality rates were 30.7 and 7.6% in the groups inoculated with Salmonella pullorum, Salmonella enteritidis, PT5A, and Salmonella enteritidis, respectively. No mortality or clinical sign were observed in other treatment groups or in uninoculated control groups. Cecal pouches are found to be the ideal organ. Mean body weights were reduced to 1.8 to 12.6% in inoculated groups compared with the uninoculated control group. The consistent gross and histopathological lesions were of peritonitis, perihepatitis, yolk sac infection, and enteritis. Subclinical Salmonella infection identified in this study resulted in reduced body weights of inoculated birds compared with uninoculated controls.

In addition to previous supportive observations Roy and others (2001) recorded the pathogenicity of one isolate of Salmonella typhimurium, four isolates of Salmonella heidelberg, three isolates of Salmonella kentucky, two isolates of Salmonella montevideo, one isolate of Salmonella hadar, and two isolates of Salmonella enteritidis (SE), one belonging to phage type PT13a and the other to PT34, was investigated in specific-pathogen-free chicks. Three hundred eighty-four chicks were separated into 16 equal groups of 24 chicks.
Thirteen groups were inoculated individually with 0.5 ml of broth culture containing $1 \times 10^7$ colony-forming units (CFU) of either *Salmonella typhimurium* (one source), *Salmonella heidelberg* (four sources), *Salmonella montevideo* (two sources), *Salmonella hadar* (one source), *Salmonella kentucky* (three sources), *Salmonella enteritidis* PT 13a (one source) or *Salmonella enteritidis* PT 34 (one source) by crop gavage. Two groups of 24 chicks were inoculated in the same way with $1 \times 10^7$ CFU of *Salmonella enteritidis* PT4 and *Salmonella pullorum*. Another group of 24 chicks was kept as an uninoculated control group. The chicks were observed daily for clinical signs and mortality. Isolation of *Salmonella* was done from different organs at 7 and 28 days post-inoculation (DPI). All the chicks were weighed individually at 7, 14, 21, and 28 DPI. Two chicks chosen at random from each group were euthanatized and necropsied at 7 and 14 DPI and all the remaining live chickens, at 28 DPI. Selected tissues were taken for histopathology at 7 and 14 DPI. Dead chicks were examined for gross lesions and tissues were collected for histopathology. Chicks inoculated with *Salmonella pullorum* had the highest mortality (66.66%), followed by *Salmonella typhimurium* (33.33%). Chicks inoculated with *Salmonella heidelberg* and *Salmonella enteritidis* PT4 had 12.5% mortality and 8.3% mortality, respectively, with *Salmonella enteritidis* PT 13a. Ceca were 100% positive for Salmonellae at acute or chronic infection compared with other organs. Mean body weight reduction ranged from 0.67% (inoculated with *Salmonella kentucky*) to 33.23% (inoculated with *Salmonella typhimurium*) in the inoculated groups at different weeks compared with uninoculated controls. Gross and microscopic lesions included
peritonitis, perihepatitis, yolk sac infection, typhilitis, pneumonia, and enteritis in some groups, especially those inoculated with *Salmonella typhimurium*, *Salmonella heidelberg*, *Salmonella enteritidis* PT4 and *Salmonella pullorum*.

The study is in agreement with Saif and others (2003) who observed the pathological changes by giving oral infection of *Salmonella enteritidis* and *Salmonella typhimurium* in Poultry and found sever enteritis accompanied by focal necrotic lesions in the mucosa of the small intestine, cheesy cecal cores, spleen and liver congested, kidneys enlarged and congested, peri-hepatitis and peri-carditis, coagulated yolk material in the yolk sack, purulent arthritis, airsaculitis. Inflation of the epithelium and lamina properia of the colon and ceca and cell death.

Similarly Beal and Co authors (2004) found that *Salmonella enterica* remains one of the most important food-borne pathogens of humans and is often acquired through consumption of infected Poultry meat or eggs. Control of *Salmonella* infections in chicken is therefore an important public health issue. Infection with *Salmonella enterica* serovar *typhimurium* results in a persistent enteric infection without clinical disease in chickens of more than 3 days of age, and represents a source for contamination of carcass at slaughter and entry into the human food chain. Data presented indicate a profound effect of age at initial exposure on the persistence of infection and a lesser effect on the development of effective immunity to re-challenge. The percentage of birds positive for Salmonella was high until 8-9 weeks of age, regardless of the age at which the birds were infected (1, 3 or 6 weeks). The birds infected at 3 and 6 weeks of age
produced a more rapid and higher antibody response than those infected at 1 week of age, but in all cases infection persisted for a considerable period despite the presence of high antibody levels. Following a re-challenge infection with *Salmonella typhimurium*, all three previously-infected groups had fewer bacteria in the gut, spleen and liver compared with age-matched birds receiving a parallel primary infection. However, the birds primary infected at 3 and 6 weeks of age cleared infection more rapidly than those infected at a younger age. Interestingly older-primed birds had higher specific T lymphocyte proliferative responses and specific circulating levels of IgY antibody at time of re-challenge. Although birds initially infected at 1 week of age and those that were previously uninfected produced a stronger antibody response following re-challenge, they were slower to clear Salmonella from the gut than the older-primed groups which expressed a stronger T lymphocyte response. The data presented indicate that clearance of Salmonella from the gut is age-dependent and we propose that this relates to the increased competence of the enteric T cell response. The findings that Salmonella persists beyond 8-9 weeks, irrespective of age at exposure, has implications for the broiler sector and indicates the need to remain Salmonella free throughout the rearing period. Moreover, the re-challenge data demonstrates that infection at a young age is less effective in producing protective immunity than in older chickens. This feature of the development of protective immunity needs to be considered when developing vaccines for the broiler sector of the Poultry industry. Moreover, the rechallenge data demonstrates that infection at young age. Van Immerseel and Co workers (2004) recorded that Poultry are very
likely to become infected with Salmonella in the early period, due to environmental contamination. The purpose of their study was to evaluate the effect of infection dose on the risk of persistent infection in laying hens. In this study, young layer chicks were orally infected with a low $10^2$ at 1 day posthatch or a high dose (10(9) cfu at one week posthatch of Salmonella enteritidis. The pattern and duration of fecal shedding was studied for 18 wk. All chickens shed Salmonella early after infection and shed Salmonella intermittently during the whole study period. There were more positive birds in the high-dose group than in the low-dose group in the first weeks following Salmonella exposure. From 10 weeks post-infection onwards, however, birds that were orally infected with the low dose of Salmonella enteritidis shed more Salmonella than the birds that received the high dose. At 18 wk of age, there was no difference in cecal colonization between the treatment groups. It can be concluded that infection of newly hatched chicks with a low dose of Salmonella enteritidis can lead to persistent infection until onset of lay, hereby excreting Salmonella bacteria intermittently. The above mentioned scientists support the work and view of the author, as their work is in line with the authors work.

The work of the following scientists up to some extent did not agree and in line with the study and work of the author and have some differences with the view of the author due to the reasons of the dose rate, geographic distribution, environmental factors and age factor of the bird.

