

**QUANTITATIVE RISK ASSESSMENT OF DEVELOPING SALMONELLOSIS
THROUGH BEEF CONSUMPTION IN LUSAKA PROVINCE, ZAMBIA**

By

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DECLARATION

I, Chabwasi Isaac Manyori do hereby declare that the contents of the dissertation being submitted herein are my own original work and they have not been previously submitted to this or any University for the award of a degree or any other qualification.

SignatureDate.....

ABSTRACT

Bacteria of the genus *Salmonella* may cause disease in many host species (including humans). Consumption of infected beef products has been linked to zoonotic transmission of diseases in humans. The aim was to quantitatively assess the risk of developing salmonellosis through consumption of beef in Lusaka province of Zambia, based on the Codex Alimentarius framework. Data used to achieve this objective were obtained from reviews of scientific literature, government reports, questionnaire survey and expert opinions. The Swift Quantitative Microbial Risk Assessment (SQMRA) model was used to analyse the data. The study was driven by lack of research-based information in this area despite the reported cases of salmonellosis in human and the prevalence of *Salmonella* in beef carcasses being 22% (Hang'ombe *et al.*, 2008).

The results of questionnaire survey on beef consumption showed that 60 percent of people in Lusaka consumed beef once every week, 16 percent consumed once in every two weeks, 15 percent consumed beef once a month and 9 percent consumed every day. Out of the 100 persons interviewed, 89 percent consumed well cooked beef, 9 percent half cooked and only 2 percent consumed raw beef. The average serving portion of beef per meal/individual was 192gm for restaurant consumer while 60gm and 83.1gm were for low and medium levels of beef consumer respectively. At ID₅₀ of 9.61×10^3 cfu/g and retail contamination concentration of 12cfu/gm, the risk of developing salmonellosis through consumption of beef prepared by consumers with low and medium levels of beef consumption was estimated at 0.06% and 0.08%, respectively, while the risk associated with restaurant consumption was estimated at 0.16% per year.

The study concludes that the risk of developing salmonellosis among residents in Lusaka province, as results of beef consumption, was generally low, mainly due to the methods used for food preparation. Further work is required to broaden the scope of the study and also undertake microbiological evaluation of ready-to eat beef from both household and restaurant risk exposure pathways.

DEDICATION

This thesis is deeply dedicated to my best, dearest friend and lovely wife Fatuma Kimanta and my beloved son Abel, my mother Rosemary who, for their sincere love, shared with me the challenges and the hardships during my studies outside the country. I sincerely thank them all immeasurably for their endurance and understanding when I could not avail myself as much as I should have done during the long period of my studies and stay in Zambia. My best friend Fatuma, you missed me a lot and to you, this is the reward of your endurance and I am proud of you my love.

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LIST OF ABBREVIATIONS

aw	Water Activity
CAC	Codex Alimentarius Commission
CC	Cross-contamination
CDC	Centers for Disease Control
cfu	Colony Forming Units
CSF	Cerebral Spinal Fluid
CSO	Central Statistics Office
DDS	Doctor of Dental Surgery
DT104	Definitive Phage Type 104
EFSA	European Food Safety Authority
FAO	Food and Agricultural Organization
Fprd	Percentage of Portion Prepared Done
Fprh	Percentage of Portion Prepared Half Done
Fprr	Percentage of Portion Prepared Raw
FSIS	Food Safety and Inspection Service
h-done	Half Done
HIV	Human Immunodeficiency Virus
ID50	Infectious Dose 50
LPS	Lipopolysaccharide
LT	Labile Toxin
NTS	Non-typhoidal <i>Salmonella</i>

OIE	International Office of Epizootics
OR	Odds Ratio
PCR	Polymerase Chain Reaction
pH	Logarithmic Measure of Hydrogen Ion Concentration
QMRA	Quantitative Microbial Risk Analysis
Sc/r	Percentage of portions that cross contaminate the environment given that they are contaminated at retail
SPI	<i>Salmonella</i> Pathogenicity Islands
SPI 1 and 2	<i>Salmonella</i> Pathogenicity Islands 1 and 2
sQMRA	Swift Quantitative Microbial Risk Analysis
TCC	Total Coliform Count
TFC	Total Faecal Coliform Count
T3SSs	Type III Secretion Systems
T3SS1 and T3SS2	Type III Secretion System 1 and 2
TVC	Total Viable Count
WHO	World Health Organization
WTO	World Trade Organization

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Under the current globalization, we are all residents of a global village. The expanding trade of food and livestock, and increased human travel and migration are means of spreading infectious diseases irrespective of national boundaries (Evans and Leighton, 2014). This makes infectious disease control and food safety important for all countries. This expansion of trade and human travel may lead to transfer of diseases to areas where originally these were not a problem. This is because disease transmission is usually associated with cultural change including eating habits, mass catering, complex and lengthy food supply procedures, increased international movement and poor hygiene practices in native communities (Evans and Leighton, 2014). One of the most widespread infectious foodborne diseases of humans is salmonellosis (Teunis *et al.*, 2010; Carrasco *et al.*, 2012; Kagambèga *et al.*, 2013).

Salmonellosis is a disease of both humans and animals caused by two species of *Salmonella* (*S. enterica* and *S. bongori*) (Kemal, 2014; OIE, 2014). The pathogens cause enteric fevers, gastroenteritis and septicemia which are of both socio-economic and public health importance (Ulaya, 2013). The majority of infections are associated with the ingestion of contaminated foods such as beef and beef products, poultry, pork, eggs, milk, cheese, seafood, fruits, juices, and vegetables (Freitas-Neto *et al.*, 2010; Jackson *et al.*, 2013), although most infections caused by multidrug-resistant *Salmonella* are acquired through contaminated foods of animal origin (Abouzeed *et al.*, 2000).

The population in Lusaka province is at higher risk of getting salmonellosis as most of the risk factors for developing this disease are present. Such risk factors include: consumption of beef in various forms such as offals, T-bone steak, cooked beef which are served with nshima or as a soup, also those which are put on skewer and dried ones

and eaten alone (*'michopo'*) and beef products such as meat pie, sausage rolls, pizza and burger are consumed in various communities. Generally, per capita consumption of beef in Zambia is estimated at 4kg, which is very low compared to other countries like Chile with per capita consumption of beef of 46 pounds (20.6 kg) having the population same as that of Zambia; and Uruguay 81.59 pounds (37kg) with a population of 3.3 million (World Bank, 2011; Rob, 2015).

