

**INCIDENCE AND CHARACTERISATION OF *SALMONELLA*
ENTERITIDIS IN POULTRY PRODUCTS AND HUMAN
DIARRHOEA CASES IN LUSAKA DISTRICT, ZAMBIA.**

BY

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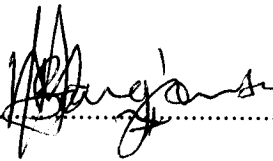
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Medicine in Veterinary Microbiology**

**School of Veterinary Medicine
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Declaration

I **Bernard Mudenda Hang'ombe** declare that the work presented in this dissertation was done by myself and has not been presented to this or any other University.

Signature:

Date:15/05/98

Dedication

I dedicate this work to my parents for their support, love and desire to see me attain higher education and to all the poultry growers and consumers of Zambia.

APPROVAL

This dissertation of **BERNARD MUDENDA HANG'OMBE** is approved as fulfilling the requirements for the award of the Degree of Master of Veterinary Medicine in Veterinary Bacteriology of the University of Zambia.



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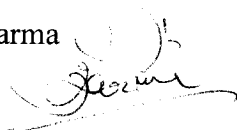
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ABSTRACT

This study was conducted to find out the incidence of *Salmonella enteritidis* in table eggs and chicken carcasses entering the consumer market in the city of Lusaka. Diarrhoea stool samples from humans admitted to the University Teaching Hospital (UTH) were also included in the study to investigate *Salmonella enteritidis* in the population.

Table eggs were collected from eight randomly selected layer farms in and around the city. Specimens from the chicken carcasses were sampled from a big processing plant operating in Lusaka city. Two thousand four hundred (2400) commercially purchased table eggs and three hundred eighty two (382) chicken carcasses were cultured on suitable media for isolation of *Salmonella enteritidis*. The shell membranes and yolks from table eggs were pooled in units of ten and cultured. Nine (3.75 per cent) of the two hundred and forty samples of table eggs were found contaminated with *Salmonella enteritidis*, while eighteen (4.7 per cent) of the carcasses sampled were found positive for *Salmonella enteritidis*. Other *Salmonella* serovars isolated from the chicken carcasses were *Salmonella infantis*, *Salmonella gallinarum* and *Salmonella mbadaka* while *Salmonella gallinarum* was the only other serovar from the table eggs. A total of eighty six (86) diarrhoea stool samples from humans obtained from UTH, Lusaka were also cultured and eight of these were found positive for *Salmonella typhimurium*.

Phage typing of the *Salmonella enteritidis* isolates showed that all the isolates from the table eggs and seven from chicken carcasses belonged to the enteropathogenic invasive PT₄, while three from the carcasses were PT₇ and eight were untypable.

Efforts were made to characterise the *Salmonella enteritidis* isolates by carrying out pathogenicity test, biochemical test and antibiogram test. The characterisation involving animal models of mice, embryonated eggs and seven days old chicks displayed that *Salmonella enteritidis* isolates from the table eggs were more virulent than the isolates from the chicken meat. Biochemical test did not reveal anything significant.

Salmonella enteritidis isolates from table eggs and chicken carcasses tested for antibiotic sensitivity had several features in common. They were sensitive to amoxycillin, ampicillin, co-trimoxazole, furazolidone, gentamycin and tetracycline while *Salmonella typhimurium* isolates from diarrhoea cases were resistant to amoxycillin and ampicillin. Several *Salmonella mbandaka* isolates showed resistance to tetracycline.

Salmonella enteritidis has been isolated from the table eggs and poultry meat carcasses for the first time in Zambia. The results of the study further demonstrated that poultry products from Zambia have a higher incidence of *Salmonella enteritidis* contamination compared to reports in other parts of the world. It warrants a planned epidemiological study of *Salmonella enteritidis* in the country to formulate appropriate control measures.

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LIST OF CONTENTS

CONTENTS	PAGE
CHAPTER ONE: INTRODUCTION	1
CHAPTER TWO: LITERATURE REVIEW	
2. 0. 0 Classification of <i>Salmonella enteritidis</i> .	4
2. 1. 0. General overview of <i>Salmonella enteritidis</i> .	5
2. 2. 0. Transmission of <i>Salmonella enteritidis</i> .	6
2. 2. 1. Factors related to transmission of <i>Salmonella enteritidis</i> .	12
2. 3. 0. Pathogenesis of <i>Salmonella enteritidis</i> in chickens.	14
2. 4. 0. Zoonosis of <i>Salmonella enteritidis</i> infection.	17
2. 4. 1. Infection in chickens.	17
2. 4. 2. <i>Salmonella enteritidis</i> in humans.	19
2. 5. 0. Justification for screening <i>Salmonella enteritidis</i> .	23
2. 5. 1. Methods of <i>Salmonella enteritidis</i> detection in chickens and eggs.	24
2. 5. 2. Diagnosis of <i>Salmonella enteritidis</i> in human cases.	27
2. 6. 0. Control and prevention of <i>Salmonella enteritidis</i> .	27
CHAPTER THREE: MATERIALS AND METHOD	
3. 0. 0. Type and source of samples collected for microbial examination.	34
3. 0. 1. Table eggs.	34
3. 0. 2. Poultry carcass swabs.	35

3. 0. 3. Human diarrhoea stool samples.	36
3. 1. 0. Microbiological examination of samples.	36
3. 1. 1. Table eggs.	36
3. 1. 2. Chicken carcass swabs.	38
3. 1. 3. Human stool samples.	38
3. 2. 0. Identification of <i>Salmonella</i> isolates.	39
3. 3. 0. <i>Salmonella enteritidis</i> characterisation.	39
3. 3. 1. Antimicrobial disc sensitivity test.	39
3. 3. 2. Minimum lethal dose determination for infectivity testing in mice.	40
3. 3. 3. Infectivity testing in mice.	41
3. 3. 4. Minimum lethal dose determination and infectivity testing of embryonated eggs.	41
3. 3. 5. Minimum lethal dose determination in seven days old chicks.	42
3. 3. 6. Infectivity testing of seven days old chicks.	43
3. 3. 7. Subjection of <i>Salmonella enteritidis</i> isolates to sugar test.	43
3. 4. 0. Statistical analysis.	44

CHAPTER FOUR: RESULTS

4. 0. 0. <i>Salmonella</i> isolation.	45
4. 1. 0. <i>Salmonella</i> isolation from chicken carcasses.	45
4. 2. 0. <i>Salmonella</i> isolation from table eggs.	46
4. 3. 0. <i>Salmonella</i> isolation from human stool.	47
4. 4. 0. Isolation of <i>Salmonella enteritidis</i> and its phage type.	47
4. 5. 0. Antibigrams of <i>Salmonella enteritidis</i> .	48

4. 6. 0. Pathogenicity of <i>Salmonella enteritidis</i> isolates in mice.	53
4. 7. 0. Pathogenicity of <i>Salmonella enteritidis</i> in embryonated eggs.	54
4. 8. 0. Virulence of <i>Salmonella enteritidis</i> isolates in seven days old chicks.	55
4. 9. 0. Sugar test results.	56

CHAPTER FIVE: DISCUSSION

5. 0. 0. <i>Salmonella</i> isolation from chicken carcasses.	57
5. 1. 0. <i>Salmonella</i> isolation from table eggs.	61
5. 2. 0. <i>Salmonella</i> isolation from human diarrhoea patients.	65
5. 3. 0. Antibiotic susceptibility pattern of <i>Salmonella</i> isolates.	66
5. 4. 0. <i>Salmonella enteritidis</i> characterisation.	68
5. 5. 0. Further investigations proposed.	73

CHAPTER SIX: CONCLUSION

REFERENCES	76
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LIST OF TABLES

Title	Page
Table 1: Frequency of <i>Salmonella</i> isolation from different samples.	45
Table 2: Percentage of different <i>Salmonella</i> serovars isolated from chicken carcasses.	46
Table 3: Percentage of different <i>Salmonella</i> serovars isolated from table eggs.	46
Table 4: Incidence of <i>Salmonella enteritidis</i> positive samples in table eggs and poultry chicken carcasses.	47
Table 5: Phage types of <i>Salmonella enteritidis</i> .	48
Table 6: Antibigrams of <i>Salmonella enteritidis</i> isolates.	50
Table 7: <i>Salmonella infantis</i> antibiogram.	51
Table 8: Selected <i>Salmonella mbadaka</i> antibiogram from chicken carcasses.	51
Table 9: <i>Salmonella typhimurium</i> isolates from human patients	52
Table 10: Pathogenicity of <i>Salmonella enteritidis</i> PT ₄ from table eggs in mice.	53
Table 11: Pathogenicity of <i>Salmonella enteritidis</i> from chicken carcasses in mice.	54
Table 12: Pathogenicity of <i>Salmonella enteritidis</i> isolates from table eggs & chicken carcasses in chicken embryos.	55
Table 13: Pathogenicity of <i>Salmonella enteritidis</i> PT ₄ from table eggs in chicks.	56

CHAPTER ONE

INTRODUCTION

Modern poultry industry, besides being a source of income to poultry farmers, provides a food of high nutritious value. To meet these demands, the poultry industry in Zambia has developed very rapidly in the last two decades. As a result, large and small poultry units have been set up in different parts of the country. Since the profitability of the poultry industry to a larger extent depends on simultaneous disease control programs, the impact of poultry diseases on the industry is quite obvious. Of the many diseases enumerated as hazards in the development of poultry industry, salmonellosis has always been named the most important all over the world. *Salmonella* infection affects the economic viability of the poultry industry by causing death and poor growth of chickens, decreased egg production, poor hatchability and increased cost of production (Jackson *et al.* 1971).

Report on avian salmonellosis report in Zambia, dates back to 1931 when four outbreaks of fowl typhoid were reported in native breed fowls (Gasper and Hrabeta 1977). So far there are 18 serovars of *Salmonella* reported from poultry in Zambia and these include; *S. bonn*, *S. dublin*, *S. enteritidis*, *S. gallinarum*, *S. heidelberg*, *S. infantis*, *S. kaufmanii*, *S. kisarawe*, *S. oranienburg*, *S. rubislaw*, *S. typhimurium*, *S. weltevreden*, *S. worthington*, *S. agona*, *S. schwarzengrund*, *S. alamo*, *S. haifa* and *S. virginia* (Sharma *et al.* 1991, 1996 and Tuchili *et al.* 1996a).

The importance of *Salmonella* infection does not only lie in causing disease in domestic livestock and birds, but also in the contamination of human food by carrier animals and birds, (Wray 1985) which result in losses like medical costs for affected persons, loss of man hours from productive patients and the health hazards to would be customers for any contaminated poultry product. Ubiquitous *Salmonella* serovars like *Salmonella typhimurium* and *Salmonella enteritidis* are mostly responsible for food borne infections (Le Minor 1984 and Old 1990). According to Humphrey *et al.* (1996), *Salmonella enterica* serotype *enteritidis* continues to be an internationally important human pathogen and predominates as human salmonellosis in countries both in the northern and southern hemispheres. *Salmonella* from poultry are responsible for most cases of salmonellosis in human beings (Jordan 1994). Outbreaks of disease caused by *Salmonella enteritidis* in humans have been associated with the consumption of intact shell eggs or foods that contain eggs (Poppe *et al.* 1991). The association of eggs as a major source of *Salmonella enteritidis* infections in humans has become an important international public health issue (Centers for Disease control 1988, 1990 and St Louis *et al.* 1988). In several investigations of human outbreaks, *Salmonella enteritidis* isolated were implicated to have come from laying flocks which had the same phage type (Telzak *et al.* 1990 and Mishu *et al.* 1991).

Salmonella enteritidis has been isolated from day old chicks and other chicken specimens submitted for necropsy in Zambia, but only at a low frequency of occurrence (Sharma *et al.* 1991). Recently Kabilika (1997) demonstrated the incidence of *Salmonella enteritidis* in dead-in-shell chicken embryos, which clearly indicated the possibility of infection in the breeding flocks in Zambia. Once the

breeding birds are infected the dissemination of *Salmonella enteritidis* infection to broilers and egg laying flocks is obvious. Despite the demonstration of *Salmonella enteritidis* in breeding birds in Zambia, there has been no documented information on the occurrence of *Salmonella enteritidis* in chicken table eggs and other associated poultry products on the Zambian market. Similarly there is little information on human salmonellosis in the Zambian population.

The main objectives in this study are therefore, as follows:

- i) To investigate the incidence of *Salmonella enteritidis* contamination in table eggs and processed chicken carcasses from the retail outlets and poultry processing plants in Lusaka, Zambia.
- ii) To attempt Isolation of *Salmonella* from human patients suffering from diarrhoea and find a correlation for poultry and human serotypes.

CHAPTER TWO

LITERATURE REVIEW

2. 0. Classification of *Salmonella enteritidis*

Salmonella is a gram-negative rod shaped, motile bacterium with exception of *Salmonella gallinarum* and *Salmonella pullorum* serovars which are non-motile. *Salmonella choleraesuis* forms part of the 2500 serovars which is separated, both phenotypically and genetically, into six subspecies (Holt *et al.* 1994). All serovars in subspecies *choleraesuis* are named. These include serovar *choleraesuis*, serovar *gallinarum*, serovar *paratyphi* A, serovar *pullorum* and serovar *typhi*. *Salmonella enterica* serovar *enteritidis* (*Salmonella enteritidis*) belong to *Salmonella choleraesuis*. It is a serotype closely related to *Salmonella pullorum* and *Salmonella gallinarum* with the antigenic formula of 1, 9, 12: g, m.