Girard-Santosuosso and others (1988) observed genetic resistance of four chicken lines against Salmonella enteritidis (SE) phage type 4 (PT4) systemic
colonization was investigated. Thirteen-week-old chickens were intravenously inoculated with 10(6) SE colony-forming units, and the levels of SE colonization were determined at various time intervals after inoculation in liver, spleen, genital organs, and ceca. The course of SE infection showed a rapid contamination of liver, spleen, and genital organs, whereas the ceca were infected later. A significant (P < 0.001) effect of the chicken line on levels of SE was detected on day 3 post-inoculation (PI) in liver and ceca, on day 10 PI in ceca, and on day 15 PI in spleen. Because an early control of systemic Salmonella infection by the Ly/Nramp1 gene has been demonstrated in mice, we aimed to study the early resistance of chickens to SE. As a consequence, we then focused our study on the between- and within-line variabilities of SE levels on day 3 PI. According to the SE levels in liver on day 3 PI, the chicken lines could be classified as susceptible (Y11 and L2) or resistant (PA12 and B13). This early variability was explored in resistant B13 and susceptible L2 lines. Differences between these two lines were confirmed in liver but not in ceca. A large within-line variability was observed in all organs of these two lines. The genetic origin of this variability will have to be determined as a prerequisite to an eventual selection.

Buhr and Co workers (2001) challenged broilers with 10^9/ml Salmonella typhimurium and then were provided with 0 to 15 glucose during feed with drawl on litter. After feed with drawl broilers were processed and their crops were aseptically removed and weighed. It was found that, inhibition of the growth of Salmonella typhimurium in the crops of broilers provided that cocktail
supplemented with 7.5% glucose was generally associated with increased growth and lactic acid bacteria and decreased crop.

Boldur and Co author (2002) reported that Salmonella infections originating from Poultry are one of the major causes of food-borne disease. For the control of Salmonella in Poultry a multifactorial approach is more likely to be effective, and the genetic resistance of Poultry breeds to Salmonella infections may be a valuable contribution. Experimental Salmonella enteritidis infections were examined in three different broiler outbred lines: the FC line, which had been selected for feed conversion efficiency; the R line, which had been selected for growth rate; and the C line, a commercially available line. The FC line had the highest mortality rate after intramuscular inoculation with $5 \times 10^5$ colony forming units (CFU) of Salmonella enteritidis at 2 weeks of age (40% versus 21 and 20% in the other lines). However, at slaughter age, the number of birds carrying Salmonella in caecal contents, and the concentration of Salmonella in the caecal contents, was lowest in the FC line. The FC and R lines were compared by inoculation with doses ranging from $10^2$ to $10^7$ CFU Salmonella enteritidis. At sublethal doses ($10^5$ CFU or less), the FC line carried significantly less Salmonella in caecal contents and the rate of systemic infection was lower. The start of shedding was also delayed compared with the R line. At doses of $10^6$ CFU Salmonella enteritidis or higher, there were no differences in Salmonella carriage between the lines, and the FC line showed higher mortality. In conclusion, resistance to mortality and resistance to the carriage of Salmonella enteritidis do not necessarily coincide within lines, as the FC line showed high
mortality but low carriage, both in survivors of high infection doses and in all birds at lower infection doses.

Lima-Filho and Co workers (2004) experimentally exposed plasmid free non pathogenic *Escherichia coli* (EMO) or not (control) 10 days before challenge with *Salmonella typhimurium* (10²) colony forming units (CFU)/mouse). Survival after challenge was higher (P < 0.05) in the experimental group (16%) than in the control animals (0%). Histopathological examination of the colon and ileum mucosa of the experimental group showed less extensive lesions such as edema, cell inflammatory infiltration and hyperemia. The epithelial cells of the mucosal surface and the production of the mucous layer were also better preserved in the experimental group. The population levels of *Salmonella typhimurium* in the feces were initially 10-fold lower (P < 0.05) in the experimental groups. However, 3 days after challenge both experimental and control groups showed similar population levels ranging from 10⁸ to 10⁹ CFU/g of feces. The intestinal contents of total and anti-*Salmonella typhimurium* sIgA were higher in the experimental groups 10 days after inoculation of *E. coli* EMO strain. Translocation of *Salmonella typhimurium* to the spleen was 10-fold lower (P < 0.05) in the experimental group only on day 3 after infection. This was not related to an increase in the bacterial blood clearance of the animals, as shown by experimental venous challenge with *E. coli* B41. In conclusion, treatment of mice with *E. coli* EMO strain promoted a relative protection against experimental infection with *Salmonella typhimurium*. This protection was not due to the
reduction of the population of pathogens in the intestine but was probably related to stimulation of the immune response.

**Typhoid Fever**

According to World Health Organization an incidence of typhoid fever was 365/100,000 with 16 to 20 million cases accruing world wide annually. The incidence of typhoid fever in developing countries was 541/100,000 and 7.7 million cases in Asia alone, with the total of 600,000 deaths due to typhoid fever in developing countries every year. The increasing tendency of typhoid fever cases has been attributed due to the resistance developed by the pathogen with the passage of time and increased from 10% in 1987 to 60% in 1990 and then continues report from different centers regarding resistance was ranging from 10% to 70% (Khan, 2001).

In order to estimate the distribution and incidence of *Salmonella enteritidis* and *Salmonella typhimurium* in the general population of Lahore, a hospital population of 400 patients suspected from typhoid fever from four different hospital of Lahore Area were selected i.e, 100 from each hospital. Stool and blood samples from each patient were collected as detailed in the methodology (material and method) which was subjected to PCR test for the detection of *Salmonella enteritidis* and *Salmonella Typhimurium*. A total of 14% and 10% stool samples were found positive for *Salmonella enteritidis* and *Salmonella Typhimurium* respectively.
Similarly, 6% and 2% blood samples were found positive for *Salmonella enteritidis* and *Salmonella Typhimurium* respectively. There was a significant difference (P< 0.05) between the sero-positivity of stool and blood samples in the suspected typhoid fever patients as far as *Salmonella enteritidis* and *Salmonella typhimurium* were concerned. However, there was no significant difference (P> 0.05) between the hospitals (Appendix-V).

The results are comparable and agreeable at a different angle and at different spectrum as compared to Milakovic and Co workers, (1990) and Rzedzicki and Co workers (2000), and Kraus and Co workers (1999). Most of the previous work has incriminated *Salmonella Typhi* and *Salmonella paratyphi* as main cause of typhoid fever but the results of Milakovic and Co workers (1990) and Kraus and Co workers (1999) Rzedzicki and Co workers (2000) have mentioned that *Salmonella enteritidis*, *Salmonella paratyphi* and *Salmonella typhi* being the most common sero types for typhoid fever in human beings.

