DETERMINATION OF VIRULENCE FACTORS IN *SALMONELLA* ISOLATES OF HUMAN, POULTRY AND DOG ORIGIN IN LUSAKA DISTRICT, ZAMBIA

BY

WILLIAM DANDAULO ULAYA

A thesis submitted to the University of Zambia in fulfillment of the requirements of the degree of Master of Science in Microbiology

THE UNIVERSITY OF ZAMBIA
SCHOOL OF VETERINARY MEDICINE
DEPARTMENT OF PARACLINICAL STUDIES
LUSAKA

DECLARATION

I, **William Dandaulo Ulaya**, do hereby declare that the contents of this thesis being submitted herein are my original work and they have not been previously submitted to any University for the award of a degree or any other qualification.

Signature: …… Date:…….…….

CERTIFICATE OF APPROVAL

This thesis submitted by **William Dandaulo Ulaya,** is approved as fulfilling the requirements for the award of the degree of **MASTER OF SCIENCE IN MICROBIOLOGY** of the University of Zambia.

Dr Bernard M. Hang'ombe Supervisor	---------- Signature	---------- Date
Prof. Lily C. Bebora External examiner	---------- Signature	---------- Date
Prof. Boniface Namangala Internal examiner	---------- Signature	---------- Date
Dr. Careen Hankanga Internal examiner	---------- Signature	---------- Date

ABSTRACT

This study was conducted in Lusaka, Zambia to determine the distribution of the *invA, invF, hilA, hilC, hilD, sipC* and *spiC* virulence genes in *Salmonella* isolates from dogs, chickens and humans using both the Polymerase chain reaction (PCR) and dot blot hybridization methods. In the study, 243 dog fecal samples and 136 chicken specimens were analyzed by the culture and isolation method. A total of 18 *Salmonella* isolates (prevalence 7.6%) belonging to sero groups E, B, C and untypable serovars from dogs and sero group D (*S. enteritidis*) from chickens (prevalence 16.2%) were subjected to virulent gene analysis. A further 25 *Salmonella* isolates belonging to sero group B, C, D and E from humans were also examined for the presence of the virulence genes. The *invA* gene was found present in all the 66 isolates using both methods. However, using the Dot blot hybridization assay it was observed that isolates from the chicken, dog and human sources varied in the possession of the *hilD* gene (13%, 55.5% and 72%, respectively) and *sipC* gene (13%) only from chicken isolates. Using the Polymerase chain reaction (PCR) assay the *invF* gene was distributed in varying percentages (16.7%, 40%, 20%) as well as the *sipC* gene (8%, 8.7%, 4%) among isolates from dog, chicken and human sources, respectively. The *hilD* gene was possessed only by isolates from dogs. Also examined were the antimicrobial susceptibility patterns of the isolates, which showed that dog *Salmonella* isolates were susceptible to amoxicillin (100%) and nitrofurantoin (94.4%), those from chicken showed susceptibility to nitrofurantoin (100%), amoxicillin (95.7%), ampicillin and tetracycline (82.6% respectively) and those from human showed susceptibility to nitrofurantoin (79.6%) and amoxicillin (68%).

It was concluded from the results of the study that dogs and chickens could be important carriers of invasive *Salmonella* in Lusaka, Zambia. There was no significant difference in the distribution of *Salmonella* virulence genes in dogs, chickens and humans. However, their degrees of expression varied from one *Salmonella* source to another showing their pathogenic potential in the infected hosts. Humans were shown to have similar *Salmonella* serogroups as those found in dogs and chickens, signifying the zoonotic potential of the bacteria. The multiple antimicrobial drug resistance observed from *Salmonella* in dogs and chickens may be potential sources of human salmonellosis in Zambia which could be difficult to treat.

DEDICATION

This work is dedicated to **my beloved wife Patricia, my children Chimwemwe, Daliso** and **Nthokozo** for their patience, encouragement, emotional and spiritual support during the time of my study. Above all I thank **God Almighty,** for making all things possible.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to Dr. Bernard M. Hang'ombe for the remarkable supervision, material and moral support he rendered throughout the research work and write up of this dissertation, as well as for going out of his way to ensure that the research work was completed successfully and on schedule.

I am thankful to Dr. King Nalubamba and Dr Victor Zulu for co-supervising my study, for their guidance in the planning, execution of my research and write up of the dissertation. The Dean of the School of Veterinary Medicine Prof Aaron Mweene, for his encouragement and support.

My gratitude also goes to the academic and technical staff of the School of Veterinary Medicine departments of Clinical studies and Paraclinical studies, Mr. E. M. Mulenga, Mr. J. Chilundu and Mr. L. M. Moonga for their invaluable assistance and technical support. Members of staff of the Bacteriological laboratory of the Department of Pathology and Microbiology at the University Teaching Hospital for accommodating me in their busy laboratory and permitting me to use human *Salmonella* isolates from their storage bank.

Further, I wish to thank Prof H. Isogai and Prof E. Isogai of the Sapporo Medical University and Tohoku Agriculture University in Japan, respectively for providing PCR reagents and antiserum for the Dot blot hybridization analysis.

Finally but not the least I would like to thank the University of Zambia for affording me an opportunity to carry out this study under the Non-academic staff training award.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
CSF	Cerebral spinal fluid
CSO	Central statistical office
DNA	Deoxyribonucleic acid
DVO	District veterinary officer
EFSA	European food safety agency
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization
GDP	Gross domestic product
HIV	Human immunodeficiency virus
hilA	Hyper-invasive locus A
hilB	Hyper-invasive locus B
hilC	Hyper-invasive locus C
invA	Invasion A gene
invF	Invasion F gene
LPS	Lipopolysaccharide
NTS	Non-typhoid Salmonellosis
PCR	Polymerase chain reaction
SPI	*Salmonella* pathogenicity island
sipC	*Salmonella* invasion protein C
spiC	*Salmonella* pathogenicity island protein C
URS	Upstream repressing sequence
USDA	United States Department of Agriculture
XLD	Xylose lactose deoxycholate agar
WHO	World Health Organisation

CHAPTER ONE

INTRODUCTION

Among the various pathogenic bacteria known to cause mass food poisoning incidents, the most notorious are members of the genus *Salmonella* (Krieg and Holt, 1984). They are highly adaptive and potentially pathogenic to humans and animals. They are the causative agents of enteric fevers, gastroenteritis and septicemia which are of both socio-economic and public health importance. This therefore makes the genus of *Salmonella* one of the most important bacterial zoonoses in the world. The disease prevalence is increasing as a result of industrialization and intensive production of livestock and poultry through small scale farmers (Nambota *et al*., 2000). In addition, selective pressure imposed by the use of antimicrobials in both human and veterinary medicine promotes the spread of multiple antimicrobial resistance resulting in the growing problem of *Salmonella* infections that are difficult to treat (Carattoli, 2003).

Salmonella has been declared by the World Health Organisation (WHO) and the Food Agriculture Organisation (FAO) as the most common and important zoonosis since 1950. This has led to its inclusion in the terrestrial animal health code of the World Animal Health Organisation (Anon, 2004). Worldwide estimates of non-typhoid *Salmonella* cases range from 200 million to 1.3 billion, with an estimated death toll of 3 million people each year (Coburn *et al*., 2007). Most human salmonellosis cases are foodborne, but each year infections are also acquired through direct or indirect animal contact in homes, veterinary clinics, zoological

gardens, farm environments or other public, professional or private settings. Majowicz *et al*., (2010) estimated that approximately 80.3 million of 93.8 million human *Salmonella*-related gastroenteritis cases that are estimated to occur globally each year are foodborne, thus representing approximately 86% of human salmonellosis cases. Another study estimated that approximately 55% of human *Salmonella* cases were foodborne, 14% were travel-related, 13% are acquired through environmental sources, 9% occur due to direct human-to-human transmission and 9% were attributable to direct animal contact (EFSA, 2008; Vargas-Galindo, 2007).

Salmonella serotypes have a broad host range and clinical manifestations that result from the combination between serotype and host species involved (Santos *et al.*, 2002), are prevalent in the warm-blooded animal population (Jones, 2005), including rodents (Porwollik *et al*., 2005), snakes (Solari *et al*, 2003), and free living terrestrial and aquatic turtles (Vila *et al*., 2008). Although most infections cause mild to moderate self-limiting disease, serious infections leading to deaths do occur (Jong and Ekdahl, 2006). The zoonotic transmission of *Salmonella* is not limited to those originating from food animals alone; pets, especially dogs that have close interaction with humans, may be responsible for *Salmonella* transmission (Kozak *et al*., 2003), veterinarians and public health officials have recognized shedding of *Salmonella* by dogs as a possible source of infection for dog owners and their communities (Kozak *et al*., 2003; Sanchez *et al*., 2002; Kahrs *et al*., 1978). *Salmonella* poisoning in dogs is primarily from ingestion of any food contaminated by the *Salmonella* bacteria. Dogs can also get salmonellosis from other animals through infected feces and saliva. Dogs that eat trash or dead animals are at high risk for *Salmonella* food poisoning. Birds often carry *Salmonella* bacteria, and dogs can catch

salmonellosis from eating infected birds or by having contact with bird feeders, bird houses or other objects that have come into contact with infected birds.

Non-typhoidal *Salmonella* has the ability to cause infection in 2 ways; gastroenteritis and systemic infection (Marcus *et al*., 2000). *Salmonella* pathogenesis begins with the ingestion or uptake of the bacteria into the host. In *Salmonella* associated with gastroenteritis, the adhesion and invasion of the intestinal cells results in a series of inflammatory and secretory responses in the host, causing disease (Hansen-Wester and Hensel, 2001). On the other hand, *Salmonella* serotypes that cause systemic disease establish infection through their ability to survive and replicate in phagocytic macrophage cells (Ohl and Miller, 2001). The bacteria migrate to the local mesenteric lymph nodes and then to the spleen and liver via phagocytic cells, resulting in the spread of the disease (Ohl and Miller, 2001; Marcus *et al*., 2000). In order for *Salmonella* to initiate the above described processes of gastroenteritis and systemic infection, a wide variety of virulence factors are required.

In Zambia *Salmonella* serovars associated with human salmonellosis have been isolated from poultry and its products (Hang'ombe *et al*., 1999a; Kabilika *et al*., 1999; Tuchili *et al.*, 1996). There is, however, no information regarding *Salmonella* prevalence or serovars found in dogs to appreciate the public health risks associated with dog keeping, as well as the risk of dogs acquiring *Salmonella* from poultry. There is also scarcity of information with regard to the association of virulence factors in isolates from these closely related species.

Justification of the study

Salmonella in humans is acquired through contact with infected materials of animal origin. Poultry and dogs have a close interaction with humans as source of food and as pets respectively. These have been cited as important reservoirs and transmitters of *Salmonella* resulting in severe infection in humans (Morse and Duncan, 1975), thereby raising public health concern. This concern has increased in Zambia due to Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) cases and an ever increasing poultry industry. Recently, Zambia's economy has been on the upswing resulting in the increase of fast food outlets selling chicken products and an increase in households keeping dogs. It is therefore important to determine the presence of virulence determinants (*sipC, spiC, hilA, hilC, hilD invA* and *invF*) in isolates from human, poultry and dogs in order to understand any link and pathobiology of this important zoonotic organism in various animal species. It is hoped that the outcomes of the study would help in the understanding of the epidemiology and public health implication of this zoonotic organism in these closely interacting species, have implications on the prevention, control and treatment strategies of human and animal salmonellosis to policy makers, as well as create awareness on the need of increased good hygienic practices in the poultry industry and households rearing dogs.

The objectives of this study were as follows:

1. Isolate and identify *Salmonella* from dogs and poultry in Lusaka, Zambia.
2. Characterize *Salmonella* isolates of human, dog and poultry sources based on the presence of virulence genes.
3. Determine the antimicrobial susceptibility pattern of *Salmonella* isolates obtained from human, poultry and dog sources.

4. CHAPTER TWO

5. LITERATURE REVIEW

2.1.0 General overview of Salmonellosis

Salmonella is the aetiological agent of both human and animal salmonellosis, a very common and widely spread enteric disease. It is a significant cause of acute and chronic diarrhoea resulting into death of numerous animal species and human beings (McGavin *et al.*, 2001). Salmonellosis is therefore of significant importance both in animal production and in public health. It can occur as a severe systemic disease responsible for heavy economic losses to the commercial poultry industry through morbidity, mortality and reduced egg production (Barrow, 1989). The emergence of the serotype *enteritidis* in poultry has raised concern, due to the zoonotic transmission to humans and its ability to cause severe disease in man (Rabsch *et al.*, 2001; Lee, 1974). This transmission association of humans and animals was typically reported by Sato *et al*., (2000) when *Salmonella*, isolated from a diarrheic child and the dog living in the same house, were found to be of similar strain, highlighting the public health risk posed by the association between man and dogs as companion animals.

2.2.0 The genus *Salmonella*

The genus *Salmonella* was named after Dr. Daniel Salmon, a veterinary bacteriologist at the United States Department of Agriculture (USDA) (Gast, 2003; Salyers and Whitt, 2002). *Salmonella* are short Gram-negative bacilli and nonsporulating. Most of them move using peritrichial flagella, although serotypes such as *S. pullorum* and *S. gallinarum* are nonmotile.

They are either aerobic or facultative anaerobic, and grow between 5 and 45°C. Optimum growth occurs at 37°C. The ideal pH for multiplication is 7, but *Salmonella* survives in pH values between 4 and 9. They grow in culture medium for enterobacteria and on blood agar. Colonies are 2 to 4 mm in diameter, with smooth and round edges. They are slightly raised in medium containing carbon and nitrogen. Colonies may remain viable for a long time when stored in peptone broth (Gast, 1997). Biochemically, *Salmonella* strains have the ability to catabolize nutrients, and catabolize D-glucose and other carbohydrates, except lactose and sucrose, with production of acid and gas. They are catalase positive and oxidase negative, they do not ferment malonate, do not hydrolyze urea and do not produce indole, they can use citrate as a sole source of carbon, reduce nitrate to nitrite, and may produce hydrogen sulfide (Quinn *et al*., 2002). The bacterium itself is surrounded by a mucus layer, which contributes to its resistance to phagocyte digestion, and has a fringe of fimbria located around its outer surface that are used in cell adhesion (Hirsh *et al*., 2004; Quinn *et al*., 2002). They belong to the bacterial family *Enterobacteriaceae*, which along with other major pathogens in this group are often attributed to causing illness within the small intestine, from which the bacteria can migrate and result in progression to full systemic body disease (Hirsh *et al*., 2004).

The genus *Salmonella* comprises two species, *Salmonella bongori* and *Salmonella enterica.* Within *Salmonella enterica* there are six subspecies: *Salmonella enterica* subspecies *enterica* (I), *Salmonella enterica* subspecies *salamae* (II), *Salmonella enterica* subspecies *arizonae* (IIIa), *Salmonella enterica* subspecies *diarizonae* (IIIb), *Salmonella enterica* subspecies *houtenae* (IV) and *Salmonella enterica* subspecies *indica* (VI) (Solari *et al.,* 2003). These

subspecies can be further classified into approximately 50 serogroups based on their lipopolysaccharide (LPS) O antigen component (Sabbagh *et al*., 2010). *Salmonella bongori* and most subspecies of *Salmonella enterica* colonize the environment of cold-blooded animals and in some cases can cause disease in these animals. However, the most biomedical relevant subspecies is *S. enterica* subspecies *enterica*, whose serovars have special clinical significance in both veterinary and human diseases (Brenner *et al*., 2000). *Salmonella enterica* subspecies *enterica* can be further divided into over 2500 serovars based on their flagellar (H) antigen and lipopolysacharide (LPS) (O) antigen structures (Sabbagh *et al*., 2010; Coburn *et al*., 2007; Tindall *et al*., 2005; Brenner *et al*., 2000).

2.3.0 Host adaptability of *Salmonella*

In spite of their close genetic relationship, there are significant differences in virulence, host adaptation, and host specificity between strains belonging to different *S. enterica* subspecies (I) serovars (Uzzau *et al*., 2000). There is a large body of epidemiologic data on the host specificity of different *S. enterica* subspecies (I) serovars, some serovars show preferences for certain hosts but are not entirely restricted to them. The serovars *paratyphi A, gallinarum* and *pullorum* are restricted to specific hosts. The serovar *paratyphi A* causes a systemic disease (paratyphoid) in humans (Uzzau *et al.*, 2000), the serovar *pullorum* causes systemic pullorum disease in poultry especially in freshly hatched chicks where high mortality and intestinal inflammation are recorded (Buxton and Fraser, 1977) and the serovar *gallinarum* causes the severe systemic fowl typhoid disease in poultry and a few other avian species (Barrow *et al*., 1994). The serovar *dublin* is adapted to cattle, where it causes systemic and

enteric disease. Infrequently, the serovar *dublin* causes septicemia and enteric disease in humans (Kingsley and Baumler, 2000).

Serovars *typhimurium* and *enteritidis* infect a broad range of host animals. Interestingly, they cause different diseases in different animal species. In calves, serovar *typhimurium* and rarely *enteritidis* (Petrie *et al*., 1977) causes enterocolitis and the animals can succumb to dehydration (Tsolis *et al*., 1999; Watson *et al*., 1998). In newly hatched chicks, serovars *enteritidis* and *typhimurium* cause systemic disease and diarrhea, whereas older chickens are asymptomatic carriers (Beal *et al*., 2004; Withanage**,** 2004). In immunocompetent humans, serovars *enteritidis* and *typhimurium* cause localized self-limiting enterocolitis. Systemic disease may develop in immunocompromised individuals (Mastroeni *et al*., 2003). Serovars *enteritidis* and *typhimurium* also cause a systemic typhoid fever-like disease (Tsolis *et al*., 1999; Baumler *et al*., 1998) in susceptible mouse strains, but no diarrhea. The mechanisms determining which type of disease is caused and in which host, by serovars *enteritidis* and *typhimurium* are still poorly understood.

2.4.0 Salmonellosis and public health

Salmonellosis is an important global public health problem causing substantial morbidity. In spite of the improvement in hygiene, food processing, education of food handlers and information to the consumers, food borne diseases still dominate as the most important public health problem in most countries. There are reports of foodborne salmonellosis in humans since the 19th century, caused by the ingestion of contaminated bovine meat (Barrow, 1993). It is one of the most problematic zoonosis in terms of public health all over the world

because of the high endemicity and its difficulty to control (Antunes *et al*., 2003; Santos *et al*., 2002) and the resulting significant morbidity and mortality rates (Cardoso *et al*., 2002). *Salmonella* is one of the most important agents involved in food borne outbreaks reported in several countries (Tessari *et al*., 2003), accounting for 19% in the fresh and frozen poultry products in South Africa (Nierop *et al*., 2005), 3.1% and 2.8% in chicken and turkey meat respectively in Ireland (Jordan *et al*., 2006), 6.5% in Albania (Beli *et al*., 2001), 60% in Portugal (Antunes *et al*., 2003) and 49% in Spain (Capita *et al*., 2003). In a study carried out by Hang'ombe *et al*., (1999a), the occurrence of *S. enteritidis* was elucidated in pooled table eggs and market-ready chicken carcasses in Zambia. Apart from the food borne transmission of infection, the other major epidemiological development in salmonellosis is the emergence of multiple antibiotic resistant *Salmonella* strains, particularly in the developing countries (Okeke *et al*., 2005). In the past, the main motivations for controlling *Salmonella* infections in poultry were the losses caused by clinical (pullorum disease and fowl typhoid) and subclinical diseases (paratyphoid infections) (Calnek, 1997). Nowadays, due to the public health implications, prevention of foodborne transmission of *Salmonella* is a priority for the poultry sector (Oliveira and Silva, 2000). Apart from being foodborne, each year infections are also acquired through direct or indirect animal contact in homes, veterinary clinics, zoological gardens, farm environments or other public, professional or private settings (Hoelzer *et al*., 2011). Therefore, contacts with dogs in homes, veterinary clinics and shelters clearly represent potential threats to human health. Even though dog to human transmission of *Salmonella* has been recorded (Sato *et al*., 2000; Morse and Duncan, 1975), the potential public health risks associated with companion animals such as dogs are difficult to quantify.

2.5.0 Economic importance of Salmonellosis

Historically, *Salmonella typhimurium* was the most common agent of foodborne disease in humans. However, in the past decades, *Salmonella enteritidis* has been most frequently involved in salmonellosis outbreaks (Berchieri and Freitas, 2009). It causes millions of dollars in losses to the industry, mainly in cattle, swine and poultry production, both in local and international trade. Salmonellosis represents a significant cost to society in many countries. Very few countries though report data on economic cost of the disease. Estimated costs of medical expenses, sick leaves and loss of productivity related to the high incidence of salmonellosis in the US range from US$1.3 to US$4.0 billion a year (Taitt *et al*., 2004). In Denmark, the annual estimated cost of food borne salmonellosis was US$ 15.5 million in 2001, representing approximately 0.01% of the country's Gross Domestic Products (GDP) while data related to the cost of food borne disease are generally not available from developing countries, Zambia included (WHO, 2005).