Although the domestic market is small and under-developed in Zambia, demand for beef products has grown steadily in Lusaka province, the capital region, now home to almost 2.7 million people (CSO, 2015). Shifting consumption patterns are associated with an emerging middle class with increasing purchasing power. There is also an increase in domestic beef production in both commercial and traditional sectors and a rising import of beef and beef products to cover for the increased demand in the country (World Bank, 2011). Further, producers have resorted to importation of beef from other countries to meet local demand. The increase in production may have negative impacts in terms of food safety, especially in traditional production, as the country does not have enough slaughter facilities (Lubungu *et al.*, 2015).

Indeed, Zambia, like other low and middle income countries of Africa, has few formal abattoirs compared to a large number of informal slaughterhouses associated with poor hygienic practices (Haileselassie *et al.*, 2013). There is higher risk of fecal spillage on the meat because of slaughtering on the floor (slaughter slabs). Given this scenario, the chances of producing contaminated carcasses are high, since contamination of carcasses may occur throughout the value chain (from production through to consumption). This might lead to the introduction of *Salmonella* into the food chain if there was an early exposure of domestic animals to the organism that results in long-term persistent infections (Muma, 1998; Isogai *et al.*, 2005; Haileselassie *et al.*, 2013; Ndalama and Mdegela 2013).

Salmonella has been previously detected in human samples in Lusaka; out of the 200 clinical diarrhoea stool samples, 9 (4.5%) were found to be bacteriological culture positive for *Salmonella* (Hang'ombe, 1998). 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 Nontyphoidal Salmonella (NTS) species, respectively. In an earlier study at the University Teaching Hospital (UTH), Lusaka, 45 strains of various NTS species were isolated from stool samples, blood, and cerebral spinal fluid (CSF) (Hang'ombe, 1998). About 93% of the strains were isolated from infants less than two years old. *SalmonellaHeidelberg* was the most common species isolated from stool and revealed a multi-drug resistant character. This shows that Salmonella is present and that there is a risk of getting salmonellosis once an individual consumes contaminated food, including beef, or gets otherwise exposed (e.g. direct contact with infected animals).

This shows that pathogenic *Salmonella* is present in Lusaka and hence there is a risk of human exposure through various means including consumption of contaminated beef.

There is paucity of information on the risk of developing salmonellosis through the consumption of beef in Lusaka province. This study therefore aims to quantitatively assess the risk of developing human salmonellosis through beef consumption. It is hoped that the outcomes of the study would help in the understanding of the risk associated with exposure of this zoonotic organism through consumption of beef and its public health implications.

1.2 Problem statement

Beef is among the most highly nutritious foods as it is a good source of proteins, fats and minerals for humans. However, both raw and cooked beef are good substrate for the growth and multiplication of harmful microorganisms like salmonella species and hence can serve as source of infection to humans (Krauss *et al.*, 2003). Unhygienic handling of beef that is contaminated can be a source of infection and diseases to humans. Apart from health, this zoonotic disease may also have effect on social life and economic capacity of the community.

Recently, Zambia's economy has been growing and so is the economy of Lusaka province resulting in the increase of outlets selling beef products. Therefore, demand for

beef products has grown steadily, due to shifting consumption patterns associated with an increase in population and an emerging middle class with increasing purchasing power (Sinkala *et al.*, 2014). There is also an increase in the number of smaller private slaughter facilities that have poor hygiene standards and sell beef at cheaper prices into informal markets; these particularly poses a serious health hazard to the beef consumers (Lubungu *et al.*, 2015). Beef sold in these informal markets is often not subjected to food safety controls which could present a risk of zoonotic diseases such as salmonellosis to humans. Though hygiene is better informal markets compared to that in the informal markets, even these could still poses some risk since there is no abattoir or butchery which can give 100 percent safety assurance.

Under such circumstances, increased production and consumption of beef including ground beef, dried beef and offals together with other beef products like beef sausage, meat pie, beef burger, beef pizza which are consumed by Zambians increases the risk of getting *Salmonella* infection. In addition, the possible cross-contamination during preparation such as using the same knife and cutting board across different products, and mixed handling of cooked and uncooked meat, especially in the restaurants and other eating points.

Presently, little is known about the risk of human salmonellosis that could occur through consumption of beef in Lusaka Province and Zambia in general. Previous studies have also indicated that among the microbiological hazards in the beef value chain, *Salmonella* has a great public health significance (Plym and Wierup, 2006; Dhanoa and Fatt, 2009; Kemal, 2014). Muma (1998) isolated *Salmonella* from beef carcasses in a survey involving abattoirs in Lusaka and Copperbelt provinces, whose results demonstrated that there was a high level of contamination on carcasses due to poor hygiene status in abattoirs (Muma, 1998; Ntanga 2013). Further, diarrhoeal cases have been reported in Lusaka, some of which were due to *Salmonella* infections (Mwansa *et al.*, 2002; Hang'ombe *et al.*, 2011). It is therefore important to assess whether the increase in beef consumption has a bearing on the public health risk in terms of foodborne hazards. It is hoped that the outcomes of this study would help in the understanding of the risk associated with human exposure to *Salmonella* due to beef consumption and public health implication of this zoonotic organism. The result will also

provide information needed for prevention, control and treatment strategies for human and animal salmonellosis, as well as create awareness on the need to improve good hygienic practices in the beef industry.

1.3 Study justification

Lusaka province is one of the most leading provinces in the country in terms of beef consumption apart from the Copperbelt province. Meat is an important source of protein in the *Zambian* communities. However, while meat is a rich nutrient source, it can also be a potential vehicle of human foodborne illnesses including Salmonellosis. Health risks associated with consumption of beef is little known by the community because in most cases *Salmonella* contaminated beef results in illnesses that do not last long. Despite a number of risk factors for developing salmonellosis in Lusaka such as inappropriate slaughtering processes, improper retail operations and consumption of beef and beef products, no study has been done to assess the risk of this zoonotic disease in the province. Further, salmonellosis has been reported in both beef and poultry products offloaded on the *Zambian* market, suggesting a possible source of human exposure through the beef value chain as well (Mudenda *et al.*, 2008; Munang'andu *et al.*, 2012).