Vallenas and Co workers, (1985) made comparative study in 118 pediatric patients (2 to 13 years old) with suspected typhoid fever. Only 47% of children 2 to 6 years, as compared with 89% in children 7 to 13 years. At least one was positive for *Salmonella typhi* in 43 patients. Bone marrow cultures were positive in 84% of the confirmed cases, a sensitivity significantly greater then for duodenal contents (42%), blood (44%) and stool (65%) cultures. Higher recovery rates for blood cultures were found during the first week of illness than later. Bone marrow cultures remain the most effective method for the recovery of *Salmonella typhi*. Stool cultures appear to be more effective in children than in
adults. A partial agreement in result was due to the detection of different and specific sero-types by both the projects. The sero-types of *Salmonella enteritidis* and *Salmonella typhimurium* were not included in the laboratory of tests by most of the research workers. This study is innovative in this respect that instead of *Salmonella typhi* and *Salmonella paratyphi*, *Salmonella enteritidis* and *Salmonella typhimurium* were selected. Therefore, the results were comparable but not exactly tallied.

Similarly, Milakovic and Co workers (1990) observed that in the 4 year period there was a considerable increase in both human and Poultry infections with *Salmonella enteritidis*. Of the 299-627 Poultry organ samples examined annually, 48.6% were infected in 1986, 60% in 1987, 20.4% in 1988 and 33.1% in 1989, compared with 0.6% of 849 in 1985. Of the 26118 in 3345/human fecal samples examined annually, 41.4% were infected in 1986, 63.6% in 1987, 41.4% in 1988 and 44.6% in 1989, compared with 7.1% in 1985. The main sources of the human infections were Poultry products.

There was good attempt by Luby and Co authors (1998) who conducted a study to evaluate risk factors for developing typhoid fever in a setting where the disease is endemic in Karachi, Pakistan. They enrolled 100 cases with blood culture-confirmed Salmonella typhi between July and October 1994 and 200 age-matched neighbor hood controls. Cases had a median age of 5.8 years. In a conditional logistic regression model, eating ice cream, eating food from a roadside cabin during the summer months taking antimicrobials in the 2 weeks preceding the onset of symptoms and drinking water at the work-site were all
independently associated with typhoid fever. There was no difference in the
microbiological water quality of home drinking water between cases and controls.
Typhoid fever in Karachi resulted from high-dose exposures from multiple
sources with individual susceptibility increased by young age and prior
antimicrobial use. Improving commercial food hygiene and decreasing
unnecessary antimicrobial use would be expected to decrease the burden of
typhoid fever.

Kraus and Co workers (1999) reported that enteric fever is a systemic
illness caused by Salmonella infection, with Salmonella typhi, Salmonella
paratyphi and Salmonella enteritidis being the most common sero types. Humans
are the only reservoir for Salmonella typhi and its predilection for the ileum is due
to the fact that organisms enter the body by translocation across specialized
Peyer's patch epithelium and then proliferate in the mucosal macrophages.
Although rare in developed countries, enteric fever should be considered in any
patient with recent travel to endemic areas and in the context of illness thought to
be related to contaminated foods.

Rzedzicki and Co workers (2000) recorded that a growing increase of
Salmonella infections in humans. Most of these infections were caused by
Salmonella enteritidis. An important factor in preventing human Salmonellosis
was interrupting the infection chain which originated in Poultry. Bacteriological
methods imposed by current regulations were laborious and time consuming. In
these cases, serological tests were of great value. Among the many serological
methods, the Elisa has was used in the control of Salmonella infections in poultry
flocks because it was a sensitive and quick test. Antibiotic therapy was considered an important factor that can influence bacterial variability. Antibiotic therapy may promote the spread of resistant strains.

Bishop and Co workers (2001) reported that Salmonellosis was the major cause of illness in the USA. A total of 441,863 Salmonella isolates were reported. Annual isolation rates decreased from 19 to 13/100,000 persons, however, trends varied by sero type. Salmonella infections continue to be an important cause of illness especially among infants. Recent declines in food associated sero type may reflect changes in the meat, Poultry and eggs. Waqar and his Fellows (2002) conducted a descriptive study in 75 patients, admitted with suspected typhoid fever. Out of 75, 47 were male and 28 were female patients. Maximum incidents was seen in 5-15 years age group (73%). Forty (53%) patients had duration of fever of 7-14 days. The fever was mostly at high grade (67%) with a history of gastro intestinal complaints like diarrhea, vomiting, abdominal pain etc. This study has also been done during 2001 in Sheikh Zaid Hospital Lahore.

Sufficient attempts could not be made to identify the other sero types like Salmonella enteritidis and Salmonella typhimurium from typhoid fever cases of human beings. But the authors attempt is unique in his nature and perhaps this type of study has been made to open up the new avenues and all the focus was given to detect the Salmonella enteritidis and Salmonella typhimurium from the typhoid fever and food poisoning cases of human suspected patients. Attempts were made to establish an association if any between the consumption of poultry
and poultry biproducts for the development and precipitation of typhoid fever and food poisoning.

The literature about Salmonella enteritidis and Salmonella typhimurium is meager, scanty and insufficient because very few scientists have attempted to detect salmonellae as mentioned earlier. This is why the authors study is the 1st one in its nature for the detection of Salmonella enteritidis and Salmonella typhimurium from suspected human cases of typhoid and food poisoning through PCR experiment. The author has focused on those sero types which were highly prevalent in poultry and poultry biproducts and wanted to see if the same organisms could be found in human beings as a pathogen for typhoid fever and food poisoning. This is why a fast, although expansive, but highly sensitive test of PCR was preferred as compared to eliza and conventional cultural and biochemical test for the detection of Salmonellae particularly Salmonella enteritidis and Salmonella typhimurium.

The author therefore has the recommendation that further investigational research should be continued in order to find the relationship of other sero-types of Salmonella as a cause of Salmonellosis in human beings i.e typhoid fever and food poisoning.

**Food Poisoning**

Salmonellae are the etiological agents of variety of disease problems in poultry, they remained essentially a concern for public health as a cause of out breaks of food poisoning (Humbert and Salvat 1997).
Thorns (2000) recorded in many countries of the world that bacterial food born zoonotic infections were the most common cause of human intestinal diseases. Salmonella and Campylobacter accounted for over 90% of all reported cases of bacteria-related food poisoning world-wide. Poultry and Poultry products had been incriminated in the majority of traceable food borne illnesses caused by these bacteria, although all domestic livestock were the chief reservoirs of the infections. In contrast to the enzootic nature of most Salmonella and Campylobacter infections, Salmonella enteritidis caused a pandemic in both Poultry and humans during the latter half of the 20th Century. Salmonella typhimurium and campylobacter appeared to be more ubiquitous in the environment. The dissemination of Salmonella enteritidis along the food chain has been fairly well understood, and control programmes had been developed to target key areas of Poultry meat and egg production. Recent evidence indicates that these control programmes were associated with an overall reduction of Salmonella enteritidis along the food chain. Future control strategies needed to consider variations in the epidemiology of food-borne zoonotic infections, and apply a quantitative risk analysis approach to ensure that the most cost-effective programmes were developed.