2.6.0 Salmonellosis in humans

Within *Salmonella enterica* subspecies I (*Salmonella enterica* subspecies *entericae*), the most common O-antigen serogroups are A, B, C1, C2, D and E. Strains within these serogroups cause approximately 99% of *Salmonella* infections in humans and other warm-blooded animals (Velge *et al.,* 2005). In high-income countries, non-typhoidal *Salmonella* (NTS) predominantly cause a self-limiting diarrheal illness in healthy individuals while bloodstream or focal infections are rare and mainly happen in individuals with specific risk factors (Gordon, 2008). By contrast, in sub-Saharan Africa, NTS are consistently the most common bacterial bloodstream isolates in both adults and children presenting with fever

(Reddy *et al*., 2010; Sigaúque *et al*., 2009; Gordon *et al*., 2008; Gilks *et al*., 1990) and case fatality estimates for invasive NTS disease among hospitalized patients in Africa have been in the range of 4.4% to 27% for children (Enwere *et al*., 2006) and 22% to 47% for adults (Gordon *et al*., 2008). Invasive, recurrent, and multiple-site NTS infections are seen in adults with advanced HIV disease and in African countries with a high prevalence of HIV infection. NTS isolates have become the most common blood culture isolates among febrile adults admitted to the hospital (Gordon *et al*., 2001; Archibald *et al*., 1998; Gilks *et al.*, 1990). Clinical associations have been consistently noted with anemia, malaria, malnutrition and more recently, HIV infection (Berkley *et al*., 2005).

In rural Kenya, the annual estimated minimum incidence of bacteremia was 505 cases per 100,000 persons in the age group of less than 5 years old, of which 88 cases per 100,000 persons were NTS bacteremia (Susan *et al*., 2009). In rural Mozambique, the annual incidence of childhood bacteremia was 425 cases per 100,000 persons among children aged less than 15 years, and within this category, NTS annual incidence accounted for 120 cases per 100,000 persons (Sigauque *et al*., 2009). In the Gambia, an incidence of NTS bacteremia of 262 cases per 100,000 persons among children aged 29 months was demonstrated (Enwere, 2006). In Southern Africa, salmonellosis may be considered as a serious public health problem as well as being a frequently occurring zoonosis. Human carrier rates of 4.3%, 8.08%, 29.3%, 44% and 72% in different geographical areas of South Africa were reported (Meara, 1973).

In a study carried out in Lusaka, Zambia, to detect *Salmonella* from human samples, out of the 200 clinical stool samples, 9 (4.5%) were found to be bacteriological culture positive for S*almonella* (Hang'ombe *et al*., 2011). Mwansa *et al*., (2002) reported that of 124 adults and 105 children with persistent diarrhea in Zambia, 6 (5%) and 21 (20%) were infected with non-typhoidal *Salmonella* spp., respectively. In a study by Chintu *et al*., (1993), *Salmonella* spp. was found present at 1% among enteric pathogens identified in 73 patients with chronic diarrhea. And in an earlier study at the University Teaching Hospital, Lusaka, Zambia, 45 strains of various NTS species were isolated from stool samples, blood and Cerebral Spinal Fluid (CSF). About 93% of strains were isolated from infants less than two years old. *S. heidelberg* was the most common species isolated from stool and revealed a multi-drug resistant character. Only *S. typhimurium* and *S. bovis-morbificans* were isolated from blood and CSF (Dube and Bhagwat, 1983). The above findings show the significance of *Salmonella* as an important pathogen in Zambia with potential of causing serious impact on human health leading to morbidity and/or death.

2.7.0 Salmonellosis in animals

A number of animal species including ruminants, carnivores, birds and reptiles can play a major role as carriers in the spread of *Salmonella* and transmit them to other healthy animals and humans (Sanchez *et al*., 2002). Salmonellosis in animals is characterized clinically by one or more of 3 major syndromes; septicemia, acute enteritis and chronic enteritis. The incidence has increased with the intensification of livestock production. Young calves, piglets, lambs, and foals usually develop the septicemic form. Adult cattle, sheep, and horses commonly develop acute enteritis, and chronic enteritis may develop in growing pigs and

occasionally in cattle. Pregnant animals may abort (Zeiko, 2011). The clinically normal carrier animal is a serious problem in all host species. While many other *Salmonella* spp. may cause disease, an example of the more common ones in each species are as indicated in Table 1.

Table 1: Examples of some of the most common *Salmonella* subspecies I serotypes in selected animal species (CDC, 2009).

Natural host	*Salmonella* serotype.
Cattle	*typhimurium, dublin, newport, enteritidis, hadar, infantis, stanley, paratypi B*
Dogs	*typhimurium, heidelberg, kentucky, anatum, newport, paratyphi B, derby, enteritidis*
Horses	*typhimurium, anatum, newport, enteritidis, abortus, equi, paratyphi B, derby*
Humans	*typhi, typhimurium, anatum, infantis, newport, enteritidis, abortus, equi, paratyphi B, derby, enteritidis*
Sheep and Goats	*typhimurium, dublin, anatum, montevideo, paratyphi B, derby, enteritidis*
Pigs	*typhimurium, paratyphi B, choleraesuis, abortus, ovis derby, infantis, anatum, enteritidis*
Poultry	*typhimurium, enteritidis, gallinarum, pullorum*
Snakes	*typhimurium, anatum, newport, enteritidis, abortus, equi, paratyphi B, derby, enteritidis, javiana*
Fish	*newport, paratyphi B, paratyphi B*

Dogs generally seem to be resistant to *Salmonella* infection and most cases are latent and non-clinical (Kozak *et al*., 2003). Dogs can harbour many serovars of *Salmonella* (Morse and Duncan, 1975). The estimated prevalence of *Salmonella* organisms in normal, healthy dogs is 1% to 36% (Sanchez *et al*., 2002). Ojo and Adetosoye (2009) in their study of *Salmonella* infection in diarrheic and non-diarrheic dogs in Ibadan, Nigeria found the incidence of *Salmonella* in faeces to be 3.7%. Clinical cases of salmonellosis have been reported in dogs which if severe, can result in diarrhea, vomiting, fever, depression, abortion and death (Morse and Duncan, 1975). In Southern Africa, the situation is less clear with regard to companion animals. No outbreaks of clinical salmonellosis had previously been recorded among dogs contrary to what has been found in other countries. There is furthermore scanty data available on the carrier rate among dogs.

In Zambia, the dog population is generally unknown and remains a source of concern, although some districts and towns have some estimates. However, according to record compilation by the District Veterinary Office, Lusaka, for the number of dogs that were vaccinated against Rabies in 2009, the office recorded 2,069 (Personal communication, Dr. M. C. Kanemanema, DVO, Lusaka). The University of Zambia Veterinary Clinic recorded a total of 2,721 dogs that were vaccinated against Rabies for the year 2009 (unpublished data). These figures however, are not indicative of the total dog population in Lusaka urban as figures of other dogs vaccinated from various veterinary clinics within Lusaka may not have been captured. In Zambia, information on status of *Salmonella* in dogs as well as the implication of dogs as sources of *Salmonella* in humans is lacking as no previous studies in this area have been undertaken.

2.8.0 Salmonellosis in poultry

Worldwide, birds are kept for meat or egg production, companionship, sports, scientific or educational purposes. In 2007, an estimated 17.9 billion chickens, 1.1 billion ducks, 447 million turkeys and 343 million geese and guinea fowl were farmed world-wide (Tomley and Shirley, 2009). Although potentially all livestock animals can be infected with *Salmonella* and therefore pose a risk for humans, poultry-derived products, particularly chicken eggs, are considered a major source of human infection with *Salmonella* (Hald *et al*., 2004). *Salmonella* prevalence varies considerably by poultry type, differs between serotypes and biovars, and intestinal carriage often appears to be lower than isolation rates from egg shells, dead birds, and environmental samples. *Salmonella* prevalence in hatcheries is estimated between 0 and 17% for chickens, compared to approximately 25% for geese, and 20 to 60% for ducks (Chao *et al*., 2007; Bailey *et al*., 1994). The reported prevalence of *Salmonella* in chicken carcasses in South Asian countries varies from country to country. Studies in northern Thailand revealed 57% prevalence in chicken meat at the market during the period 2002 to 2003 (Padungtod and Kaneene, 2006), 14.5% prevalence in Kathmandu, Nepal (Maharjan *et al*., 2006), and 42.6% prevalence in Ho chi Minh city, Vietnam (Bao, 2005). Sero-prevalence of poultry *Salmonella* in Bangladesh has been reported to be 23.5% (Sikder *et al*., 2005).

Chickens can be infected with many different serovars of *Salmonella*. Of these, *S. enterica* serovars *pullorum* and *gallinarum*, are host specific and represent a major concern for the poultry industry but have no impact on public health. Other *S. enterica* serovars frequently

isolated from chickens, such as *typhimurium*, *kentucky*, *enteritidis* and *heidelberg*, can infect a wider range of hosts and frequently reach the human food chain, posing an important risk for human health. *Salmonella* biovars *pullorum* and *gallinarum* have been eradicated in commercial poultry productions in the developed world, but are still important in backyard flocks as well as the developing world (Lutful, 2010).

Poultry production has steadily expanded in Zambia from an estimated 16 million birds in 2000 to about 26 million in 2008. It is estimated that 1.3 million chickens and six millions eggs were produced every month in 2008 (CSO, 2008). Reports of avian salmonellosis in Zambia date back to 1931 when four outbreaks of fowl typhoid were reported in native breed fowls (Gasper and Harbeta, 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. typhimurium, S. heidelberg, S. infantis, S. kaufmanii, S. kisarawe, S. orarnienberg, S. weltverden, S. worthington, S. agona, S. schwarzengrund, S. aloma, S. haifa, S. virginia* and *S. rubislaw* (Tuchili *et al*., 1996). In a study carried out by Kabilika *et al*., (1999), the incidence of *Salmonella enteritidis* was demonstrated in dead-in-shell chicken embryos, which clearly indicated the possibility of infection in the breeding flocks in Zambia. In another study, Hang'ombe *et al*., (1999b) found the frequency of *Salmonella* spp. from broiler carcasses to be 20.5% and the occurrence of *Salmonella enteritidis* in pooled table eggs and market-ready chicken carcasses to be 3.1% and 4.7%, respectively. The importance of chicken meat as a vehicle for human salmonellosis has been highlighted by national surveys in Zambia which reveal that many of the *Salmonella* serovars that can be recovered

from chicken meat can also be found in infected humans (Hang'ombe *et al*., 1999a; Tuchili *et al*., 1996).

2.9.0 Transmission patterns of *Salmonella*

Animals are a primary reservoir for NTS associated with human infections. The main source of human *Salmonella* infections is poultry-derived food, mainly eggs and egg products and chicken meat (EFSA, 2006). Faeces of nearly all animal species may be potential sources of *Salmonella*; therefore, the zoonotic transmission of *Salmonella* is not limited to food animals alone. Pets, especially dogs that have close interaction with humans, may be responsible for *Salmonella* transmission. Veterinarians and public health officials have recognized the shedding of *Salmonella* as a source of infections for dog handlers, dog owners and their communities (Kozak *et al*., 2003; Sanchez *et al*., 2002). Infected dogs may remain carriers and fecal shedders and thus serve as sources of *Salmonella* for man and other animals (Kozak *et al*., 2003). Contact with sick livestock is not an uncommon method of exposure for farm workers. Although these sources are not usually responsible for large outbreaks, they may be responsible for sporadic cases. The CDC reported several outbreaks of multidrug-resistant *S. typhimurium* infection associated with veterinary facilities (CDC, 2001).

Fecal-oral transmission from person to person in areas with poor sanitation and contaminated water is the route for enteric or typhoid fever. Humans are the only known carriers of *Salmonella typhi* (Ryan and Ray, 2004). The broad host-range *Salmonella* serovars are prevalent within warm-blooded animal populations that make up the human food supply, and bacterial transmission generally results from consumption of raw or undercooked food

products (Jones, 2005), with poultry being one of the most important reservoirs of *Salmonella* species.

In dog salmonellosis, various potential sources of *Salmonella* have been cited. Raw meat obtained from rendering plants and used to feed dogs can be contaminated with *Salmonella* and have been associated with canine salmonellosis (Cantor *et al*., 1997; Galton *et al.*, 1955). In addition to raw meat, these diets may contain vegetables, grains and fruits which may also include ground bones and are served raw to dogs as their main meal (Moon, 2003). Dogs may also access the organism from environmental exposure such as garbage, untreated sewage, and contaminated kennels or hospitals (Tsai *et al*., 2007). A considerable number of *Salmonella* serotypes have been isolated from domestic dogs around the world. Scavenging dogs exposed to many contaminated materials are likely to harbor more *Salmonella* serovars than non-scavengers kept under hygienic conditions.

In chickens the main route of transmission of *Salmonella* is vertical by the egg. Infection of breeder flocks with *Salmonella* leads to a rapid dissemination of the organism to progeny broiler and commercial egg laying flocks. *Salmonella* is also spread between birds horizontally by the fecal-oral route. The bacterium survives for long periods in the environment and has been isolated from litter and dust in poultry houses (Lister, 1988).

2.10.0 Clinical signs of Salmonellosis

With respect to human disease, *Salmonella* serotypes can be divided into three groups that cause distinctive clinical syndromes: typhoid fever, bacteremia and enteritis (Santos *et al*.,

2002). The NTS serotypes can cause protean manifestations in humans, including acute gastroenteritis, bacteremia and extraintestinal localized infections involving many organs (Chiu *et al*., 2004). The clinical course of human salmonellosis is usually characterized by acute onset of fever, abdominal pain, diarrhoea, nausea and sometimes vomiting. In some cases, particularly in the very young and in the elderly, the associated dehydration can become severe and life-threatening. In such cases, as well as in cases where *Salmonella* causes bloodstream infection, effective antimicrobials are essential drugs for treatment. Serious complications occur in a small proportion of cases. Although outbreaks usually attract media attention, studies indicate that more than 80% of all salmonellosis cases occur individually rather than as outbreaks (WHO, 2005). Typhoid/enteric fever is characterized by the sudden onset of a sustained and systemic fever, severe headache, nausea and loss of appetite. Other symptoms include constipation or diarrhea, enlargement of the spleen, possible development of meningitis or general malaise. Untreated typhoid fever cases result in mortality rates ranging from 12 to 30% while treated cases allow for 99% survival.

Salmonellosis in animals is characterized clinically by either septicemia, acute enteritis or chronic enteritis. However, the clinically normal carrier animal is a serious problem in all host species. In dog salmonellosis clinical signs include hyperpyrexia, anorexia, diarrhea, bloody stools, weight loss, abdominal pain, diarrhea, abortion and death (Morse and Duncan, 1975). Cardiovascular collapse and shock can occur, as can systemic infection, generally in pediatric or immunocompromised patients (Marks and Kather, 2003; Greene, 1998). The acute phase of the infection may last from four to ten days. Chronic intermittent diarrhea for three or four weeks may be the sequel. Probably the mortality in clinical cases does not

exceed ten percent. It should be emphasized, however, that most *Salmonella*-infected dogs are asymptomatic and are long term, sporadic fecal shedders of the pathogen (Sanchez *et al*., 2002; Morse *et al*., 1976).

In poultry, salmonellosis can occur as a severe systemic disease responsible for heavy economic losses to the commercial poultry industry through morbidity, mortality and reduced egg production (Barrow *et al*., 1989). A good example is *S. enteritidis* which may produce a clinical disease in chickens of up to six weeks of age and occasionally in adult laying birds, showing signs of profuse watery diarrhea and dehydration in acute cases, labored breathing, pasting of vent and huddling together but also transferable to human causing gastroenteritis, enteric /typhoid fever and diarrhea (Anjun, 1997). Mortality may be high in chicks under one week of age. Older chicks may show uneven growth and stunting. This could lead to birds being rejected at slaughter with lesions of pericarditis, perihepatitis, perinephritis and septicaemia (Lister, 1988).

2.11.0 Molecular pathobiology of *Salmonella*

Salmonella are capable of producing serious infections that are often foodborne and present as gastroenteritis. However, a small percentage of these infections may become invasive and result in bacteremia and serious extra intestinal disease (Fluit, 2005). Some infectious disease texts recognize three clinical forms of salmonellosis that include gastroenteritis, septicemia and enteric fevers. Here the focus is on the two extremes of the clinical spectrum: gastroenteritis and enteric fever.

2.11.1 Enteritis and septicemia due to *Salmonella*

Non-human-adapted serovars commonly cause enteritis. Following ingestion of contaminated food or water, the pathogenesis of both typhoid and enteritis begins with an intestinal phase, while only typhoid progresses to a systemic phase. The intestinal phase involves colonization of the intestine and penetration of the intestinal epithelium through separate mechanisms. The first involves active bacterial invasion (Jones *et al*., 1994), while the second involves passive uptake of *Salmonella* (Rescigno *et al*., 2001). To initiate disease in the gastrointestinal tract, *Salmonella* must adapt to the hypoxic, acidic and alkaline environments that they encounter en route from the stomach to the small intestine. The bacteria must then swim through mucin overlying the enterocytes, attach themselves to the intestinal columnar epithelial or specialized M-cells overlying Peyer's patches to invade the host epithelial cells. Host adaptation exhibited by *Salmonella* serovars is believed to be caused, in part, by the distribution of specific adhesins among *Salmonella* serovars that are involved in the organism's colonization of the gastrointestinal tract (Baumler *et al*., 1997).

Salmonella organisms exist as intracellular pathogens in diseased animal hosts. *Salmonella* organisms produce hair-like projections, resembling pilli, which upon contact with epithelial cells "inject" bacterial proteins into the host cell's cytoplasm (Kubori *et al*., 2000). This event triggers a signal transduction cascade that results in the transient, disassembly, and reorganization of the cytoskeleton and uptake of *Salmonella* organisms by enterocytes or M cells (Galan and Zhou, 2000). Following internalization, *Salmonella* are confined to endocytic vacuoles, but these infected vacuoles move from the apical to the basal surfaces of the host cell releasing *Salmonella* organisms to the submucosal compartment (Boyd *et al*.,

1997). Successful pathogenesis of *Salmonella* requires sophisticated virulence factors which may be genetic, biochemical or structural features that enable it to produce disease in a host **(**Rychlik and Barrow, 2005).

The septicemic form of *Salmonella* infection can be an intermediate stage of infection in which the patient is not experiencing intestinal symptoms and the bacteria cannot be isolated from fecal specimens. The severity of the infection and whether it remains localized in the intestine or disseminates to the bloodstream may depend on the resistance of the patient and the virulence of the *Salmonella* isolate (Giannella, 1996). Several translocated *Salmonella* pathogenicity island 1 (SPI-1) proteins contribute to intestinal inflammation, fluid secretion and diarrhea by disrupting the epithelial barrier and increasing water flux by an exudative mechanism. Innate immune system activation also contributes to intestinal inflammation. The organisms have virulence factors such as virulence-plasmids, toxins, fimbriae and flagella that help in establishing an infection (Alphons *et al.,* 2005).

Human-adapted serovars of *Salmonella* cause typhoid fever, a systemic and life-threatening infection. For *Salmonella* organisms to cause systemic infection, the organism must resist being killed by the White Blood Cells. *Salmonella* does not resist phagocytosis but adapts to the hostile milieu of the phagolysosome. It is the outcome of this interaction between phagocyte and microbe that determines whether the disease develops into a potentially fatal enteric fever (Henderson *et al*., 1999). After invading M cells of the Peyer's patches in the intestine (Jones *et al.*, 1994), the bacteria migrate to the local mesenteric lymph nodes and then to the spleen and liver via phagocytic cells, resulting in the spread of the disease (Ohl

and Miller, 2001; Marcus *et al*., 2000). In order for *Salmonella* spp. to initiate both gastroenteritis and systemic infection, a wide variety of virulence factors are required. *Salmonella* invasion genes are organized into contiguous and functionally related loci in the bacterial chromosome. These genes are evolutionarily conserved among *Salmonella* species, making them useful targets in polymerase chain reaction-based detection schemes for *Salmonella* (Kubori *et al*., 2000).