Salmonella enteritidis comprises of over 50 phage types (Humphrey *et al.* 1996), with phage type 4 (PT₄) appearing to be the most widespread. Outbreaks of *Salmonella enteritidis* food poisoning cases have been associated with PT₄, PT₈ and PT_{13a} (Rowe 1989). These phage types involved in epidemic infections usually, vary between geographic regions. PT₄ was more isolated in Europe (Ward *et al.* 1987, Fantasia and Filetici 1994), where as PT₈ and PT_{13a} are more isolated in United States of America (Rowe 1989 and Hickman *et al.* 1991). In the United Kingdom, most egg associated human *Salmonella enteritidis* outbreaks were due to PT₄ (Hopper and

Mawer 1988) whilst in the USA, PT₈ isolates were the most prevalent from both human and poultry sources (Rodrigue *et al.* 1992).

Currently PT₄ has been isolated in the USA (Kinde *et al.* 1996), South Africa (L Mare 1996., personal communication) and Zambia (Kabilika 1997) where it was not reported before. This shows the diverse of PT₄ world wide at the moment.

2. 1. General overview of *Salmonella enteritidis*.

Salmonella enteritidis is a well recognised pathogen, that causes food poisoning in man and is commonly among the 'top 10' prevalent serotypes in many countries world-wide (Old 1990). A review by the WHO *Salmonella* surveillance data indicated that the prevalence of *Salmonella enteritidis* infection has increased in North and South America, Europe and possibly Africa (Cox 1995). It has also been reported in Japan (Anon 1989) and Australia, (Cox 1995). In general, it has been reported in all continents of the world and that it is internationally spreading fast (Rodrigue *et al.* 1990).

Salmonella enteritidis causes food poisoning following consumption of contaminated food. A number of *Salmonella enteritidis* food poisoning outbreaks have been associated with consumption of poultry products (Mitchell 1989). Eggs or foods containing eggs have been implicated in significant proportions of *Salmonella enteritidis* outbreaks (Anon 1988a and St. Louis *et al.* 1988). In England and Wales poultry meat was seriously considered as a source of human salmonellosis (Humphrey *et al.* 1988). Rampling *et al.* (1989) also noted that chicken meat was commonly

contaminated with *Salmonella enteritidis*. Therefore, it is clear that *Salmonella enteritidis* presence in the poultry population, should be considered to be of significant importance to public health concern.

2. 2. Transmission of *Salmonella enteritidis*

Salmonella enteritidis infection in chicken flocks can be transmitted from various sources either directly or indirectly. It is therefore, important to understand the source of infection and the mechanisms of transmission in order to formulate the right control programs.

A. Ovarian transmission in chickens.

Salmonella enteritidis organisms may be directly transmitted through the ovaries, to the egg as a result of infection of the ovary and ovarian follicles following systemic infection in hens (Timoney *et al.* 1989). Thiagarajan *et al.* (1994) demonstrated that *Salmonella enteritidis* can colonise the pre-ovulatory follicles by interacting with the ovarian granulosa cells. This is a form of vertical transmission of the infection. Occasionally, the agent gains entrance to the egg as a result of contamination of the free ovum in the peritoneal cavity, or by contact in the oviduct (Zander 1984). Once the shell and membranes are added, the organism enjoys a protected location where it is not easily destroyed. Vertical transmission in broilers has been suggested from epidemiological observations (O'Brien 1988) and from the reported identification of *Salmonella enteritidis* in ovarian tissue from a parent flock (Lister 1988).

Alternatively, contamination of the egg may occur *in vivo* because of dissemination of the organism to the ovaries following localisation and colonisation of the large intestine by *Salmonella enteritidis*, as well as through migration to their reproductive organs (Timoney *et al.* 1989 and Shivaprasad *et al.* 1990). Ovarian infection by *Salmonella enteritidis* will result not only in the laying of contaminated eggs but also, gives rise to infected chicks even before hatching (Jordan 1994). Eventually, such infected chicks will grow to become broilers or pullets which will subsequently also lay contaminated eggs (Hopper and Mawer 1988., Lister 1988 and O'Brien 1988).

Salmonella enteritidis infection of the vagina can result into frequent incidence of contaminated eggs as the *Salmonella enteritidis* adhere to the egg shells and may eventually pass through the shell and shell membranes (Miyamoto *et al.* 1997). Reiber *et al.* (1995), Reiber and Conner (1995) reported that insemination of hens with *Salmonella enteritidis* contaminated semen resulted in genital infection and consequently, sporadic production of contaminated eggs. Miyamoto *et al.* (1997) recovered *Salmonella enteritidis* from the isthmus and uterus following intra-vaginal inoculation and from the uterus/vagina following intra-cloacal inoculation. Therefore, ascending contamination of the lower parts of the oviduct may occur under field conditions (Miyamoto *et al.* 1997)

B. Horizontal transmission

i) Faecal carrier transmission

Faecal contamination of egg shells with organisms during the process of laying is very important in the spread of the disease (William's 1984). Adult laying hens infected

with *Salmonella enteritidis* may carry the organism in their large intestines and shed it in their faeces, which may lead to contamination of the egg shell surface. Penetration of the egg shell by the organism during storage can lead to contamination of the egg contents (Coyle *et al.* 1988).

The faecal carriers may transmit the disease directly to young chicks, when they get into contact. On the other hand older birds can be the chronic intestinal carriers (Williams 1984).

ii) Hatchery and brooder transmission

The hatchery and brooder environments contaminated with *Salmonella enteritidis* can lead to an introduction of the disease in the life line of the birds. Contaminated egg shells and other debris of the hatch may also serve as a source of infection in the hatchery incubator. From the incubator, *Salmonella enteritidis* may be distributed by air currents throughout the hatchery, establishing a high level of *Salmonella enteritidis* aerosol infection. Contaminated dust may carry *Salmonella enteritidis* and be inhaled by susceptible young birds (Williams 1984).

iii) Equipment

Farm equipment and vehicles used for cleaning on the farm usually have accumulations of litter and faeces. These can play a vital role in transmission especially when they are hired or are required to clean a number of farms. Lahellec *et al.* (1985) reported that drinkers had the highest incidence of *Salmonella*

contamination in broiler flocks. Recycled egg trays can also transmit the disease (Williams 1984).

Poultry hauling equipment can also disseminate *Salmonella enteritidis* through feathers, faeces, blood, exudates and skin encrustation's left in crates or picked up at the slaughter plant (Simmons and Byrnes 1972). This can lead to contamination of poultry meat right in the processing plant.

iv) Humans

Humans are the greatest potential introducers of *Salmonella enteritidis* infection because of their mobility, duties, curiosity, ignorance, indifference and carelessness (Williams 1984). They can transmit the disease through footwear and clothing after being in contact with contaminated dust, feathers and excrement (Jordan 1994). Neighbours, contract work crews and visitors play a major role in *Salmonella enteritidis* lateral transmission (McIlroy *et al.* 1989). The zoonotic significance of *Salmonella enteritidis* make humans a major player in the maintenance and transmission of *Salmonella enteritidis* to the chicken flocks.

v) Feed

Contaminated feed can be a possible source of transmission for *Salmonella enteritidis* (Lahellec *et al.* 1985). Broiler chickens may become infected with *Salmonella enteritidis* through consumption of such contaminated feed (McIlroy *et al.* 1989 and Jordan 1994).

vi) Aerosols and Conjunctival transmission

Aerosols containing *Salmonella enteritidis* can be a possible mechanism of transmission (Holt 1995). Baskerville *et al.* (1992), showed that low numbers of *Salmonella enteritidis* administered by aerosol could result in a disseminated infection. Similar results of *Salmonella* transmission have been reported by Clemmer *et al.* (1960). Airflow patterns can affect the rapidity of horizontal transmission of *Salmonella enteritidis* with particles carrying *Salmonella enteritidis* floating with the flow of air (Masayuki *et al.* 1997).

Another possible means of *Salmonella enteritidis* transmission is through the conjunctiva. Humphrey *et al.* (1992), infected laying hens with *Salmonella enteritidis* through conjunctival challenge. He noted that the organisms drain with secretions into the lachrymal duct, reach the pharynx and are then swallowed. Some of the organisms may also invade the conjunctival blood vessels directly, thus infecting the chicken.

vii) Rodents

Rodents contaminate feed and litter with their excrement (Williams 1984). Mice are one of the major reservoirs for introduction of *Salmonella enteritidis* into poultry house environments (Gast and Beard 1993). Mice are highly susceptible to *Salmonella enteritidis* infection and may serve as amplifiers by excreting large numbers of *Salmonella enteritidis* in their faeces (Henzler and Opitz 1992., Gast and Beard 1993 and Jordan 1994). Davies and Wray (1995) demonstrated the possible role of wild mice in the epidemiology of *Salmonella enteritidis* infection on broiler breeder and layer breeder units by bacteriological examination of local mice. Wild

mice infected naturally were found to excrete *Salmonella enteritidis* intermittently, in their droppings.

Davies and Wray (1995) noted that droppings of mice can easily be sought out by birds when mixed in their feed and bedding because of their seed like size and colour. An individual dropping may contain large numbers of *Salmonella* and if ingested by a bird it could lead to *Salmonella* infection which then can spread to other birds in the batch. *Salmonella* can also be encased in a mouse dropping there by, getting protected from antimicrobial action of the crop. This protection enable *Salmonella* to survive and reach the intestine, where they colonise and invade the intestines. This direct infection with organisms passaged through a living host like mice may enhance their invasiveness in chickens through the activation of genes regulating heat shock proteins (Murray and Young 1992).

viii) Household pets

Dogs and cats, like rodents, are capable of transmitting *Salmonella enteritidis* in a poultry unit. When these pets are not confined to the household areas but roam continually among the poultry pens may be possible transmitters of *Salmonella enteritidis* (Zander 1984). Bhaiyat *et al.* (1997) isolated *Salmonella enteritidis* from dogs, suggesting their possible role as reservoirs.

ix) Wild Birds

It has become increasingly clear in recent years that wild birds cannot only be infected with *Salmonella* but can also mechanically carry material contaminated with bacteria

on their claws (Jordan 1994). The wild birds can also transmit *Salmonella* including *Salmonella enteritidis* by contaminating drinking water through uncovered water tanks on the farm.

x) Insects

Domestic flies and beetles are both capable of transmitting *Salmonella* and infection can persist through the insects from one generation to another via eggs and larvae (Jordan 1994). Ticks have been found to harbour *Salmonella enteritidis* (Steinhaus 1949). Cockroaches can also be a source of *Salmonella* contamination. Kopanic *et al.* (1994) in their study found that, cockroaches are capable of acquiring and infecting other cockroaches and objects, therefore implicating them as potential vectors of food-borne pathogens in poultry production and processing facilities.

2. 2. 1. Factors related to transmission of *Salmonella enteritidis*

i) Environmental stress

Environmental stress can enhance *Salmonella enteritidis* transmission in chickens. Environmental stress can be caused by a number of factors like: transportation, chilling weather conditions, excessive heat, poor nutrition, housing, and poor sanitation which leads to ammonia build up, exacerbating the *Salmonella* situation in the flock. Ammonia gas concentrations of 170ppm can cause keratoconjunctivitis, deciliation of trachea and tracheitis. In lower concentrations, it predisposes to more severe respiratory disease associated with a variety of respiratory pathogens (Jordan 1994). These complications can lead to a decreased cell mediated immunity, which can exacerbate the *Salmonella* situation.

Transportation of broiler birds for slaughter in closely packed crates may lead to a small number of birds sub-clinically infected, build up and eventually cause contamination of large numbers of poultry carcasses (Jordan 1994). This effect was also noted by Rigby and Petit (1980) in broilers subjected to transport stress. Following 24 hours of simulated transport (crating, truck transport, etc.) the percentage of cecal *Salmonella* carriers and shedders increased dramatically (Rigby and Petit 1980).

ii) Molting

Molting, a procedure used to achieve a second egg lay from aging flocks has been found to be a significant amplifier of *Salmonella enteritidis* (Holt 1995). Previous work has shown that feed removal to induce a molt in older hens increased the severity of an infection by *Salmonella enteritidis*. Molted hens have a shortened time of onset with more severe intestinal inflammation (Holt and Porter 1992a and Macri *et al.* 1997) and shed significantly more *Salmonella enteritidis* (Holt 1993., Holt and Porter 1992a., 1992b., 1993). Increase in susceptibility of molted hens suggest that molting may increase the horizontal transmission of *Salmonella enteritidis* to infected hens in adjacent cages. Small scale studies (Holt and Porter 1992b., 1993) have shown that infected molted hens rapidly transmitted *Salmonella enteritidis* to molted hens in adjacent cages and the number of organisms shed was higher than in unmolted hens. In the same study, Holt and Porter (1992b) indicated that induced molting can cause the recurrence of a previous *Salmonella enteritidis* infection. The recurrence of a previous *Salmonella enteritidis* infection may be as a result of an impairment of

intestinal immunity associated with lymphopenia, decreased cell mediated immunity and prolonged bacteremia (Ben-Nathan *et al.* 1981 and Holt 1992). This recurrence of infection might affect the microbiological quality of the eggs coming from these hens (Holt and Porter 1993). This could clearly suggest the importance of molting in the maintenance cycle of *Salmonella enteritidis* in the flocks.