In order to explain the hypothesis, Mead (2000) reported that in newly hatched chicks, the rapid establishment of an adult-type intestinal microflora, via the oral route, produced almost immediate resistance to colonization by any food poisoning Salmonellae that gain access to the rearing environment. Exploitation of the ‘competitive exclusion’ (CE) effect was now an accepted part of the overall
strategy by which Poultry-associated Salmonellas are being controlled in some countries.

Zhang-Barber and Co workers (1999) reported that Salmonella species are facultative intracellular pathogens causing localized or systemic infections, in addition to a chronic asymptomatic carrier state. They were of worldwide economic and public health significance. In Poultry, which represented important sources of cheap protein throughout the world, fowl typhoid and pullorum disease continue to cause economic losses in those parts of the world where the Poultry industries were continuing to intensify and where open sided housing was common. A number of serotype that caused human gastro-enteritis were also increasing. The costs or impracticality of improvements in hygiene and management together with the increasing problems of antibiotic resistance suggested that vaccination in Poultry would become more attractive as an adjunct to existing control measures.

Sarna and Co authors (2002) A supportive report about epidemiology after investigation of an outbreak of gastroenteritis amongst attendees of a local community dinner in a Perth suburb. Of the 98 people interviewed (response rate 98%), 53 reported gastrointestinal symptoms (attack rate 54%). Faecal cultures from 11 cases, 2 food preparers, 1 waitress and leftover mock ice-cream dessert grew Salmonella typhimurium PT135. Of the 3 food handlers, one was asymptomatic, another gave an unclear history of onset of illness and the waitress claimed illness onset 9 days after the dinner. Eggs used to make the mock ice-cream dessert were supplied directly from the producer who used
inappropriate shell cleaning methods. The method of preparation of the dessert encouraged contamination. The cause of this outbreak was almost certainly the ice-cream dessert with contamination most likely resulting either from the eggs used to make the dessert or one or both of the food preparers, coupled with inadequate cooking of the dessert. The pasteurized egg products or egg pulp should be used in the preparation of uncooked or minimally cooked dishes.

Similarly, Ortega-Benito and Langridge (1992) recorded an outbreak of food poisoning comprising of 68 cases in 1989 occurring at a private club in Teddington (London), eating sandwiches continuing mayonnaise microbiologically investigation found Salmonella typhimurium DT4 in 36 of 68 fecal samples taken. The probable source was identified as a flock of one of the egg supplier. Muramatsu and Nishizawa (1992) recorded an out break of food poisoning in which the major symptoms observed in the 680 patients in 1989. Diarrhhea was recorded in 70.9%, abdominal pain 51.2% fever 44.6%, headache 27.9%, nausea 5.9% and vomiting 5.7%.

In the authors study 100 human of suspected food poisoning (as defined in the methodology) were selected from 4 different hospitals of Lahore (Pakistan). From each patient, stool and blood sample was selected and tested for bacterial DNA of Salmonella enteritis and Salmonella typhimurium through PCR. On the average 14 and 10 stool samples were found positive against Salmonella enteritis and Salmonella typhimurium from each of the 25 patients of each hospital respectively. Similarly on an average 5% and 6% blood samples were found positive from 25 patients of each hospital respectively. There was a
significant difference ($P < 0.05$) between the sero-positivity of stool and blood samples in the suspected food poisoning patients as far as *Salmonella enteritidis* and *Salmonella typhimurium* were concerned. However, there was no significant difference ($P > 0.05$) between the hospitals (Appendix-VI). In a retrospective epidemiological question based study, it was concluded that more than 50% of the patient the food poisoning was associated with the consumption of egg and meat of poultry. The odds ratio in terms of relative risk is equal to 10.52 which is greater than 1 and food poisoning significantly associated with the consumption of food and poultry and poultry biproducts.


Kupek and Co workers (2001) described *Salmonella enteritidis* infection through food involving 7802 people with sign and symptoms consisting with the food poisoning, most frequent symptoms were diarrhea (92%), abdominal pain (73%), fever (70%), vomiting (49%) and nausea (45%). The out breaks most frequently occurred at home (60%) but the largest infections resulted from industrial kitchens (78%). The age group between 20 and 30 years was most affected. No relationships between the number of out breaks and out side temperature or humidity were found. The attack rate should significant difference between men and women. Better attention to storage of food products at home
and in industrial kitchens was needed to control this common gastrointestinal illness.

Dohtsu and Co workers (2001) investigated Salmonella food poisoning due to infected eggs. The partially cooked omelette would permit for the growth of a sufficient inoculum to cause disease. To prevent food poisoning, the food should be cooked well at 75°C for more than 1 minute and should not have omelets during the hot summer session.

A clinico-epidemiological and microbiological investigation was conducted by Godoy and his fellows (2000). An outbreak of gastrointestinal infection due to Salmonella enteritidis, which is due to most likely food vehicle. An historic study was conducted out among persons exposed to menus at a school canteen. Data were gathered on age, sex, foods consumed and clinical symptoms. School premises and menus were inspected, food samples obtained (spaghetti and meatballs), and stool samples taken from 30 affected subjects and 8 food handlers. Isolated strains were studied using pulsed-field electrophoresis. Attack rates were computed, and the odds ratio adjusted for the remaining foodstuffs (ORa) used to calculate the independent contribution made by the respective foods to risk of infection. Study coverage was 75.7% (140/185). The overall attack rate was 72.1% (101/140), with 12.9% of those affected requiring hospitalization. Salmonella enteritidis was isolated in stool cultures from 28 affected subjects, and in 2 blood and 6 stool cultures from food handlers. Moreover, Salmonella enteritidis was also isolated in the food samples. They concluded that, this outbreak served to underscore the gravity of Salmonella
species food poisoning, the danger of using inadequately cooked eggs, and the importance of interviewing food handlers to ensure proper classification as patients or carriers. Pasteurized egg products should be used for eating purpose.

Parry and Co workers (2004) believed that food hygiene precautions in domestic kitchens were an important strategy in efforts to reduce the incidence of sporadic food poisoning handle the same types of foods and adopt similar food hygiene precautions in their kitchens to the rest of the population. This suggested the need to examine other factors. A case-control study of sporadic Salmonella food poisoning was conducted to investigate several domestic kitchen risk factors. Measures of perception of risk, knowledge, and control associated with food poisoning in case and control respondents are reported here. It was found that perceived personal risk from food poisoning in the home was less than perceived risk to other people. In contrast, ratings of personal knowledge about food poisoning and personal control over food poisoning in the home were seen to be greater than other people's knowledge and control. There were no differences between the cases and the controls in their ratings of knowledge about food poisoning or their control over food poisoning. *Salmonella enteritidis* had been incriminated after epidemiological analysis in causing food poisoning out breaks by using pulsed-Field gel electrophoresis (PFGE) of fragments of chromosomal DNA isolates (Murase and Co workers 1996).
CHAPTER 6

SUMMARY

The present research *endeavour* was made to study and investigate the prevalence of *Salmonella enteritidis* and *Salmonella typhimurium* from poultry feed, poultry meat and poultry eggs and their role in the chain of transmission of salmonellae to *human* beings. The objective was to generate data to improve the quality of poultry products and human health *awareness*.