2.11.2 Virulence determinants of *Salmonella*

Salmonella express a variety of virulence factors which mediate the organism's pathogenicity. These factors may include polymorphic surface carbohydrates, multiple fimbrial adhesins, phase-variable flagella and well structured mechanisms for invasion and survival in host macrophages and other cells. There are approximately 200 genes including those on the five chromosomal pathogenicity islands (SPI-1 to SPI-5) on *Salmonella* chromosomes that are essential for virulence. A pathogenicity island is a genetic element that occurs as a distinct and separate unit in the bacterial chromosome (Hensel, 2004; Hentschel and Hacker, 2001). All pathogenicity islands share a few common features such as not being found in a closely related, nonpathogenic reference species or strain, often encompassing large DNA regions (10-200 kB) that contain genes which often confer virulence to bacteria. Furthermore they have Guanine Cytocine content lower than the rest of the bacterial genome and are inserted into tRNA sites, where they are often flanked by short sequences (direct repeats). They are also usually associated with elements like inverted repeats, transposases and integrases (van Asten and van Dijk, 2005; Hacker and Kaper, 2000; Marcus *et al.*, 2000). SPI-1 and SPI-2 encode two of the type III secretion systems that facilitate invasion by

Salmonella species. Although SPI-2 is conserved among and specific to *Salmonella* spp., it is not present in all of the *Salmonella* subspecies groups (Hensel *et al.*, 1997a; 1997b; Ochman and Groisman, 1996). While SPI-1 is necessary for bacterial invasion and internalisation, SPI2 is required for establishment of systemic infection and intracellular replication (Paesold *et al.*, 2002; Ohl and Miller, 2001; Hensel *et al.*, 1997b; Ochman and Groisman, 1996). Many of the genes required for intestinal penetration and invasion of host cells are carried on the 40-kb region at centisome 63 of the *Salmonella* pathogenicity island 1 (Mills and Finlay, 1994). In order for *Salmonella* spp. to cause disease, the presence of an intact and functional SPI1 is required. Ginnocchio *et al.*, (1997) found that environmental isolates of *Salmonella* carrying naturally occurring deletions in the SPI-1 region lack the ability to invade mammalian cells. SPI-1 contains at least 37 genes, which encode various components of the type III secretion systems (T3SSs), its regulators and its secreted effectors that give *Salmonella* isolates the ability to colonise and invade intestinal epithelial cells and transport virulent effector proteins from the bacteria into the host cell cytosol (Hansen-Wester and Hensel, 2001; Ohl and Miller, 2001; Marcus *et al.*, 2000).

Following invasion of host cells, SPI-2 genes are expressed and are necessary for intracellular survival of *Salmonella* in both epithelial cells and macrophages (Haimovich and Venkatesan, 2006). Mutants lacking SPI-2 genes resulted in a reduction of virulence and the inability to colonize the spleens of infected animals (Anthea *et al*., 2000). Some of the secreted effector proteins include *sipA, sipB, sipC, sifA, hilA, hilC, hilD invA, spiC* and *invF* (Reffatelu *et al.*, 2005; Takaya *et al*., 2005). These clusters of chromosomal virulence genes

are found only within the genus *Salmonella* (Amavisit *et al*., 2003). A summary of these genes and their possible significance in *Salmonella* pathogenicity is indicated in Table 2.

Table 2: *Salmonella* secreted effector proteins and their possible significance in disease

Salmonella gene	Possible significance	Reference
invA	necessary for invasion of epithelial cells	Darwin and Miller, 1999a; Rahn *et al*., 1992
invF	chaperone protein	Darwin and Miller, 2000
sipC	translocase mediating bacteria entry into epithelial cells	Hayward and Koronakis, 1999
hilA	central transcriptional regulator of the invasion genes	Darwin and Miller, 2000
hilC	modulate invasion gene expression	Schechter and Lee, 2000
hilD	modulate invasion gene expression	Schechter and Lee, 2000
spiC	modulate invasion gene expression	Schechter and Lee, 2000
sipA	interferes with host cell actin cytoskeleton	Zhou *et al*., 1999
sipB	involved in the translocation process of effector proteins	Darwin and Miller, 1999
sifA	recruitment of lysosome-associated membrane protein 1 (LAMP1), membrane growth, and maintenance of the SCV.	Stein *et al*., 1996; Garcia del-Portillo *et al*., 1993

In vitro characterization of *hilA, hilC, hilD* and *invF* suggests that these regulators operate in a hierarchy to modulate invasion gene expression with *hilA* acting as the central regulator in this cascade. The *hilA*, is required for expression of SPI-1 invasion genes (Schechter and Lee, 2000). The *invA* gene codes for proteins in inner membranes of bacteria which are necessary for invasion of epithelial cells (Darwin and Miller, 1999a). The *invF* and its putative chaperone protein, *sicA* (along with *hilA*) is responsible for activating and controlling the expression of TTS-associated proteins encoded within and outside of SPI-1 (Darwin and Miller, 2000; Eichelberg and Galan, 1999). The secreted effector s*ipA* together with s*opA, sopB, sopD,* and s*opE* act in concert to induce diarrhea (Zhang *et al*., 2002), whereas the s*ipB* protein has been identified as the bacterial effector responsible for induction of apoptosis (Hersh *et al.*, 1999). The s*ipC* acts as a translocase, mediating bacterial entry into epithelial cells and is also translocated into the host cytosol (Hayward and Koronakis, 1999). It has been suggested that *sipA,* along with the proteins *sipB* and s*ipC* form a translocation complex that delivers effector proteins into the host cells (Zhang *et al.,* 2003; Cherayil *et al.,* 2000; Darwin and Miller, 1999b).

Salmonella bacteria containing mutations in these genes have been shown to be attenuated in mice infected orally but fully virulent after intraperitoneal administration, presumably because of their inability to gain access to the intestinal epithelium (Galan and Curtiss, 1989) and a variety of environmental and regulatory factors affect *hilA* expression, which in turn affects the invasion phenotype of *Salmonella* strains. It has been hypothesised that an unidentified repressor protein binds to an upstream repressing sequence (URS) identified in

the *hilA* promoter region to stop *hilA* expression under conditions unfavourable for bacterial invasion (Schechter *et al.*, 1999). The proteins of *hilC* and *hilD* have been demonstrated to depress *hilA* expression by binding directly to the URS in the *hilA* promoter region (Schechter *et al.*, 1999; Schechter and Lee, 2001). While mutation in *hilC* only modestly affects bacterial invasion, it has been shown that the presence of a functional *hilD* is essential for the expression of *hilA*, highlighting the minor and major role these genes play in SPI 1 regulation (Boddicker *et al.*, 2003). SPI-1 expression is a complex series of interactions between different genes in response to the host environment, allowing *Salmonella* species to initiate invasion and bacterial internalization.

2.12.0 Diagnosis of *Salmonellosis*

It is important that an infection by *Salmonella* is confirmed before any treatment is undertaken. Diagnosis can be confirmed by bacteriological tests such as culture and isolation. Furthermore, *Salmonella* isolates can be differentiated from one another in a wide variety of ways and the number of *Salmonella* species continues to increase. Epidemiologically, it is important to be able to distinguish *Salmonella* isolates, because definitive typing of *Salmonella* isolates may assist in tracing the source of an outbreak and monitoring trends in antimicrobial resistance associated with a particular type (Yan *et al.*, 2003).

In addition to the conventional antigen-based serotyping, there are advanced techniques for *Salmonella* typing currently being used to enhance the tracing of the individual isolates and to understand the virulence characteristics of the organisms. The following techniques are currently available for *Salmonella* diagnosis:

(a) Serotyping

(b) Phage typing

(c) Molecular typing that may include restriction enzyme digestion, amplification and nucleotide sequencing based techniques.

2.12.1 Culture and isolation of *Salmonella*

The conventional technique for the detection of the *Salmonella* microorganism includes the following steps: pre-enrichment, selective enrichment, isolation and selection, biochemical characterization, serological characterization and final identification. This technique requires at least four days for a negative result and six to seven days for the identification and confirmation of positive samples (Soumet *et al.*, 1997).

2.12.2 Serotyping of *Salmonella*

Conventional serotyping of *Salmonella* is the most commonly used method to differentiate strains (Yan *et al*., 2003). *Salmonella* isolates are serotyped based on the antigenic properties of three surface structures from the lipopolysaccharide (LPS) layer of the cell wall; the O (somatic), H (flagellar) and Vi (capsular) antigens (Nataro *et al.*, 2007). Many *Salmonella* show diphasic production of flagellar antigens and each strain can spontaneously and reversibly vary between these two phases with different sets of H antigens. In phase I or the specific phase, the different antigens are designated by small letters, and in phase 2 or the group phase, the antigens first discovered are numbered. In a single cell, usually only one antigen is expressed at a time (Yan *et al*., 2003). *Salmonella* serotyping has been invaluable in epidemiological investigations of disease outbreaks (Ethelberg *et al.*, 2004; Uesugi *et al.*,

2007). Since *Salmonella* serotyping is done worldwide, this has assisted in the surveillance and identification of international outbreaks (Nataro *et al.*, 2007). Conventional serotyping using the auto-agglutination method however has some limitations, such as detection of Vi antigens (Wain *et al*., 2005) and strains which are not typeable (Rasschaert *et al*., 2005). This allows detection of a single antigen-antibody reaction at a time and requires well-experienced technologists to perform. It also consumes relatively high amount of reagents and takes a longer time (Cai *et al*., 2005).

2.12.3 Rapid methods for *Salmonella* identification

Rapid immunological identification and confirmation tests based on latex agglutination, enzyme immunoassay (EIA) and enzyme-linked immunosorbent assay (ELISA) have been developed for *Salmonella*, and simple-to-use lateral flow test strips using immunochromatographic technology have also been developed. Molecular methods are also available, notably DNA hybridization and Polymerase chain reaction (PCR) assays for the identification of *Salmonella enterica*. PCR testing utilizes gene-specific primers to amplify and examine variation between strains (Millemann *et al.*, 2000; Kardos *et al.*, 2007) and offers the possibility to improve detection and characterization of pathogenic bacteria, since one can target species-specific DNA regions and specific traits of pathogenicity, especially genes coding for toxins, virulence factors, or major antigens. Studies of gene expression are extremely important to the understanding of numerous bacterial processes including virulence (Krieg and Holt, 1984).

Other typing methods that have been used to investigate and study *Salmonella* strains include the use of antibiotic resistance genes as epidemiological markers (Bessa *et al.*, 2007; Matsumoto *et al.*, 2006; Ward *et al.*, 2005), multilocus sequence typing, which analyses the DNA sequences of a series of housekeeping, ribosomal and/or virulent genes (Foley *et al.*, 2006; Kotetishvili *et al.*, 2002), multilocus variable-number tandem repeats analysis, which makes use of short sequence repeat motifs as a target to type isolates (Lindstedt *et al.*, 2004) and multiple amplification of phage typing locus, which detects presence/absence of a range of bacteriophage in the genome (Ross *et al.*, 2008; Ross and Heuzenroeder, 2005). In this study, virulence genes were targeted for detection of *Salmonella* isolates of human, poultry and dog origin in Lusaka, Zambia

2.13.0 Prevention, control and treatment of salmonellosis

Salmonella are difficult to eradicate from the environment. However, because the major reservoirs for human infection are poultry and livestock, reducing the number of *Salmonella* harbored in these animals would significantly reduce human exposure. Treatment of animal feeds to kill *Salmonella* before distribution may result in a marked reduction in salmonellosis. Changing animal slaughtering practices to reduce cross-contamination of animal carcasses; protecting processed foods from contamination; providing training in hygienic practices for all food-handling personnel in slaughterhouses, food processing plants, and restaurants; cooking and refrigerating foods adequately in food processing plants, restaurants, and homes; and introducing or expanding of governmental enteric disease surveillance programs are some of the ways by which salmonellosis could be prevented and controlled.

Prevention of salmonellosis in dogs can be done by ensuring that food sources are free from infection and stored correctly. Prevention of transmission from dogs shedding *Salmonella* in their faeces to other animals and humans should be considered by means of hygienic measures and careful disposal of faeces. Dogs may pose a risk to humans where close contact between dogs and their owners occurs in households. Children and immunosuppressed individuals may be particularly at risk (Sato *et al.*, 2000). However, the same contaminated food sources may infect coincidentally both dogs and humans. Normally animals with salmonellosis are not treated with antibiotics unless they are systemically ill or immunosuppressed, as there is some evidence suggesting that antibiotics can actually favor the growth of antibiotic resistant strains of *Salmonella* and thereby prolong shedding.

In poultry, effective prevention of *Salmonella* infection requires that breeding stocks are free from the organism to prevent transmission through the egg to progeny (Wray *et al.*, 1996). The key strategy in *Salmonella* control and prevention lies mainly in the prevention of the introduction and reintroduction of *Salmonella* (Mallinson *et al*., 2001). These measures include good personal hygiene and sanitary practices, thorough cleaning and disinfection of poultry houses and equipment between flocks, effective manure and sewage disposal, effective control of rodents and other pests, exclusion of poultry by-products from feedstuffs, use of properly processed feed and use of eggs/chicks from monitored breeding flocks. Control often begins with surveillance of *Salmonella* carriage in the parental flocks, since the hatching eggs may be contaminated with faeces during laying. Egg disinfection and hatching cabinet sanitization may be included to reduce aerosol contamination of uninfected eggs and

chicks. In addition to bio-security, feed and drinking water management, *Salmonella* control in breeders often includes the use of autogenous vaccines to reduce or eliminate intestinal colonization. Although permanent eradication of *Salmonella* from poultry flocks is difficult, realistic control measures if put in place could assist in reducing the infection levels

Salmonellosis in humans caused by non-typhoid *Salmonella* strains usually results in a self-limiting diarrhea that does not warrant antimicrobial therapy. However, there are occasions when these infections can lead to life-threatening systemic infections that require effective chemotherapy (Helms *et al.,* 2002). The antimicrobials most widely regarded as optimal for the treatment of salmonellosis in adults is the group of fluoroquinolones. They are relatively inexpensive, well tolerated, have good oral absorption and are more rapidly and reliably effective. Third-generation cephalosporins are widely used in children with serious infections, as quinolones are not generally recommended for this age group. The earlier drugs chloramphenicol, ampicillin, amoxicillin and trimethoprim-sulfamethoxazole are occasionally used as alternatives (WHO, 2005).

In Veterinary Medicine, antimicrobial agents are used in therapy, metaphylaxis, prophylaxis, and as growth promoters (Scharwz *et al*., 2001). However, prophylactic and curative use of antibiotics to control *Salmonella* is not recommended for different reasons. Firstly, antibiotic resistant *Salmonella* strains have emerged through prophylactic antibiotics use to control *Salmonella* (Bywater, 2004). Secondly, there is a concern about the presence of antibiotic residues in meat. Thirdly, most antibiotics fail to eliminate *Salmonella* from the animals although shedding is (temporarily) decreased. The use of subtherapeutic doses of antibiotics

as growth promoters is a public health problem, because many resistant microorganisms may transfer resistance to microorganisms found in bird faeces. This kind of use may be responsible for selective pressure that generates resistant bacteria, due to the risk of dissemination of pathogens and transfer of resistance genes, via food chain, to pathogenic and commensal microorganisms of humans, decreasing the treatment options for infections (Medeiros, 2011). It is clear that antibiotics are not the primary choice to control *Salmonella* in poultry flocks.

CHAPTER THREE

MATERIALS AND METHODS

3.1.0 Study site and sampling

The study was conducted at the School of Veterinary Medicine, University of Zambia, which has facilities for culturing, isolation and identification of bacteria. The laboratory also has facilities for molecular diagnosis and characterization of *Salmonella*.

3.2.0 Study design

A prospective cross sectional study design was conducted, where *Salmonella* isolates for serotyping and virulence factors determination were obtained from human, dog and chicken sources.

3.3.0 Sample size

The sample size was calculated using the following formula for quantitative Veterinary Epidemiology at 95% level of confidence:

$n = [1- (1 – \alpha)\ 1/D][N- (D – 1)/2]$

Where n = required sample size

D = estimated prevalence

N = population size

α = confidence level

Estimated prevalence of *Salmonella* in dogs was taken at 10% (Greene, 1998; Galton, 1969) and therefore, the calculated sample size was 200 (n = 200). In poultry, the estimated prevalence was 23% (Hang'ombe *et al.*, 1999a) and the sample size was calculated to 150 (n = 150). As for human *Salmonella*, 25 isolates from clinical fecal samples were kindly provided by the University Teaching Hospital, Microbiology laboratory, Lusaka, Zambia.

3.4.0 Type and source of samples

Samples for culture and isolation were obtained from dogs and chickens, while isolates from humans were readily provided by the Microbiology unit of the University Teaching Hospital.

3.4.1 Dog fecal samples

Fresh rectal fecal samples were collected from both young and adult dogs of both sexes using well labeled examination gloves from dogs attending four different Veterinary clinics located within the metropolitan city of Lusaka, Zambia. From these veterinary clinics a total of 243 rectal samples were collected from dogs between October 2009 and September 2010. The clinical history was obtained before fecal sample collection. The samples were stored at 4ºC while awaiting bacteriological analysis.

3.4.2 Poultry samples

Samples were collected from chicken carcasses that had been submitted for post mortem at the University of Zambia, School of Veterinary Medicine pathology laboratory from several farms around Lusaka. The carcasses were sampled with sterile cotton swabs in the inside of

the visceral and cloacal area surface; visceral organs were also aseptically collected for bacteriological examination. The samples were placed in 10 ml of selenite broth directly after sampling. The broth was incubated aerobically at 37ºC for 18 hours after which bacterial isolation and identification was done.

3.4.3 Human *Salmonella* isolates

Human *Salmonella* isolates were obtained from the bacteria storage bank of the University Teaching Hospital, Microbiology laboratory, Lusaka. These isolates were re-cultured and re-confirmed using *Salmonella* 'O' antisera (Laboratory Diagnostic Products Ltd., Middlesex, United Kingdom) by slide agglutination test.

3.5.0 *Salmonella* isolation and virulence factors determination

Salmonella was isolated from dog and chicken specimens and confirmation of the isolates was done as described below.

3.5.1 *Salmonella* culture, isolation and confirmation

For the isolation and identification of *Salmonella* from dog and chicken specimens, standard conventional methods were used as described by Hang'ombe *et al*., (1999a). Briefly, 1 gram of each rectal fecal and organ sample was inoculated into selenite broth (Biotec, Suffolk, UK) for enrichment and incubated aerobically at 37ºC for 18 hours. Swab samples were treated as for fecal and organ samples. Subcultures were then made from each broth culture onto MacConkey agar (Oxoid, Hampshire, England) and Xylose lysine deoxycholate agar (XLD) (Oxoid, Hampshire, England). The agar plates were incubated aerobically at 37ºC for

18 hours. After incubation, *Salmonella* suspected colonies appearing colorless and non-lactose fermenting on MacConkey agar and with a black spot on XLD agar were identified and selected. The colonies were Gram stained and subjected to biochemical tests as described by Barrow and Feltham (1993). Briefly, the center of the test colony was lightly picked with a sterile inoculating needle and inoculated into Triple sugar iron agar (TSI) and Lysine indole agar (LIA) (Himedia, India), by streaking the slant and stubbing the butt and incubated at 37^{o}C for 18 hours. Caps of the tubes were made loose to retain the aerobic condition.

Isolates which produced alkaline (red) slants and acid (yellow) butts, with or without production of H_2S (blackening of agar) in TSI and also produced an alkali (purple) reaction in LIA were retained as potential *Salmonella* isolates and were further subjected to other biochemical tests. They were inoculated into the following media; simmons citrate agar, urea agar, phenol red mannitol broth, phenol red lactose broth, phenol red sucrose broth and sulfide indole motility media (SIM) (Nissui, Tokyo, Japan) and then incubated at 37^{o}C for 18 hours. All isolates that produced a negative reaction for urease, indole, lactose and sucrose but a positive reaction for dulcitol and mannitol; and/or variable reaction on citrate were tentatively diagnosed as *Salmonella*.

Isolates with characteristics consistent with those of *Salmonella* were serologically confirmed with *Salmonella* somatic polyvalent (O) grouping antisera (Denka Seinken, Japan) which binds to the surface antigen of the microorganism to form a visible antigen-antibody precipitation. This was followed by a range of specific O antisera groups to differentiate them into serogroups.

3.5.2 Determination of virulence factors of *Salmonella*

The determination of *Salmonella* virulence factors was done using, the Dot blot Hybridization method and the Polymerase chain reaction assays, as described by Hang´ombe *et al.* (2008) and Tuchili *et al.* (1996).

3.5.2.1 Polymerase chain reaction (PCR)

To prepare the DNA for PCR assay, *Salmonella* isolates from trypticase soy agar were cultured in 0.5 ml Brain Heart Infusion (BHI) broth and incubated at 37°C for 24 hours. Samples were then treated at 95°C for 10 min to obtain bacterial cell extracts which were then directly used as template DNA and virulence genes were detected by PCR targeting the *invA, invF, sipC, hilA, hilC* and *hilD* gene sequences, respectively. The virulence gene sequences and the size of amplified fragments are listed in Table 3.