Despite evidence of presence of *Salmonella* species in beef from previous research, very little is known about the risk of salmonellosis through consumption of beef in Lusaka Province and *Zambia* in general. It is therefore important to assess whether the increase in beef consumption increases public health burdens due to exposure to foodborne hazards. To address this information gap, this study used a Swift Quantitative Microbiological Risk Assessment (sQMRA) model to quantify these risks (Evers and Chardon, 2010). There is a paucity of published literature that demonstrates a quantitative risk of developing salmonellosis through the consumption of beef using sQMRA food safety risk analysis tool. This study illustrates scenarios where both the household and restaurant risk pathways have been used to assess the risk of developing salmonellosis through the consumption of beef prepared in three different ways. This

study will also be important in stimulating further research on this important public health problem.

1.4 Objectives of the study

1.4.1 General objective

The main objective of this study was to quantitatively assess the risk of developing Salmonellosis through consumption of beef in Lusaka Province of Zambia.

1.4.2 Specific objectives

1. To identify hazards associated with consumption of beef
2. To characterize *Salmonella* as a hazard associated with beef consumption
3. To assess human exposure to *Salmonella* through consumption of beef
4. To characterize risk of Salmonellosis in human associated with consumption of beef

CHAPTER TWO

LITERATURE REVIEW

2.2 General Overview of the Beef Sector in Zambia

Zambia has a total population of three and half million cattle. Approximately 17percent of commercial beef production is mainly concentrated in areas along the line of railway, i.e. parts of Central province (Chisamba, Kabwe and Mkushi districts), and Southern province (Kalomo, Choma, Monze and Mazabuka districts) (Davison and Ndiyoi, 2008; GRZ, 2016). The traditional cattle sector accounts for about 83percent of the national herd (Sinkala *et al.*, 2014). Therefore, the national domestic beef supply is largely reliant on the traditional farmer's willingness to sell. Among transitional farmers, off-take rates are very low (8-9% per annum); average slaughter weights are also low; conception rates are equally poor while mortality rates are high (World Bank, 2011). The veterinary extension service system is ineffective, and many traditional cattle farmers have not adopted good animal husbandry practices related to feeding, breeding, and animal health management which can make cattle to be exposed to diseases and present risk to consumers of beef (Chaabila, 2012). Due to low offtake rates, beef processors struggle to access sufficient numbers of animals to meet the growing demand for beef products. Farmers only sell animals when they need money or when animals are sick (Chaabila, 2012).

2.3 Beef processing

The increased demand for beef in the Country have caused proliferation of a large

number of informal slaughterhouses compared to formal abattoirs which are few in number. This increased number of informal slaughterhouses poses a risk of releasing onto the market contaminated carcasses as most of them do not have good sanitary conditions. A study done in Zambia by Muma (1998) on slaughterhouses hygiene and bacterial contamination of carcasses revealed that slaughterhouses had lowest hygiene score ranging from 18.7percent to 21.3percent and that *Salmonella* contamination was detected on beef carcasses (Muma, 1998).

In most Zambian urban areas, raw beef is sold in local market where hygiene is generally poor. In such places, meat is sold in open spaces which makes beef liable to contamination. Further, the handling of meat itself is not often good as the sellers do not often wash hands before and after touching meat which can present a risk to *Salmonella* infection.

A study done in Tanzania by Ntanga (2013) to assess microbial contamination in the beef production chain from abattoir to retail meat outlets showed that there were higher mean values for Total Viable Counts (TVC), Total Coliform Counts (TCC) and Total Faecal Coliform Counts (TFC) being 7.24, 5.55 and 5.27 log CFU/gm, respectively. The bacterial counts were high in abattoir compared to retail meat outlets. The study also showed that practices for personal and environmental hygiene were not adhered to and there were low hygienic standards (Ntanga, 2013).

Another study done in Ethiopia on food safety knowledge and practices of abattoir and butchery shops and the microbial profile of beef showed that 15.4percent of the abattoir workers had no health certificates and also lacked essential basic facilities for a slaughterhouse such as potable running water, separated areas for clean and dirty operations, stunning facilities and lacked proper working tools (Haileselassie *et al.*, 2013). It was also noted that abattoir and butcher shop workers had food safety knowledge gaps, and bacteria was isolated on worker's clothes which meant that they could serve as sources of carcass contamination (Haileselassie *et al.*, 2013). This could lead to the introduction of *Salmonella* into the food chain if there was early exposure of domestic animals to the organism that results in long-term persistent infections (Muma, 1998; Isogai *et al.*, 2005; Haileselassie *et al.*, 2013; Ndalama and Mdegela 2013). Studies

have shown that *Salmonella* infections can also spread through international trade in animal feed, live animals and food (Plym and Wierup 2006). All of this can lead to *Salmonella* spp being transmitted from cattle to beef and hence presenting a risk to humans if not prepared well at household level (EFSA, 2008).

In addition, contamination during food processing, catering and domestic environment in households, restaurants and other eating points can also occur where exposure of pathogens on surfaces may take place either by direct contact with the contaminated objects or indirectly through airborne particles (Kusumaningrum *et al.*, 2003). Studies have shown that meat processing is likely to contribute to the higher levels of contamination in minced beef products compared to beef carcasses. When meat is cut into pieces, more microorganisms are added to the surfaces of exposed tissue surfaces hence, meat cut into small pieces particularly minced meats have very high total counts of microorganisms and *Salmonellae* are likely to be present in large numbers (Bayleyegn and Daniel, 2003; Ejeta *et al.*, 2004). Cutting of meat into pieces which is commonly done in the kitchen preparation of beef; and using the same knife when cutting different food items without disinfecting in between; and using same cutting boards for cooked and uncooked meat could lead to microbial contamination (Kusumaningrum *et al.*, 2003; Redmond and Griffith, 2004). This is because *Salmonella* can survive on stainless steel and on washing sponges which could lead to cross contamination in the kitchen environment. The study done in Netherland by Kusumaningrum showed that *Salmonella enteritidis* can survive on stainless steel surface for about nine hours and cross-contamination of washing sponges were at 21percent to 43percent (Kusumaningrum *etal.*, 2003).

2.4 Salmonellosis

2.4.1 General overview of Salmonellosis

Salmonellosis is an important global public health problem causing substantial morbidity and hence has a significant economic impact both in animal production and public health. Although most infections cause mild to moderate self-limiting diseases, serious infections leading to deaths do occur (Birgitta de Jong and Ekdahl, 2006). In cattle the

disease manifests clinically as a syndrome of septicemia, acute or chronic enteritis and abortion. In humans, *Salmonella* is one of the most common causes of bacterial gastroenteritis, enteric fevers, and septicemia. Therefore, Salmonellosis is a disease of both socio-economic and public health importance as it affects animal production and public health (Ulaya, 2013).