Salmonellosis is one of the most *wide spread* food borne *zoonoses*. The etiological agents *Salmonella enteritidis* and *Salmonella typhimurium* not only produce the disease but during the convalescent phase (after the recovery of disease) remain carriers for *indefinite* period of time. In this study 400 samples were collected and were distributed and detailed as; poultry feed (n=100), poultry intestines (n=100 Small and n=100 Large intestines) and eggs (n=100) were collected for the identification of the organism through polymerase chain reaction (PCR). The Positivity *percentage* as tested through PCR for *Salmonella enteritidis* in the poultry feed was 20,15,10,15 and 10 for *layer starter*, *layer grower*, *layer finisher*, *broiler starter* and *broiler finisher* respectively (P>0.05). The positivity percentage as tested through PCR for *Salmonella typhimurium* for *layer starter*, *layer grower*, *layer finisher*, *broiler starter* and *broiler finisher* feed was 15,10,10, 10, and 10 respectively (P>0.05). There was no significant difference between layers feed and broilers feed as far
as identification of *salmonella enteritidis* and *salmonella typhimurium* was concerned (P>0.05) but the prevalence range of *salmonella enteritidis* and *salmonella typhimurium* from poultry feed was 10-20% which was biologically significant. The positivity percentage rate of *Salmonella enteritidis* for small and large intestine in Desi birds (local breed) was 2 and 16 % respectively. Where as for broilers in small and large intestine it was 4 and 18% respectively. The positivity of *Salmonella typhimurium* in small and large intestine of Desi birds was 2 and 14% where as in broilers it was 4 and 16% in the small and large intestine respectively. There was a significant difference (P <0.05) between the positivity of percentage of *salmonella enteritidis* and *salmonella typhimurium* as far as identification of *Salmonellae* from Desi and broiler meat was concerned.

It was found that 16%, 8%, 16% and 16% egg albumin was found positive for *Salmonella enteritidis* in layer egg albumin, Desi (local breed) egg, albumin, double yolk albumin and broken egg albumin respectively. In each case 25 egg albumin were collected and tested for the detection of *Salmonellae*. Similarly the egg yolk from layers, Desi (local breed) double yolk and broken eggs was taken and positivity rate for *Salmonella enteritidis* was found 12%, 4%, 12% and 12% respectively. It was found that 12%, 4%, 12% and 12% egg albumin was found positive for *Salmonella typhimurium* in layer egg albumin, Desi egg albumin, double yolk albumin and broken egg albumin respectively. In each case 25 egg albumin were collected and tested for the detection of Salmonella. Similarly the egg yolk from layers, desi double yolk and broken eggs was taken and positivity rate for *Salmonella enteritidis* was found
8%, 4%, 8% and 4% respectively. The positively rate for *Salmonella typhimurium* in both albumin and yolk was relatively less in both albumin and yolk of layers, desi double yolk and broken eggs. Statistically there was no significant difference (P > 0.05) but the prevalence of *Salmonella enteritidis* and *Salmonella typhimurium* from different eggs ranged between 4-16% and 4-12% respectively which was biologically significant.

The *Salmonella enteritidis* and *Salmonella typhimurium* were isolated, identified and grown on the artificial and selective media. The virulence of the organisms of *Salmonella enteritidis* and *Salmonella typhimurium* were estimated through calculation of LD$_{50}$. It was found as $10^{-3.58}$/ml and $10^{-3}$/ml for *Salmonella enteritidis* and *Salmonella typhimurium* respectively, having significant difference (P < 0.05). In order to understand the pathogenesis and carrier states of salmonella organisms in poultry, a group of 300 broiler birds were procured and divided into three groups were studied upto the age of 3 months. The infection was orally given on the 7th day of their age. As an average 86.74% of the birds were maintaining the organism of the *Salmonella enteritidis* in the large intestine during the entire experimental period in contrast to the small intestine in which 0% were found positive (P < 0.05). Similarly an average 94.94% of the birds were maintaining the organism of the *Salmonella typhimurium* in the large intestine during the entire experimental period in contrast to the small intestine in which 0% were found positive (P < 0.05) but non of the samples of Small and Large intestine of control group (Group-C) were found positive for *Salmonella enteritidis* and *Salmonella typhimurium*.,
There was a significant difference between *Salmonella enteritidis* and *Salmonella typhimurium* in large intestine of poultry (*P* < 0.05). The histopathology of different organs of broiler chickens i.e. liver, lung, spleen, kidney, small intestine, large intestine, bursa of fabracious and lean muscles at different phases of disease was also conducted for the better understanding of pathogenesis due to salmonellosis. The principal lesions in the liver at the age of 14 to 28 days in groups A and B were leukocytic infiltration, necrosis and haemorrhage. No lesions were recorded in liver after 28 days of age in groups A and B. No lesions were recorded in group C. The principal lesions of the lungs at the age of 14 to 28 days in groups A and B were leukocytic infiltration, mild necrosis, vascular congestion and haemorrhages. No lesions were recorded in lungs after 28 days of age in groups A and B. No lesions were recorded in group C. The principal lesions of the spleen were mild leukocytic infiltration, necrosis and congestion at the age of 14 to 28 days in groups A and B. No lesions were recorded in spleen after 28 days of age in groups A and B. No lesions were found in group C. The principal lesions of the kidneys were marked tubular necrosis with glomerular degeneration and leukocytic infiltration and haemorrhages at the age of 14 to 28 in groups A and B. No lesions were recorded in kidneys after 28 days of age in groups A and B. No lesions were found in group C. The principal lesions of the small intestine were degeneration of mucosa with inflammatory cells, necrosis, inflammation, superficial ulceration on mucosal lining of intestine at the age of 14 to 21 days. No lesions were recorded in small intestine after 21 days of age in group A and B. No lesions
were recorded in control group C. The principal lesions of the large intestine were leukocytic infiltration with necrosis and inflammation at the age of 14 to 91 days. The lesions were recorded up to 91 days of age in group A and B. No lesions were recorded in control group C. The principal lesions of Bursa of fabricious were atrophy & necrosis of bursal follicles and leukocytic infiltration at the age of 14 to 21 in groups A and B. No lesions were recorded in Bursa of fabricious after 21 days of age in groups A and B. No lesions were found in group C. The principal lesions of lean muscle were muscular degeneration and necrotic areas at the age of 14 to 21 days in groups A and B. No lesions were recorded in lean muscles after 21 days of age in groups A and B. No lesions were found in group C.