The PCR was done using the Phusion flash high fidelity PCR master mix (Finnzymes instrument, Finland). The reactions were performed in a final volume of 10 µl containing 5 µl phusion flash PCR master mix, 0.5 µM of primer sets in 1 µl volume of each and 2 µl of PCR water. The Piko thermal cycler (Finnzymes Instruments Oy, Finland) was programmed at 95^{o}C for 30 seconds for initial denaturation, followed by 35 cycles consisting of 95^{o}C for one second, 58^{o}C for 5 seconds, 72^{o}C for 15 seconds with final extension at 72^{o}C for 1 minute. The PCR products were electrophoresed in 1.5 % agarose gel, stained with ethidium bromide and visualized by UV transilluminator.

.

Table 3: Targeted genes of investigation in this study

Target Gene	Primer sequences used in the study	References
hilA	*F*: 5-GAGAATACACTAGATCTATGCCACAT-3 *R*: 5-TCATCGCCGATTCCAGCTGGGCGATA-3	Darwin and Miller, 2000
invA	*F:* 5- GCT GCG CGC GAA CGG CGA AG-3 *R*: 5- TCC CGG CAG AGT TCC CAT T -3	Rahn *et al.*, 1992
sipC	*F*: 5-GGGAGGGATCCATGTTAATTAGTAATGTGGGA-3 *R*: 5-CCTTTAAGCTTAGCGCGAATATTGCCTGC-3	Hayward and Koronakis, 1999
hilD	*F*: 5-GCTGTTAAGTCGACGAACTATGTATGGC-3 *R*: 5-TCCGTAAGCTTCCACTGTCTGGGTG-3	Schechter and Lee, 2000
hilC	*F*: 5-TGCTCCCGGGTATTTGTCAAAAGTG-3 *R*: 5-GCCCTTCTAGATCGCTCATAC-3	Schechter and Lee, 2000
invF	*F*: 5-AAGGGATCCATGTCATTTTCTGAAAGCGACAC-3 *R*: 5-GTTGTAGGGAAAGCTTCTCCAGTAATG-3	Darwin and Miller, 2000

3.5.2.2 Dot Blot hybridization assay

The samples were subjected to the dot blot hybridization system for the detection of *Salmonella invA, invF, hilA, hilC, hilD, sipC* and *spiC* genes. Briefly, for whole cell extracts preparation, the isolated *Salmonella* were incubated in 0.5 ml BHI broth overnight. A culture of hemolytic *Escherichia coli* isolated from a clinical case was cultured as a negative control. This was done to clearly show the existence or non-existence of the effector proteins under specific study for *Salmonella*. Following incubation, the BHI broth was centrifuged at 2000

xg for 10 minutes to harvest bacterial cells. The resultant pellet was mixed with 100 µl of SDS buffer containing 1% SDS in 50 mM Tris-HCl, pH 8.0 and the mixture was heated at 95°C for 3 minutes in order to obtain whole cell extracts which were used as DNA samples for dot blot hybridization.

The test DNA samples plus positive and negative control samples were dot spotted on nitrocellulose membrane (Schleicher and Schuell Bioscience, Dassel, Germany) using a copy plate capable of transferring 25 µl of the sample supernatant extract. The copy plate transfers 48 samples at a time. After transferring samples to the membrane, it was then air dried at room temperature. This was followed by blocking with 5% skim milk in phosphate buffer saline (PBS) for 1 hour at room temperature on a shaker. The blocking solution was then washed off using PBS washing buffer, 5 times in 10 minutes and then incubated on a shaker for 1 hour at room temperature with the first rabbit antibody against *invA, invF, hilA, hilC, hilD, sipC* and *spiC genes* (1: 2,500 dilution) for each respective membrane in 0.5% skimmed milk. The membranes were then washed 5 times in 30 min with PBS. This was followed by treatment with goat anti-rabbit antibody (1: 5,000 dilution) for a further 1 hour at room temperature. After washing, the membranes were developed in 5 ml TMB (tetramethylbenzidene) stabilized substrate for horseradish peroxidase (Promega Co., Madison, WI) until a positive reactive spot was visible in 5 min. To stop the reaction, the membranes were rinsed in distilled water and then air-dried. A known positive control sample of *Salmonella enteritidis* was included from a previous study (Hang'ombe *et al*., 1999a).

3.5.3 Antimicrobial susceptibility test for *Salmonella* isolates

Antibiograms of all the *Salmonella* isolates obtained from dog, chicken and human sources were determined by the Kirby-Bauer technique disc diffusion method on Mueller–Hinton agar (Bauer *et al*., 1966). The isolates were cultured on trypticase soy agar for 18 hours at 37^{o}C. A loopful of bacterium for each *Salmonella* isolate was suspended in 1ml trypticase soy broth and incubated for about six hours to march McFarland 1.0 standard, after which 100 µl of broth was placed onto Mueller–Hinton agar (Difco; Becton, Dickinson and Co, Franklin Lakes, NJ, USA) and spread thoroughly and evenly using a glass spreader.

Commercial antimicrobial paper discs which included amoxicillin, ampicillin, Co-trimoxazole, erythromycin, gentamycin, nitrofurantoin, tetracycline and vancomycin (Oxoid) were placed on the sensitivity test agar plates. These were fully secured to the agar by applying gentle pressure using a fine alcohol flamed forceps. The agar plates were incubated at 37^{o}C overnight after which the diameters of bacteria growth inhibition were measured and noted using a ruler on the undersurface of the petri dish.

3.6.0 Statistical analysis

The data base was established in Excel Spread sheets and for statistical analysis it was transferred to STATA SE/11 for windows statistical package (Stata Corp. College Station, Texas. USA). A p-value of <0.05 was considered indicative of a statistically significant difference.

CHAPTER FOUR

RESULTS

4.1.0 *Salmonella* isolation

Out of a total of 243 dog fecal and 136 chicken samples that were examined for *Salmonella*, 18 and 23 were found to be positive for *Salmonella* respectively. Therefore, the incidence of *Salmonella* in dog and chicken samples was 7.4% and 16.9% respectively (Table 4). The 243 dog samples included 30 specimens from diarrhoeic dogs. The total isolation percentage of *Salmonella* from diarrhoeic and non-diarrhoeic dogs was 10% and 7.04%, respectively.

Table 4: Incidence of *Salmonella* isolation in dog and chicken samples collected from Lusaka, Zambia

Source of samples	Number of samples tested (*n)*	*Salmonella* positive samples	Per cent positive (%)
Dog	243	18	7.4
Chicken	136	23	16.9
Total	379	41	10.8

The isolated *Salmonella* were serotyped to determine serovars circulating in dogs and chickens. Out of the 18 *Salmonella* isolated from dog fecal samples, 4 different serovars were obtained (Table 5). Of the 18 *Salmonella* isolated, 3 belonged to group B, 5 to group C, 4 belonged to group E, and 6 were untypable. In case of chickens, all the 23 *Salmonella* isolates (100%) were

found to belong to the serovar *S. enteritidis*, following reaction to 1,9,12 *Salmonella* O grouping antisera.

Table 5: *Salmonella* serovars isolated from dogs

Salmonella group	*Salmonella* serovar antigen reactions	Number isolated	Per cent (%)
B	04	3	38.9
C	07, 08	5	16.7
E	03, 10	4	27.8
Untypable		6	22.2
Total number		18	100

The 25 human *Salmonella* isolates collected from the University teaching Hospital, Lusaka, Zambia were re-cultured and re-confirmed using the *Salmonella* polyvalent O antisera. Out of the 25 human *Salmonella* isolates, 4 different serovars were obtained (Table 6). Of these *Salmonella* isolates, 9 belonged to group B, 4 to group C, 6 belonged to group E and another 6 belonged to group D.

Table 6: *Salmonella* serovars from human

Salmonella group	*Salmonella* serovar antigen reactions	Number isolated	Per cent (%)
B	04	9	36
C	07, 08	4	16
E	03, 10	6	24
D	9,12	6	24
Total number		25	100

4.2.0 Confirmation of *Salmonella*

The *invA* gene was detected in all isolates from chicken, dog and human and was used to confirm the suspected *Salmonella* isolates. As shown in Table 7, comparisons were made on the confirmation of *Salmonella* based on culture and biochemical tests, serum reaction and finally PCR. On culture and biochemical tests, all the isolates exhibited the classical pattern of biochemical reactions, such as oxidase negative, catalase positive, indole and Voges- Proskauer negative, methyl red and simmons citrate positive, lysine and ornithine decarboxylase positive. Hydrogen sulphide was produced and urea was not hydrolysed. Carbohydrates such as arabinose, maltose, mannitol, mannose, sorbitol trehalose and xylose were fermented.

Table 7: Confirmation of *Salmonella* serovars isolated from chicken, dog and human

Salmonella origin	Culture and biochemical tests	*Salmonella* poly 'O' reaction	PCR invA confirmation
Human	25	25	25
Chicken	23	23	23
Dogs	18	18	18
Total	66	66	66

Of the suspected isolates, PCR confirmed the isolates through the presence of the *invA* gene with a 284 bp amplicon (Fig. 2) despite some isolates being untypable, but reacting to *Salmonella* poly 'O'. All the *Salmonella* isolates from dog, chicken and human sources possessed the *invA* gene.

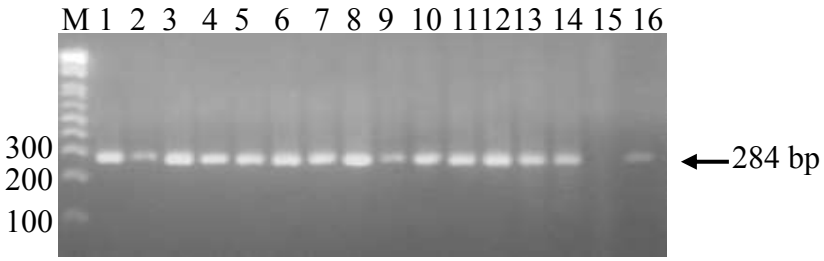


Figure 1: Gel electrophoresis of *Salmonella invA* gene specific PCR products (indicated by arrow). Lanes 1 to 8, Group D *Salmonella* from poultry; lanes 9 to 14: isolates from dog, Lane 9 to 10: Group B, Lane 11 Group C, Lane 12 Group E, Lanes 13 and 14 being the untyped group;. Lane 15 and 16 are the negative and positive controls, respectively; Lane M: 100 bp marker.

4.3.0 Virulence genes determination using PCR

The effector proteins that may be responsible in the pathogenesis of *Salmonella* were investigated and determined by PCR. The effector proteins investigated were *invA, invF, hilA, hilC, hilD* and *sipC.* The PCR results are shown in Figs. 2 - 4. The *invF* gene was detected in dog (16.7%), chicken (40.1%) and human (20%) while the *sipC* gene was 8% in dogs, 8.7% in chickens and 4% in humans. The *hilD* gene was only detected in dog samples (5.6%), the *hilA* and *hilC* were not detected in any of the samples.

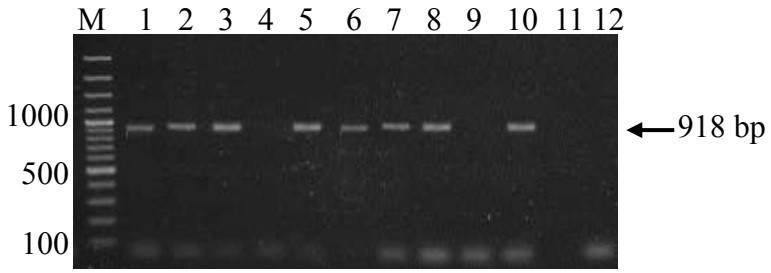


Figure 2: Gel electrophoresis of *Salmonella invF* gene specific PCR products (indicated by arrow). Lanes 1 – 3: dog *Salmonella* isolates from Group C, E and the untyped group; Lanes 5, 6, 7 and 8: positive isolates from poultry; Lanes 9 and 10: negative and positive controls, respectively; Lane M: 100 bp marker.

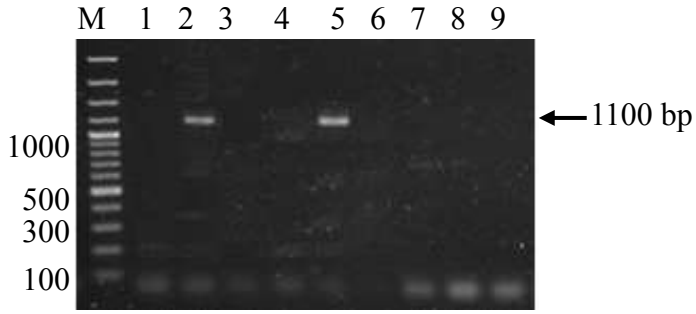


Figure 3: Gel electrophoresis of *Salmonella sipC* gene specific PCR products (indicated by arrow). Lanes 2 – 5: positive bands for *sipC*. Lane M: 100 bp marker while Lane 9 is the negative control.

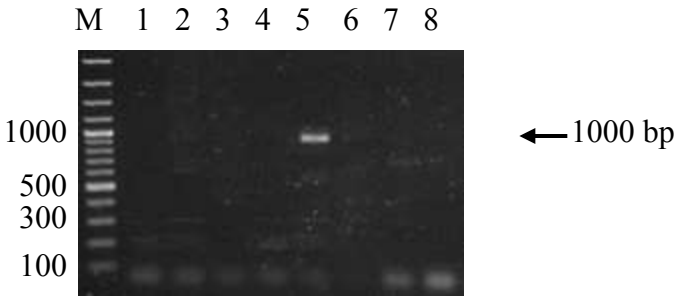


Figure 4: Gel electrophoresis of *Salmonella hilD* gene specific PCR products (indicated by arrow).Detection. Lane 5: *hilD* positive band; Lane M: 100 bp marker and Lane 8 is the negative control.

Table 8: Summary of virulence genes determined by PCR

	Virulence factors +ve (%)						
Source of *Salmonella*	*invA*	*invF*	*hilA*	*hilC*	*hilD*	*sipC*	*spiC*
Dog (*n=18*)	18 (100)	3 (16.7)	0	0	1 (5.6)	2 (8)	0
Chicken (*n=23*)	23 (100)	10 (40)	0	0	0	2 (8.7)	0
Human (*n=25*)	25 (100)	5 (20)	0	0	0	1 (4)	0

Using the PCR method it was observed that there was no significant difference ($P < 0.01$) in the distribution of virulence genes in the *Salmonella* isolates from dogs, chickens and humans. The isolates from the three different sources all had presence of the *invA* gene but had the *invF* and *sipC* genes in varying percentages (Table 8).

4.4.0 Virulence genes determination using Dot blot hybridization

The dot blot hybridization was also used to assess the presence of virulence genes. From the study, the *invA* gene was detected in all the isolates (Fig. 6) whereas varying percentages of possession were observed with *hilD* gene at 55.5% in dogs, 13% in chickens and 72% in humans (Fig. 7a and 8). As for the *sipC* gene, 13% were detected in chickens and the *spiC* gene was detected at 4% in humans (Fig. 7b and 7c).

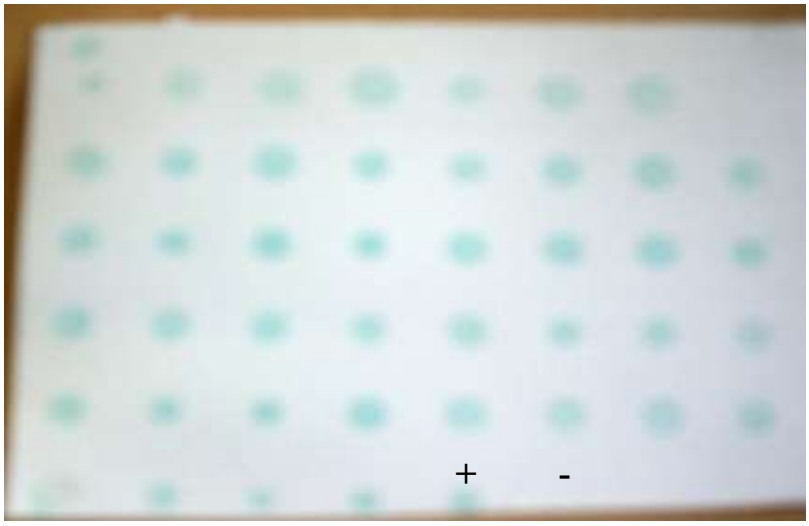
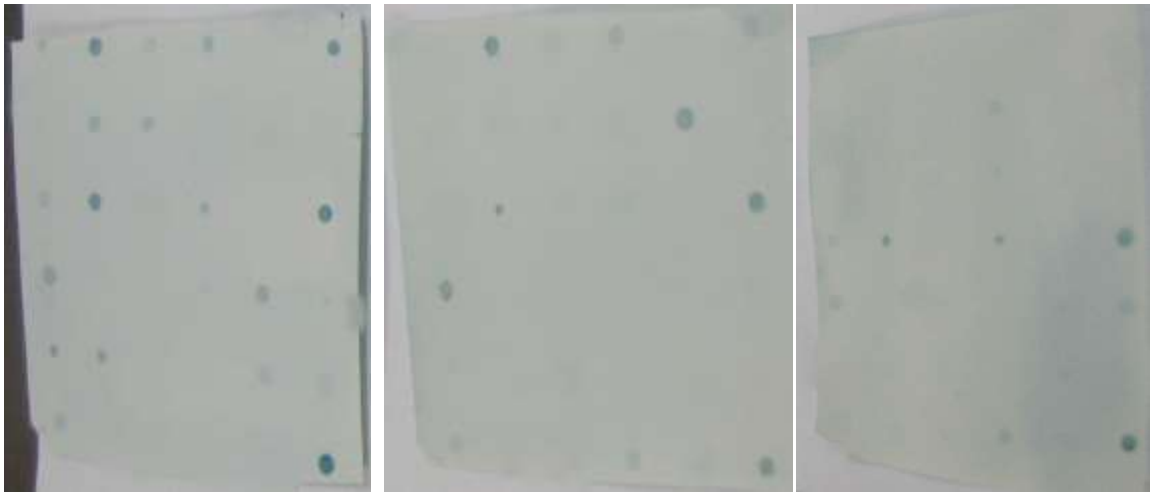


Figure 5: Visual detection of *invA* from antigen extracts dot spotted on the nitrocellulose membranes, typical positive reactions are shown with deep staining spots. The positive (+) and negative (-) controls are also highlighted.



a. b.

c.

Figure 6: Visual detection of *hilD*, *sipC* and *spiC* in *Salmonella* isolates from dog (a), chicken (b) and human (c) respectively. Typical positive reactions are shown with deep staining spots.

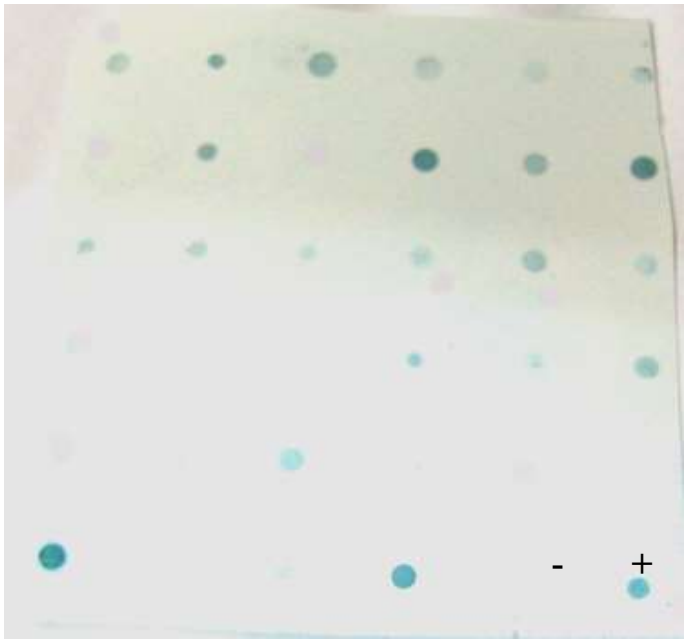


Figure 7: Visual detection of, *hilD* in *Salmonella* isolates of human origin. Typical positive reactions are shown with deep staining spots. The positive (+) and negative (-) controls are also highlighted.

Table 9: Summary of virulence genes detection by Dot blot hybridization

	Virulence factors +ve (%)						
Source	*invA*	*invF*	*hilA*	*hilC*	*hilD*	*sipC*	*spiC*
Dog (*n=18*)	18 (100)	0	0	0	10 (55.5)	0%	0
Chicken (*n=23*)	23 (100)	0	0	0	3 (13)	6 (26)	0
Human (*n=25*)	25 (100)	0	0	0	22 (88)	0	1 (4)

The results of the Dot blot hybridization assay showed that there was a significant difference ($P < 0.001$) in the distribution of virulence genes in the *Salmonella* isolates from dogs, chickens and humans. Even though all isolates possessed the *invA* gene, the *hilD* gene was possessed in varying percentages by isolates from the three different sources but isolate from chickens and human also possessed the *sipC* and *spiC* genes, respectively (Table 9).