2.4.2 The genus Salmonella

This genus is named after Daniel E. Salmon who first reported the isolation of *Salmonella* from a pig in 1885 and named the organism *Bacterium choleraesuis* (currently known as *Salmonella enterica* serovar Choleraesuis. Generally, there are two main species of *Salmonella* (*S. enterica* and *S. bongori*) (Kemal, 2014). *Salmonella* is the causative agent of gastroenteritis and typhoid fever, one of the major foodborne pathogens of significant public health concern (Fluit, 2005). The genus is prevalent and is one of the most widespread foodborne zoonoses in industrialized as well as developing countries even though the incidences seem to vary (Bayleyegn and Daniel, 2003). Being a member of the family *Enterobacteriaceae*, the *salmonellae* are straight rods usually motile with peritrichous flagella (except *S. pullorum* and *S. gallinarum*), facultative anaerobe, only capable of fermenting glucose with production of gas except *S. typhi* and *S. dublin*, reduce nitrate to nitrite and most are phototropic. The organisms multiply optimally at a temperature range of 35°C to 37°C and pH of about 6.50-7.50 and water activity (aw) range of 0.940-0.840. They are chemo-organotrophic organisms, having both a respiratory and a fermentative type of metabolism (de Souza Sant'Ana, 2012). They are also able to multiply in the environment with low level or no oxygen (European Commission, 2000). The bacteria are sensitive to heat and will not survive at temperature above 70°C; so it is sensitive to pasteurization, but resistant to drying even for years. Although freezing can be detrimental to *Salmonella* species survival, it does not guarantee destruction of the organism. There is an initial rapid decrease in the number of viable organisms at temperatures close to the freezing point as a result of the freezing damage. However, at lower temperatures *Salmonella* species have the ability to survive long term frozen storage (Food Standard Australia, 2013).

2.4.3 Salmonellosis in beef cattle

Studies have shown that *Salmonella* can be isolated in cattle faeces, hides and beef (Barkocy-Gallagher *et al.*, 2003; Rhoades *et al.*, 2009). Serotypes that are most commonly found in beef cattle are *Salmonella dublin* (*S. dublin*) and *Salmonella typhimurium* (*S. typhimurium*) though *S. enteritidis* is also implicated in some studies (McEvoy *et al.*, 2003; EFSA, 2008). Salmonellosis manifest clinically as a syndrome of septicemia, acute or chronic enteritis and abortion in cattle. Animals get infections from others of the same species, especially in the case of the host adapted serovars. In adult cattle, there are important differences in the behavior of *S. dublin* and *S. typhimurium*. Those animals which recover from clinical *S. dublin* infection may become persistent excretors, shedding up to 10^6 organisms per gram of faeces daily (Kemal, 2014).

2.4.4 Bacterial Contamination of beef meat

Meat is one of the sources of animal proteins, essential amino acids, vitamin B complex and minerals in the human diet though it is highly susceptible to microbial contaminations, which may lead to its spoilage and food borne infections in human hence resulting into economic and human health challenges (Komba *et al.*, 2012; Ba'aba, 2014). The fact that meat is rich in protein and fat, low carbohydrate content and have sufficient water activity, it provides sufficient environment for growth of both spoilage and pathogenic bacteria which includes *Salmonella*. Generally, muscles of healthy animals are sterile. Meat tissues get contaminated during the various stages of slaughter such as during the process of removing the gastrointestinal tract, on the slaughter floor and transportation (Stopforth *et al.*, 2006; Ercolini *et al.*, 2006; Ba'aba, 2014). Abattoir facilities can be a source of contamination to the slaughtering processes hence abattoirs can be a source of beef contamination (FAO/WHO, 2002; Okoli Chidi *et al.*, 2006). The presence of non-typhoid

Salmonella serotypes in cattle and the cross contamination of beef carcass tissue is one of the most common causes of *Salmonella* infection in humans once the contaminated beef is consumed (Gomez *et al.*, 1997). The Food and Agricultural organization (FAO), the World Health Organization (WHO) and other epidemiological studies have documented that illness due to contaminated food such as meat is the most widespread

health challenges to humans and is an important cause of reduced economic productivity and hence a major public health concern (FAO/WHO, 2002; Käferstein, 2003). Such studies include a study done in Nigeria by Tafida that reported occurrence of *Salmonella* in retail raw beef and pro roasted meat, barbequed meat, spiced sun dried meat and shredded fried meat which can serve as sources of infection leading to salmonellosis (Tafida *et al.*, 2013).

The study also showed that *Salmonella* can survive cooking temperatures and hence the potential for infection (Blankenship 1978; Tafida *et al.*, 2013). Ahmed *et al.* (2013), revealed that microbial load of raw meat from abattoirs and retail shops in Lahore, Pakistan was high which indicated a possibility of bacterial spoilage and food-borne illnesses on consuming the meat. Another study done in European countries reported salmonellosis outbreak attributable to consumption of beef. (EFSA, 2008). Consumption of beef burger and hamburger have been implicated as sources of human salmonellosis (European Commission, 2000; Food Standard Australia, 2013). Also a study done by Noël in Southern France showed that eating beef sausages had increased risk (OR= 9.3) of developing salmonellosis (Noël *et al.*, 2006).

2.4.5 Salmonellosis in humans

Salmonella enterica causing human disease are divided into human-restricted typhoidal serovars (Typhi and Paratyphi) which causes typhoid fever, and non-typhoidal *Salmonella* (NTS) serovars with varied host-range and most of them are zoonotic. These zoonotic *Salmonella* serovars have emerged as an important cause of invasive bloodstream infection in sub-Saharan Africa, especially among young children with malaria and malnutrition, and among adults with HIV (Mtove *et al.*, 2010; Feasey *et al.*, 2012). These *Salmonella* species are considered to be among the most important foodborne pathogens in the world. They are highly adaptive and potentially pathogenic to humans (Kemal, 2014). There are more than 2500 different *Salmonella* serotypes exist currently which are potentially pathogenic to humans (Popoff *et al.*, 2003). In humans, it causes bacterial gastroenteritis and deaths do occur from *Salmonella* food poisoning (Mead *et al.*, 1999). Humans get *Salmonella* generally through consumption of

contaminated food, both of non-animal and animal origin such as inadequately cooked or raw beef, minced beef and ground beef; also beef products like beef sausage, meat pie, beef burger (Ejeta *et al.*, 2004; Meyer *et al.*, 2010; Yang *et al.*, 2010; Hassanein *et al.*, 2011; Duggan *et al.*, 2012; Food Safety Authority of Ireland, 2013; Obeng *et al.*, 2013; Tafida *et al.*, 2013). On the other hand, Water, fruits, vegetables and products like cheese and ice cream have also a potential of causing salmonellosis (Foley and Lynne, 2008; Duggan *et al.*, 2012; Mughini-Gras *et al.*, 2014). Animal contact and foodhandlers have been implicated in *Salmonella* contamination and hence causing disease in humans (Hoelzer *et al.*, 2011; Obeng *et al.*, 2013).