The carrier state was not only the source of spread of disease with in the poultry but also caused typhoid fever and food poisoning in humans. The chain of transmission started from poultry feed to poultry meat and ultimately to humans as dead end host. Finally, the 400 samples of stool and blood from 200 human patients (100 suspected of typhoid fever and 100 suspected of food poisoning) were also collected from four different hospitals from urban area of Lahore for the identification of Salmonella enteritidis and Salmonella typhimurium through PCR method in order to see the public health impact of Salmonellosis through consuming the meat and eggs of the carrier birds. A total of 14% and 10% stool samples were found positive for Salmonella enteritidis and Salmonella Typhimurium in case of suspected typhoid fever patients respectively. Similarly 6% and 2% blood samples were found positive for
Salmonella enteritidis and Salmonella Typhimurium. There was a significant difference ($P < 0.05$) in the sero positivity of stool and blood samples of suspected typhoid fever patients and also as for as Salmonella enteritidis and Salmonella typhimurium was concerned. However there was no significant difference ($P > 0.05$) between the hospitals On the average 14 and 10 stool samples were found positive against Salmonella enteritidis and Salmonella, typhimurium from each of the 25 patients of each hospital respectively in case of suspected food poisoning patients. Similarly on an average 5% and 6% blood samples were found positive from 25 patients of each hospital respectively. There was a significant difference ($P < 0.05$) in the sero positivity of stool and blood samples of suspected food poisoning patients as far as Salmonella enteritidis and Salmonella typhimurium was concerned. However there was no significant difference ($P > 0.05$) between the hospitals.
CONCLUSION

A series of five experiments were conducted and carried out to study and explore the project "Pathogenesis of Salmonellosis with respect to carrier states in poultry and its public health impact."

For this purpose, in the 1st phase, identification, isolation and characterization of Salmonella enteritidis and Salmonella typhimurium was attempted. It was followed by the estimation of LD 50 and carrier states and histopathological study at different phases of disease in broiler chickens experimentally infected with Salmonella enteritidis and Salmonella typhimurium to ascertain the nature of carrier states in terms of maintenance of the Salmonellae by different organs leading to histopathological changes and finally to the stage of shedding of the organism through the feces in the environment. Dissemination to human beings and the Public health impact of Salmonellosis was studied in the human subjects who consumed the meat and eggs of the carrier birds which were followed by testing their stool and blood samples through polymerase chain reaction (PCR). In this way the pathogenesis and chain of Salmonellae enteritidis and Salmonella typhimurium infection through poultry feed, meat, eggs and humans beings was transmissible. However, the humans were considered as dead end host. It was concluded that Salmonella enteritidis and Salmonella typhimurium was maintained in the large intestine of the poultry and has transmitted from poultry feed, poultry meat and poultry eggs to human beings and thus, causing typhoid fever and food poisoning.
RECOMMENDATIONS / SUGGESTIONS

Major aim of this research endeavour was to help in understanding the basic principles involved in the chain of infectious cycle of Salmonellosis. In addition to that the application of the quality control of poultry products with respect to Salmonella infection to broiler chicks and broiler meat available in the market for human consumption is the ultimate goal of this project. The objective was to reduce the risk of Salmonellosis in poultry and humans. The following measures are suggested.

1. PREVENTION AND CONTROL OF SALMONELLOSIS IN POULTRY/ ANIMALS

A. Monitoring
   • The poultry and their environment should be monitored by frequent testing of Salmonellae.
   • Bacteriological profile of poultry house environment.
   • Serological testing of flock and removal of infected birds.
   • Culturing of tissues from selected birds.
   • Egg shells, egg albumin & egg yolk culturing.

B. Hygiene and Sanitation
   • Eggs from infected layer flocks should be pasteurized before consumption.
   • Salmonella positive breeder flocks should be given pellet feed.
   • Hatching sanitation
   • Proper disinfection of hatching eggs.
   • Proper sanitation and disinfection of farm premises.
   • The provision of salmonella-free feed i.e. pellet feed is of prime importance for the prevention of salmonella infections of poultry flocks and parent flocks.
   • Control of rodent, insects and wild birds
C. Managemental

- For routine treatment of eggs and progeny, only those antibiotics should be used that do not cause microbial resistance against drugs widely used in humans.
- Resistance of *Campylobacter* spp. and *Salmonella* spp to fluoroquinolones has become a public health risk. This does not exclude well targeted and transient use of antibiotics as essential measures in salmonellosis control programmes.
- Vaccination of breeder flock is recommended for decrease of the *salmonella* infection pressure.

1. MEASURES FOR THE PREVENTION AND CONTROL OF SALMONELLOSIS IN HUMANS

A. Meat and Eggs

- Wrap fresh meat in plastic bags at the market to prevent blood from dripping on other foods.
- Cook poultry products at temperature of 170°F for breast meat and at 180°F for thigh meat.
- Avoid eating raw or under cooked meat and egg.
- Cook poultry meat and egg thoroughly.
- Purchase only inspected grade AA eggs and animal food products.
- Handle raw eggs carefully.
- Keep eggs refrigerated.
- Throw away cracked or dirty eggs.
- Do not eat half fried and half boiled eggs.
- Wash hands immediately after handling raw poultry or raw eggs.
- *Full fried and full boiled* eggs should be used for eating to prevent food borne Salmonellosis problem.
B. PERSONNEL HYGIENE MEASURES

- Washing of hands with soap and warm water before and after handling foods, after using the bathroom.
- Refrigerate foods properly.
- Use bleach to wash cutting boards and counters used for preparation immediately after use to avoid cross contamination of other foods.
- People who have Salmonellosis should not prepare food for others.
- Educate the food handlers and persons who prepare food.
- Educational programmes covering pre- and post harvest food safety procedures, especially salmonella control, should be initiated in the animal and food production sectors for the public awareness.
LITERATURE CITED
LITERATURE CITED


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Jennifer, D. Boddicker and B. D. Jones (2004). Lon Protease Activity Causes Down-Regulation of Salmonella Pathogenicity Island 1 Invasion Gene Expression after Infection of Epithelial Cells Department of Microbiology, Roy J. and Lucille A. Carver School of Medicine, University of Iowa, Iowa City, Iowa 52242


APPENDIX-I

Summary of Statistical analysis and probability values of type of feed in layers & broilers with regard to *Salmonella enteritidis* and *Salmonella typhimurium*

Table: A

<table>
<thead>
<tr>
<th>Type of Feed</th>
<th>No of Positive samples for <em>Salmonella enteritidis</em> (n=20)</th>
<th>No of Positive samples for <em>Salmonella typhimurium</em> (n=20)</th>
<th>Z value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Layer</td>
<td>Starter 4</td>
<td>3</td>
<td>0.379 NS</td>
<td>0.3322 NS</td>
</tr>
<tr>
<td></td>
<td>Grower 3</td>
<td>2</td>
<td>0.449 NS</td>
<td>0.3265 NS</td>
</tr>
<tr>
<td></td>
<td>Finisher 2</td>
<td>2</td>
<td>0.000 NS</td>
<td>0.5000 NS</td>
</tr>
<tr>
<td>Broiler</td>
<td>Starter 3</td>
<td>2</td>
<td>0.449 NS</td>
<td>0.3265 NS</td>
</tr>
<tr>
<td></td>
<td>Finisher 2</td>
<td>2</td>
<td>0.000 NS</td>
<td>0.5000 NS</td>
</tr>
</tbody>
</table>