The *invA* gene was detected in all the isolates (100%) by using both the PCR and Dot blot hybridization methods (Table 10). Similarly *hilA*, *hilC* and *spiC* genes were not detected in any of the isolates by either of the methods except in the human isolates which had 4% detection of the *spiC* gene using the dot blot hybridization method. The *invF* gene was detected at varying levels of 16, 40 and 20% in isolates from dog, chicken and human sources respectively by the

PCR method only (Table 10). The *sipC* gene was detected at varying levels of 8, 8.7 and 4% in isolates from the three respective sources of dog, chicken and human by the PCR method, whereas 26% was recorded from chicken isolates by use of the Dot blot hybridization method which also recorded detection of *hilD* gene at varying levels of 55.5, 13 and 88% in isolates from the dog, chicken and human sources as opposed to PCR method which recorded detection of 5.6% in isolates from dog sources only (Table 10).

Table 10: Overall results from Dot blot hybridization and PCR methods for the determination of virulence genes of *Salmonella* isolates from dogs, chickens and humans.

		Virulence factors determined						
Source	Methods used	*invA* +ve (%)	*invF* +ve (%)	*hilA* +ve (%)	*hilC* +ve (%)	*hilD* +ve (%)	*sipC* +ve (%)	*spiC* +ve(%)
Dog (*n=18*)	PCR	18(100)	3(16.7)	(0)	(0)	1(5.6)	2(8%)	(0)
	Dot blot	18(100)	(0)	(0)	(0)	10(55.5)	(0)	(0)
Chicken (*n=23*)	PCR	23(100)	10(40)	(0)	(0)	(0)	2(8.7)	(0)
	Dot blot	23(100)	(0)	(0)	(0)	3(13)	3(13)	(0)
Human (*n=25*)	PCR	25(100)	5(20)	(0)	(0)	(0)	1(4)	(0)
	Dot blot	25(100)	(0)	(0)	(0)	18(72)	(0)	1(4)
Total % + ve	PCR	100%	25%	0%	0%	1.8%	6.9%	0%
	Dot blot	100%	0%	0%	0%	1.7%	4.3%	1.3%
Sensitivity of protocol	PCR	100%	27.3%	0	0	1.5%	7.6%	0%
	Dot blot	100%	0	0	0	47%	4.6%	1.5%

4.5.0 Antibiotic susceptibility patterns

The antibiograms of *Salmonella* from dogs, chickens and humans were analyzed by the disc diffusion methods as shown in table11; the antibiotic susceptibility pattern of *Salmonella* isolates from dog feacal samples showed high percentages of susceptible to amoxycilin (100%) and nitrofurantoin (94.4%). Resistance was observed to ampicillin (50%), gentamycin (55.6%), tetracycline (61%), Co-trimoxazole (83.3%), vancomycin (100%) and erythromycin (100%).

Table 11: Antibiograms of *Salmonella* from dogs

	No. of susceptible and resistant isolates per antibiotic							
	AML 10 µg	TE 30 µg	NITR	AMP 10 µg	VAN 5 µg	ERY 5 µg	CN 10 µg	SXT 25 µg
Sensitive (%)	18 (100)	7 (38)	17 (94.4)	9 (50)	0 (0)	0 (0)	8 (44.4)	3 (16.7)
Resistant (%)	0 (0)	11 (61)	1 (5.6)	9 (50)	18 (100)	18 (100)	10 (55.6)	15 (83.3)

Note: AMP= Ampicillin; AML= Amoxicillin; CN= Gentamycin; ERY= Erythromycin; NITR= Nitrofurantoin; TE= Tetracyclin; SXT= Co-trimoxazole; VAN= Vancomycin.

Salmonella enteritidis isolates from the chicken samples showed sensitivity to nitrofurantoin (100%), amoxicillin (95.7%), tetracycline and ampicillin (82.6%) (Table 12). Resistance was observed to co-trimoxazole (73.9%), gentamycin (82.6%), vancomycin and erythromycin (100%).

Table 12: Antibiograms of *Salmonella* from Chickens

	No. of susceptible and resistant isolates per antibiotic							
	AML	TE	NITR	AMP	VAN	ERY	CN	SXT
Sensitive (%)	22 (95.7)	19 (82.6)	23 (100)	19 (82.6)	0 (0)	0 (0)	4 (7.4)	6 (26)
Resistant (%)	1 (4.3)	4 (7.4)	0 (0)	4 (7.4)	23 (100)	23 (100)	19 (82.6)	17 (73.9)

Note: AMP= Ampicillin; AML= Amoxicillin; CN= Gentamycin; ERY= Erythromycin; NITR= Nitrofurantoin; TE= Tetracyclin; SXT= Co-trimoxazole; VAN= Vancomycin.

The human *Salmonella* isolates showed sensitivity to nitrofurantoin (68%) and amoxillin (48%). Resistance was observed to tetracycline (52%), co-trimoxazole (56%), gentamycin (60%), ampicillin (68%), vancomycin and erythromycin (100%) (Table 13).

Table 13: Antibiograms of *Salmonella* from humans

	No. of susceptible and resistant isolates per antibiotic							
	AML	TE	NITR	AMP	VAN	ERY	CN	SXT
Sensitive (%)	17 (68)	12 (48)	19 (76)	8 (32)	0 (0)	0 (0)	10 (40)	11 (44)
Resistant (%)	8 (32)	13 (52)	6 (24)	17 (68)	25 (100)	25 (100)	15 (60)	14 (56)

Note: AMP= Ampicillin; AML= Amoxicillin; CN= Gentamycin; ERY= Erythromycin; NITR= Nitrofurantoin; TE= Tetracyclin; SXT= Co-trimoxazole; VAN= Vancomycin.

CHAPTER FIVE

DISCUSSION

5.1.0 Prevalence of *Salmonella* in dogs and chickens

The results of this study clearly indicate the presence of *Salmonella* in dogs in Lusaka, Zambia where 7.4% of the dogs were found positive for *Salmonella*. The isolation rate is in agreement with those of other studies which reported the frequency of fecal isolation of *Salmonella* species from dogs to be between 0 and 43.0% (Sanchez *et al*., 2002; Carter and Quinn, 2000). In a study conducted by Seepersadsingh *et al*., (2004); the prevalence of *Salmonella* spp. in non-diarrheic dogs was reported to be 3.6% and Ojo and Adetosoye, (2009), reported the prevalence of *Salmonella* isolation to be 3.7% from fecal samples collected from both diarrheic and non-diarrheic dogs. In Japan, 5.9% of stray or unwanted apparently healthy dogs were positive for the presence of *Salmonella* in their intestinal contents (Fukushima *et al*., 1985). On the other hand; Morse and Duncan, (1975) reported that 20% of the dog population was infected with *Salmonella*. The differences in the sample sizes of dogs, year of sampling, type of fecal sample, geographical properties, and sampling strategies and isolation methods performed in the various countries may affect the prevalence (Seepersadsingh *et al*., 2004). Dogs have been reported to be carriers of *Salmonella* spp. worldwide they thus have the potential to serve as sources of exposure or infection for humans (Carter and Quinn, 2000). This report highlights that *Salmonella* - contaminated dogs in Zambia pose a threat to human health due to being potential sources for *Salmonella* transmission as observed in other countries (Sanchez *et al*., 2002; Kahrs *et al*., 1978).

Risk factors for *Salmonella* carriage in dogs may include type of diets, exposure to livestock and scavenging. There are few published studies looking at the associations between *Salmonella* carriage and other pet management factors, such as antimicrobial use, probiotics, environmental exposures, and exposure to other animals. This study did not look at the association between prevalence and risk factors of *Salmonella* in dogs. However, it was observed that most of the dogs in the study were not confined and therefore capable of feeding freely through scavenging. The findings of *Salmonella* prevalence in dogs in this study were the first as no similar studies have been undertaken previously in Zambia.

In this study the prevalence of *Salmonella* in chickens was found to be 16.9%. This prevalence was in agreement with previous studies of *Salmonella* in poultry, with contamination percentages ranging from 13.7% to 66% (Uyttendaele et al., 1998; Jerngklinchan *et al*., 1994; Baumgartner *et al*., 1992). There are also studies reporting *Salmonella* detection rates as low as 0 to 17.0% (Ata and Aydın, 2008) and 9.9 to 17.9% (Van Hoorebeke *et al*., 2010). Goncagul *et al*., (2005) isolated *Salmonella* and other nontypable *Salmonella* from 7 out of 8 broiler carcass producers at prevalence levels of 8.6% and 9.5% respectively. In a study carried out in China, of 1152 chicken samples analyzed for the presence of *Salmonella* from three types of retail markets, the overall prevalence was 52.2% (Yang *et al*., 2010). In a cross-sectional study, undertaken to determine the presence and prevalence of *Salmonella* in retail raw chicken meat and giblets (gizzard and liver) in supermarkets in Addis Ababa (Ethiopia), *Salmonella* were detected from 54 of the 301 (17.9%) samples examined (Tibaijuka *et al*., 2003). In this study, the prevalence was found to be 16.7%, which is slightly lower as indicated in other studies (Yang *et al*., 2010,

Tibaijuka *et al*., 2003). Conditions that could have affected isolation may include the time lapse from when the birds died and when they were sampled, the storage of the sample before examination and whether the birds were subjected to treatment with antibiotics before slaughter. Other factors could have been the age as young birds are more susceptible to *Salmonella* colonization, level of pathogen exposure and whether the strains carried genetic factors that facilitate evasion of host defenses (Bailey, 1988).

5.2.0 Determination of *Salmonella* serovars in dogs, chickens and humans

Group typing of the *Salmonella* isolates from dogs showed that they belonged to 3 different serogroups namely, group E (22.2%), B (16.7%) and C (27.8%), while others were untypable (33.3%). The results are in agreement with other studies which found that dogs can harbor many serovars of *Salmonella*. Moran (1961) found 23 different serotypes of *Salmonella* among 51 isolates, while Seepersadsingh *et al*., (2004) reported 28 different serovars of *Salmonella* in dogs. The number and occurrence of serotypes isolated from dogs vary considerably from country to country and reflect the animal's diet or the general environment (Carter and Quinn, 2000). A number of medically important serotypes for humans have been isolated from domestic dogs and cats and several studies have reported the isolation of multidrug-resistant *Salmonella* (Guardabassi *et al*., 2004). *Salmonella* most frequently isolated from dogs proved to belong to similar sero-types to those seen most in man as was the case in this study. Some of the *Salmonella* isolates in serogroup B (*S. paratyphi B, S. derby, S. agona, S. typhimurium, S. heidelberg, S. haifa,* and *S. abortusequi*), serogroup C (*S. edinburg, S. paratyphi* C, *S choleraesuis, S. typhisuis, S. livingstone, S. infantis, S. inganda, S. Newport* and *S. Kentucky*) and some in serogroup E (*S. anatum, S. pietersburg, S. ngor* and *S. dortmun*) are zoonotic. The

presence of *Salmonella* in dogs in this study therefore makes them potential sources of infection to their human companions. Dogs can also disseminate the organism by contaminating the environment, thereby exposing the general public and other animals to the risk of infection. Cases of dog – to - human transmission of *Salmonella* resulting in severe infection in the latter have been previously reported (Morse and Duncan, 1975). In Zambia, however, dog to human transmission of *Salmonella* have not been established as no studies in this area have previously been undertaken.

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. heldelberg, S. infantis, S. kaufmanii, S. kisarawi, S. oranienburg, S. rubislaw, S. typhimurium, S. weltverden, S. worthington, S. agona, S. schwarzengrund, S. alamo, S. haifa* and *S. virginia* (Tuchili *et al*., 1996). However the isolates in this study were all found to belong to serogroup D *Salmonella,* including *S. enterica* subsp *enterica* serovar *enteritidis* (100%). The high prevalence of *S. enteritidis* observed in this present study is comparable with the findings described by other investigators (Kilinc and Aydin, 2006). Bennasar *et al.,* (2000), observed that the presence of *Salmonella* in the chicken meat is a particular health risk for the consumer and is related to the presence of epidemiologically important serotypes such as *S. enteritidis* and *S. typhimurium.* The isolation of only *S. enteritidis* in clinical samples from chickens in Zambia is of great significance and raises great public health concern due to the zoonotic transmission to humans and is indicative that hatcheries and poultry farms in Zambia have zoonotic organisms that have the potential of entering the food-chain. The isolation also sounds a message to poultry veterinarians that *S. eEnteritidis* must be considered seriously in poultry disease prevention and for their own safety. In another study in Zambia,

Hang'ombe *et al*., (1999a) isolated *S.* e*nteritidis* from pooled table eggs and chickens carcasses processed for human consumption, indicating that eggs and chickens are potential sources of infection to humans. Considering the upswing in the consumption of chicken meat and eggs in Zambia (CSO, 2008), surveillance and monitoring of *Salmonella* in chicken is important. Although potentially all livestock can be infected with *Salmonella,* contaminated poultry meat and eggs may be the main source of *Salmonella* for humans. Especially whre *S. enteritidis* is implicated, human infections can be traced back to eggs and poultry meat (Plummer *et al*., 1995; Altekruse *et al*., 1993; Telzak *et al*., 1990).

Human *Salmonella* serotypes in this study belonged to serogroups B, C, D and E. *Salmonella* serogroup B was the highest serotype followed by serogroups D and E, with serogroup C being the least. The results suggest that serogroup B *Salmonella* is the most common cause of gastroenteritis in humans in Lusaka. Reports on *Salmonella* serovars isolated from human patients with diarrhea diagnosed at UTH, indicated serogroup B *Salmonella* as the predominant isolate (Mwansa 1997, personal communication). Serogroup B and serogroup D *Salmonella* have been reported to account for 79 to 95% of all bacteremic non-typhoidal *Salmonella* infections in sub-Saharan Africa (Brent *et al*., 2006). With the advent of AIDS in Zambia, identification of serogroup D (*S. enteritidis*) in this study is a definite risk factor for *Salmonella* non - typhoidal bacteremia. The fact that the human *Salmonella* serotypes in this study were found to be similar to those found in dogs and chicken indicate that these animal species represent potential threats to human health.

5.3.0 Determination of *Salmonella* virulence genes

The PCR assay carried out in this study for the detection of the *invA, invF, hilA, hilC, hilD* and *sipC* genes in 66 *Salmonella* isolates from three different sources (dog, chicken and human) revealed that the *invA* gene was present in all the isolates. These findings are in agreement with earlier reports (Nashwa *et al*., 2009; Trafny *et al*., 2006; Zahreai *et al*., 2006). This gene is involved in the invasion of the cells of the intestinal epithelium and is present in pathogenic *Salmonella*. Therefore for salmonellosis to occur it is important that a gene responsible for invasion must be present.

The *invF* gene was found distributed among 3 (16.7%), 10 (40%) and 5 (20%) of the dog, chicken and human isolates respectively, This gene is a transcriptional regulator required for the expression of several genes encoding type III secretion system SPI-1 effector proteins (Darwin and Miller, 1999a, 2000). On the other hand, the *sipC* gene was distributed among the dog (8%) and chicken (8.7%) isolates, respectively as well as among human human 1 (4%) isolates. The *sipC* gene is an actin-binding protein that interferes with host cell actin cytoskeleton and is required for an efficient bacterial internalization by the host cell (Hayward and Koronakis, 1999).

The *hilD* gene was only distributed among dog 1 (5.6%) isolates. This gene is an essential transcriptional regulator that binds to the upstream repressing sequences (URS) and appears to repress *hilA* expression (Schechter *et al*., 1999). The observation in this study was that despite the diversity of *Salmonella* serotypes, there were some virulence genes common to isolates from

animals and humans, suggesting that opportunity exists for genetic exchange between human and animal isolates to create the next emerging pathogen. The chicken isolates, despite belonging to the same serogroup, showed variations in the expression of the *hilD* and *sipC* genes. This could infer to intrinsic differences in their pathogenic potential. The same could be said for the serotypes belonging to isolates from dogs and humans which also showed variation in the expression of various genes.

Similarly the dot blot hybridization assay carried out for the detection of the *invA, invF, hilA, hilC, hilD, spiC* and *sipC* genes in 66 *Salmonella* isolates from the three different sources (dog, chicken and human) also revealed that the *invA* gene was present in all the isolates (100%). This was demonstrated by the deeply staining spots on the nitrocellulose paper. The *hilD* gene was also found distributed among isolates from dog (55.5%), human (18%) and poultry (13%) in varying percentages respectively. The *sipC* gene was found distributed only among 13% of *Salmonella* isolates from poultry and the *spiC* gene was also found distributed only among 4% of isolates from human. Hang'ombe *et al*., (2008) in a similar study also found *invA* to be distributed in all the isolates form poultry sources but a variance was found with regards the distribution of other investigated genes that included *spiC, sipC, hilA* and *invF*. As in any experimental work, some external factors may have influenced detection of the targeted genes. These factors may include low levels of *Salmonella* antigens, none - specific inhibitors in the sample as well as other none specific environmental factors at different stages of the assay.

The results of this study show that *invA* gene was detected in all the isolates (100%) by using both the PCR and Dot blot hybridization methods. The *hilA* and *hilC* genes were not detected

except for the *spiC* gene which was detected in 4% of the human isolates by use of the dot blot hybridization method. The *invF* gene was detected in varying percentages at 16% in dog, 40% in chicken and 20% in human isolates respectively by the PCR method. The *sipC* gene was also detected at varying percentages at 8% in dog, 8.7% in chicken and 4% in human isolates, respectively by the PCR method. On the other hand 13% detection rate was recorded from chicken isolates by use of the Dot blot hybridization method which also recorded detection at varying percentages of 55.5% in dog, 13% in chicken and 72% human isolates respectively as opposed to the PCR method which recorded detection at 5.6% in isolates from dogs only.

The observations from this study suggest that the *invA* gene is widely distributed among the *Salmonella* isolates irrespective of their serovars and source of isolation. This therefore makes this gene a suitable target for the detection of *Salmonella* from different types of biological specimens as earlier observed (Jamshidi *et al*., 2008). The *invA* gene, located on the pathogenicity island 1 of *Salmonella* species, is essential for invasion of epithelial cells (Collazo and Galán, 1997). It is present in all invasive strains of *Salmonella* (Galán, 1996) and absent from closely related genera such as *Escherichia* c*oli* (Bäumler *et al.*, 1998). This gene contains sequences unique to this genus and has been proved to be a suitable PCR target with potential diagnostic application (Jamshidi *et al*., 2008). Other genes (*invF, hilA, hilC, hilD, sipC* and *spiC*) which are also essential in the pathogenesis of *Salmonella* were found present in varying degrees among the isolates from the three sources. These variations may have an influence on the type of *Salmonella* infection produced, either systemic due to the presence of all the essential virulence factors mediating migration of the organisms to other organs of the body or enteritis due to localization in the intestine.

Salmonella is able to detect environmental stimuli in its surroundings and activate/repress virulence-associated genes accordingly. Many environmental factors have been reported to influence SPI1 gene expression and/or effector protein secretion, including low oxygen tension, neutral pH, acetate and other short-chain fatty acids, cationic peptides, and bile (Altier, 2005; Bajaj *et al*., 1996). If any one of these requirements is not met, invasion gene expression is suboptimal. Galan and Curtiss (1990), explored the effect of high concentrations of salt (300 mM and higher) and concluded that the osmoinducibility of *invA* depends on changes in DNA supercoiling but not on the osmoregulator *ompR*. On the other hand, Bajaj *et al*., (1996) showed that osmolarity indirectly activates SPI1 gene expression and that the process is mediated by *hilA*. And according to Lucas *et al*., (2000), virulence genes are thought to be regulated in the host such that they are expressed only at those sites where their products are needed. Unregulated production of virulence factors at inappropriate sites may inhibit the bacteria's ability to cause disease (Heithoff *et al*., 1999). Majority of research have focused on bacteria grown in laboratory media and subjected to environmental stimuli designed to mimic conditions found in the host. However, this strategy has limitations. Although certain host environmental parameters can be mimicked in *vitro* to induce a subset of virulence genes, the full repertoire is only expressed in vivo (Michael *et al*., 2000). This probably explains the low and different percentages observed during this study in the detection of the various virulence genes.