2. 3. Pathogenesis of *Salmonella enteritidis* in chickens

Although *Salmonella enteritidis* colonizes the intestinal tract of chickens, it invades to reach internal tissues which provokes a specific antibody response, and is deposited in the contents of eggs (Gast and Beard 1993). Invasion of *Salmonella* into the cells of the intestinal epithelium is an important step in the infection process. This initial invasion is followed by colonisation of other organs throughout the body (Porter and Curtiss 1997). Phage type 4 infections in the United Kingdom have been responsible for increased morbidity and mortality in broiler chickens (Lister 1988). *Salmonella enteritidis* phage type 4 is invasive in chickens and the extra-intestinal spread has been considered important in this problem (Rowe 1989 and Hinton *et al.* 1989). Jordan (1994), reported an unusual feature of *Salmonella enteritidis* in broilers, where there was an appearance of clinical signs of disease in birds over four weeks of age. Such birds at five and six weeks of age were uneven, stunted and badly feathered.

It is important to note that, following the transmission of *Salmonella enteritidis* to the chicken, it undergoes various transformations to manage itself in the host. The ability of *Salmonella enteritidis* to invade tissues is quite important (Gast and Beard 1993). Invasiveness, which encompasses mechanisms for colonization (adherence and initial

multiplication), ability to by-pass or overcome host defense mechanisms, and the production of extracellular substances (“invasins”) which facilitate invasion are important. Adhesion is a likely prerequisite for the establishment and maintenance of *Salmonella* in the gut (Old, 1990). Pathogenic bacteria have several virulence-associated surface structures, such as lipopolysaccharide, capsules, surface layers, flagella and fimbriae (Brubaker 1985). *Salmonella enteritidis* also has these virulent structures which contribute to its invasiveness and pathogenicity. In particular, these structures for *Salmonella enteritidis* include; lipopolysaccharide, flagella, surface layers, fimbriae and plasmids (Cox 1995, Petter 1996).

Different *Salmonella enteritidis* strains have considerable variations in pathogenicity for chickens. *Salmonella enteritidis* isolates from chickens and humans have been found to colonize the ovaries and oviducts of inoculated hens more frequently than isolates from horses or cows (Gast and Beard 1993). *Salmonella enteritidis* phage type 4 is highly invasive (Rowe 1989 and Hinton *et al.* 1989). Hinton *et al.* (1990) in a study of the invasiveness of *Salmonella enteritidis* PT₄ found that all the five test strains had colonised the caecum and invaded the liver of young chicks. In another study, Barrow (1991) determined that *Salmonella enteritidis* phage type 4 invaded to reach the spleen more often than did other phage types. Barrow and Lovell (1991), also reported heavy invasion in the ovary of layers infected with *Salmonella enteritidis* PT₄.

Gast and Beard (1992a) evaluated the effects of four *Salmonella enteritidis* isolates (three from poultry and one from man) in experimentally infected chicks and laying

hens. Significant differences between strains were observed in mortality rates among chicks and in total egg production. The difference in production frequency of *Salmonella enteritidis* contaminated eggs and the serum antibody response among hens was also observed between strains.

Efforts to explain the differences between *Salmonella enteritidis* strains (and thus the basis for *Salmonella enteritidis* pathogenesis) have been focused on the following key issues;

a) O-antigen variation and lipopolysaccharide structural variation between *Salmonella enteritidis* isolates has been found to correlate with organ invasiveness especially in five day old chicks and contaminating eggs after oral inoculation of hens (Petter *et al.* 1996). Petter *et al.* (1996) in his study demonstrated that the variants that completely lacked O-antigen were no more efficient at contaminating eggs after oral inoculation of hens while the lacy phenotype was more organ invasive than isogenic variants producing low molecular weight O-antigen suggesting that the structure of O-antigen might influence the secretion and function of *enteritidis* cell surface proteins leading to variations.

b) The presence of plasmids has been the most intensively investigated possible explanation for pathogenic behavior of *Salmonella enteritidis* strains. A 38 megadalton (MDa) plasmid was previously observed to be essential for the full expression of virulence of *Salmonella enteritidis* PT₄ in mice (Chart *et al.* 1989). A plasmid of similar size was identified in most isolates (of a variety of phage types) from poultry and poultry houses in Maine (Singer *et al.* 1992).

Another important factor in *Salmonella enteritidis* pathogenesis is the possible variations in the host susceptibility caused by exposure to potentially immunocompromising treatments. Prior infection with infectious bursal disease virus increased mortality, due to persistence of *Salmonella enteritidis* organ colonization in chicks subsequently infected with *Salmonella enteritidis* (Opitz *et al.* 1990). In another study, induced molting was associated with increased bowel inflammation and increased numbers of *Salmonella enteritidis* shed in alimentary secretions after oral inoculation of hens (Holt and Porter 1991). This is as a result of an impairment of intestinal immunity.

2. 4. Zoonosis of *Salmonella enteritidis* infection

2. 4. 1. Infection in chickens

Salmonella enteritidis is particularly pathogenic to chickens. It can affect layers, broilers and broiler breeder flocks. The organism has been isolated from broilers, breeders and commercial egg laying flocks (Lister 1988., Hopper and Mawer 1988). It has also been isolated from eggs, yolk sac of baby chicks, ovaries, carcasses from abattoirs and sick birds (El-Amin *et al.* 1984., Laszlo *et al.* 1985., O'Brien 1988, Paul and Batchelor 1988, Rampling *et al.* 1989, Shivaprasad *et al.* 1990., Sharma *et al.* 1991., Barrow and Lovell 1991).

In chickens it can cause a clinical disease leading to heavy mortalities of birds (Lister 1988 and Jordan 1994). In the United Kingdom, it has been shown that PT₄ caused increased mortality in broiler chicks of less than one week of age and stunted growth

in up to five per cent of the flock (Lister 1988 and O'Brien 1988). Infected broilers of two weeks old were found with lesions of toxic indurated yolk sac, pericarditis and necrotic foci in the liver (O'Brien 1988). *Salmonella enteritidis* PT₄ was isolated in pure culture from pericardial sacs of the 22 out of 30 (73%) broiler sampled at the time of slaughter (O'Brien 1988). At broiler processing plants in the UK, an average of one in 1000 birds were condemned because of gross pericarditis due to *Salmonella enteritidis* (Rampling *et al.* 1989). In this study Rampling *et al.* (1989) was able to isolate *Salmonella enteritidis* from other tissues such as heart, spleen and caecum of broilers and swabs of the carcass cavity.

Salmonella enteritidis infection can be established in older birds without any apparent clinical signs of reduced production (McIlroy *et al.* 1989, Gast and Beard 1990a) or can become firmly established in breeder and commercial egg laying flocks at any stage of production and remain undetected unless effective monitoring procedures are used (McIlroy *et al.* 1989).

In experimentally infected laying hens, isolates of several phage types have been observed to invade internal organs, and a few isolates have caused reduced egg production, depression, anorexia, diarrhoea, and infrequent mortality (Gast and Beard 1990a, 1990b., Shivaprasad *et al.* 1990 and Humphrey *et al.* 1992). Shivaprasad *et al.* (1990) have further reported that lesions in adult birds were minimal or absent 4 and 18 days post-inoculation of *Salmonella enteritidis* organisms.

Salmonella enteritidis has been isolated from the contents of clean, intact eggs produced by both naturally and experimentally infected laying hens (Humphrey *et al.* 1989a, 1989b; Timoney *et al.* 1989, Gast and Beard 1990a). Both naturally and experimentally infected hens have been observed to lay eggs with *Salmonella enteritidis* in the yolk or albumen (Gast and Beard 1993).

Naturally infected hens have generally been observed to produce contaminated eggs at very low frequencies (Mawer *et al.* 1989) or intermittently (Humphrey *et al.* 1989b). Humphrey *et al.* (1991b) isolated *Salmonella enteritidis* from contents of less than one per cent eggs from a total of 5,700 obtained from 15 infected British commercial laying flocks. The contaminated eggs, generally contained very small numbers of *Salmonella enteritidis* cells (Humphrey *et al.* 1991b). Evidence deduced from experimentally infected hens has also shown that contaminated eggs do not often harbour large numbers of *Salmonella enteritidis* (Gast and Beard 1992b). Eggs can be contaminated by layers previously infected with as low as 10^3 *Salmonella enteritidis* cells (Humphrey *et al.* 1991a).

2. 4. 2. *Salmonella enteritidis* in Humans

Salmonellosis is primarily a disease affecting very young, the old and the debilitated persons (Hadfield *et al.* 1985). Mature adults may succumb to infections when resistance is lowered by stress, viral disease, inadequate diet, or grossly unsanitary surroundings (George 1978). People who are generally at a higher risk are those with weakened immune systems like Acquired Immune Deficiency Syndrome (AIDS). AIDS patients suffer from salmonellosis more easily and frequently (estimated 20-

fold) than the general population, such patients suffer from recurrent episodes of attacks (Anon 1990). A very small number of *Salmonella* is enough to cause severe illness in AIDS patients.

Scragg *et al.* (1978) reported an increased incidence of *Salmonella enteritidis* infections in African and East Indian children in South Africa. However, the disease syndrome was not severe. In Liberia, Monson *et al.* (1984) reported *Salmonella enterica* serotype *enteritidis* replacing *Salmonella typhi* as the most common organism isolated from patients in a pediatric ward. In Zambia no reports of *Salmonella enteritidis* in humans has been documented although a higher percentage of acute bacterial diarrhoea cases are due to *Salmonella* (Dube 1984). In his study, Dube (1984) reported that forty per cent of diarrhoea cases observed were caused by *Salmonella*, but no serotyping was done on the isolates. *Salmonella* was also isolated from food handlers in Lusaka (Dube 1983). The *Salmonella* isolated from the University Teaching Hospital Bacteriology diagnostic laboratory in Lusaka, Zambia, recently that have been serotyped in 1997 included the following: *Salmonella enteritis*, *Salmonella infantis*, *Salmonella heidelberg*, *Salmonella munche*n and *Salmonella typhimurium* (Mwansa and Degroote 1997., personal communication).

Salmonella enteritidis remains an important cause of outbreaks and sporadic cases of food poisoning gastroenteritis in man (Carter and Chengappa 1991). A number of *Salmonella enteritidis* outbreaks in humans have been documented in a number of countries of the world. The implication of eggs as a major source of *Salmonella enteritidis* infections in humans has become an important international public health

issue (Centers for Disease control 1988, 1990 and St. Louis *et al.* 1988). Grade A whole shell eggs are a major source of *Salmonella enteritidis* infections in humans in the United States of America and the United Kingdom (Coyle *et al.* 1988).

The multiplication of *Salmonella enteritidis* in eggs held at warm temperatures has been identified as an important factor in many *Salmonella enteritidis* outbreaks (Gast and Beard 1993). It has also been found that *Salmonella enteritidis* multiplies rapidly in eggs at temperatures as low as 10 to 15°C (Humphrey 1990). *Salmonella* organisms can survive normal light cooking as noted by Scott (1930). The exhibition of wide range of heat tolerance by *Salmonella enteritidis* organisms seriously implicates food poisoning due to eggs, particularly in institutional settings. Lin *et al* (1988) traced a *Salmonella enteritidis* outbreak back to a restaurant breakfast bar. Incompletely cooked scrambled eggs were allowed to continue cooking slowly at the bar's maintenance temperature. A larger nosocomial outbreak of *Salmonella enteritidis* infection due to consumption of raw eggs and the improper handling of eggs in a kitchen was reported in the USA (Telzak *et al.* 1990). This involved mayonnaise made from raw eggs, which was added to a tuna-macaroni salad and left unrefrigerated for five hours before being served and consumed. In this case, nine patients with confirmed salmonellosis died, one of them was an AIDS patient while the other two had diabetes mellitus. *Salmonella enteritidis* PT₈ was isolated from the patients. This evidence epidemiologically implicated raw eggs and the ovary of a hen from a farm that supplied the eggs. This clearly shows an association between consumption of contaminated eggs and *Salmonella enteritidis* infections in humans.

Contaminated chicken meat, can also be a source of *Salmonella enteritidis* infection in humans. *Salmonella* serovars isolated from broiler carcasses are also frequently found in human isolates (Poppe *et al.* 1991). Isolation of *Salmonella* organisms in a processing plant is a good indicator of possible chicken carcasses contamination while being dressed in the processing plant and thus providing a major source of infection to humans (Simmons and Byrnes 1972., Poppe *et al.* 1991). Simmons and Byrnes (1972) isolated *Salmonella* from rinse samples of chicken carcasses.

A person infected with *Salmonella enteritidis* usually has fever, nausea, malaise abdominal cramps and diarrhoea beginning 12 to 72 hours after consuming contaminated food or beverage. The illness usually lasts four to seven days, and most people may even recover without antibiotic treatment (Anon 1995). However, certain diarrhea can become very severe, to require hospitalisation and treatment (Anon 1995). The elderly, infants and those with impaired immune systems tend to have a more severe illness which may lead to death if not promptly treated. In such patients, the infection becomes systemic by spreading from the intestines to the bloodstream and other body sites (Anon 1995).

Salmonella enteritidis once it invades and become systemic can cause a number of complications such as; septiceamia and endocarditis (Gill 1979), empyema (Burney *et al.* 1977), meningitis (Chusid *et al.* 1980), bone and joint infections (Ortiz-Neu *et al.* 1978) and subcutaneous abscesses (Gremillon *et al.* 1977). Although any serotype of *Salmonella* may cause these complications, the clinical presentation of enteric fever, spinal meningitis , or subcutaneous abscesses (or all three symptoms) is unusual

for *Salmonella enteritidis* but may cause such complications which may require lengthy hospitalisation even in healthy people (Anon 1990).