The infectious dose of *Salmonella* differs for *Salmonella typhi* and non-typhoid *Salmonella*. For *Salmonella typhi*, it is about 10^5 bacilli by ingestion and 10^3 bacilli for non-typhoidal but the dose is usually low in the elderly, immunocompromised people, antibiotic users and those with achlorhydria or regular use of antacid and related medication (Blaser and Newman, 1982; Kemal, 2014). Children, elderly and immunocompromised individuals are the high risk groups with case fatality rate of 20% - 25% in children and adults, respectively (Morpeth *et al.*, 2009; Feasey *et al.*, 2012). Infections due to non-typhoidal *Salmonella* causes severe diseases in HIV patients while for *Salmonella typhi*, infections show little association with immunocompromised status (Gordon, 2008).

Generally, *Salmonella* infection in humans present clinically as syndromes which are divided into typhoid fever, which is caused by *typhi* and *paratyphi*, and a range of clinical syndromes, including gastroenteritis which presents as nausea, vomiting and watery foul smelling diarrhea occurring 6-48 hours after ingestion with a more rapid onset occurring once higher inoculum is consumed; or a compromised case which is caused by a large number of non-typhoidal salmonella serovars (NTS). Typhoid and paratyphoid is a human-restricted disease which is a highly adapted invasive disease, but shows little association with immunocompromised people; but non-typhoidal salmonella serovars (NTS) have a broad vertebrate host range and epidemiologically often involves food animals, with dramatically more severe and invasive presentation in immunocompromised particularly HIV patients (Krauss *et al.*, 2003; Gordon, 2008). The incubation period of the disease depends on the number of bacteria ingested and varies

from 5- 72 hours. Diarrheal stool may contain blood and or mucus if the colon is affected. Fever up to 39°C is not uncommon. Convalescences occur within 1-2 days but the illness may last for 5-7 days (Krauss *et al.*, 2003). Symptoms are more severe in people who are at high risk like those extreme age groups (the young, because their immune system is immature and the elderly, because the immune system is declining), person with decreased gastric acidity and the immune compromised (Gordon, 2008; Crum-Cianflone, 2008; Sigaúque *et al.*, 2009; Mtove *et al.*, 2010; Huang *et al.*, 2012). Generally, diarrhoea, septicemia, gastroenteritis, blood stool, mixed infection, extraintestinal infection, longer course of antibiotics are among the clinical features.

Most cases of salmonellosis caused by Non -Typhoid *Salmonella* presents as uncomplicated gastroenteritis that seldom require antimicrobial therapy though invasive infection such as bacteremia, osteomyelitis and meningitis may occur leading to febrile systemic illness which mimic typhoid fever and other features are nonspecific with sometimes no diarrhoea. The disease is more severe in immunocompromised people (Mwansa *et al.*, 2002; Gordon, 2008; Huang *et al.*, 2012; Feasey *et al.*, 2012; Chen *et al.*, 2013).

Globally, Non Typhoidal *Salmonella* (NTS) is a major cause of gastroenteritis and diarrheal disease though most infection presents as uncomplicated gastroenteritis that seldom requires antimicrobial treatment. The disease sometimes invades other organs causing bacteremia, osteomyelitis, and meningitis where they are termed invasive NTS infection which antimicrobial therapy is mandatory (Majowicz *et al.*, 2010; Chen *et al.*, 2013; Ao *et al.*, 2015).

These zoonotic bacteria lead to high morbidity rates both in the developing world and affluent countries though high mortality is reported in the poorest third world countries where NTS serovars (mainly *S. enterica* serovars *Typhimurium* and *Enteritidis*) predominantly cause bloodstream and focal infection for adults with HIV infection and children with HIV, malaria, and malnutrition while the incidence in industrialized world is decreasing (Sánchez-Vargas *et al.*, 2011; Chen *et al.*, 2013). A study done in Asia revealed that NTS invasive disease is infrequent in Asia except in severe

immunocompromised cases (Dhanao and Fatt, 2009; Khan *et al.*, 2010).

It is estimated that tens of millions of human NTS cases occur worldwide every year and the disease results in more than hundred thousand deaths (Coburn *et al.*, 2007; WHO ,2013a). Salmonellosis cases in humans are mainly foodborne, but direct or indirect animal contact at home, veterinary clinics, zoological gardens, farm environments and human-to-human transmission have been reported (EFSA, 2008; Havelaar *et al.*, 2008; Freitas Neto *et al.*, 2010; Majowicz *et al.*,2010).

In Europe and North America, *Enteritidis* and *Typhimurium* have drawn most attention and in North America, *Enteritidis* and *Typhimurium* lead to approximately 1.4 million salmonellosis cases each year, with atleast 22percent of cases requiring hospitalization for medical treatment. However, other serovars are often more prevalent in other parts of the world and result in more severe infections with higher morbidity (Hendriksen, 2010).

In Africa, invasive NTS disease is endemic to rural and urban areas, especially in sub-Saharan Africa, and has consistently been reported as a leading cause of gastroenteritis and bacteremia among immuno-compromised people, infants and newborns (Bryce *et al.*, 2005; Morpeth *et al.*,2009). Lack of coordinated national epidemiological surveillance systems in developing countries is the major hindrance for identifying sources and transmission routes of *Salmonella* infections (Kariuki *et al.*,2006). In Mozambique and Tanzania, studies done in children admitted in rural hospitals reported the hospital based prevalence of invasive non-typhoidal *Salmonella* ranging from 29percent to 64percent and *Salmonella typhi* was 9percent among the children admitted in both hospitals (Kariuki *et al.*, 2006; Sigaúque *et al.*, 2009; Mtove *et al.*, 2010).

Salmonellosis present with a-non-specific febrile illness making presumptive diagnosis and treatment a challenge for clinicians in low-resource settings like most of developing countries.