The results of this study using both the Dot blot hybridization and PCR methods showed that four virulence genes out of seven targeted genes were detected in both dog and chicken *Salmonella* isolates respectively whereas five virulence genes detected in human isolates by the

dot blot hybridization and PCR methods. This is in agreement with the assertion that many of the virulence genes are elicited and expressed during active infection and not expressed when cells are grown on laboratory medium (Michael *et al*., 2000). On the other hand, the gastro intestinal environments of dogs, chickens and humans are different and this may also have had an obvious effect on the expression of virulence genes from the *Salmonella* isolates. No attempt was made in this study to simulate physiological gastro - intestinal environments of the three hosts *in vitro* in order to enhance gene expression. Even though all the isolates from the three sources in this study showed the presence of the *invA* gene which is essential for *Salmonella* invasion of the intestinal epithelial cells, progression to clinical disease would only be possible if other virulence genes in the hierarchy are expressed. There was no significant difference in the distribution of virulence genes between diarrheic and non-diarrheic dogs, implying that the diarrhea in dogs in this study could have been induced by other factors other than *Salmonella* virulence genes. Dogs may have developed ways of suppressing the expression of some of the genes to avert progression to clinical disease compared to chickens and humans.

The study observed that genes not expressed in some of the isolates may not be suitable for the detection of *Salmonella* from different types of biological specimens because of their uneven distribution depending on the clinical type of *Salmonella*.

In the present study, it was observed on average that using the PCR method the percentage of distribution of the targeted genes in dog, chicken and human *Salmonella* isolates was found to be higher (15.2%) as opposed to using the dot blot hybridization which yielded a lower percentage (12.5%). This therefore implies that the PCR method has more sensitivity and effectiveness in

determining the distribution of virulence determinants possessed by *Salmonella* isolates from different biological sources. The PCR method was quicker and user friendly as opposed to the dot blot hybridization method. According to the PCR data there was no significant difference in the presence of the targeted genes in *Salmonella* isolates from dogs, chickens and humans. However, using the Dot blot hybridization method, a significant difference was observed in the presence of virulence genes in the isolates from the three sources. This difference may be attributed to false positives due to some none - specific reactions in the Dot blot hybridization assay. According to Knowles and Gorham, (1990), while hybridization tests serve some specific purposes quite well, relatively low sensitivity and technical difficulties have limited the application of these tests in clinical laboratories. However, the high sensitivity of PCR, makes this technique a true candidate to replace many of the procedures currently in use for the rapid detection of pathogens. A study by Malinen *et al*., (2002) also observed that in comparison to dot-blot hybridization, real-time PCR was easier and faster to perform and also proved to have a superior sensitivity. The results suggest that PCR has a great potential for analysis of the fecal microflora and other biological specimens. The PCR method therefore, could be a suitable tool to yield a better understanding on the evolution of pathogenic serovars and the role of virulence genes in pathogenesis and for further epidemiological studies in Zambia. However, factor which may limiting the use of the PCR method for routine diagnosis in developing countries, are high costs for the equipment and reagents as well as lack of adequate skilled man power.

5.4.0 Antimicrobial patterns of *Salmonella* isolates

The results of antibiograms of *Salmonella* isolates from dogs, chickens and humans in this study showed susceptiblity to amoxicillin and nitrofurantoin, while those of chickens were also susceptible to ampicillin and tetracycline. A high percentage of *Salmonella* isolates from humans

in this study were resistant to tetracycline, ampicillin, gentamycin, erythromycin, vancomycin and co-trimoxazole, those of chickens showed resistance to co-trimoxazole, gentamycin, vancomycin and erythromycin, while those of dogs showed resistance to gentamycin, tetracycline, Co-trimoxazole, vancomycin and erythromycin. According to the isolates from human, dog and chicken sources were resistant to six, five and four antibiotics, respectively. The isolates from the three sources were commonly resistant to at least four (co-trimoxazole, gentamycin vancomycin and erythromycin) out of the eight antibiotics tested. This is not surprising as an increased incidence in the occurrence of multidrug-resistant *Salmonella* has been widely reported in the past decade, presumably attributed to the extensive use of antimicrobial agents in human and veterinary medicine (Helms *et al*., 2002). The antibiotic resistance pattern observed among the *Salmonella* isolates from dogs and chickens in this study could have chemotherapeutic implications in humans should they become infected by serotypes of *Salmonella* from these sources. This is because according to the results of our study, the serovars isolated from either dogs or poultry are capable of infecting humans as well. Wright *et al.*, (2005) have suggested the potential for zoonotic transmission of multidrug-resistant *Salmonella* from stray dogs to humans.

In Africa and most other developing regions, multidrug resistance, particularly to commonly available antibiotics like tetracycline, remains a major challenge for the healthcare system (Kariuki *et al.*, 2005; Bonfiglio *et al.*, 2002; World Health Organization, 2000). Fadlalla *et al*., (2012), in their study observed that the frequency of resistance to antimicrobials among isolates from human was higher than that among animal isolates and high resistance to ampicillin and tetracycline alongside other antibiotics was observed. In particular, multidrug-resistant NTS have

caused life-threatening invasive disease outbreaks in children in many African countries, including Democratic Republic of Congo (Cheesbrough *et al.*, 1997, Green and Cheesbrough, 1993), Rwanda (Lepage *et al.*, 1990), Nigeria (Adejuyigbe *et al.*, 2004) and Malawi (Milledge *et al.*, 2005; Graham, 2002). Mwansa *et al*., (2002), observed that antimicrobial sensitivity patterns in their study indicated that resistance was a substantial problem among enterobacteria in humans in, Zambia. And Nyeleti *et al*., (2004), in their study observed that the *Salmonella* isolates tested from the poultry production environment in Zambia displayed multiple antibiotic resistances to a number of antibiotics used to treat both humans and animals. The resistance may be attributed to indiscriminate use of antibiotics in human and animal medical practices leading to the emergence of resistant strains of bacteria which may be hard to treat (Wegner *et al.*, 1999).

The findings in this study indicate potential public health threats posed by both dogs and poultry and highlight the importance of implementing preventive measures to reduce *Salmonella* contamination which may ultimately affect humans. The use of antibiotics should therefore be well regulated and instituted only when it is absolutely indicated. In Zambia, laws are not strictly followed on usage and purchase of drugs, and as such some drugs have been subject to abuse especially in poultry production (Pandey and Sharma, 1994). The need for continued surveillance is emphasized to determine local antimicrobial susceptibility data to zoonotic and invasive *Salmonella* in order to identify changing patterns of resistance. Such data is essential for developing appropriate treatment of salmonellosis. Moreover, the prevalence of highly susceptible *S. enteritidis* strains suggests the limited use of antibiograms as an epidemiological marker.

CHAPTER SIX

CONCLUSIONS

1. The isolation of zoonotic *Salmonella* in both diarrhoeic and non-diarrhoeic dogs in Lusaka, Zambia makes them a potential source of infection to humans.

2. The isolation of zoonotic *Salmonella* from poultry in this study reaffirmed reports of other similar studies in Zambia indicating significant presence of *Salmonella* e*nteritidis* in poultry.

3. Results suggest that *Salmonella* serotypes isolated from dogs and chickens do not belong to the same sero groups.

4. The study demonstrated that the *invA* gene which is present and functional in all virulent *Salmonella* strains was detected in all the *Salmonella* isolates from dog, chicken and human sources by PCR and Dot Blot Hybridization.

5. The fact that other virulence genes subject to investigation in this study were found absent or present in varying degrees in the isolates may imply that their expressions could have been affected by different environmental factors in the host.

6. The expression of some virulence genes of *Salmonella* vary from one animal species to another due to the possible adaptation of the bacteria in the affected hosts.

7. Antibiotic susceptibility pattern of *Salmonella* isolates from chicken and dogs demonstrated sensitivity to easily available antibiotics in Zambia while human *Salmonella* isolates only showed limited sensitivity.

CHAPTER SEVEN

RECOMMENDATIONS

1. In this study, the isolation of *S. enteritidis* in clinical samples from chickens raises great public health concern due to its zoonotic transmission to humans and ability to cause severe disease in man. There is need for public awareness campaigns and notifications to be incorporated in the routine strategic processes of the Governments Public Health Department, which should also include educating the food business entrepreneurs and people involved in food handling.

2. In view of the research finding, that dogs in Zambia can harbor *Salmonella* whose serotype may be similar to those that are found in man and therefore present a domestic and occupational hazard, there is need to develop comprehensive policies to ensure good hygiene practices, environmental infection control, strict quarantine procedures, personal protective equipment and other biosecurity measures to reduce the risk of human infections wherever dogs are kept, especially free range dogs.

3. Veterinarians can be an important link to reducing the incidence of non-typhoidal salmonellosis in humans by assisting in the development and implementation of control strategies to reduce carriage of *Salmonella* by food-producing and companion animals.

4. Routine PCR based procedures can have a major benefit in rapid disease diagnosis and epidemiological investigations.

5. Regular epidemiological investigations may assist in monitoring the occurrence of salmonellosis and in development of strategies for its prevention.

6. A better understanding of the interplay of factors (virulence genes) that contribute to the incidence, distribution and establishment of salmonellosis may be helpful in eliminating or reducing the presence and survival of *Salmonella* in the environment thereby protecting and promoting public health.

REFERENCES

Adejuyigbe, E. A., Ako-Nai, A. K. and Adisa, B. (2004). Bacterial isolates in the sick young infant in Ile-Ife, Nigeria. J. Trop. Ped. **50:**323-327.

Alphons, J. A. M., van Asten, A. J. and van Dijk, J. E. (2005). Mini review: Distribution of "classic" virulence factors among *Salmonella* species. FEMS Immun. Med. Micro. **44:**251-259.

Altekruse, S., Koehler, J., Hickman-Brenner, F., Tauxe, R. V. and Ferris, K. (1993). A comparison of *Salmonella enteritidis* phage types from egg-associated outbreaks and implicated laying flocks. Epid. and Infec. **110:**17-22.

Altier, C. (2005). Genetic and environmental control of *Salmonella* invasion. J. Micro. **43:**85-92.

Amavisit, P., Lightfoot, D., Browning, G. F. and Markham, P. F. (2003). Variation between pathogenic serovars within *Salmonella* pathogenicity islands. J. Bact. **185:**3624-3635.

Anjum, A. D. (1997). *Poultry Diseases*. 2nd Ed. Pakistan: Veterinary and Agriculture, Publications, Faisalabad, pp 23-25.

Anonymous, (2004). Terrestrial Animal health code. 3rd Ed. World Health Organisation for Animal Health, 12, ruedeprony, 75017, Paris, France.

Anthea, K. L., Detweiler, C. S. and Falkow, S. (2000). OmpR regulates the two-omponent system srA-SsrB in *Salmonella* Pathogenicity Island 2. J. Bact. **182:**771-781.

Antunes, P., Reu, C., Sousa, J. C., Peixe, L. and Pestana, N. (2003). Incidence of *Salmonella* from poultry and their susceptibility to antimicrobial agents. Int. J. Food Micr. **82:**97-103.

Archibald, L. K., den Dulk, M. O., Pallangyo, K. J. and Reller, L. B. (1998). Fatal *Mycobacterium tuberculosis* bloodstream infections in febrile hospitalized adults in Dar es Salaam, Tanzania. Clin. Infect. Dis. **26:**290-296.

Ata, Z. and Aydin, N. (2008). Isolation of *Salmonella* spp. In Ankara region poultry plants. J. Ankara Univ. Vet. Fac. **55:**161-166.

Bailey, J. S. (1988). Integrated colonization control of *Salmonella* in poultry. Poult. Sci. **67:**928-932.

Bailey, J. S., Cox, N. A. and Berrang, M. E. (1994). Hatchery-acquired *Salmonella* in broiler chicks. Poult. Sci. **73:**1153-1157.

Bajaj, V., Lucas, R. L., Hwang, C. and Lee, C. A. (1996). Co-ordinate regulation of *Salmonella typhimurium* invasion genes by environmental and regulatory factors is mediated by control of *hilA* expression. Mol. Microbiol. **22:**703-714.

Bao, V. N. (2005): Prevalence of *Salmonella* and *Campylobacter* spp. from broiler meat in abattoirs at Ho Chi Minh city, Vietnam. Free University, Berlin dissertation.

Barrow, P. A. (1989). Salmonellosis-Prospects for microbiological control in poultry. Av. Path. **18:**557-561.

Barrow, P.A. (1993). A *Salmonella* control-past, present and future. Av. Path. **22:**651-69.

Barrow, G. I. and Feltham, R. K. A. (1993). Cowan and Steel's Manual for the identification of Medical bacteria. Cambridge, England.

Barrow, P. A., Huggins, M. B. and Lovell, M. A. (1994). Host Specificity of *Salmonella* Infection in Chickens and Mice is expressed in Vivo Primarily at the Level of the Reticuloendothelial System. Infect. Immun. **62:**4602-4610.

Bauer, A. W., Kirby, W. M., Sherris, J. C. and Turck, M. (1966). Antibiotic sensitivity testing by a standardized single disc method. Am. J. Clin. Pathol. **45:**493-496.

Baumgartner, A., Heurnann, P., Schmid, H., Liniger, M. and Simmen, A. (1992). *Salmonella* contamination of poultry carcasses and human salmonellosis. Archv. Fur. Leben. **43:**123-124.

Baumler, A. J., Tsolis, R. M., Ficht, T. A. and Adams, L. G. (1998). Evolution of host adaptation in *Salmonella enterica*. Infect. Immun. **66:**4579-4587.

Baumler, A. J., Gilde, A. J. and Tsolis, R. M. (1997). Contribution of horizontal gene transfer and deletion events to development of distinctive patterns of fimbrial operons during evolution of *Salmonella* serotypes. J. Bacteriol. **179:**317-322.

Baumler, A. J., Tsolis, R. M., Ficht, T. A. and Adams, L. G. (1998). Evolution of host adaptation in *Salmonella enterica*. Infect. Immun. **66:**4579-4587.

Beal, R. K., Wigley, P., Powers, C., Hulme, S. D., Barrow, P. A. and Smith, A. L. (**2004).** Age at primary infection with *Salmonella enterica* serovar *typhimurium* in the chicken influences persistence of infection and subsequent immunity to re-challenge. Vet. Immunol. Immunopathol. **100:**151-164.

Beli, E., Duraku, E. and Telo, A. (2001). *Salmonella* serotypes isolated from chicken meat in Albania. Inter. J. Food Micro. **71:**263-266.

Bennasar, A., de Luna, G., Cabrer, B. and Lalucat, J. (2000). Rapid identification of *Salmonella typhimurium*, *Salmonella enteritidis* and *Salmonella virchow* isolates by Polymerase Chain Reaction based fingerprinting methods. Inter. Micro. **3:**31–38.

Berchieri Jr, A. and Freitas Neto, O. C. S. (2009). *Salmoneloses aviárias*. In: Doenças das aves, Berchieri Jr, A.; Silva, E. N.; Di Fábio, J.; Sesti, L.; Zuanaze, M. A. F. 2nd Ed. Seção **4:**435-454, FACTA, Campinas, SP.

Berkley, J. A., Lowe, B. S. and Mwangi, I. (2005). Bacteremia among children admitted to a rural hospital in Kenya. N. Engl. J. Med. **352:**39-47.

Bessa, M. C., Michael, G. B., Canu, N., Canal, C. W., Cardoso, M., Rabsch, W. and Rubino, S. (2007). Phenotypic and genetic characterization of *Salmonella enteric* subsp. *enterica* serovar *typhimurium* isolated from pigs in Rio Grande do Sul, Brazil. Res. Vet. Sci**.** **83:**302-310.

Bonfiglio, G., Simpore, J., Pignatelli, S., Musumeci, S. and Solinas, M. L. (2002). Epidemiology of bacterial resistance in gastro-intestinal pathogens in a tropical area. Int. J. Antimicrob. Agents. **20:**387-389.

Boddicker, J. D., Knosp, B. M. and Jones, B. D. (2003). Transcription of the *Salmonella* invasion gene activator, *hilA*, requires *hilD* activation in the absence of negative regulators. J. Bacteriol **185:**525-533.

Boyd, E. F., Li, J. and Ochman, H. (1997). Comparative genetics of the inv-spa invasion gene complex of *Salmonella enterica*. J. Bacteriol. **179:**1985-1991.

Brenner, F. W., Villar, R. G., Angulo, F. J., Tauxe, R. and Swaminathan, B. (2000). *Salmonella* nomenclature. J. Clin. Micro. **38:**2465-2467.

Brent, A. J., Oundo, J. O., Mwangi, I., Ochola, L. and Lowe, B. (2006). *Salmonella* bacteremia in Kenyan children. Pediatr. Infect. Dis. J. **25**:230-236.

Buxton, A. and Fraser, G. (1977). Animal microbiology. Blackwell Scientific Publications, Oxford, United Kingdom.

Bywater, R. J. (2004). Veterinary use of antimicrobials and emergence of resistance in zoonotic and sentinel bacteria in the EU. J. Vet. Med.. **51:**361-363.

Cai, H. Y., Lu, L., Muckle, C. A., Prescott, J. F. and Chen, S. (2005). Development of novel protein microarray method for serotyping *Salmonella enterica* strains. J. Clin. Micro**.** *Diseases of Poultry*. 10th Ed. Iowa State University Press.

Cantor, G. H., Nelson, S. and Vanek, J. A. (1997). *Salmonella* shedding in racing sledge dogs. J. Vet. Diagn. Invest. **9:**447-448.

Capita, R., Alvarez-Astorga, M., Alonso-Calleja, C., Moreno, B. and Garcia-Fernandez, M. D. C. (2003). Occurrence of *Salmonella* in retail chicken carcasses and their products in Spain. Inter. J. Food Micro. **81:**169-173.

Carattoli, A. (2003). Plasmid-Mediated Antimicrobial Resistance in *Salmonella enterica.* Mol. Biol; **5:**113-122.

Cardoso, A. L. S. P., Tessari, E. N. C., Castro, A. G. M., Kanashiro, A. M. I. and Gama, N. M. S. Q. (2002). Pesquisa de *Salmonella* spp. em ovos comerciais, analisados no Laboratório de Patologia avícola de Descalvado, SP. Revista Higiene Alimentar, São Paulo. **16:**76-79.

Carter, M. E. and Quinn, J. P. (2000). *Salmonella* infections in dogs and cats. In: Wray, C., and Wray, A. *Salmonella* in Domestic Animals. CAB International, Wallingford, UK. pp. 231-244.

Centers for Disease Control and Prevention, (2009). Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food - 10 states. Morb. Mortal. Wkly. Rep. **5**9:418-422.

Centers for Disease Control and Prevention, (2001). Outbreaks of multidrug-resistant *Salmonella typhimurium* associated with veterinary facilities: Idaho, Minnesota, and Washington, 1999. MMWR Morbid Mortal Wkly Rep. **50:**701-704.

Central Statistics Office (CSO) (2008). Livestock and Housing Population Census of Zamia, Government of the Republic of Zambia, Government Printers, Lusaka, Zambia.

Chao, M. R., Hsien, C. H., Yeh, C. M., Chou, S. J., Chu, C., Su, Y. C. and Yu, C. Y. (2007). Assessing the prevalence of *Salmonella enterica* in poultry hatcheries by using hatched eggshell membranes. Poult. Sci. **86:**1651-1655.

Cheesbrough, J. S., Taxman, B. C., Green, S. D., Mewa, F. and Numbi, I. (1997). A clinical definition for invasive *Salmonella* infection in African children. Pediatr. Infect. Dis. J. **16:**277-283.

Cherayil, B. J., McCormick, B. A. and Bosley, J. (2000). *Salmonella enterica* Serovar *typhimurium*-Dependent Regulation of Inducible Nitrite Oxide Synthase Expression in Macrophages by Invasins *sipB, sipC, sipD* and Effector *sopE*2. Infect. Immun. **68:**5567-5574.

Chintu, C., Zumla, A., Luo, N., Mathewson, J., Perera, C. and Ngwenya, B. (1993). Acute and chronic diarrhea (AD, CD) in HIV-1 infected patients, Lusaka, Zambia and Houston, Texas. Int. Conf. AIDS. **9:**64.

Chiu, C. H., Su, L. H. and Chu, C. (2004). *Salmonella enterica* serotype *choleraesuis*: epidemiology, pathogenesis, clinical disease, and treatment. Clin. Microbiol. Rev. **17:**311-322.

Coburn, B., Grassl, G. A. and Finlay, B. B. (2007). *Salmonella*, the host and disease: a brief review. Immunol. Cell Biol. **85:**112-118.

Collazo, C. M. and Galán, J. E. (1997). The invasion-associated type III protein secretion system in *Salmonella.* Gene. **192:**51-59.

Darwin, K. H. and Miller, V. L. (1999a). *InvF* is required for expression of genes encoding proteins secreted by the SPI1 type III secretion apparatus in *Salmonella typhimurium*. J. Bacteriol. **181:**4949-4954

Darwin, K. H. and Miller, V. L. (1999b). Molecular basis of the interaction of *Salmonella* with the intestinal mucosa. Clin. Microbiol. Rev. **12:**405-428.