Hadfield *et al.* (1985), reported an outbreak of antibiotic resistant *Salmonella enteritidis* in Liberia, West Africa. In this case, *Salmonella enteritidis* was resistant to streptomycin, tetracycline, chloramphenicol, carbenicillin, penicillin, ampicillin and sulfadiazine. The illness was presenting itself as an enteric fever but also as meningitis, gastroenteritis, empyema, subcutaneous abscesses, chronic otitis media, or a combination of these conditions. The predisposing factors were young age and debilitation from malnutrition, marasmus, kwashiorkor or postmeasles complications. The mortality of infected patients was 27.8 per cent.

2. 5. Justification for screening *Salmonella enteritidis*

The food industry requires rapid and sensitive methods for the detection of *Salmonella enteritidis* in a variety of products and environmental samples.

Effective control of *Salmonella enteritidis* depends largely on the accurate detection of infected breeders and layer hens (O'Brien 1990). Therefore, detection of *Salmonella enteritidis* infected laying flocks has become a vital part of efforts to reduce the frequency of egg borne transmission of *Salmonella enteritidis* to human consumers (Gast and Beard 1993). This can also be applied to primary breeding stock (pedigree and grandparent flocks). In developed countries like the USA *Salmonella enteritidis* task force has been established to evaluate the infection status of flocks

implicated by epidemiological investigations as the source of eggs responsible for outbreaks among human (Gast and Beard 1993).

2. 5. 1. Methods of *Salmonella enteritidis* detection in chickens and eggs

A number of methods and techniques have been developed specifically for detection of *Salmonella enteritidis* in chickens and associated foods. These methods include;

i) Serological testing

This is an antibody detection test. Rapid serological tests such as slide agglutination using *Salmonella pullorum* or *Salmonella enteritidis* antigen have been employed because these pathogens share major antigens (Mallinson and Snoeyenbos 1989). Detection of antibodies in egg yolks could be achieved as early as nine days after infection of hens using either *Salmonella enteritidis* or *Salmonella pullorum* antigens in a microantiglobulin test (Gast and Beard 1991).

Many tests based on Enzyme linked immunosorbent assay (ELISA) technology have been developed, using a range of antigens for the detection of homologous antibody. The antigens used include gm-flagellin (Timoney *et al.* 1990), lipopolysaccharide (Chart *et al.* 1990 and Dadrast *et al.* 1990), heat extracted antigen (Nicholas and Cullen 1991) and outer membrane proteins (Dadrast *et al.* 1990 and Kim *et al.* 1991). Heat extracted protein antigen detected more positive samples than lipopolysaccharide antigen using sera or egg yolk extract (Nicholas *et al.* 1990). Generally, the sensitivity of ELISA has proved superior to other more conventional tests for detection of antibodies to *Salmonella enteritidis* (Cox 1995). Gast *et al.* (1997) has shown that egg yolk antibody testing can infact be a rapid and effective screening method for

identifying *Salmonella enteritidis* infected laying flocks. Cox (1995), suggested that the agglutination test is more definite and that the agglutination test is the test of choice because of cost and labour intensity.

ii) Bacteriological tests

Bacteriological tests complements serological tests. Isolation and culturing of *Salmonella enteritidis* organisms from poultry house and abattoirs by the drag swab technique is very sensitive indicator that a house is infected with *Salmonella enteritidis* (Opara *et al.* 1992). All suspected environmental samples like chick box liners, fluff samples and rodent droppings must be cultured in order to isolate *Salmonella enteritidis*. Confirmation of infection status in poultry houses and abattoirs can be done by isolating *Salmonella enteritidis* from internal organ samples.

Another bacteriological testing option involves culturing of *Salmonella enteritidis* from egg samples. *Salmonella* organisms are mainly grown on selective media following enrichment. This approach has the advantage of being a direct measure of the criterion of ultimate epidemiological significance, whereas, tests which measures the flock infection parameters only, indirectly assess the potential of a flock to produce *Salmonella enteritidis* contaminated eggs (Gast and Beard 1993). The most efficient way of isolating small numbers of *Salmonella enteritidis* cells is by pooling 10 to 30 eggs. The homogenised pooled egg contents is incubated before culturing to allow multiplication of *Salmonella enteritidis* at detectable levels (Gast 1993a, 1993b). Preliminary screening to select eggs with blood spots for culturing has been

noted to improve the chances of isolating *Salmonella enteritidis* from eggs (Gast and Beard 1993).

Culturing of *Salmonella enteritidis* from organs of dead chickens is also an essential tool in *Salmonella enteritidis* detection. Selected organs like yolk sac from chicks, liver, spleen, intestine and gonads from mature birds are used for isolation. The gonads should include interstitial ovarian tissue and a section of the oviduct.

Detection of *Salmonella enteritidis* can also be done at the abattoir and at the hatchery by collecting dead in-shell embryos for culture. Samples from the abattoir can include, runoff water, neck skins and swabs from the visceral and thoracic cavities.

iii) Other *Salmonella* detection techniques

A variety of rapid detection systems are commercially available. They include; immunodiffusion, immunofluorescence, bioluminescence and other novel applications of modern biotechnology (Mallinson and Snoeyenbos 1989), like the Polymerase Chain Reaction (PCR) technique.

The PCR is a method of gene amplification that can be used for highly sensitive and specific detection of microorganisms in a wide range of samples (Olive 1989 and Cohen *et al.* 1994). Cohen *et al.* (1994) have reported that *Salmonella enteritidis* can be identified in faeces from hens using the PCR and oligonucleotide primers specific for all members of the genus *Salmonella*. Microbiological culture was less sensitive

than the PCR assay, in the detection of *Salmonella enteritidis*, it is therefore a more useful diagnostic tool despite the cost of running the whole procedure. Woodward and Kirwan (1996) also demonstrated the detection of *Salmonella enteritidis* cells in egg contents using the PCR technique. This technique has also been used in Zambia to detect *Salmonella* DNA in chicken embryos and environmental samples (Tuchili *et al.* 1996b).

2. 5. 2. Diagnosis of *Salmonella enteritidis* in human cases

In humans, *Salmonella enteritidis* diagnosis is mainly done by isolating *Salmonella enteritidis* bacteria from stool. Serological identification of *Salmonella enteritidis* cultured isolate from stool is the most basic method used. In some cases, blood, cerebrospinal fluid, and pus from abscesses can also be used for *Salmonella enteritidis* isolation (Hadfield *et al.* 1985).

2. 6. Control and Prevention of *Salmonella enteritidis*

Control of *Salmonella enteritidis* lies largely in preventing the introduction of *Salmonella enteritidis* in any poultry set up and avoiding extraneous contaminants of all poultry products. Such products include eggs and chicken meat. A number of control and preventive measures have been applied synonymously in any set up that produces chicken meat and eggs. Although permanent eradication of *Salmonella* from poultry flocks is difficult, realistic control measures if put in place could assist in reducing the infection levels. Some of these measures include:

i) Farm management

At a farm level, control of *Salmonella enteritidis* is by strict adherence to well established biosecurity measures. In the past, control measures which were used to combat emerging pandemics was by slaughtering of the entire flock affected. This approach proved ineffective because *Salmonella enteritidis*, like other *Salmonella* persists for a very long time in the environment (Cox 1995). Currently different control measures are being used by poultry farmers, depending on their resources.

a) Antibiotic treatment

Prophylactic and therapeutic use of certain antibiotics is very effective in preventing losses from salmonellosis. Provision of furazolidone in the feed for 10 days at a level of 0.04 per cent is one of the most commonly used treatment regimens. Furazolidone has also been reported to confer prophylactic protection against *Salmonella enteritidis* when incorporated in the feed at a lower level of 0.01 per cent (Jordan 1994). Antibiotic treatment has two major problems of leaving a high proportion of carrier birds among the survivors and the development of drug resistant strains of *Salmonella enteritidis*.

b) Clean feed

Provision of uncontaminated animal proteins in the ration can prevent the introduction of *Salmonella* in the flock. It has been reported that pelleted feeds are more likely to be free of *Salmonella* because of the high temperature used during pellet feed manufacturing (Whiteman and Bickford 1989., Jordan 1994).

Treatment with short-chain fatty acids has been reported to reduce or eliminate *Salmonella* contamination of feed (Kamata 1992 and Cox 1995). Treating feed with chemicals such as formic or propionic acid or fumigating with formaldehyde or methyl bromide can significantly reduce *Salmonella* in the feed (Jordan 1994). Treatment of feed given to laying hens with 0.5 per cent formic acid reduced the isolation rate of *Salmonella*, this was subsequently associated with a reduction in the incidence of infection in newly hatched chicks (Humphrey and Lanning 1988).

c) Cleaning and disinfection

One of the most critical control factors in the poultry industry is decontamination of an infected breeding site (Davies and Wray 1996). Thorough cleaning and disinfection of premises is therefore essential. The houses and all equipment must be thoroughly cleaned and disinfected, with a one in ten solution of iodophor and fumigated with formaldehyde (McIlroy *et al.* 1989). It should be borne in mind that the effectiveness of cleaning and disinfection procedures has been observed to vary with the composition of the surfaces being treated. In one study, chemical and hot water disinfection effectively removed *Salmonella* from galvanised steel, but not from wood (EL-Assaad *et al.* 1990). Fundamental errors such as over dilution and inconsistent application of disinfectants has been observed by Davies and Wray (1996).

In some cases it is important to have the entire water system drained and the tanks, lines and drinkers thoroughly cleansed and disinfected with an iodophor solution. Wooden nest boxes which are too difficult to clean must be incinerated. Apparent clean up failures can result, in some instances where pond or stream water is used.

Thorough cleaning and disinfection should be applied and verified by negative culture results. Hatcheries, incubators, buildings, vehicles, consumables, equipment and protective clothing must all be cleaned and disinfected accordingly.

d) Control of rodents, pets and wild birds

A permanent rodent control system is very important. A system to be implemented must achieve a 95 per cent reduction in mice (Gast and Beard 1993). Rodent control is particularly important because mice play a major role in the environmental persistence of *Salmonella enteritidis*. It is difficult to prevent the access of mice to the poultry house completely. As a result the objective should be to monitor and control the rodent population continuously to avoid the build up of the large population which is capable of maintaining a significant residual *Salmonella enteritidis* infection (Henzler and Opitz 1992). Bait and trapping points should be strategically located around the perimeter of the poultry unit and around the houses. All spilled feed, stored equipment and vegetation around the houses should be removed to reduce mice habitat. To avoid mice or rats coming from other nearby premises or structures, a joint rodent control policy should amicably negotiated. Adherence to effective rodent control, combined with efficient cleaning and disinfection regimens, may be all that is needed to bring *Salmonella enteritidis* problem under control.

ii) Immunisation

Immunisation control is a promising means of reducing or eliminating infection with *Salmonella enteritidis* (Cox 1995). A range of live, killed and component immunogens have been tested, including *Salmonella gallinarum*, 9R vaccine (Barrow

et al. 1990., 1991), rough and *aroA* mutants of *Salmonella enteritidis* (Barrow *et al.* 1991) and an acetone killed oil emulsion of *Salmonella enteritidis* (Gast *et al.* 1992). Cooper *et al.* (1993) did further studies on the application of live *Salmonella enteritidis* aromatic auxotrophic mutant vaccine in chickens. This vaccination conferred worthwhile protection in intensively reared broiler chickens and egg laying hens in a field trial.

Several vaccines have since been developed and put into use. In Germany, Zoosaloral H vaccine has been developed for commercial purposes (Anon 1993). Zoosaloral H protects hens from *Salmonella typhimurium* infections and produces a stable heterologous immunity to *Salmonella enteritidis* infections in laying hens, because of their cross immunity. Excretion of pathogens is reduced while horizontal and vertical infectious chains are interrupted after three oral doses of the vaccine. Humoral and cellular defense mechanisms are stimulated by application of the live vaccine. Immunity has been proved under field trials in laying hens to have lasted for about 62 weeks. The vaccine has no known side effects.

iii) Competitive exclusion

The use of competitive exclusion (CE) to reduce the rate of colonisation by *Salmonella* in chickens was first described in 1973 (Rantala and Nurmi 1973). This competitive exclusion method of control (Probiotics) aims at establishing an adult type flora in the intestines of chicks (Jordan 1994). This technique has been widely investigated and applied commercially in broiler chickens, with significant field success when employed as part of an integrated control program (Seuna *et al.* 1978

and Cox *et al.* 1992). This approach (CE) has been demonstrated in laboratories in various countries to be an effective method of reducing *Salmonella* colonisation in the intestinal tract of chickens (Snoeyenbos *et al.* 1978., Blanchfield *et al.* 1982., Bailey *et al.* 1988 and Corrier *et al.* 1991).

Anaerobic bacterial caecal culture administered to day old chicks has shown to reduce caecal colonisation from 82 per cent to 8 per cent and liver colonisation from 52 per cent to 13 per cent after exposure to *Salmonella enteritidis* (Cox 1995). Persistence of *Salmonella enteritidis* contamination and other *Salmonella* in the environment and the consistent contamination of feed is the major limitation to the successful exploitation of this technique in the field (Cox 1995). The presence and persistence of *Salmonella* in commercial hatcheries (Cox *et al.* 1990, 1991) limits the effectiveness of CE applied after chick placement in growout houses (Goren *et al.* 1988), because colonization of the chicks can occur before CE treatment. The other problem is the obvious risk of infecting chicks with other pathogens (Jordan 1994).

iv) Consumer education

No method of control at the production level has yet proven to be effective. Consumer education is therefore the best means of minimising the public health risk (Cox 1995). The general public must realise that eggs are a raw food and that it is a risk to consume raw eggs, such publicity would significantly reduce the incidence of *Salmonella enteritidis* infection. Egg-containing dishes prepared for any of the high risk persons in hospitals, nursing homes, restaurants or at home should be thoroughly

cooked and served promptly. Eggs stored for more than three weeks should be well cooked, especially before being eaten by babies, the sick or the elderly (Anon 1995).