***Salmonella* diagnosis**

Diagnosis of *Salmonella* infection is done commonly by bacterial culture, polymerase chain reaction (PCR), ELISA and serological tests where vaccination is not practiced

(Hang'ombe *et al.*,2011). Although the incidence of *Salmonella* on carriers in developing countries is known to be high, proper bacteriological diagnosis of *Salmonella* infection in these countries is not adequate as clinical laboratory infrastructure is lacking in resource limited countries of Africa. Although culture and isolation is labor-intensive, expensive, and time-consuming, it is important to perform these so that proper diagnosis is attained for better treatment of the disease (Hang'ombe *et al.*, 2008).

***Salmonella* treatment**

The treatment of salmonellosis in humans depends on whether an individual has non-typhoidal or typhoidal infection. Non-typhoidal *Salmonella* is often self-limiting, hence routine use of antibiotics is not required. For this type of *Salmonella* infection, fluid and electrolyte replacement should be considered for prolonged cases (Crum-Cianflone, 2008; Kemal, 2014). For invasive non-typhoidal infection and enteric fever, antibiotic therapy is mandatory to save life. Frequently, drugs used includes fluoroquinolones, chloramphenicol, ampicillin, amoxicillin and trimethoprim-sulfamethoxazole though studies have reported resistance in some countries suggesting that prevention of this disease is important (Fluit, 2005; Foley and Lynne 2008; Dhanoa and Fatt 2009; Hidayah, 2011).

***Salmonella* prevention and control**

Salmonellosis prevention should start from beef production to consumption. Ensuring beef production is done in a clean environment starting from reducing *Salmonella* prevalence in cattle by employing comprehensive control strategy in animals including treating the infected herds. In addition, mandatory testing before slaughter can be applied like the one implemented in Sweden to reduce carcass contamination (Boqvist and Vågsholm, 2005). Improvement in farm animal hygiene, in slaughter process, in food harvesting and in packaging operation can help to prevent salmonellosis (Hans, 2006). At household level, prevention is achieved by good hand hygiene and adhering to food preparation guidelines, also avoiding eating raw or uncooked meat, drinking of raw milk or unpasteurized dairy products, and avoiding cross contamination of food. Separation of

uncooked meat from ready-to-eat ones; thorough washing of hands, cutting boards or knives and other utensils are also important in prevention of salmonellosis. Generally, education of food handlers and the general population is a key component for prevention of this disease (Ejeta *et al.*, 2004; Crum-Cianflone, 2008; Ravishankar *et al.*, 2010; Soares *et al.*, 2012).

Targeted surveillance of the level of *Salmonella* contamination in different food products and environment is necessary to control spread of pathogens. Ensuring safe food production requires knowledge on the nature and origin of animal, animal feed, the health status of animals at the farm, the use of veterinary medicinal data regarding antemortem and postmortem findings and the risk association with post harvests production strategies (Snijders and Knapen 2002).

Although there is no 100 percent vaccine effective to prevent salmonellosis in human whereas, vaccine against *Salmonella typhi* has been developed, especially in children, but is only 60percent effective. Even after one is vaccinated by this vaccine, there is still a high chance of developing the disease. It is not expected that there will be a single vaccine that is effective against all the different forms of *Salmonella* soon, though there are ongoing researches investigating what can be done to produce a useful human vaccine for *Salmonella* (Danielle, 2006).

2.5 Risk assessment of Salmonella in beef

2.5.1 Introduction

The effects of globalisation on health and increasing international trade made World Trade Organization (WTO) to establish the Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) in 1995. Under global free trade and nondiscriminatory trading systems of WTO, SPS agreement requires the member states to justify their sanitary and phytosanitary measures with scientific evidence (WTO, 1995). This is the foundation of risk assessment on food related animals and products and hence the development of the quantitative microbiological risk assessment (QMRA) tools in the 1990's which have included stages and procedures described by the Codex Alimentarius Commission (CAC, 1995).

2.5.2 The general overview of Codex Alimentarius Commission Risk Assessment Framework

The Codex Alimentarius Commission (CAC) is the Joint FAO/WHO Food Standards setting organization with the mandate of protecting the health of the consumers from being exposed to food hazards and ensuring fair practices in international food trade. In promoting fair trade, CAC encourages member states to adopt the Codex standards. However, in the absence of standards or where a member state seeks higher standards than those provided by the CAC, member states are allowed to adopt higher levels of protection as long as they are justified by a risk assessment.

Risk assessment being a science-based investigation consist of four steps under Codex Alimentarius Commission (CAC) framework, which is the international standard-setting body for foods in international trade (Codex Alimentarius Commission, 1997). The steps include hazard identification, exposure assessment, hazard characterization and risk characterization (Fig. 1). These steps describe a systematic process for identifying and evaluating the significance of microbial hazards in the food(s) of concern and gives the outcomes in terms of a risk estimate, a measure of the magnitude of risk, based on current scientific knowledge and understanding. Risk assessment is only one element of risk analysis, other elements includes risk management and risk communication (Lammerding and Paoli, 1997). Risk analysis has been incorporated by the CAC, and other international organizations, for the management of public health risks for hazards in food though there are few studies on *Salmonella* risk assessment in beef (FAO/WHO 1995,1997,1998).

2.5.3 Risk assessment studies

Studies on *Salmonella* risk assessment are scarce generally but some of the examples of *Salmonella* risk assessment done include a study done in France on risk assessment of humansalmonellosis from the consumption of ground beef where the risk of salmonellosis per 100 g serving ranged from 0 to 2.33×10^6 though the risk varied

depending on the type of cooking and the fat content; the same study showed that risk of salmonellosis was close to zero when ground beef was consumed well done (Abdunaser *et al.*, 2009). Also Guillier (Guillier *et al.*, 2013) revealed that consumption of beef burger was a risk factor of getting salmonellosis as high contamination levels of *Salmonella* in beef burgers were found in that study.

Furthermore, *salmonella* outbreak following food consumption and ground beef was implicated in 65 (1.6%) of the total bacterial outbreaks reported in France, and the most common serotype isolated in these outbreaks was *Salmonella typhimurium* leading to hospitalization of some people involved. Delarocquein-Astagneau *et al.* (2000) revealed that children less than 15 years old who consume raw or uncooked ground beef were five times more likely to get salmonellosis (OR=5) compared to those who eat well cooked beef.