Darwin, K. H. and Miller, V. L. (2000). The putative invasion protein chaperone *sicA* acts together with *invF* to activate the. expression of *Salmonella typhimurium* virulence genes. Mol. Microbiol. **35:**949-960.

Dube, S. D. and Bhagwat, A. G. (1983). Non-typhoidal *Salmonella* infection in Zambian infants. Transactions of the Royal Society of Tropical Medicine and Hygiene. **77:**336-337.

EFSA (European Food Safety Authority), (2006). Analysis of the baseline study on the prevalence of *Salmonella* in laying hen flocks of Gallus. EFSA Journal. **81:**1-71.

EFSA (European Food Safety Authority), (2008). Scientific opinion of the panel on Biological hazards on a request from the European Commission on a quantitative microbiological risk assessment on *Salmonella* in meat: Source attribution for human salmonellosis from meat. EFSA Journal. **625:**1.

Eichelberg, K. and Galan, J. E. (1999). Differential regulation of *Salmonella typhimurium* type III secreted proteins by pathogenicity island 1 (SPI-1)-encoded transcriptional activators *invF* and *hilA*. Infect. Immun. **67:**4099-4105.

Enwere, G., Biney, E. and Cheung, Y. B. (2006). Epidemiologic and clinical characteristics of community acquired invasive bacterial infections in children aged 2-29 months in the Gambia. Pediatr. Infect. Dis. J. **25:**700-705.

Ethelberg, S., Lisby, M., Torpdahl, M., Sørensen, G., Neimann, J., Rasmussen, P., Bang, S., Stamer, U., Hansson, H. B., Nygård, K., Baggesen, D. L., Nielsen, E. M., Mølbak, K. and Helms, M. (2004). Prolonged restaurant-associated outbreak of multidrug-resistant *Salmonella typhimurium* among patients from several European countries. Clin. Microbiol. Infect**. 10:**904-910.

Fadlalla, I. M. T., Hamid, M. E., Rahim, A. G. A. and Ibrahim, M. T. (2012). Antimicrobial susceptibility of *Salmonella* serotypes isolated from human and animals in Sudan. J. Pub. Heal. Epid. **4:**19-23.

Fluit, A.C. (2005). Mini review: Towards more virulent and antibiotic-resistant *Salmonella*? FEMS Imm. Med. Microbiol. **43:**1-11.

Foley, S. L., White, D. G., McDermott, P. F., Walker, R. D., Rhodes, B., Fedorka- Cray, P. J., Simjee, S. and Zhao, S. (2006). Comparison of subtyping methods for differentiating *Salmonella enterica* serovar *typhimurium* isolates obtained from food animal sources. J. Clin. Microbiol. **44:**3569-3577.

Fukushima, H., Nakamura, R., Iitsuka, S., Ito, Y. and Saito, K. (1985). Presence of zoonotic pathogens (*Yersinia* spp., *Campylobacter jejuni*, *Salmonella* spp. and *Leptospira* spp.) simultaneously in dogs and cats. Zentralbl. Bakteriol. Mikrobiol. Hyg. **181:**430-440.

Galan, J. E. and Curtiss, R. (1989). Cloning and molecular characterization of genes whose products allow *Salmonella typhimurium* to penetrate tissue culture cells. Proc. Natl. Acad. Sci. USA. **86:**6383-6387.

Galan, J. E. and Curtiss, R. (1990). Expression of *Salmonella typhimurium* genes required for invasion is regulated by changes in DNA supercoiling. Infect. Immun. **58:**1879-1885.

Galán, J. E. (1996). Molecular genetic bases of *Salmonella* entry into host cells. Mol. Microbiol. **20:**263-71.

Galan, J. E. and Zhou, D. (2000). Striking a balance: modulation of the actin cytoskeleton by *Salmonella.* Proc. Natl. Acad. Sci. USA. **95:**8754-8761.

Galton, M. M., Harless, M. and Hardy, A. V. (1955). *Salmonella* isolation from dehydrated dog meats. J. Amer. Vet. Med. Assoc. **127:**57-58.

Galton, M. M. (1969). Humans and pets as sources of *Salmonella*. J. Am. Chem. Soc. **46:**230-232.

Garcia del-Portillo, F., Zwick, M. B., Leung, K. Y. and Finlay, B. B. (1993). *Salmonella* induces the formation of filamentous structures containing lysosomal membrane glycoproteins in epithelial cells. Proc. Natl. Acad. Sci. USA. **90:**10544-10548.

Gaspar, P. and Hrabeta, P. (1977). A survey of salmonellosis in domestic animals in Zambia. Bulletin of Animal Health and Production in Africa. **25:**61-64.

Gast, R. K. (2003). *Salmonella* Infections, In: *Diseases of Poultry*, Saif, Y. M., Iowa State Press, Ames, Iowa, pp. 567-614.

Gast, R. K. (1997). Paratyphoid infections. In: *Disease of poultry*, Calnek, B. W.; Barnes, H. J.; Beard, C. W.; Mcdougald, L. R.; Saif, Y. M. Tenth edition. Ames: Iowa State University Press, pp. 97-129.

Gilks, C, F., Brindle, R. J. and Otieno, L. S. (1990). Life-threatening bacteraemia in HIV-1 seropositive adults admitted to hospital in Nairobi, Kenya. Lancet. **336:**545-549.

Giannella, R. A. (1996). *Salmonella*. In: Baron S. Medical Microbiology. 4th Ed. Galveston (TX): University of Texas Medical Branch at Galveston.

Ginocchio, C. C., Rahn, K., Clarke, R. C. and Galan, J. E. (1997). Naturally occurring deletions in the centisome 63 pathogenicity island of environmental isolates of *Salmonella* spp. Infect. Immun. **65:**1267-1272.

Goncagul, G., Gunaydin, E. and Carli, K. T. (2005). Prevalence of *Salmonella* serogroups in chicken meat. Turk. J. Vet. Anim. Sci. **29:**103-106.

Gordon, M. A., Walsh, A. L. and Chaponda, M. (2001). Bacteraemia and mortality among adult medical admissions in Malawi: predominance of non-Typhi *Salmonella* and *Streptococcus pneumoniae*. J. Infect. **42:**44-49.

Gordon, M. A., Graham, S. M. and Walsh, A. L. (2008). Epidemics of invasive *Salmonella enterica* serovar *enteritidis* and *S. enterica* serovar *typhimurium* infection associated with multidrug resistance among adults and children in Malawi. Clin. Infect. Dis. **46:**963-969.

Graham, S. M. (2002). Salmonellosis in children in developing and developed countries and populations. Curr. Opin. Infect. Dis. **15:**507-512.

Green, S. D. and Cheesbrough, J. S. (1993). *Salmonella* bacteraemia among young children at a rural hospital in western Zaire. Ann. Trop. Paediatr. **13:**45-53.

Greene, C. E. (1998). Enteric bacterial infections - Salmonellosis. In: Greene CE, ed. Infections of dog and cat, 2nd Edition, Toronto: WB Saunders, pp. 235-240.

Gordon, M. A. (2008). *Salmonella* infections in immunocompromised adults. J. Infect. **56:**413-422.

Gordon, M. A., Graham, S. M. and Walsh, A. L. (2008). Epidemics of invasive *Salmonella enterica* serovar *enteritidis* and *S. enterica* serovar *typhimurium* infection associated with multidrug resistance among adults and children in Malawi. Clin. Infect. Dis. **46:**963-969.

Guardabassi, L., Schwarz, S. and Lloyd, D. H. (2004). Pet animals as reservoirs of antimicrobial-resistant bacteria: Review. J. Antimicrob. Chemother. **54:**321-332.

Hacker, J. and Kaper, J. B. (2000). Pathogenicity islands and the evolution of microbes. Annu. Rev. Microbiol. **54:**641-679.

Haimovich, B. and Venkatesan, M. M. (2006). *Shigella* and *Salmonella*: Death as a means of survival. Micro. and infec**. 8:**568-577.

Hald, T., Vose, D., Wegener, H. C. and Koupeev, T. (2004). A Bayesian approach to quantify the contribution of animal-food sources to human salmonellosis. Risk Anal. **24:**255-269.

Hang'ombe, B. M., Sharma, R. N., Skjerve, E. and Tuchili, L. (1999a). Occurrence of *Salmonella enteritidis* in pooled table eggs and market-ready chickens in Zambia. Avian Dis. **43:**597-599.

Hang'ombe, B. M., Sharma, R. N., Tuchili, L. and Skjerve, E. (1999b). Isolation of bacteria from table eggs in Zambia. Ind. J. Anim.Sci. **69:**445-447.

Hang'ombe, B. M., Isogai, E., Mubita, C., Isogai, N., Silungwe, M., Chisenga, C., Moonga, L., Mulenga, E., Yabe, J., Takaya, Y.T., Kurebayashi, Y. and Isogai, H. (2008). Detection of *invA, spiC, sipC, invF, and hilA* in *Salmonella* isolated from beef and poultry by Dot Blot Hybridization in Zambia. J. Appl. Res. Vet. Med. **6:**1-6.

Hang'ombe, B. M., Ulaya, W., Mwansa, J. C. L., Mubita, C., Isogai, N., Mulenga, E., Moonga, L., Isogai, H. and Isogai, E. (2011). Feasibility of using dot blot hybridization to

detect *Salmonella invA, spiC* and *sipC* directly from clinical specimens. Afr. J. Microbiol. Res. **5:**857-860

Hansen-Wester, I. and Hensel, M. (2001). *Salmonella* pathogenicity islands encoding type III secretion systems. Microbes Infect. **3:**549-559.

Hayward, R. D. and Koronakis, V. (1999). Direct nucleation and bundling of actin by the *SipC* protein of invasive *Salmonella*. EMBO Journal. **18:**4926-4934.

Hensel, M., Shea, J. E., Bäumler, A. J., Gleeson, C., Blattner, F. and Holden, D. W. (1997a). Analysis of the Boundaries of *Salmonella* Pathogenicity Island 2 and the Corresponding Chromosomal Region of *Escherichia coli* K-12. J. Bacteriol. **179:**1105-1111.

Hensel, M., Shea, J. E., Raupach, B., Monack, D., Falkow, S., Gleeson, C., Kubo, T. and Holden, D. W. (1997b). Functional analysis of *ssaJ* and *ssaK/U* operon, 13 genes encoding components of the type III secretion apparatus of *Salmonella* Pathogenicity Island 2. Mol Microbiol. **24:**155-167.

Hensel, M. (2004). Evolution of pathogenicity islands of *Salmonella enterica*. Int. J. Med. Microbiol. **294:**95-102.

Hentschel, U. and Hacker, J. (2001). Pathogenicity islands: the tip of the iceberg. Microbes Infect. **3:**545-548.

Heithoff, D. M., Sinsheimer, R. L., Low, D. A. and Mahan, M. J. (1999). An essential role for DNA adenine methylation in bacterial virulence. Science. **284:**967-970.

Helms, M., Vastrup, P. and Gerner-Smidt, P. (2002). Excess mortality associated with antimicrobial drug-resistant *Salmonella typhimurium*. Emerging Infectious Diseases. **8:**490-495.

Henderson S. C., Bounous D. I. and Lee M. D. (1999). Early events in the pathogenesis of avian Salmonellosis. Infect. Immun. **67:**3580-3586.

Hersh, D., Monack, D. M., Smith, M. R., Ghori, N., Falkow, S. and Zychlinsky, A. (1999). The *Salmonella* invasin s*ipB* induces macrophage apoptosis by binding to caspase-1. Proceedings of the National Academy of Sciences, USA, **96:**2396-2401.

Hirsh, D. C., Machachlan, N. J. and Walker, R. L. (2004). Veterinary Microbiology, 2nd edition. Blackwell Publishing, Oxford.

Hoelzer, K., Isabel, A., Switt, M. and Wiedmann, M. (2011). Animal contact as a source of human non-typhoidal salmonellosis. Vet. Res. **42:**34.

Jamshidi, A., Bassami, M. R. and Afshari-Nic, S. (2008). Identification of *Salmonella* serovars *typhimurium* by a multiplex PCR-Based assay from Poultry carcasses in Mashhad-Iran. Int. J. Vet. Res. **3:**43-48.

Jerngklinchan, J., Koowatananukul, C., Daengprom, K. and Saitanu, K. (1994). Occurrence of *Salmonella* in raw broilers and their products in Thailand. J. Food. Protect. **57:**808-810.

Jones, B. D. (2005): *Salmonella* gene invasion regulation: A story of environmental awareness. J. Micro**. 43:**110-117.

Jong, B de. and Ekdahl, K. (2006). Human salmonellosis in travellers is highly correlated to the prevalence of *Salmonella* in laying hen flocks. Eurosurveillance.

Jordan, E., Egan, J., Dullea, C., Ward, J., McGillicuddy, K., Murray, G., Murphy, A., Bradshaw, B., Leonard, N., Rafter, P. and McDowell, S. (2006). *Salmonella* surveillance in raw and cooked meat and meat products in the Republic of Ireland from 2002 to 2004. Inter. J. F. Micro. **112:**66-70.

Kabilika, S., Musonda, M. and Sharma, N. R. (1999). Bacteria flora from dead-in-shell chicken embryos from Zambia. Ind. J. Vet.Res. **8:**1-6.

Kardos, G., Farkas, T., Antal, M., Nógrády, N. and Kiss, I. (2007). Novel PCR assay for identification of *Salmonella enterica* serovar Infantis. Lett. Appl. Microbiol. **45:**421-425.

Kahrs, R. F., Holmes, D. N. and Poppensiek, G. D. (1978). Diseases transmitted from pets to man: An evolving concern for veterinarians. Cornell Vet. **68:**442-459.

Kariuki, S., Revathi, G., Muyodi, J., Mwituria, J., Munyalo, A., Kagendo, D., Murungi, L. and Hart, C. A. (2005). Increasing prevalence of multidrug-resistant non-typhoidal *Salmonella*, Kenya, 1994–2003. Int. J. Antimicrob Agents. **25:**39-45.

Kilinc, U. and Aydin, F. (2006). Kayseri yoresindeki tavukculuk isletmelerinden toplanan tavuklardan izole edilen Salmonella turlerinin antibiyotiklere duyarliliklari. Erciyes. Univ. Saglik. Bil. Derg. **15:**35-40.

Kingsley, R. A. and Baumler, A. J. (2000). Host adaptation and the emergence of infectious disease: the *Salmonella* paradigm. Mol. Microbiol. **36:**1006-1014.

Knowles, D. P. and Gorham, J. R. (1990). Diagnosis of viral and bacterial diseases. Rev. Sci. Tech. Off. Int. Epizool. **9:**733-757.

Kotetishvilli, M., Stine, O. C., Kreger, A., Morris Jr, J. G. and Sulakvelidze, A. (2002). Multilocus sequence typing for characterization of clinical and environmental *Salmonella* strains. J Clin Microbiol**. 40:**1626-1635.

Kozak, M., Horosova, K., Lasanda, V., Bilek, J. and Kyselova, J. (2003). Do dogs and cats present a risk of transmission of salmonellosis to humans? Bratise. Lek. Listy. **104:**323-328

Krieg, N. R. and Holt, J. G. (1984). *Salmonella*. In: Bergey's manual of systematic bacteriology Williams and Wilkinson, Baltimore, London.

Kubori, T., Sukhan, A. and Aizawa, S. (2000). Molecular characterization and assembly of the needle complex of the *Salmonella typhimurium* type III protein secretion system. Proc. Natl. Acad. Sci. USA. **97:**10225-10230.

Lee, J. A. (1974). Recent trends in human salmonellosis in England and Wales: the epidemiology of prevalent serotypes other than *Salmonella typhimurium*. J. Hyg. **72:**185-95.

Lepage, P., Bogaerts, J., Van Goethem, C., Hitimana, D. G. and Nsengumuremyi, F. (1990). Multiresistant *Salmonella typhimurium* systemic infection in Rwanda. Clinical features and treatment with cefotaxime. J. Antimicrob. Chemother. **26:**53-57.

Lindstedt, B. A., Vardund, T., Aas, L. and Kapperud, G. (2004). Multiple-locus variable-number tandem-repeats analysis of *Salmonella enterica* subsp. enterica serovar *typhimurium* using PCR multiplexing and multicolor capillary electrophoresis. J. Microbiol. Methods. **59:**163-172.

Lister, S.A. (1988). *Salmonella enteritidis* infection in broilers and broiler breeders. Veterinary Record. **123:**350.

Lucas, R. I., Lostroh, C. P., DiRusso, C. C., Spector, M. P., Wanner, B. L. and Lee, C. A. (2000). Multiple Factors Independently Regulate *hilA* and Invasion Gene Expression in *Salmonella enterica* Serovar *typhimurium*. J. Bacteriol. **182:**1872-1882.

Lutful Kabir, S. M. (2010). Avian colibacillosis and salmonellosis: a closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. Int. J. Environ. Res. Public Health.**7:**89-114.

Maharjan, M., Joshi, V., Joshi, D. D. and Manandhar, P. (2006). Prevalence of *Salmonella* species in various raw meat samples of a local market in Kathmandu. Part II. Trends in the study of disease agents. **1081:**249-256.

Majowicz, S. E, Musto, J., Scallan, E., Angulo, F. J., Kirk, M., O'Brien, S. J., Jones, T. F., Fazil, A. and Hoekstra, R. M. (**2010**). The global burden of nontyphoidal *Salmonella* gastroenteritis. Clin Infect Dis. **50:**882-889.

Malinen, E., Kassinen, A., Rinttilä, T. and Palva, A. (2003). Comparison of real-time PCR with SYBR Green I or 5′-nuclease assays and dot-blot hybridization with rDNA-targeted oligonucleotide probes in quantification of selected faecal bacteria. Microbiology. **149:**269-277.

Mallinson, E. T., Joseph, S. M. and deRezende, C. L. E. (2001). *Salmonella* control and quality assurance at the farm end of the food safety continuum. J. Am. Vet. Med. Assoc. **218:**1919-1922.

Marcus, S. L., Brumell, J. H., Pfeifer, C. G. and Finlay, B. B. (2000). *Salmonella* pathogenicity islands: big virulence in small packages. Microbes Infect. **2:**145-156.

Marks, S. L. and Kather, E. J. (2003). Bacteria–associated diarrhea in dogs: A critical appraisal. J. Pract. **33:**1029-1060.

Mastroeni, P., Ugrinovic, S., Chandra, A., MacLennan, C., Doffinger, R. and Kumararatne, D. (2003). Resistance and susceptibility to *Salmonella* infections: lessons from mice and patients with immunodeficiencies. Rev. Med. Microbiol. **14:**53-62.

Matsumoto, Y., Yamada, M., Suzuki, M., Kitazume, H., Muto, T. and Toba, M. (2006). An epidemiological study of outbreaks of *Salmonella enterica* serovar *paratyphi* A occurred in two Chinese families after traveled to different areas of China. Kansenshogaku Zaashi**. 80:**647-649.

Mc Gavin, D. M., Carlton, W. W. and Zachary, J. F. (2001), Thompson special Veterinary Pathology, 3rd Ed. Mosby, an affiliate of Elsevier's (Health) Sciences Rights Department, Philadelphia, USA., pp. 43-46.

Meara, P. J. (1973). Salmonellosis in slaughter animals as a source of human food poisoning. Jnl. S. Afr. vet. Ass. **44:**152-233.

Medeiros, M. A. N. (2011). Prevalência de *Salmonella* spp. e resistência antimicrobiana dos isolados em carcaças de frango congelado no varejo. Brasil, 2004 a 2006. In: Seminário Internacional de Salmoneloses Aviárias**,** Rio de Janeiro, R. J. Anais.

Michael, J., Angelichio, D. and Andrew, C. (2000). In Vivo Expression Technology. Infec. Immun. **70:**6518-6523.

Milledge, J., Calis, J. C. and Graham, S. M. (2005). Aetiology of neonatal sepsis in Blantyre, Malawi: 1996–2001. Ann. Trop. Paediatr. **25:**101-110.

Millemann, Y., Gaubert, S., Remy, D. and Colmin, C. (2000). Evaluation of IS200-PCR and Comparison with Other Molecular Markers to Trace *Salmonella enterica* subsp. *enterica* Serotype *typhimurium* Bovine Isolates from Farm to Meat. J. Clin. Microbiol. **38:**2204-2209.

Mills, S. D. and Finlay, B. B. (1994). Comparison of *Salmonella typhi* and *Salmonella typhimurium* invasion, intracellular growth and localization in cultured human epithelial cells. Microb. Pathog. **17:**409-423.

Moon, C. (2003). Table scraps to kibble and back again. Minneapolis, USA: Twin Cities Dog. **4:**8-10.

Moran, A. B. (1961). Occurrence and Distribution of *Salmonella* in Animals in the United States. U.S. Livestock Sanitary Association's Proceedings, 65th Annual Meeting. pp. 441-448.