Frozen poultry should always be completely thawed before cooking and then should be well cooked. Public awareness campaigns on the hazards of *Salmonella enteritidis* infection appears to be the best approach to the problem.

CHAPTER THREE

MATERIALS AND METHODS

3. 0. 0. Type and Source of Samples collected for microbial examination:

Three types of samples were used:

3. 0. 1. Table eggs

Wholesome eggs meant for human consumption from different retail outlets of eight farms were included in the study. The farms producing table eggs were purposively selected from 15 such farms around Lusaka. Eggs from these farms are packed in paper trays soon after collection from layer houses. No grading or processing of any kind is done to these eggs. Packed egg trays are then sent to retail outlets for sale to consumers. There are no refrigeration storing facilities for eggs at retail outlets therefore eggs are kept at room temperature until they are bought.

Eggs were collected on a weekly basis from the first three sample farms until a maximum of 300 eggs were examined. Ninety eggs were collected at each collection visit. This was to take into account any intermittent excretion of any *Salmonella* if at all the bacteria was in the flock.

A total of 2400 eggs from the eight farms were collected. These eggs formed a sample size of 240 as they were pooled in sample units of ten.

3. 0. 2. Poultry carcass swabs

Poultry carcass cotton swabs were collected from the processing plant located two kilometers from Lusaka city center. The processing plant chosen in this study is the largest privately owned chicken processing plant in the country. The plant is capable of processing more than 1000 chickens per hour. Different broiler farms around Lusaka usually hire the plant to process chickens for them. At the time of this study, the plant was working at one third of its normal operating capacity. Ten thousand birds were slaughtered every day. The plant exports chickens to neighboring countries like Mozambique and the Democratic Republic of Congo.

The processing plant is composed of different sections like the off-loading bay, killing and bleeding section, scalding, picking, evisceration and washing section, and finally chilling and packaging section.

The final washing after evisceration is done in chlorinated water, using calcium hypochlorite (Olin Corporation, South Africa). Initially 200grams of Calcium hypochlorite is added to 500 litres of water, and then 50grams was added roughly after every three hours. The period of which the carcasses stay in chlorinated water was not monitored. The swabs were collected from carcasses after chilling at the packing point destined to enter the market. All chicken carcasses were swabbed in the visceral cavity and cloacal area surfaces.

Chicken carcasses were randomly selected from the packaging section, by collecting a bird every ten minutes for a period of six hours on each sampling day. A maximum of

36 sample swabs were collected each day. Sampling was done once every week. A total of 382 sample swabs were obtained from the carcasses for the entire study period.

3. 0. 3. Human diarrhoea stool samples

Faecal samples were obtained from the University Teaching Hospital (UTH) from patients with diarrhoea. The UTH is the largest general hospital in the country. It is used as a referral hospital as well as a teaching hospital for medical students. It is the only general hospital in Lusaka.

The faecal samples were collected at random from the patients with diarrhoea for microbiological investigations at the University Teaching Hospital diagnostic laboratory. Eighty six (86) diarrhoea patients were selected in this study for a period of ten weeks. The patients were involved in the study on the basis of having diarrhoea and fever.

3. 1. 0. Microbiological examination of Samples

Microbiological examination, in this study, was largely confined to culture and detection of *Salmonella species*.

3. 1. 1. Table eggs

a) Shell decontamination

Shell decontamination was done in the laboratory by dipping the eggs in five per cent tincture of Iodine to ensure that organisms present on the outer surface of the shell did

not contaminate the egg contents. The eggs were dipped for five to ten minutes after which they were dried in the safety cabinet class II.

b) Inoculum preparation

Cracked eggs were not sampled to avoid extraneous contamination. Following cleaning, the eggs were opened by puncturing the shell using sterile forceps. The yolk was collected into a sterile beaker by allowing the albumin to drain out without breaking the vitelline membrane. The vitelline membrane was cut using a sterile scissors. The yolks of ten eggs were pooled together and then homogenised by beating using a sterile kebab stick. The shell membranes were peeled off the shells aseptically using a sterile fine forceps. The shell membranes were placed in the sterile petri dishes, where they were pooled together to form one sample per ten eggs.

During the examination or breaking of the eggs, separate pairs of clean disposable gloves for each unit were worn, to avoid cross contamination of samples.

c) Culture and isolation of *Salmonella*

Two separate samples were prepared and cultured from table eggs.

i) Egg yolk:

The pooled yolks were incubated at 38°C for 24 hours. After incubation, 1ml of the yolk inoculum was cultured in 10 ml of selenite broth at 37.5°C for 24 hours to 48 hours and then subcultured on Xylose Lysine Deoxycholate agar (XLD; Oxoid) for

incubation at 37.5°C for 24 hours. Plates which did not show any growth, were incubated for a further 24 hours.

ii) Shell membranes:

The pooled shell membranes of ten eggs, were placed in 10ml of selenite broth and then incubated at 37.5°C for 24 to 48 hours. The broth was then subcultured on XLD (Biotec, UK) for 24 hours at 37.5°C.

3. 1. 2. Chicken carcass swabs

a) swab and inoculum preparation

The sterile swabs were placed directly into the selenite broth after swabbing the carcasses. These were returned to the laboratory in cool boxes for culturing. Swabbing was done aseptically, by breaking the end part of the swab stick into the media and throwing away the part that was held for swabbing.

b) Culture and Isolation of *Salmonella*

The selenite broth with the swabs was incubated at 37.5°C for 48 hours. XLD agar was used for subculturing of the broth. The broth was incubated at 37.5°C for 24 hours.

3. 1. 3. Human stool samples

The faecal samples were routinely collected from patients with diarrhoea. From the faecal sample, one gram was enriched in 9ml selenite broth by incubation at 37.5°C

for 48 hours. The selenite broth was subcultured on XLD agar and incubated at 37.5°C for 24 hours.

3. 2. 0. Identification of *Salmonella* Isolates

All *Salmonella*-like colonies from the media petri dishes were confirmed by biochemical and serological testing according to standard procedures (Ewing 1986). The biochemical testing involved inoculation of three *Salmonella* suspect colonies into triple sugar iron slants (TSI; Biotec) and were confirmed by biochemical tests on the basis of hydrogen sulphide production, methyl red positive, voges-proskauer negative, indole negative, citrate positive, urease negative, lysine positive, motility and gas production. Isolates showing typical reactions were screened using *Salmonella* polyvalent 'O' antisera (Denka Seiken co., Tokyo, Japan). Further confirmation and serotyping of all *Salmonella* cultures and phage typing of *Salmonella enteritidis* was done at Onderstepoort Veterinary Institute, Onderstepoort, South Africa and at the National Veterinary Laboratory, Iowa (USA).

3. 3. 0. *Salmonella enteritidis* characterisation

Following *Salmonella enteritidis* phage types identification, the following studies were used to characterise the *Salmonella enteritidis* isolates.

3. 3. 1. Antimicrobial disc sensitivity test.

Antibiograms of all *Salmonella enteritidis* isolates were determined by the Kirby-Bauer procedure. The isolates were first cultured on trypticase® soy agar (TSA; Becton Dickinson) for 18 hours at 37°C. A loopful of bacterium for each *Salmonella*

enteritidis isolate was suspended in 1ml trypticase soy broth (Oxoid) and incubated for six hours, after which 100µl of broth was placed on the sensitivity test agar (Biotec). The broth was spread thoroughly and evenly using a glass spreader.

The antimicrobial discs were placed on the sensitivity test agar plates with a fine alcohol flamed forceps. The discs were fully secured to the agar by applying firm gentle pressure. The sensitivity test agar plates were incubated at 37°C overnight and were measured for the diameters of bacterial growth inhibition using a ruler on the undersurface of the petri dish from the edge of the disc to the edge of the zone in millimeters. The diameters of each isolate and antimicrobial agent used were recorded. Strains of *Salmonella infantis*, *Salmonella mbadaka* and *Salmonella typhimurium* isolated in the study were also included in this test.

3. 3. 2. Minimum lethal dose determination for infectivity testing in mice

The minimum lethal dose (MLD₅₀) was first determined using one of the *Salmonella enteritidis* PT₄ isolate from the table eggs i.e. *Salmonella enteritidis* PT₄ reference number 19. This was determined as detailed by Reed and Muench (1938). A 15 hours old trypticase soy broth culture was used as inoculum. Three groups of four(4) mice, each weighing 20g were inoculated intraperitoneally with 100µl (5.6×10^7 c.f.u/ml), 200µl (1×10^8 c.f.u/ml) and 300µl (2×10^8 c.f.u/ml) of neat inoculum respectively. MLD₅₀ was found to be 100µl (5.6×10^7 c.f.u/ml). This was then used for all isolates in the experiment. The groups of mice were caged individually and fed on an autoclaved feed. Food and water were supplied *ad libitum*

3. 3. 3. Infectivity testing in mice

A total of 26 isolates of *Salmonella enteritidis* were used under the infectivity test. Five mice were assigned to each isolate and were inoculated with 100µl of the neat culture intraperitoneally. Five other mice were inoculated with only normal saline to serve as controls.

All mice were observed daily for signs of illness such as ruffled fur, hunched backs, inactivity and lethargy. Virulence of the *Salmonella enteritidis* isolate was based on the mortality of the mice. Bacteriological cultures of liver, spleen and blood were taken from dead mice using aseptic techniques and cultured for *Salmonella* using standard pre-enrichment, enrichment and plating techniques. Mice remaining alive seven days after infection were euthanised with chloroform and incinerated.

3. 3. 4. Minimum lethal dose determination and infectivity testing of embryonated eggs

Fertile specific pathogen free eggs were obtained from Central Veterinary Research Institute (Balmoral). The eggs were incubated in a Showa incubator (Showa incubator Laboratories; Japan) for seven days after which they were used for infectivity test.

A six hours old trypticase soy broth culture of *Salmonella enteritidis* PT₄ reference number 19 was used. One millilitre of the trypticase soy broth culture was diluted ten fold with phosphate buffered saline (PBS). Two dilution's of 10⁻¹ and 10⁻² dilutions were used.

The MLD₅₀ for embryonated eggs, was determined as detailed by Reed and Muench (1938). Three groups of embryos comprising four eggs each were inoculated with 10⁻¹ and 10⁻² dilution's respectively while the other group was inoculated with PBS. 100µl of inoculum was inoculated into the yolk sac (yolk sac route) per embryo.

The 10⁻² dilution exhibited the MLD₅₀ and was used for the rest of the experiment. Mortality of the embryos were monitored up to 48 hours after inoculation. All dead embryos were cultured for *Salmonella* to determine the specificity of the test.

3. 3. 5. Minimum lethal dose determination in seven days old chicks

Specific pathogen free one day old chicks were obtained from the Central Veterinary Research Institute (Balmoral). The chicks were housed in iron cages at an optimal temperature till they were seven days old.

The minimum lethal dose (MLD₅₀) was determined using *Salmonella enteritidis* PT₄ reference number 19 isolate from the table eggs as indicated by Reed and Muench (1938). A four hours trypticase soy broth culture was used as an inoculum. Three groups of four chicks each were used. Two groups were inoculated intraperitoneally with 200µl and 100µl of neat inoculum respectively while the other group was used as a control. The control chicks were inoculated with 200µl normal saline. Each group of chicks was placed in separate compartments after inoculation. The autoclaved feed and water were provided *ad libitum*. A 50 per cent mortality was observed in the group inoculated with 200µl inoculum.

3.3.6. Infectivity testing of seven days old chicks

The dose of 200µl was used for all *Salmonella enteritidis* PT₄ isolates to test infectivity. Five chicks were used for all *Salmonella enteritidis* PT₄ isolates, isolated in this study. The chicks used for the all the *Salmonella enteritidis* PT₄ were supplied by the local hatcheries.

Following inoculation, the birds were observed twice a day. Bacteriological cultures of liver, spleen, heart, caeca and blood where available, were taken from dead chicks and examined as described in mice experiment. All birds remaining viable two weeks after infection were euthanised using carbon dioxide asphyxia and then incinerated.

3.3.7. Subjection of *Salmonella enteritidis* isolates to sugar tests

The *Salmonella enteritidis* PT₄ isolates from the chicken carcasses and table eggs were subjected to sugar tests to observe whether or not a growth of the bacterium would ferment particular sugars with the production of acid, determined by the colour change (from purple to yellow).

Liquid media of phenol red (Difco) containing the following sugars was used; Glucose, Lactose, Sucrose, Mannitol, Mannose, Sorbitol, Salicin, Galactose, Arabinose, Raffinose, Trehalose and Salicin. Esculin hydrolysis test was also done.

3. 4. 0. Statistical analysis

Calculations of the mean and standard error (S.E) estimations for the mean per cent mortality and other analysis was done as described by Bland (1989). The incidence per cent of *Salmonella enteritidis* was calculated as follows:

$$I(\%) = r/n$$

Where I(%) = Incidence per cent.

r = Positive samples for *Salmonella enteritidis* .

n = Total number of samples examined.