Beef contamination with *Salmonella* can also occur in the kitchen while preparing food. Retention of bacteria on food contact surfaces increases the risk of cross-contamination of these microorganisms to food and hence can lead to *Salmonella* on food (Kusumaningrum *et al.*, 2003). However, the risk is lowered when the surfaces are dry because bacterial growth and survival is reduced on dry environment (Kusumaningrum *et al.*, 2003). Mughini-Gras *et al.*, (2014) found that cleaning sponges are potential vehicles of pathogen in domestic kitchen since pathogen are able to survive in kitchen sponges for at least weeks without dieing hence the potential to transfer bacteria to other surfaces in contact with food. The study showed that, at 50-65% w/w water activity the transfer rate of pathogens by these sponges is 21-43% (Ravishankar *et al.*, 2010; Mughini-Gras *etal.*, 2014).

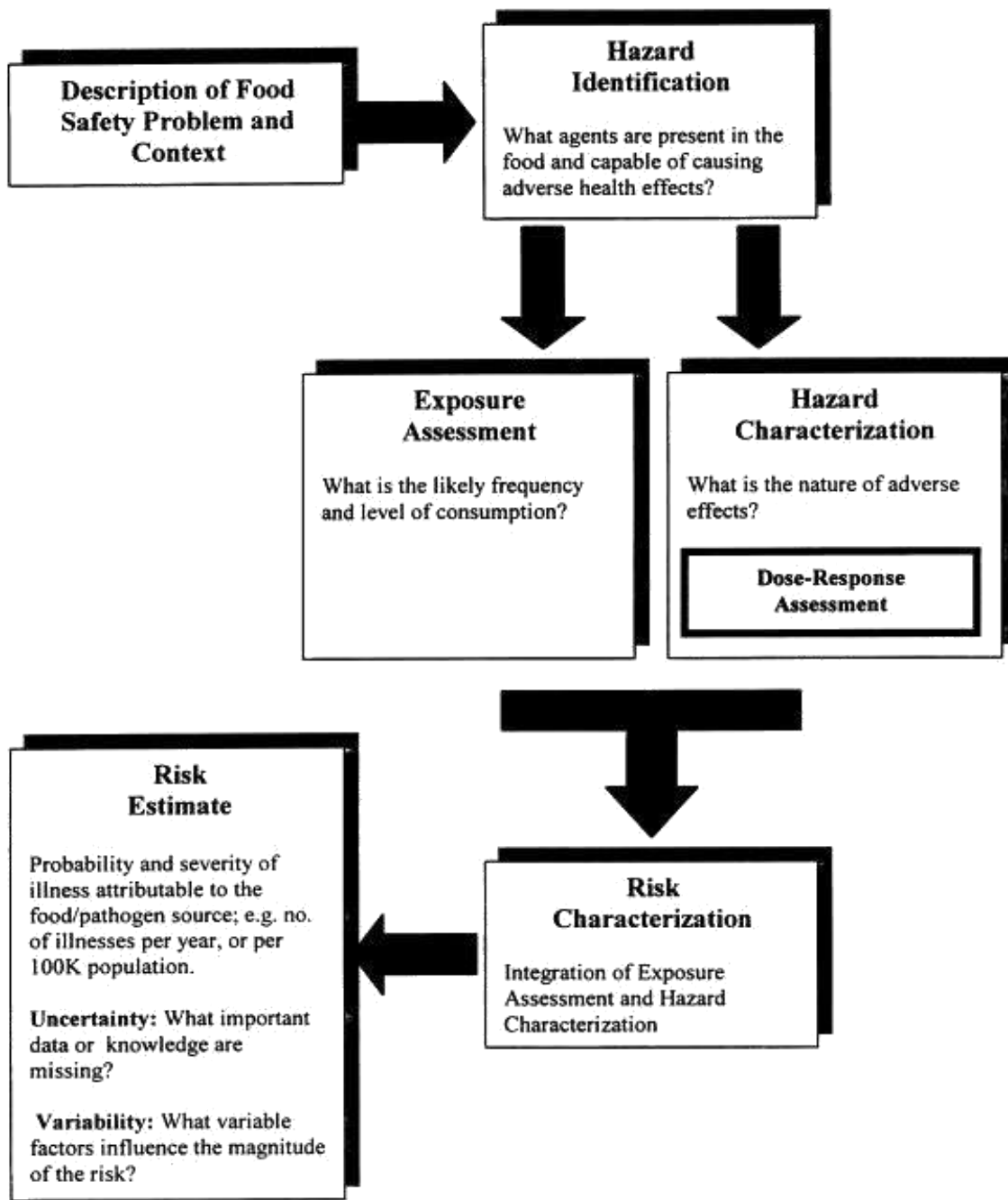


Figure 1: Steps of microbial food safety risk assessment developed by Ann *et al.*, (2000)

CHAPTER THREE

DATA SOURCES AND METHODOLOGY

3.2 Study area

This study was conducted in the Lusaka province of Zambia, an area with relatively high beef consumption due to high purchasing power (Sinkala *et al.*, 2014).

3.3 Swift Quantitative Microbiological Risk Assessment (sQMRA) model

The sQMRA-model model was developed by Evers and Chardon (2010). It is implemented in a Microsoft Excel spreadsheet. Deviating from a full-scale Quantitative Microbiological Risk Assessment (QMRA), where pathogen numbers are followed through the whole food chain, this model starts at retail and ends with the number of human cases of illness. The model is deterministic and includes cross-contamination and preparation (heating) in the kitchen and as well as dose response relationship. The general setup of the sQMRA tool consists of consecutive questions for values of each of the 11 parameters, always followed by intermediate model output broken down into categories of contamination, cross-contamination and preparation, as show in Fig. 3 under the results section. Model input and output are summarized and exposure as well as cases are attributed to the distinguished categories. As a relative risk measure, intermediate and final model outputs are always compared with results from a full-scale

QMRA of *Campylobacter* on chicken fillet as shown in Figs. 4-6 under the results section. The model allows results of the research to be quickly interpreted in terms of public health risk, given that pathogen concentration is determined from the model. It is also more accessible and understandable for scientists that are new to the QMRA research area or are not very mathematically inclined (Evers& Chardon, 2010).

3.4 Study design and data sources

The study used a cross sectional design which depended on both secondary and primary data sources.

Secondary data: This was a risk analysis desktop study which mainly depended on review of scientific peer reviewed papers and grey literature (secondary data). The literature review was guided by research questions based on the sQMRA model as shown in Table 1. Literature search was conducted on major electronic databases including Web of Science and Pub Med (NLM) using The University of Zambia (UNZA) library database.