Morse, E. V., Duncan, M. A., Estep, D. A., Riggs, W. A. and Blackburn, B.O. (1976). Canine salmonellosis: a review and report of dog to child transmission of *Salmonella enteritidis*. Am J Public Health. **66:**82-84.

Morse, E. V. and Duncan, M. A. (1975). Canine Salmonellosis: prevalence, epidemiology, signs, and public health significance. J. Am. Vet. Med. Assoc. **167:**817-820.

Mwansa, J., Mutela, K., Zulu, I., Amadi, B. and Kelly, P. (2002). Antimicrobial Sensitivity in Enterobacteria from AIDS Patients, Zambia. Emerg. Infect. Dis. **8:**92-93.

Nambota, A. M., Mweene, A. S. and Muma, J. B. (2000). Training manual for the 2nd third country training course (Zoonosis module). Printed by the School of Veterinary Medicine, University of Zambia, Lusaka, Zambia, in conjunction with Japan International Cooperation Agency, pp. 33-34.

Nashwa, H. M., Mahmoud, A., Sami, H. and Adawy, S. (2009). Application of Multiplex Polymerase Chain Reaction (M-PCR) for Identification an Characterization *Salmonella enteritidis* and *Salmonella typhimurium.* J. Appl. Sci. Res. **5:**2343-2348.

Nataro, J. P., Bopp, C. A., Fields, P. I., Kaper, J. B. and Strockbine, N. A. (2007). *Escherichia*, *Shigella*, and *Salmonella*. In Manual of Clinical Microbiology. 9th edition. P. R.

Nierop, W. V., Duse, A. G., Marais, E., Aithma, N., Thothobolo, N., Kassel, M., Stewart, R., Potgieter, A., Fernandes, B., Galpin, J. S. and Bloomfield, S. F. (2005). Contamination of chicken carcasses in Gauteng, South Africa, by *Salmonella*, *Listeria monocytogenes* and *Campylobacter*. Inter. J. F. Microbio. **99:**1-6.

Nyeleti, C., Cogan, T. A., Kabilika, S. H., Katongo, J. C., Nyirenda, S., Silomba, S., Sinyangwe, P. G. and Humphrey, T. J. (2004). Antibiotic and disinfectant resistance of *Salmonella* serovars isolated from the poultry production environment in Zambia. Bull. Anim. Hlth. Prod. Afr. **52:**168-175.

Ochman, H. and Groisman, E. A. (1996). Distribution of Pathogenicity Islands in *Salmonella* spp. Infect. Immun. **64:**5410-5412.

Ohl, M. E. and Miller, S. I. (2001). *Salmonella*: a model for bacterial pathogenesis. Ann. Rev. Med. **52:**259-274.

Ojo, O. E. and Adetosoye, A. I. (2009). *Salmonella typhimurium* infection in diarrhoeic and non-diarrhoiec. Veterinarski Arhiv. **79:**371-377.

Okeke, I. N., Laxminarayan, R., Bhutta, Z. A., Duse, A. G., Jenkins, P., O'Brien, T. F. and Pablos-Mendez, A. (2005). Antimicrobial resistance in developing countries. Part I: recent trends and current status. Lanc. Infec. Dis.. **5:**481-493.

Oliveira, D. D. and Silva, E. N. (2000). *Salmonella* in table eggs: occurrence in retails, storage conditions and eggshell disinfections. Arq. Bras. Med. Vet. Zootec. **52**: 655-661.

Padungtod, P. and Kaneene, J. B. (2006). *Salmonella* in food animals and humans in northern Thailand. Inter. J. Food Micro. **108:**346-354.

Paesold, G., Guiney, D. G., Eckmann, L. and Kagnoff, M. F. (2002). Genes in *Salmonella* pathogenicity island 2 and the *Salmonella* virulence plasmid are essential for *Salmonella*-induced apoptosis in intestinal epithelial cells. Cell Microbiol. **4:**771-781.

Pandey, G. S. and Sharma, R. N. (1994). Antimicrobial susceptibility patterns of *Salmonella* isolates from cattle and chicken in Zambia. Ind.Vet. Med. J. **18:**175-178.

Petrie, L., Selman, I. E. Grindlay, M. and Thompson, H. (1977). Salmonellosis in young calves due to *Salmonella enteritidis*. Vet. Rec. **101:**398-402.

Plummer, R. A. S., Blissett, S. J. and Dodd, C. E. R., (1995). *Salmonella* Contamination of Retail problems. Microbes and Infection. **3:**237-247.

Porwollik, S., Santiviago, C. A., Cheng, P., Florea, L. and McClelland, M. (2005). Differences in gene content between *Salmonella* enteric serovar *enteritidis* isolates and comparison to closely related serovars *gallinarum* and *dublin.* J. Bacteriol. **187:**6545–6555.

Quinn, P. J., Markey, B. K., Carter, M. E., Donnelly, W. J. and Leonard, F. C. (2002). Veterinary Microbiology and Microbiology Disease, Blackwell Publishing, Oxford.

Rabsch, W., Tschape, H. and Baumler, A.J. (2001). Non-typhoidal salmonellosis: emerging problems. Microbes Infect. **3:**237-247.

Raffatellu, M., Wilson, R. P., Chessa, D., Andrews-Polymenis, H., Tran, Q. T., Lawhon, S., Khare, S., Adams, L. G. and Bäumler, A. J. (2005). *sipA, sopA, sopB, sopD,* and *sopE2* Contribute to *Salmonella enterica* serotype *typhimurium* Invasion of Epithelial Cells. Infect. Immun. **73:**146-154.

Rahn, K., De Grandis, S. A., Clarke, R. C., Curtiss, R. and Gyles, C. L. (1992). Amplification of an *invA* gene sequence of *Salmonalla typhimurium* by polymerase chain reaction as a specific method of detection of *Salmonella*. Mol. Cell. Probes. **6:**271-279.

Rasschaert, G., Houf, K., Imberechts, H., Grijspeerdt, K., De Zutter, L. and Heyndrickx, M. (2005). Comparision of five repetitive-sequence-based PCR typing methods for molecular iscrimination of *Salmonella enterica* isolates. J. Clin. Micro. **43:**3615-3623.

Reddy, E. A., Shaw, A. V. and Crump, J.A. (2010). Community-acquired bloodstream infections in Africa: a systematic review and meta-analysis. Lancet. Infect. Dis.**10:**417-432.

Rescigno, M., Urbano, M., Valzasina, B., Francolini, M. and Rotta, G. (2001). Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. Nat. Immunol. **2:**361-367.

Ross, I. L. and Heuzenroeder, M. W. (2005). Discrimination within phenotypically closely related definitive types of *Salmonella enterica* serovar *typhimurium* by the multiple amplification of phage locus typing technique. J. Clin. Microbial. **43:**1604-1611.

Ross, I. L., Parkinson, I. H. and Heuzenroeder, M. W. (2008). The use of MAPLT and MLVA analyses of phenotypically closely related isolates of *Salmonella enterica* serovar *typhimurium*. Int. J. Med. Microbiol. **10:**1016.

Ryan, K. J. and Ray, C. G. (2004.). Sherris Medical Microbiology: An Introduction to Infectious Disease. 4th Ed. New York**:** McGraw-Hill.

Rychlik, I. and Barrow, P. A. (2005). *Salmonella* stress management and its relevance to behaviour during intestinal colonisation and infection. FEMS Microbiol. Rev. **29:**1021-1040.

Sabbagh, S. C., Forest, C. G., Lepage, C., Leclerc, J. M. and Daigle, F. (2010). So similar, yet so different: uncovering distinctive features in the genomes of *Salmonella enterica* serovars *typhimurium* and *typhi.* FEMS Microbiology Letters. **305:**1-13.

Salyers, A. and Whitt, D. (2002). Bacterial Pathogenesis: A Molecular Approach. 2nd Ed: ASM Press. pp. 539.

Sanchez, S, Hofacre, C, L., Lee, M. D., Maurer, J. J. and Doyle, M. P. (2002). Animal sources of salmonellosis in humans. J. Am. Vet. Med. Assoc. **221:**492-497.

Santos, R. L., Tsolis, R. M., Bäumler, A. J. and Adams, L. G. (2003). Pathogenesis of *Salmonella*-induced enteritis. Braz. J. Med. Biol. Res. **36:**3-12.

Sato, Y., Mori, T., Koyama, T. and Nagase, H. (2000). *Salmonella virchow* infection in an infant transmitted by household dogs. J. Med. Vet. Sci. **62:**767-769.

Santos, L. R., Nascimento, V. P., Flores, M. L., Rosek, H., D'Andrea A., Albuquerque M. C., Rampanelli Y., Machado N. P., Rios S. and Fernandes, S. A. (2002). *Salmonella enteritidis* isoladas de amostras clinicas de humanos e de alimentos envolvidos em episódios de toxinfecções alimentares, ocorridas entre 1995 e 1996, no Estado do Rio Grande do Sul**.** Revista Higiene Alimentar, São Paulo. **6:**93-99.

Schechter, L. M., Damrauer, S. M. and Lee, C. A. (1999). Two AraC/XylS family members can independently counteract the effect of repressing sequences upstream of the *hilA* promoter. Mol. Microbiol. **32:**629-642.

Schechter, L. M. and Lee, C. A. (2001). *AraC/XylS* family members, *hilC* and *hilD*, directly bind and derepress the *Salmonella typhimurium hilA* promoter. Mol. Microbiol. **40:**1289-1299.

Schechter, L. M. and Lee, C. A. (2000). *Salmonella* invasion of non-phagocytic cells. In Subcellular Biochemistry, Oelschlaeger, T., and Hacker, J. (eds). New York: Kluwer Academic/Plenum Publishers. **33:**289-320.

Schwarz, S., Kehrenberg, C. and Walsh, T. R. (2001). Use of antimicrobial agents in veterinary medicine and food animal production. Inter. J. Antimicrob. Agents. **17:** 431-437.

Seepersadsingh, N., Adesiyun, A. A. and Seebaransingh, R. (2004). Prevalence and antimicrobial resistance of *Salmonella* spp. in nondiarrhoeic dogs in Trinidad. J. Vet. Med. B Infect. Dis. Vet. Public Health. **51:**337-342.

Sigauque, B., Roca, A. and Mandomando, I. (2009). Community-acquired bacteremia among children admitted to a rural hospital in Mozambique. Pediatr. Infect. Dis. J. **28:**108-113.

Sikder, A. J., Islam, M. A., Rahman, M. M. and Rahman, M. B. (2005). Seroprevalence of *Salmonella* and *Mycoplasma gallisepticum* infection in the six model breeder poultry farms at Patuakhali district in Bangladesh. Inte. J. Poult. Sci. **4:**905-910.

Solari, C. A., Mandarino; J. R., Panizzutti; M. H. M. and Farias, R. H. G. (2003). A new serovar and a new serological variant belonging to *Salmonella enterica* subspecies *diarizonae* Mem. Inst. Oswaldo Cruz. **98:**501-502.

Soumet, C., Ermel, G., Salvat, G. and Colin, P. (1997). Detection of *Salmonella* specie in food products by polymerase chain reaction and hybridization assay in micro plate format. In Letters of Applied Microbiology. **24:**113-116.

Stein, M. A., Leung, K. Y., Zwick, M., Garcia del-Portillo, F. and Finlay, B. B. *(*1996*)*. Identification of a *Salmonella* virulence gene required for formation of filamentous structures containing lysosomal membrane glycoproteins within epithelial cells. Mol. Microbial. **20:**161-164.

Susan, C. M., Habib, O. R. and John, A. C. (2009). Invasive Non-Typhi *Salmonella* Disease in Africa. Clin. Infect. Dis. **49:**606-611.

Taitt, C. R., Shubin, Y. S. and Angel, R. (2004). Detection of *Salmonella enterica* Serovar *typhimurium* by using a Rapid, Array-Based Immunosensor. Applied and Environmental Microbiology. **70:**152-158.

Takaya, A., Kubota, Y., Isogai, E. and Yamamoto, T. (2005). Degradation of the *hilC* and *hilD* regulator proteins by ATP-dependent on protease leads to down regulation of *Salmonella* pathogenicity island 1 gene expression. Mol. Microb. **73:**146-154.

Trafny, E., Kozlowska, A. and Szpakowska, K. (2006). A novel Multiplex PCR assey for the detection of *Salmonella enterica* serovar e*nteritidis* in human faeces. Lett. Appl. Microbiol. **43:**673-679.

Tessari, E.N.C., Cardoso, A.L.S.P. and Castro, A.G.M. (2003). Prevalência de *Salmonella enteritidis* em carcaças de frango industrialmente processadas. Revista Higiene Alimentar.**17:**52-55.

Tibaijuka, B., Molla, B., Hildebrandt, G. and Kleer, J. (2003). Occurrence of *Salmonella* in retail raw chicken products in Ethiopia. Berliner. und Munchener. Tierar. Fiche. Wochen. **116:**55-58.

Tomley, F, M. and Shirley, M. W. (2009). Livestock infectious diseases and zoonoses. Philos. Trans. R. Soc. Lond. B. Biol. Sci. **364:**2637-2642.

Telzak, E. E., Budnick, L. D., Greenberg, M. S., Blum, S., Shayegani, M., Benson, C. E. and Schultz, S. (1990). A nosocomial outbreak of *Salmonella enteritidis* infection due to the consumption of raw eggs. New England Journal of Medicine. **323:**394-397.

Tindall, B. J., Grimont, P. A., Garrity, G. M. and Euzeby, J. P. (2005). Nomenclature and taxonomy of the genus *Salmonella.* International Journal of Systematic and Evolutionary Microbiology. **55:**521-524.

Tsai, H. J., Huang, H. C., Lin, C. M., Lien, Y. Y. and Chou, C. H. (2007). *Salmonella* and *Campylobacters* in household and stray dogs in northern Taiwan. Vet. Res. Comm. **31:**931-939.

Tsolis, R.M., Adams, L.G., Ficht, T.A. and Baumler, A.J. (1999). Contribution of *Salmonella typhimurium* virulence factors to diarrheal disease in calves. Infect. Immun. **67:**4879-4885.

Tuchili, L., Ulaya, W., Kato, Y. and Kaneuchi, C. (1996). Recent characterization of *Salmonella* strains isolated from chickens in Zambia. J. Vet. Med. Sci. **58:**77-78.

Uesugi, A. R., Danyluk, M. D., Mandrell, R. E. and Harris, L. J. (2007). Isolation of *Salmonella enteritidis* phage type 30 from a single almond orchard over a 5-year period. J. Food Prot**. 70:**1784-1749.

Uyttendaele, M. R., Debevere, J. M., Lips, R. M. and Neyts, K. D. (1998). Prevalence of *Salmonella* in poultry and their products in Belgium. International Journal of Food Microbiology.**40:**1-8.

Uzzau, S., Gulig, P. A., Paglietti, B., Leori, G., Stocker, B. A. and Rubino, S. (2000). Role of the *Salmonella abortusovis* virulence plasmid in the infection of BALB/c mice. FEMS Microbiol. Lett. **188:**15-18.

van Asten, A. J. and van Dijk, J. E. (2005). Distribution of "classic" virulence factors among *Salmonella* species. FEMS Immunology and Medical Microbiology. **44:**251-259.

Van Hoorebeke, S., Van Immerseel, F., Schulz, J., Hartung, J., Harisberger, M., Barco, L., Ricci, A., Theodoropoulos, G., Xylouri, E., De Vylder, J., Ducatelle, R., Haesebrouck, F., Pasmans, F., de Kruif, A. and Dewulf, J. (2010). Determination of the within and between flock prevalence and identification of risk factors for *Salmonella* infections in laying hen flocks housed in conventional and alternative systems. Prev. Vet. Med. **94:**94-100

Vargas-Galindo Á. (2007). Probabilistic inversion in priority setting of food borne pathogens MSc thesis, Delft University of Technology, Department of Applied Mathematics and Risk Analysis.

Velge, P., Cloeckeart, A. and Barrow, P. (2005). Emergence of *Salmonella* epidemics: The problem related to *Salmonella enterica* serotype *enteritidis* and multiple antibiotic resistance in other major serotypes. Veterinary Research**. 36:**267-288.

Vila, J. H., Paniagua, C. D., Santigosa, N. P., Escobar, C.D. and Herrero, A. H. (2008). *Salmonella* in free-living exotic and native turtles and in pet exotic turtles from SW Spain Research in Veterinary Science. **85:**449-452.

Wain, J., House, D., Zafar, A., Baker, S., Nair, S., Kidgell, C., Bhutta, Z., Dougan, G. and Hasan, R. (2005). Vi antigen expression in *Salmonella enterica* serovar Typhi clinical isolates from Pakistan. Journal of Clinical Microbiology. **43:**1158-1165.

Ward, M. P., Brady, T. H., Couëtil, L. L., Liljebjelke, K., Maurer, J. J. and Wu, C. C. (2005). Investigation and control of an outbreak of salmonellosis caused by multidrug resistant *Salmonella typhimurium* in a population of hospitalized horses. Vet. Microbiol. **107:** 233-240.

Watson, P. R., Galyov, E. E., Paulin, S. M., Jones, P. W. and Wallis, T. S. (1998). Mutation of *invH*, but not *stn*, reduces *Salmonella*-Induced enteritis in Cattle. Infect. Immun. **66:**1432-1438.

Withanage, G. S., Kaiser, P., Wigley, P., Powers, C., Mastroeni, P., Brooks, H., Barrow, P., Smith, A., Maskell, D. and McConnell, I. (2004). Rapid expression of chemokines and proinflammatory cytokines in newly hatched chickens infected with *Salmonella enterica* serovar *typhimurium*. Infect. Immun. **72:**2152-2159.

World Health Organisation, (2000). Overcoming antimicrobial resistance. Publication code WHO/CDS/2000.2. Geneva**:** World Health Organization.

World Health Organisation, (2005). WHO media centre, http://www.who.int/mediacentre/factsheets/fs139/en/print.html.

Wegner, H. C., Arestrup, F. M. and Gerner- Smidt, P. (1999). Transfer of antibiotic resistant bacteria from animal to man. Acta Veterinaria Scandinavica Supple. **92:**51-57.

Wray, C., Davies, R. H. and Corkish, J. D. (1996). Enterobacteriaceae. In: Jordan F. T. W. and Pattison M. (Eds). Poultry Diseases. 4th Ed. W.B. Saunders Company Ltd., London.

Wright, J. G., Tengelsen, L. A., Smith, K. E., Bender, J. B., Frank, R. K., Grendon, J. H., Rice, D. H., Thiessen, M., Gilbertson, C. J., Sivapalasingam, S., Barrett, T. J., Besser, T. E., Hancock, D. D. and Angulo, F. J. (2005). Multidrug-resistant *Salmonella typhimurium* in four animal facilities. Emerg. Infect. Dis. **11:**1235-1241.

Yan, S. S., Pandrak, M. L., Abela-Rider, B., Punderson, J. W., Fedorko, D. P. and Foley, S. L. (2003). An overview of *Salmonella* typing public health perspectives. Clin, App. Immuno. Rev. **4:**189-204.

Yang, B., Qu, D., Zhang, X., Shen, J., Cui, S., Shi, Y., Xi, M., Sheng, M., Zhi, S. and Meng, J. (2010). Prevalence and characterization of *Salmonella* serovars in retail meats of marketplace in Shaanxi, China. Inter. J. Food Micro. **141:**63-72.

Zahraei, T., Mahzoonae, M. R. and Ashrafi, A. (2006). Ampllification of *invA* gene of *Salmonella* by polymerase chain reaction (PCR) as a specific method for detection of *Salmonella*. J. Fac. Vet. Med. Univ. Tehran. **61:**195-199.

Zeiko, G. (2011). The Merck Veterinary Manual. 10th Ed. Kahn, M. C. (ed). Merck and Co. Inc, WhiteHouse Station, N.J., USA.

Zhou, D., Mooseker, M. S. and Galan, J. E. (1999). Role of the *S. typhimurium* actin-binding protein *sipA* in bacterial internalization. Science. **283:**2092-2095.

Zhang, S., Santos, R. L., Tsolis, R. M., Stender, S, Hardt, W. D., Bäumler, A. J. and Adams, L. G. (2002). The *Salmonella enterica* serotype *typhimurium* effector *proteins sipA, sopA, sopB, sopD* and *sopE* act in concert to induce diarrhea in calves. Infec. Imm. **70:**3843-3855.

Zhang, S., Kingsley, R. A., Santos, R. L., Andrews-Polymenis, H., Raffatellu, M., Figueiredo, J., Nunes, J., Tsolis, R. M., Adams, L.G. and Bäumler, A. J. (2003). (Minireview) Molecular pathogenesis of *Salmonella enterica* serotype *typhimurium*- induced diarrhea. Infect. Immun**. 71:**1-12.