CHAPTER FOUR

RESULTS

4. 0. 0. *Salmonella* isolation

Table 1 shows the frequency of *Salmonella* in different samples collected in Lusaka, Zambia. These results reveal the presence of *Salmonella* bacteria in chicken carcasses, egg yolks, egg shell membranes and in human stool.

Table 1: Frequency of *Salmonella* isolation from different samples

Type of samples	Total number of samples tested	Number of positive samples for <i>Salmonella</i>	Per cent(%)
Chicken carcasses	382	107	28.01
Egg yolks	240	34	14.16
Egg shell membranes	240	10	4.16
Human stool	86	8	9.30

It is clearly evident that the level of *Salmonella* contamination is significantly higher in the egg yolks than in the shell membranes. The chicken carcasses exhibited a high infection rate compared to other samples.

4. 1. 0. *Salmonella* isolation from chicken carcasses:

Four *Salmonella* serovars isolated from the chicken carcasses are presented in Table 2.

Of these serovars *Salmonella mbadaka* has been isolated and documented for the first time in Zambia.

Table 2: Percentage of different *Salmonella* serovars isolated from chicken carcasses:

<i>Salmonella</i> serovars	Number isolated	Per cent(%)
<i>S. Mbadaka</i>	82	76.63
<i>S. Enteritidis</i>	18	16.82
<i>S. Infantis</i>	2	1.87
<i>S. Gallinarum</i>	2	1.87
Rough type	3	2.80
Total	107	100.00

4. 2. 0. *Salmonella* isolation from table eggs:

Two serovars were isolated from table eggs, i.e. *Salmonella enteritidis* and *Salmonella gallinarum* as shown in Table 3. Thirty four *Salmonella* isolates were isolated from the egg yolk and ten *Salmonella* isolates were isolated from the shell membrane. All isolates from the Shell membranes were *Salmonella gallinarum*. The egg yolk isolates included twenty five *Salmonella gallinarum* and nine *Salmonella enteritidis*. Six *Salmonella gallinarum* isolates were isolated from the same egg samples i.e. from the shell membranes and yolks of the same sample. It is also important to note that all the *Salmonella enteritidis* isolates were isolated from different samples as to those yielding *Salmonella gallinarum*. There were no multiple isolations in the egg yolk.

Table 3: Percentage of different *Salmonella* serovars isolated from table eggs

<i>Salmonella</i> serovar	Sample source		per cent of isolation	
	Egg yolk	Shell membrane	egg yolk	Shell membrane
<i>Salmonella gallinarum</i>	25	10	73.53	100
<i>Salmonella enteritidis</i>	9	-	26.47	0
Total	34	10	100.00	100.00

4. 3. 0. *Salmonella* isolation from human stool:

Eight *Salmonella* isolates were isolated from the human stool samples. All these isolates belonged to the serovar *Salmonella typhimurium*.

4. 4. 0. Isolation of *Salmonella enteritidis* and its phage types:

A total of 27 *Salmonella enteritidis* isolates were obtained in this study: eighteen were from chicken carcasses and nine from table eggs. No *Salmonella enteritidis* was detected in the human stool samples. The incidences of *Salmonella enteritidis* positive samples in table eggs and poultry chicken carcasses are as indicated in Table 4. The incidence of *Salmonella enteritidis* isolation in chicken carcasses is higher than in table eggs.

Table 4: Incidence of *Salmonella enteritidis* positive samples in table eggs and poultry chicken carcasses.

Type of sample.	No. of samples examined.	No. of samples positive for <i>Salmonella enteritidis</i>	Incidence per cent (%)
Egg Yolk	240	9	3.75
Egg shell membranes	240	0	0
Chicken carcasses	382	18	4.7

2,400 table eggs were pooled in units of 10 to give 240 samples (N=240) of yolk and shell membranes. *Salmonella enteritidis* was isolated in nine samples of the yolk and none was isolated from the shell membranes. All the nine *Salmonella enteritidis* , belonged to phage type 4 (PT₄).

Out of 382 swab samples of chicken carcasses (N=382), Eighteen samples were positive for *Salmonella enteritidis* from chicken carcasses. Phage typing of isolates revealed different phage types. Seven belonged to phage type 4 (PT₄), three belonged to phage type 7 (PT₇) and eight belonged to the rough type (RT). Results of phage typing are presented in Table 5.

Table 5: Phage types of *Salmonella enteritidis*

Source of sample	phage type	Number
Table egg (Yolk)	PT ₄	9
Chicken carcasses	PT ₄	7
Chicken carcasses	PT ₇	3
Chicken carcasses	*RT	8
	Total	27

*RT = Rough type

4. 5. 0. Antibigrams of *Salmonella enteritidis*

Salmonella enteritidis isolates were tested for drug sensitivity to different antibiotics. The diameters of inhibition zone, observed after incubation were used to classify the strains as sensitive (S) or resistant (R). Results of the antibigrams are presented in Table 6.

It was also observed that all the 27 *Salmonella enteritidis* isolates were sensitive to ampicillin, amoxycillin, chloramphenicol, co-trimoxazole, furazolidone, gentamycin and tetracycline.

Other *Salmonella* serovars were also subjected to drug sensitivity tests and the results are shown in Tables 7, 8 and 9. The selected isolates of *Salmonella mbandaka* showed

a number of variations to antibiotic sensitivity. Some isolates were resistant to tetracycline and one showed resistance to amoxycillin. All the eight isolates of *Salmonella typhimurium* from human patients showed multiple resistance to amoxycillin and ampicillin. They were sensitive to chloramphenicol, furazolidone, gentamycin and tetracycline.

Table 6: Antibigrams of *Salmonella enteritidis* isolates

<i>Salmonella enteritidis</i> Strain Ref NO. and Phage type.	SOURCE	ANTIBIOTICS AND DIAMETERS OF ZONE OF INHIBITION IN MILLIMETERS						
		CN 10mcg	AMP 10mcg	TE 30mcg	SXT 25mcg	AML 10mcg	FR 50mcg	C 30mcg
1 - PT4	Chicken carcass	16S	23S	20S	21S	23S	20S	26S
2 - PT4	Chicken carcass	20S	22S	20S	23S	24S	20S	25S
3 - RT	Chicken carcass	20S	25S	19S	22S	22S	20S	26S
4 - RT	Chicken carcass	15S	20S	19S	22S	21S	20S	24S
5 - PT4	Chicken carcass	15S	19S	20S	20S	20S	20S	25S
6 - RT	Chicken carcass	16S	14S	20S	22S	20S	20S	26S
7 - RT	Chicken carcass	19S	20S	19S	20S	20S	20S	25S
8 - RT	Chicken carcass	20S	22S	20S	22S	21S	20S	26S
9 - RT	Chicken carcass	16S	20S	20S	19S	23S	20S	25S
10 - RT	Chicken carcass	17S	21S	21S	21S	21S	20S	24S
11 - PT7	Chicken carcass	17S	20S	22S	23S	21S	20S	26S
12 - PT7	Chicken carcass	18S	21S	21S	23S	21S	20S	24S
13 - PT7	Chicken carcass	15S	21S	20S	22S	21S	18S	24S
14 - PT4	Chicken carcass	17S	20S	20S	23S	21S	20S	26S
15 - PT4	Chicken carcass	17S	18S	20S	23S	18S	20S	26S
16 - RT	Chicken carcass	19S	22S	20S	20S	21S	20S	24S
17 - PT4	Chicken carcass	16S	24S	20S	21S	23S	17S	26S
18 - PT4	Chicken carcass	15S	20S	19S	23S	20S	20S	26S
19 - PT4	Egg yolk	18S	22S	20S	20S	20S	18S	26S
20 - PT4	Egg yolk	20S	22S	21S	21S	23S	16S	23S
21 - PT4	Egg yolk	20S	22S	20S	20S	21S	22S	26S
22 - PT4	Egg yolk	16S	21S	20S	23S	22S	20S	26S
23 - PT4	Egg yolk	16S	20S	20S	21S	17S	18S	26S
24 - PT4	Egg yolk	20S	24S	22S	20S	25S	18S	25S
25 - PT4	Egg yolk	17S	23S	20S	22S	22S	20S	26S
26 - PT4	Egg yolk	18S	21S	20S	23S	21S	18S	26S
27 - PT4	Egg yolk	17S	22S	20S	120S	20S	20S	26S

KEY

CN = Gentamycin
AMP = Ampicillin
AML = Amoxycillin
S > Sensitive

TE = Tetracycline
SXT = Co-trimoxazole
C = Chloramphenicol
R > Resistant

FR = Furazolidone

Table 7: *Salmonella infantis* antibiogram

ANTIBIOTICS AND DIAMETERS OF ZONE OF INHIBITION IN MILLIMETERS						
<i>Salmonella infantis</i> Strain NO.	SOURCE	CN 10mcg	AMP 10mcg	TE 30mcg	AML 10mcg	FR 50mcg C 30mcg
I.	Chicken carcass	15S	20S	20S	20S	26S
II.	Chicken carcass	15S	20S	20S	20S	26S

Table 8: Selected *Salmonella mbadaka* antibiogram from chicken carcasses

ANTIBIOTICS AND DIAMETERS OF ZONE OF INHIBITION IN MILLIMETERS						
<i>Salmonella</i> strain NO.	<i>mbadaka</i>	CN 10mcg	AMP 10mcg	TE 30mcg	AML 10mcg	FR 50mcg C 30mcg
10		18S	20S	OR	20S	22S 26S
11		15S	8S	OR	OR	20S 26S
26		10S	20S	15S	18S	20S 26S
52		15S	20S	OR	15S	20S 24S

Table 9: *Salmonella typhimurium* isolates from human patients

ANTIBIOTICS AND DIAMETERS OF ZONE OF INHIBITION IN MILLIMETERS						
<i>Salmonella typhimurium</i> isolate NO.	CN 10mcg	AMP 10mcg	TE 30mcg	AML 10mcg	FR 50mcg	C 30mcg
01	10S	0R	21S	0R	20S	25S
02	8S	0R	20S	0R	20S	26S
03	8S	0R	20S	0R	18S	26S
04	8S	0R	22S	0R	22S	25S
05	8S	0R	20S	0R	20S	25S
06	11S	0R	21S	0R	19S	26S
07	9S	0R	20S	0R	22S	26S
08	8S	0R	20S	0R	18S	26S

4. 6. 0. Pathogenicity of *Salmonella enteritidis* isolates in mice

The results of the pathogenicity test of *Salmonella enteritidis* isolates from table eggs and chicken carcasses are presented in Table 10 and 11 respectively. *Salmonella enteritidis* PT₄ isolates from the table eggs killed more mice as compared to *Salmonella enteritidis* isolates from the chicken carcasses. The mean per cent mortality ($\pm$ S.E.) with *Salmonella enteritidis* PT₄ from table eggs was 64.4 ± 4.44 per cent.

Table 10: Pathogenicity of *Salmonella enteritidis* PT₄ from table eggs in mice.

<i>Salmonella enteritidis</i> PT ₄ Ref No:	Observation period.			Total number of deaths	Per cent mortality
	1 st day	2 nd day	3 rd day		
19	1	2		3	60
20	-	2	2	4	80
21	-	2	1	3	60
22	2	1	1	4	80
23	1	2		3	60
24	-	2	-	2	40
25	-	2	2	4	80
26	-	3	-	3	60
27	1	2	-	3	60

*Note: 5 mice were inoculated for each isolate

Salmonella enteritidis isolates from chicken carcasses showed different results. Three isolates under study, *Salmonella enteritidis* RT reference number 10, *Salmonella enteritidis* PT₄ reference number 17 and *Salmonella enteritidis* PT₄ reference number 18 showed more virulence. The mean per cent mortality ($\pm$ S.E) was 7.78 ± 4.89 per cent.

The mice injected with isolates from chicken carcasses recovered mostly on the fourth day after inoculation. Mice inoculated with *Salmonella enteritidis* PT₇ seemed to recover much faster than all the other phage types i.e. they did not show typical signs of being ill. The mice that died were culture positive for *Salmonella enteritidis*.

Table 11: Pathogenicity of *Salmonella enteritidis* from chicken carcasses in mice.

<i>Salmonella enteritidis</i> Ref No.	Mortality (PI) occurring in Days			Deaths (No)	Mortality (%)
	1 st day	2 nd day	3 rd day		
10 (RT)	-	3	1	4	80
17 (PT ₄)	-	2	-	2	40
18 (PT ₄)	-	-	1	1	20

* 5 mice were inoculated for each isolate. Number in day column shows mortality.

PI = Post Inoculation.

4. 7. 0. Pathogenicity of *Salmonella enteritidis* in Embryonated eggs

A selected number of *Salmonella enteritidis* isolates were used due to a limited number of specific pathogen free eggs. The results from this experiment are shown in Table 12.

Table 12: Pathogenicity of *Salmonella enteritidis* isolates from table eggs and chicken carcasses in chicken embryos.

<i>Salmonella enteritidis</i> Ref No and source.	Days of observation.					Total mortality per cent
	1 st day	3 rd day	4 th day	5 th day	6 th day	
10 (RT) carcass	-	1	-	1		100
11 (PT ₇) carcass	-	-	1	-		25
16 (RT) carcass	-	1	-	-	1	50
17 (PT ₄) carcass	-	-	2	1		100
18 (PT ₄) egg yolk	-	-	1	2		100
19 (PT ₄) egg yolk	-	-	2			100
23 (PT ₄) egg yolk	-	1	2			100

*Four embryos were inoculated for each isolate. Number in day column shows mortality.