Table: B

<table>
<thead>
<tr>
<th>Type of Feed</th>
<th>No of Positive samples for <em>Salmonella enteritidis</em></th>
<th>No of Positive samples for <em>Salmonella typhimurium</em></th>
<th>Z value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Layer (n=60)</td>
<td>9</td>
<td>7</td>
<td>0.243 NS</td>
<td>0.4041 NS</td>
</tr>
<tr>
<td>Broiler (n=40)</td>
<td>5</td>
<td>4</td>
<td>0.850 NS</td>
<td>0.1976 NS</td>
</tr>
</tbody>
</table>

Table: C

<table>
<thead>
<tr>
<th>Type of Feed</th>
<th>Layer (n=20)</th>
<th>Broiler (n=20)</th>
<th>Z value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella enteritidis</em> Starter</td>
<td>4</td>
<td>3</td>
<td>0.416 NS</td>
<td>0.3337 NS</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>0.000 NS</td>
<td>0.5000 NS</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em> Starter</td>
<td>3</td>
<td>2</td>
<td>0.478 NS</td>
<td>0.3163 NS</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>0.000 NS</td>
<td>0.5333 NS</td>
</tr>
</tbody>
</table>

Table: D

<table>
<thead>
<tr>
<th>Type of Feed</th>
<th>Layer (n=20)</th>
<th>Broiler (n=20)</th>
<th>Z value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella enteritidis</em></td>
<td>6</td>
<td>5</td>
<td>0.325 NS</td>
<td>0.3727 NS</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>5</td>
<td>4</td>
<td>0.354 NS</td>
<td>0.3617 NS</td>
</tr>
</tbody>
</table>

NS =  P> 0.05  
* =  P< 0.05
APPENDIX-II

Summary of Statistical analysis and probability values of type of Poultry Meat (in Desi and Broilers) with regard to *Salmonella enteritidis* and *Salmonella typhimurium*

Table: A

<table>
<thead>
<tr>
<th>Type of poultry meat (n = 50)</th>
<th>No of Positive samples for <em>Salmonella enteritidis</em></th>
<th>No of Positive samples for <em>Salmonella typhimurium</em></th>
<th>Z. value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desi Small intestine</td>
<td>1</td>
<td>1</td>
<td>0.000 NS</td>
<td>0.5000</td>
</tr>
<tr>
<td>Desi Large intestine</td>
<td>8</td>
<td>7</td>
<td>0.250 NS</td>
<td>0.3981</td>
</tr>
<tr>
<td>Broiler Small intestine</td>
<td>2</td>
<td>2</td>
<td>0.000 NS</td>
<td>0.5000</td>
</tr>
<tr>
<td>Broiler Large intestine</td>
<td>9</td>
<td>8</td>
<td>0.243 NS</td>
<td>0.4041</td>
</tr>
</tbody>
</table>

Table: B

<table>
<thead>
<tr>
<th>Type of poultry meat (Birds)</th>
<th>No of Positive samples for <em>Salmonella enteritidis</em></th>
<th>No of Positive samples for <em>Salmonella typhimurium</em></th>
<th>Z. value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desi (n = 100)</td>
<td>9</td>
<td>8</td>
<td>1.414 NS</td>
<td>0.0787</td>
</tr>
<tr>
<td>Broiler (n = 100)</td>
<td>11</td>
<td>10</td>
<td>0.218 NS</td>
<td>0.4136</td>
</tr>
</tbody>
</table>

Table: C

<table>
<thead>
<tr>
<th>Type of organism (n = 50)</th>
<th>Source (Small intestine)</th>
<th>Source (Large intestine)</th>
<th>Z. value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella enteritidis</em></td>
<td>Desi</td>
<td>1</td>
<td>8</td>
<td>2.446*</td>
</tr>
<tr>
<td></td>
<td>Broiler</td>
<td>2</td>
<td>9</td>
<td>2.237*</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>Desi</td>
<td>1</td>
<td>7</td>
<td>2.212*</td>
</tr>
<tr>
<td></td>
<td>Broiler</td>
<td>2</td>
<td>8</td>
<td>2.000*</td>
</tr>
</tbody>
</table>

Table: D

<table>
<thead>
<tr>
<th>Type of organism (n = 100)</th>
<th>Source (Small intestine)</th>
<th>Source (Large intestine)</th>
<th>Z. value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella enteritidis</em></td>
<td>3</td>
<td>17</td>
<td>3.300*</td>
<td>0.0005</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>3</td>
<td>15</td>
<td>2.965*</td>
<td>0.0015</td>
</tr>
</tbody>
</table>

Continued
### Table: E

<table>
<thead>
<tr>
<th>Type of organism (n=50)</th>
<th>Source</th>
<th>Desi</th>
<th>Broiler</th>
<th>Z.value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella enteritidis</td>
<td>Small intestine</td>
<td>1</td>
<td>2</td>
<td>0.586 NS</td>
<td>0.2789</td>
</tr>
<tr>
<td></td>
<td>Large intestine</td>
<td>8</td>
<td>9</td>
<td>0.266 NS</td>
<td>0.3950</td>
</tr>
<tr>
<td>Salmonella typhimurium</td>
<td>Small intestine</td>
<td>1</td>
<td>2</td>
<td>0.586 NS</td>
<td>0.2789</td>
</tr>
<tr>
<td></td>
<td>Large intestine</td>
<td>7</td>
<td>8</td>
<td>0.280 NS</td>
<td>0.3897</td>
</tr>
</tbody>
</table>

### Table: F

<table>
<thead>
<tr>
<th>Type of organism (n = 100)</th>
<th>Desi</th>
<th>Broiler</th>
<th>Z.value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella enteritidis</td>
<td>9</td>
<td>11</td>
<td>0.471 NS</td>
<td>0.3187</td>
</tr>
<tr>
<td>Salmonella typhimurium</td>
<td>8</td>
<td>10</td>
<td>0.491 NS</td>
<td>0.3106</td>
</tr>
</tbody>
</table>

NS = $P > 0.05$

* = $P < 0.05$
APPENDIX-III

Summary of Statistical analysis and probability values of type of poultry eggs with regard to *Salmonella enteritidis* and *Salmonella typhimurium*

Table: A

<table>
<thead>
<tr>
<th>Type of eggs (n= 25)</th>
<th>No of Positive samples for <em>Salmonella enteritidis</em></th>
<th>No of Positive samples for <em>Salmonella typhimurium</em></th>
<th>Z value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Layer eggs A</td>
<td>4</td>
<td>3</td>
<td>0.379 NS</td>
<td>0.3523</td>
</tr>
<tr>
<td>Y</td>
<td>3</td>
<td>2</td>
<td>0.213 NS</td>
<td>0.4155</td>
</tr>
<tr>
<td>Desi eggs A</td>
<td>2</td>
<td>1</td>
<td>0.581 NS</td>
<td>0.2805</td>
</tr>
<tr>
<td>Y</td>
<td>1</td>
<td>1</td>
<td>0.000 NS</td>
<td>0.5000</td>
</tr>
<tr>
<td>Double yolk eggs A</td>
<td>4</td>
<td>3</td>
<td>0.379 NS</td>
<td>0.3523</td>
</tr>
<tr>
<td>Y</td>
<td>3</td>
<td>2</td>
<td>0.213 NS</td>
<td>0.4155</td>
</tr>
<tr>
<td>Broken eggs A</td>
<td>4</td>
<td>3</td>
<td>0.379 NS</td>
<td>0.3523</td>
</tr>
<tr>
<td>Y</td>
<td>3</td>
<td>1</td>
<td>1.021 NS</td>
<td>0.1537</td>
</tr>
</tbody>
</table>