The results from the data in Table 12, shows that the isolates from the table eggs killed the embryos much faster than the isolates from chicken carcasses. By the fourth day after inoculation, all the embryos infected with *Salmonella enteritidis* PT₄ from the table eggs were dead. Embryos inoculated with *Salmonella enteritidis* reference number 17 (PT₄), reference number 18 (PT₄) and reference number 10 (RT) from the chicken carcasses were all dead on the fifth day.

4. 8. 0. Virulence of *Salmonella enteritidis* isolates in seven days old chicks

The pathogenicity results of *Salmonella enteritidis* PT₄ isolates from table eggs are shown in Table 13. There were no mortalities observed with isolates from the chicken carcasses

Table 13: Pathogenicity of *Salmonella enteritidis* PT₄ isolates from table eggs in chicks

<i>Salmonella enteritidis</i> PT ₄ Ref No.	Period of observation					Total No. of Deaths	Per cent mortality
	1 st day	2 nd day	3 rd day	4 th day	5 th day		
19					1	1	20
20			1			1	20
21		1				1	20
22						0	0
23						0	0
24						0	0
25			1			1	20
26				2		2	40
27		1				1	20

* 5 chicks were inoculated for each isolate. Number in day column shows mortality

The *Salmonella enteritidis* PT₄ isolates from the chicken carcasses did not kill, while the *Salmonella enteritidis* PT₄ isolates from the table eggs killed seven day old chicks, as shown in Table 13. The mean per cent mortality ($\pm$ S.E) was 15.56 ± 4.44 per cent. All the chicks that died were culture positive for *Salmonella enteritidis*.

4. 9. 0. Sugar test results

Salmonella enteritidis isolates from table eggs and chicken carcasses did not show any difference after 48 hours of incubation.

CHAPTER FIVE

DISCUSSION

5. 0. 0. *Salmonella* isolation from chicken carcasses

These results clearly indicate the presence of *Salmonella* in chicken carcasses, slaughtered and packed for human consumption in a processing plant in Lusaka. Twenty eight per cent (28%) chicken carcass samples were positive for *Salmonella* contamination. The findings of this work are in agreement with Simmons and Byrnes (1972) who had 20.87 per cent and 15.05 per cent positive samples for *Salmonella* from two different samplings. Humphrey and Lanning (1987) recorded 7.1 per cent *Salmonella* positive chickens at slaughter houses., O'Brien (1988) had 73 per cent of *Salmonella enteritidis* isolation from the pericarditis samples he collected in the processing plant, while Rampling *et al.* (1989) found that one in 1000 birds were condemned in the UK processing plants due to *Salmonella enteritidis* and Zivkovic *et al.* (1997) recorded 8.57 per cent of chicken meat contamination with *Salmonella*. The results reported in this study are higher compared to the findings of other workers with the exception of O'Brien (1988) who recorded 73 per cent of *Salmonella enteritidis* isolation from the processing plant. He however got a higher per percentage of *Salmonella enteritidis* contamination because of sampling carcasses showing pericarditis grossly.

Visceral cavity and cloacal surface area of dressed chickens were swabbed for the isolation of *Salmonella* during the study. Simmons and Byrnes (1972) also isolated *Salmonella* using swabs from the visceral and cloacal areas in their study whilst Rampling *et al.* (1989) reported chicken carcass cavity of broilers, as a site of choice for the isolation of *Salmonella*.

During this study, chicken carcasses were found to be contaminated with four *Salmonella* serovars which included *Salmonella enteritidis* (16.82 per cent), *Salmonella infantis* (1.87 per cent), *Salmonella gallinarum* (1.87 per cent) and *Salmonella mbandaka* (76.64 per cent) as opposed to six and eight *Salmonella* serovars detected by Simmons and Byrnes (1972) and Zivkovic *et al.* (1997) in similar surveys conducted in Australia and Zagreb, Croatia respectively. The six serovars isolated by Simmons and Byrnes (1972) included *Salmonella derby*, *Salmonella enteritidis*, *Salmonella seftenberg*, *Salmonella singapore*, *Salmonella typhimurium* and *Salmonella worthington*, while the eight serovars reported by Zivkovic *et al.* (1997) were *Salmonella typhimurium* (29.49%), *Salmonella hadar* (23.08%), *Salmonella virchow* (19.23%), *Salmonella saint-paul* (8.97%), *Salmonella enteritidis* (8.97%), *Salmonella agona* (5.13%), *Salmonella bovis-morbificans* (2.56%) and *Salmonella senftenberg* (2.56%). *Salmonella typhimurium* and *Salmonella hadar* accounted for more than 50 per cent of the isolates isolated by Zivkovic *et al.* (1997). In the present study *Salmonella mbandaka* was the highest in terms of isolation and has been documented for the first time in Zambia. The high incidence of *Salmonella mbandaka*, might be an indication of a clonal source. Since the main objective of the present study

was isolation of *Salmonella enteritidis* from the poultry products for human consumption, a detailed study on the predominant isolate from chicken carcasses, *Salmonella mbandaka* was not made. The isolate was only studied for its sensitivity to antibiotics. Pathogenicity test on *Salmonella mbandaka* could have been useful to know its significance. It is known that *Salmonella* isolation from carcasses, brings evidence that the number and serotypes present on poultry carcasses might be a reflection of the *Salmonella* carrier state of the birds entering the processing plant, although the hygiene in the plant may determine the degree of dissemination to the number and variety of *Salmonella* amongst the carcasses (Timoney *et al.* 1970).

Chicken meat can be Contaminated from a number of sources. It can originate from birds carrying *Salmonella* internally or externally on the feathers, claws or from within the processing plant. These sources have been noted by Simmons and Byrnes (1972) and Rampling *et al.* (1989). Transportation of birds from the growing farms to the poultry processing plant, may also have an influence on *Salmonella* dissemination as observed by Rigby and Petit (1980) and Jordan (1994). Since these factors were not studied during the present study, it was not possible to trace the source of infection. However, the contamination of chicken carcasses seems to be widespread, as the processing plant receives chickens from many poultry farms with different hygienic conditions and standards.

Isolation of *Salmonella enteritidis* from chicken carcasses destined for human consumption in Zambia being reported here for the first time, is an interesting finding. Of the *Salmonella enteritidis* isolates, frequency of enteropathogenic Phage type 4 was parallel to the rough type followed by PT₇ (Table 5). This documentation of *Salmonella enteritidis* from chicken carcasses is in agreement with a number of workers (Simmons and Byrnes 1972, O'Brien 1988, Rampling *et al.* 1989 and Zivkovic *et al.* 1997). Of these workers, O'Brien (1988) and Rampling *et al.* (1989) isolated *Salmonella enteritidis* PT₄ while Simmon and Byrnes (1972) and Zivkovic *et al.* (1997) did not phage type their *Salmonella enteritidis* isolates. Isolation of *Salmonella enteritidis* in Zambia from processed chicken carcasses might indicate its prevalence in broiler chickens up to the time of slaughter as noted by Lister (1988) and O'Brien (1988), considering that *Salmonella enteritidis* was isolated from dead in shell chicken embryos from local hatcheries in Lusaka (Kabilika 1997). This inapparency of *Salmonella enteritidis* infections in the birds is of economic importance to the industry even though nothing is done about it (Anon 1988a). This can largely apply to countries like Zambia, where resources in disease management are so scarce.

The application of Hazard Analysis Critical Control point (HACCP) approach in the processing plant is done by washing chicken carcasses in chlorinated water prior to chilling. This approach is said to reduce *Salmonella* populations by as much as 25 per cent with chlorination killing detached *Salmonella* and therefore minimises cross contamination between carcasses (Pullen 1989). For this to work well, the chlorine

concentration and water volume need to be monitored as the chlorine is easily neutralised by contact with organic matter other than bacteria. At this processing plant, chlorine concentration and water volume are not closely monitored. This could suggest the high incidence of *Salmonella* resulting from use of adulterated chlorinated water. Total elimination of *Salmonella* cells from the chicken carcasses might be difficult in the processing plant reported in this study, but it is imperative that the HACCP system be applied at one or more locations in order to effectively eliminate microbial pathogens.

5. 1. 0. *Salmonella* isolation from Table eggs

A total of 34 *Salmonella* isolates were obtained from the table eggs, with predominance of *Salmonella gallinarum* (73.53%) followed by *Salmonella enteritidis* (26.47%). The isolation of *Salmonella enteritidis* from table eggs is well documented by Paul and Batchelor (1988), Humphrey *et al.* (1989b., 1991b), Poppe *et al.* (1992) and Henzler *et al.* (1994). In addition to this, the results of this study support the conclusions of others that *Salmonella enteritidis* can be detected by culturing pools of egg contents (Gast 1993a, 1993b). Isolation of *Salmonella enteritidis* from table egg yolks might indicate internal contamination of eggs in Zambia and pose as a serious health risk to people consuming improperly cooked eggs. *Salmonella enteritidis* was only isolated from the yolk and none in shell membrane. This could suggest that contamination might have occurred *in vivo* because of dissemination of the organism to the ovaries as *Salmonella enteritidis* can be isolated from ovaries of breeding birds (Shivaprasad *et al.* 1990). This can also be supported by the work done by Kabilika *et al.* (1996) who isolated *Salmonella enteritidis*

PT₄ from dead in shell chicken embryos, suggestive of the transfer of *Salmonella enteritidis* from the hen to the egg.

Phage typing of *Salmonella enteritidis* isolates during the study showed that PT₄ predominated from table eggs as well as from chicken meat. Other phage type of *Salmonella enteritidis* was PT₇. A good number of *Salmonella enteritidis* isolates proved to be of rough type, obtained exclusively from the chicken carcasses. Theoretically, isolation of *Salmonella enteritidis* from infected eggs should give a good estimate of the risk that these eggs constitute to humans as observed by Henzler *et al.* (1994). However, even with egg culture data, only the human exposure risk can be calculated (e.g., the average number of exposures to *Salmonella enteritidis* contaminated eggs per capita). It is likely that most casual exposures do not result in significant clinical illness, because bacterial dose, host susceptibility, immune status and food preparation practices may play important roles upon consumption of *Salmonella enteritidis* infected eggs (Henzler *et al.* 1994). Other *Salmonella enteritidis* phage types like; PT₈, PT₁₃, PT_{13a}, PT₂₃, PT₂₈ and PT₃₃ have been isolated from egg contents in similar studies (Humphrey *et al.* 1989b., Telzak *et al.* 1990., Poppe *et al.* 1992., Ebel *et al.* 1993., and Henzler *et al.* 1994). *Salmonella enteritidis* PT₈ and *Salmonella enteritidis* PT_{13a} have been implicated in food poisoning cases in the USA and Canada (Rowe 1989, Hickman *et al.* 1991, Rodrigue *et al.* 1992 and Telzak *et al.* 1990) while *Salmonella enteritidis* PT₄ seems to be found world wide (Ward *et al.* 1987., Rowe 1989 and Kinde *et al.* 1996).

The incidence of *Salmonella enteritidis* PT₄ in table eggs reported in this study was high (3.75 per cent) as compared to other workers who observed that naturally infected hens generally produce contaminated eggs at very low frequencies (Mawer *et al.* 1989, Humphrey *et al.* 1989b., Humphrey *et al.* 1991b., Poppe *et al.* 1992 and Henzler *et al.* 1994). Humphrey *et al.* (1989b) reported 1.1 per cent and 0.9 per cent of *Salmonella enteritidis* positive egg contents examined from two different flocks. Humphrey *et al.* (1991b) isolated *Salmonella enteritidis* from the contents of less than one per cent of over 5700 eggs from commercial laying flocks. Poppe *et al.* (1992) in his study found the overall prevalence of *Salmonella enteritidis* contaminated eggs to be less than 0.06 per cent. In another study Henzler *et al.* (1994) found frequency estimates of *Salmonella enteritidis* contaminated eggs, ranging from 0.03 per cent to 0.90 per cent. This clearly shows the low frequency detection results of *Salmonella enteritidis* from table eggs generally.

It is quite important to observe that a lot of factors in Zambia, underlying egg production and handling are biased towards *Salmonella enteritidis* propagation. These factors may include:

- a) The practice of exchanging trays while buying eggs is common. This can be a source of *Salmonella enteritidis* transmission from eggs of one farm to another. Transmission of poultry diseases through recycled egg trays was reported by Williams (1984).
- b) The presence of mice on most poultry farms in Zambia is quite common. But no study has been done yet to trap mice on these farms in order to detect whether *Salmonella* can

be isolated from them or not. Mice are known to be carriers of *Salmonella enteritidis* on persistently infected poultry units (Davies and Wray 1995).

c) The tropical temperatures of the country allow propagation of *Salmonella enteritidis* in eggs awaiting customers. This is obvious as eggs are not refrigerated till they are sold. Muliari and Zavanella (1994) observed that, environmental factors such as storage conditions tolerate *Salmonella enteritidis* propagation, despite natural defensive mechanisms of egg shells. This has also been noted by Humphrey *et al.* (1989b, 1991b), Bradshaw *et al.* (1990), Gast and Beard (1992b), and Hammack *et al.* (1993) who suggested that eggs laid by infected hens generally contain low numbers of *Salmonella* in which their presence is a potential hazard that increases during prolonged storage at unrefrigerated temperatures. It is also possible that during very hot weather, infection can become reactivated in individual birds as suggested by Corkish *et al.* (1994).

d) The agglutination test used for *Salmonella gallinarum* - *pullorum* test is non specific for *Salmonella enteritidis* detection as they share the same somatic antigens O: 9 and O: 12.