Table: B

<table>
<thead>
<tr>
<th>Type of eggs (n= 50)</th>
<th>No of Positive samples for <em>Salmonella enteritidis</em></th>
<th>No of Positive samples for <em>Salmonella typhimurium</em></th>
<th>Z value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Layer eggs</td>
<td>7</td>
<td>5</td>
<td>0.731 NS</td>
<td>0.2322</td>
</tr>
<tr>
<td>Desi eggs</td>
<td>3</td>
<td>2</td>
<td>0.448 NS</td>
<td>0.3270</td>
</tr>
<tr>
<td>Double Yolk eggs</td>
<td>7</td>
<td>5</td>
<td>0.579 NS</td>
<td>0.2812</td>
</tr>
<tr>
<td>Broken eggs</td>
<td>7</td>
<td>4</td>
<td>0.912 NS</td>
<td>0.1808</td>
</tr>
</tbody>
</table>

Table: C

<table>
<thead>
<tr>
<th>Source (n = 100)</th>
<th>No of Positive samples for <em>Salmonella enteritidis</em></th>
<th>No of Positive samples for <em>Salmonella typhimurium</em></th>
<th>Z value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>14</td>
<td>10</td>
<td>1.034 NS</td>
<td>0.1505</td>
</tr>
<tr>
<td>Yolk</td>
<td>10</td>
<td>6</td>
<td>1.005 NS</td>
<td>0.1574</td>
</tr>
</tbody>
</table>

NS = P > 0.05

\* = P < 0.05
### APPENDIX-IV

**Summary of Statistical analysis and probability values of large and small intestines of broiler chickens experimentally infected with *Salmonella enteritidis* and *Salmonella typhimurium***

<table>
<thead>
<tr>
<th>Type of samples</th>
<th>Group A experimentally infected with <em>Salmonella enteritidis</em> (n = 100)</th>
<th>Group B experimentally infected with <em>Salmonella typhimurium</em> (n = 100)</th>
<th>Group C total-infected as control (n = 100)</th>
<th>Z value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small intestine</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Large intestine</td>
<td>72 (86.74%)</td>
<td>94 (94.94%)</td>
<td>0</td>
<td>2.010*</td>
<td>0.0222</td>
</tr>
</tbody>
</table>

NS = P > 0.05  
*= P < 0.05
APPENDIX - V

Two Factor Randomized Complete Block Design
Data Case No. 1 to 16
Factorial ANOVA for the factors
Replication (Variable 3: Hospital) with values from 1 to 4
Factor A (Variable 4: SE - 1. St1 - 2) with values from 1 to 2
Factor B (Variable 5: Stool - 1. Blood - 2) with values from 1 to 2

Variable 1: Typhoid fever
Grand Mean = 2.000 Grand sum = 32.000 Total Count = 16

Table- A: Table of Means (1) (Comparison between Hospitals)

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>2.000</td>
<td>8.000</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>2.000</td>
<td>8.000</td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>2.500</td>
<td>10.000</td>
</tr>
<tr>
<td>4</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.500</td>
<td>6.000</td>
</tr>
</tbody>
</table>

Table- B: Table of Means (2) (Comparison between Salmonella enteritidis and Salmonella typhimurium)

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0</td>
<td>2.0</td>
<td>1.0</td>
<td>1.500</td>
<td>12.00</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.0</td>
<td>1.0</td>
<td>3.000</td>
<td>20.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table- C: Table of Means (3) (Comparison between Stool and Blood)

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0</td>
<td>1.0</td>
<td>3.000</td>
<td>24.00</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.0</td>
<td>2.0</td>
<td>1.000</td>
<td>8.00</td>
<td></td>
</tr>
</tbody>
</table>

Table- D: Table of Means (4) (Comparison between Stool and Blood of Salmonella enteritidis)

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0</td>
<td>1.0</td>
<td>3.500</td>
<td>14.00</td>
<td></td>
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Table E: Table of Means (5) (Comparison between Stool and Blood of *Salmonella typhimurium*

<table>
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Table F: Analysis Of Variance of Suspected Typhoid Fever Patients

<table>
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**COEFFICIENT OF VARIATION: 23.57%**

Standard Error for means group 1: 0.2357 Number of Observations: 4

Standard Error for means group 2: 0.1667 Number of Observations: 8

Standard Error for means group 4: 0.1667 Number of Observations: 8

Standard Error for means group 6: 0.2357 Number of Observations: 4
APPENDIX - VI

Variable 2: Food Poisoning
Grand mean: 2.313  Grand Sum: 37.000  Total Count: 16

**Table A:** Table of Means (1) (Comparison between Hospitals)

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**Table B:** Table of Means (2) (Comparison between *Salmonella enteritidis* and *Salmonella typhimurium*)

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**Table C:** Table of Means (3) (Comparison between Stool and Blood)

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**Table D:** Table of Means (4) (Comparison between Stool and Blood of *Salmonella enteritidis*)

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**Table E:** Table of Means (5) (Comparison between Stool and Blood of *Salmonella typhimurium*)

<table>
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</table>
Table - F: Analysis Of Variance of Suspected Food Poisoning Patients

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of Freedom</th>
<th>Sum of square</th>
<th>Mean square</th>
<th>F Value</th>
<th>P Value</th>
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<tr>
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COEFFICIENT OF VARIATION: 18.02%

Standard Error for means group 1 : 0.2083
Number of Observations: 4

Standard Error for means group 2 : 0.1473
Number of Observations: 8

Standard Error for means group 4 : 0.1473
Number of Observations: 8

Standard Error for means group 6 : 0.2083
Number of Observations: 4

Case range : 17 - 20
Variable 2: Food Poisoning
Error Mean Square = 0.1740
Error Degrees of Freedom = 9
No. of observations to calculate a mean = 4

Table - G: Least Significant Difference test (LSD Test)

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<tr>
<td>H2 2.750 A</td>
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</tr>
<tr>
<td>H3 2.500 A</td>
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</tr>
<tr>
<td>H4 1.750 B</td>
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</tr>
</tbody>
</table>

H1 = General Hospital
H2 = Jinnah Hospital
H3 = Mayo Hospital
H4 = Services Hospital
A = Salmonella enteritidis and Salmonella typhimurium
B = Stool and Blood
AB = All
α = 0.05 (P value)