The results of *Salmonella enteritidis* PT₄ obtained from this study (3.75 incidence per cent) should be interpreted conservatively as, it is based on isolations from one time of the year i.e. March, April, May, June and July (the cooler months in Zambia) and not through out the year. It is also important to note that isolation of *Salmonella gallinarum* from the shell membrane and yolk of same egg samples suggest possible contamination

of the eggs with *Salmonella gallinarum*. *Salmonella gallinarum* can also be detected from pooled egg yolks and shell membranes.

5. 2. 0. *Salmonella* isolation from Human diarrhoea patients

The isolation of *Salmonella typhimurium* from the stool samples of diarrhoea patients, at UTH, could mean that this strain is the most common cause of *Salmonella* gastroenteritis in Lusaka. Reports on *Salmonella* serovars isolated from human patients with diarrhoea diagnosed at UTH, indicate *Salmonella typhimurium* as the main isolate (Mwansa 1997, personal communication). Degroote (1997, personal communication) reported isolation of *Salmonella typhimurium* from blood cultures of the patients suffering from diarrhoea, at UTH Lusaka.

Isolation of *Salmonella* from the stool sample, does not really mean, it is the definite cause of the ailment causing fever, headache, chills and nausea. *Salmonella* carrier state do exist, hence the possibility of isolating *Salmonella* from stool cultures of patients having related problems is obvious. *Salmonella* bacteremia (non typhoidal) usually has certain risk factors like immunological status of an individual. With the advent of AIDS cases *Salmonella enteritidis* is definitely a risk factor for *Salmonella* bacteremia (Anon 1990). Degroote (1997, personal communication) while conducting a survey on *Salmonella* infection at UTH, Lusaka found that 93 per cent of the adult patients with *Salmonella* bacteremia were HIV positive compared to 31 per cent in healthy individuals. This clearly shows a definite association between HIV and *Salmonella* bacteremia.

Whether *Salmonella* infection occurs or not will largely depend on the number of organisms (quantum of infection), virulence and resistance of an individual. Persons with diseases such as liver cirrhosis, lupus, neoplastic processes, sickle cell anaemia or malaria tend to be more susceptible to *Salmonella* infection (Mwansa 1986). The fore mentioned disease conditions exacerbates the prevalence of *Salmonella* infection. However these factors were not included in our study which has revealed an incidence of 9.3 per cent *Salmonella typhimurium* from patients suffering from diarrhoea. *Salmonella enteritidis* was not isolated during this study. This serovar has not yet been documented in human patients in Zambia.

5. 3. 0. Antibiotic susceptibility pattern of *Salmonella* isolates

It was interesting to find that all the *Salmonella enteritidis* isolates tested had a number of common features in terms of antibiotic sensitivity. They were susceptible to amoxycillin, ampicillin, chloramphenicol, co-trimoxazole, furazolidone, gentamycin, and tetracycline. A similar antibiogram pattern for *Salmonella enteritidis* isolated from chicken embryos was found by Kabilika (1997). It is known that resistance to antimicrobial agents arise in a population of bacteria due to the ever increasing use of antibiotics in feeds or drinking water in chickens (Mwansa 1986). In Zambia, laws are not strictly followed on antibiotic use and purchase of drugs from shops, as such some antibiotics have frequently been used in feeds as a prophylactic measure to boost production in Zambia (Pandey and Sharma 1994). This observation has also been noted in other countries like Liberia where

antibiotic resistance in an outbreak of *Salmonella enteritidis* was reported (Hadfield *et al.* 1985).

Certain isolates of *Salmonella mbadaka* were resistant to tetracycline and amoxycillin. This resistance of *Salmonella mbadaka* is of concern as it might be the first indicator to antibiotic resistance of *Salmonella* found in chickens.

Salmonella typhimurium isolates from human cases showed resistance to amoxycillin, ampicillin and erythromycin. Degroote (1997, personal communication) in her study of *Salmonella* isolates from the blood of patients at UTH Lusaka found that the *Salmonella typhimurium* isolates from the blood were sensitive to chloramphenicol and some to gentamycin. The isolates from the faecal samples in the present study were susceptible to chloramphenicol, gentamycin, tetracycline and furazolidone. The results are in agreement with Degroote (1997, personal communication). This pattern suggests the similarity of the *Salmonella typhimurium* isolates obtained from stool and blood. It is also important to note that antibiotics for humans are readily available from a variety of sources in Zambia, and their uncontrolled use might have undoubtedly promoted the development and stabilisation of resistance although not proven.

The antibiograms in this study have clearly shown differences in antibiogram patterns of *Salmonella* from poultry and human isolates. *Salmonella enteritidis*, *Salmonella infantis* and *Salmonella mbadaka* seem to have very little differences in their antibiogram

patterns. Human isolates have shown multiple resistance as compared to poultry isolates. The differences between antimicrobial susceptibility results of poultry and human isolates seem to distinguish these two groups. Bender *et al.* (1995) have suggested that periodic monitoring of *Salmonella* isolates from animal and human sources for their resistance to antimicrobial agents would be beneficial. However, it may not be sufficient to evaluate the epidemiological relationship between *Salmonella* infections in humans and poultry or to clarify possible modes of transmission.

5. 4. 0. *Salmonella enteritidis* characterisation

Biochemical tests, antibiogram tests and pathogenicity tests were used in the present study to characterise *Salmonella enteritidis* isolates. These investigations did not suggest much differences apart from the degree of pathogenicity.

Pathogenicity test in mice showed that, *Salmonella enteritidis* isolates from the table eggs are more virulent than isolates from chicken carcasses. Three isolates tested from chicken carcasses (two *Salmonella enteritidis* PT₄ and one rough type isolate) caused mortalities, although with variable results. The mean per cent mortality with *Salmonella enteritidis* isolates from table eggs was higher than the mean per cent mortality for isolates from chicken carcasses. The mean per cent mortality of 64.4 ± 4.44 per cent for isolates from table eggs was observed as opposed to 7.78 ± 4.89 per cent for isolates from chicken carcasses. This observation suggest that there may be variation in the virulence of *Salmonella enteritidis* isolates both between and within phage types. Other workers in

for the chicks to recover from illness differed according to isolates. The chicks inoculated with isolates from the chicken carcasses recovered much faster than the isolates from the table eggs. A similar pattern has been observed in mice and embryonated egg experiments. Previous investigators have similarly noted considerable diversity in the consequences of infections of chicks with various *Salmonella enteritidis* strains. In an evaluation of a chick mortality model, Gast and Beard (1992a) reported significant differences between several PT₈, PT_{13a} and PT_{14b} *Salmonella enteritidis* strains in their virulence in experimentally infected chicks. Poppe *et al.* (1993b) found that a *Salmonella enteritidis* PT₄ strain isolated in the United Kingdom was more virulent for chicks than was a Canadian *Salmonella enteritidis* PT₄ isolate. In another study, one of three United Kingdom *Salmonella enteritidis* PT₄ isolates was found to be much less virulent for chicks than the other two (Barrow 1991). Although field reports have often linked *Salmonella enteritidis* PT₄ with high levels of morbidity and mortality in British broiler flocks (Anon 1988b and O'Brien 1988), many experimental evaluations of *Salmonella enteritidis* PT₄ isolates have failed to reveal evidence of significant pathogenicity (Hinton *et al.* 1989, Humphrey *et al.* 1989a and Humphrey *et al.* 1991a). These experimental evaluations tend to be in agreement with this study in which majority of the *Salmonella enteritidis* PT₄ isolates did not reveal significant pathogenicity.

Isolation of *Salmonella enteritidis* from the liver, spleen and blood stream of dead mice and chicks during the study shows the ability of the bacteria to invade and survive in the blood stream. This agrees with the work of other workers who have demonstrated the

invasiveness of *Salmonella enteritidis* PT₄ in young chicks and experimentally infected hens (Hinton *et al.* 1989., Gast and Beard 1990b and Shivaprasad *et al.* 1990).

In the present study, the average per cent mortality for chicks inoculated with *Salmonella enteritidis* PT₄ from the table eggs was 15.56 ± 4.44 per cent, as compared to none from the chicken carcasses *Salmonella enteritidis* PT₄. In other studies carried out in the USA, isolates of *Salmonella enteritidis* phage types isolated from poultry (such as PT₈, PT_{13a} and PT_{14b}) have occasionally been associated with morbidity or mortality in naturally or experimentally infected chickens (Poppe *et al.* 1993a and Shivaprasad *et al.* 1990), but more often these phage types have been found to colonise the intestinal tract, invade and disseminate to internal tissues, and contaminate eggs without causing any apparent clinical disease (Gast and Beard 1990a., 1990b). It is important to consider that although phage types may not unilaterally define the virulence capabilities of *Salmonella enteritidis* isolates, they may indicate epidemiologically relevant lineages. The general history of such lineage may include the acquisition of relevant virulence determinants by a particular proportion of its population (Gast and Benson 1995). Although *Salmonella enteritidis* isolates generally appear not to pose as great a risk to poultry health as do more severe pathogens such as *Salmonella pullorum* (Gast and Benson 1995), some *Salmonella enteritidis* isolates might be highly pathogenic and capable of causing significant disease losses in highly susceptible young chicks and chicken embryos (O'Brien 1988., Shawabkeh and Tarazi 1993). However the present study might have had chicks not highly susceptible to *Salmonella enteritidis* PT₄ or there susceptibility to

Salmonella enteritidis declined rapidly during the first few days of life as observed by Gast and Beard (1989). In their study Gast and Beard (1989) noted that lethal effects of *Salmonella* infections in chicks decline rapidly during the first few days of life. It may also be inappropriate to generalise too widely from the results of the present study, as it employed highly inbred lines of harco chick cockerels from the local hatcheries of Lusaka. The variations within *Salmonella enteritidis* PT₄ pathogenicity was demonstrated by Barrow (1991), Gast and Benson (1995). Some chicks generally developed clinical signs like diarrhoea, lethargy, closing of eyes and having drooped wings as reported by Williams (1994) and Jordan (1994). The chicks that recovered, might become carrier birds as noted by Jordan (1994), who observed that survivors of *Salmonella* infection usually become carriers.

The results obtained during the study from pathogenicity test of *Salmonella enteritidis* in mice, chicken embryo and seven day old chicks exhibit differences in virulence. The *Salmonella enteritidis* PT₄ isolates especially from chicken carcasses and table eggs are less pathogenic compared to some isolates from the table eggs. This can elucidate a number of suggestions:

1. The practise of washing chicken carcasses in chlorinated water might have an impact on the physiology and cellular factors of the bacteria.
2. The bacteria in broiler chickens ready for slaughter, might have undergone alteration of pathogenic factors when the broiler chickens were growing up as compared to when *Salmonella enteritidis* is found in the egg of a seemingly health laying bird.

5. 5. 0. Further investigations proposed:

1. Investigation on the tolerance of *Salmonella enteritidis* isolates from table eggs and chicken carcasses to heat, exogenous acid, Hydrogen peroxide and their ability to survive on different surfaces.
2. Investigation on the potential virulence factors of *Salmonella enteritidis* using parameters like production of aerobactin, enterobactin, colicin and haemolysin, serum resistance against serum from different animal species, binding of Congo red and crystal violet, auto-agglutination and calcium dependency and double colony morphology.
3. The use of quantitative techniques to observe gross differences in pathogenicity of *Salmonella enteritidis* isolates.
4. Continuous screening of suspect human patients for *Salmonella enteritidis* .
5. An attempt must be made to elucidate the physiological and cellular factors behind the observed differences in behaviour of isolates from chicken carcasses and table egg.
6. A detailed survey on *Salmonella* contamination in layer flocks and broiler breeder flocks supplying the Lusaka poultry industry

CHAPTER SIX

CONCLUSION

1. The study demonstrated that table eggs and poultry carcasses which reach the consumers in Lusaka, Zambia are infected with *Salmonella enteritidis*. The frequency of isolation of *Salmonella enteritidis* is much higher in Lusaka compared to reports from other countries.
2. Phage typing of *Salmonella enteritidis* isolates revealed that a majority of the isolates belonged to the most invasive phage type four (PT₄) which is of public health importance.
3. Pathogenicity of *Salmonella enteritidis* isolate tested in embryonated eggs, mice and one week old chicks showed that isolates from table eggs were more virulent compared to isolates from chicken carcasses. This difference in virulence could not be explained.
4. The antibiogram of *Salmonella enteritidis* isolates, demonstrated that *Salmonella enteritidis* from poultry isolates were sensitive to antibiotics that are easily available in Zambia as compared to *Salmonella typhimurium* isolates from humans which showed resistance to ampicillin and amoxycillin.

5. The isolation of *Salmonella enteritidis* from chicken carcasses and poultry table eggs represents a new challenge to the poultry industry of Zambia, the Veterinary advisers, the Ministry of Agriculture, Food and Fisheries.
6. The consumers of poultry products in Zambia have to be cautious while preparing poultry products for the table especially with the advent of the increasing number of fast food restaurants.
7. The search for better methods to control *Salmonella enteritidis* in poultry flocks in Zambia should be enhanced, as the incidence of isolation is high compared to the isolation per percentages reported elsewhere.
8. This study could not document any correlation of poultry and human serotypes as all the isolates isolated from human cases were *Salmonella typhimurium*, while *Salmonella enteritidis*, *Salmonella infantis*, *Salmonella gallinarum* and *Salmonella mbandaka* were isolated from poultry.

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