Further, grey literature from conference proceedings and reports from government institutions and Non-Governmental Organisations were obtained online using “Google search engine” and “Google scholar”. Search of key terms such as, “Beef consumption, Quantitative risk assessment, Salmonellosis, beef value chain, Zambia”, were used. Guided by questions in **Index 1**, literature which contained relevant data were included in the study and the rest were excluded. This was the main source of data (almost 98%).

Primary data: After an extensive literature review, it was discovered that there were information gaps on serving portions and consumption patterns of beef in Zambia. A survey was therefore undertaken to fill these information gaps. This only formed about 2% of the data. A structured questionnaire was used to address the information gaps on serving portions and consumption patterns. The study had a convenient sample size of

hundred (100) respondents. The sampling frame was composed of respondents from two areas with a different socio-economic status (40 low and 60 medium income communities), so as to obtain a representative estimate of average serving portions and consumption patterns. Residential areas were used as a proxy for socioeconomic status using the Central Statistical Office conditions of leaving survey (Mweemba and Webb, 2008; CSO, 2010). Respondents were conveniently identified and interviewed from the butcheries in low and medium/high cost residential areas where they were found buying beef and restaurants where they were found eating beef.

3.5 Data management and analysis

The data collected from the survey were coded and entered into STATA, SE/12 for Windows (StanCorp, College Station, TX). Descriptive statistics on average serving portions, consumption patterns, and kitchen preparation methods of beef were calculated. Data from the literature review were entered in the Excel version of the sQMRA model developed by Evers and Chardon (2010). This model was then run twelve times to come up with results for the exposure assessment following the household and restaurant risk exposure pathways as shown in **Fig. 2**.

3.6 Ethical clearance

Study was approved and cleared by the School of Veterinary Medicine board of graduate studies and the University of Zambia Directorate of Research and Graduate Studies (DRGS).

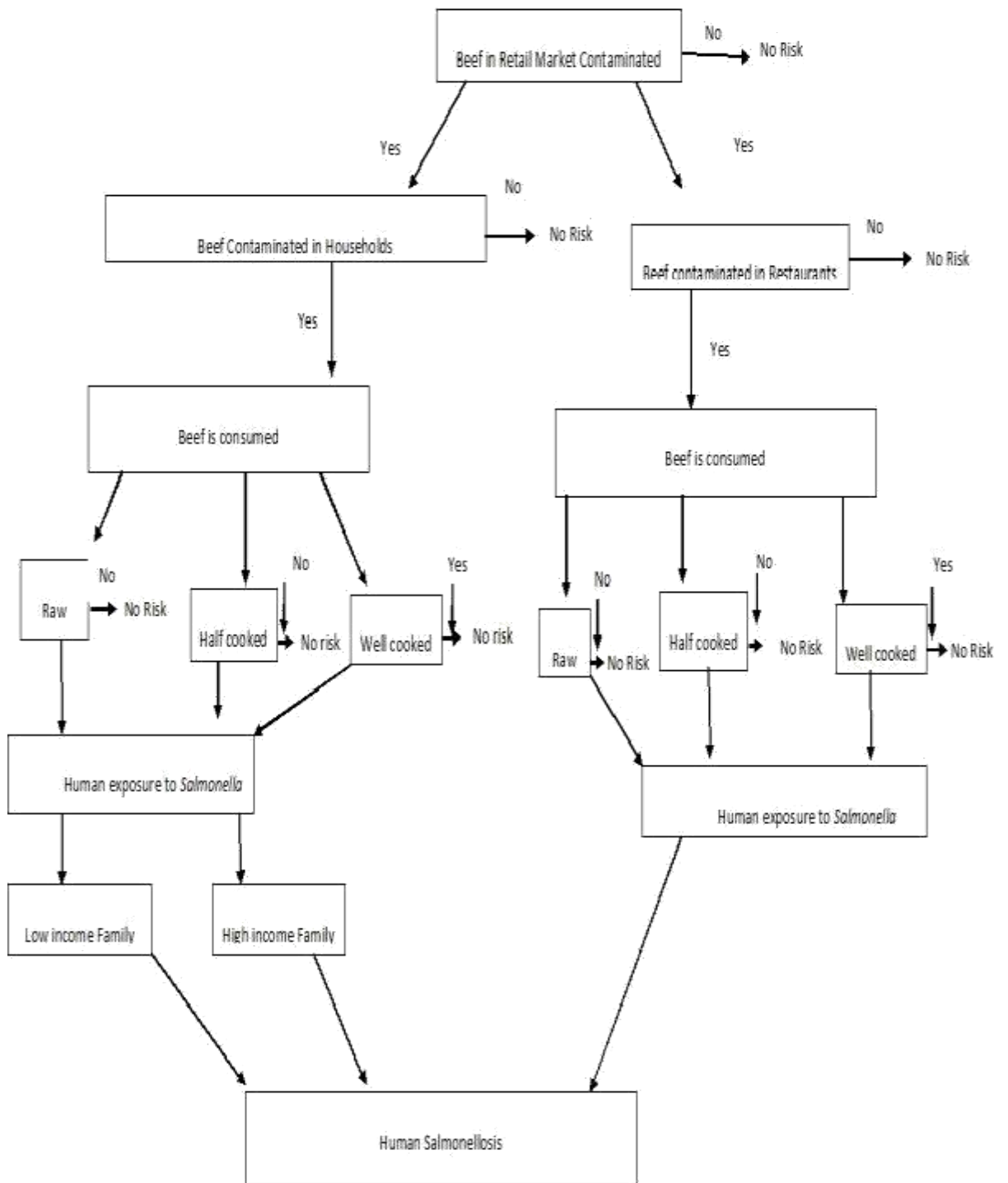


Figure 2: Exposure pathways to *Salmonella* through consumption of beef at household and restaurant levels.

CHAPTER FOUR

RESULTS

4.2 Hazard Identification

From literature reviewed in this study, *Salmonella* was identified as hazardous bacteria. It causes disease both in humans and animals. Non-typhoid *Salmonella* species are zoonotic with wide range of hosts. The pathogens cause different diseases in different hosts. In humans the pathogen causes a range of clinical syndromes including gastroenteritis, enteric fever and sometimes septicemia. In cattle the pathogen manifest clinically as a syndrome of septicemia, acute or chronic enteritis and abortion. *Salmonella* can be transmitted from cattle to human through consumption of contaminated beef.

4.3 Hazard Characterization

This is the quantitative evaluation of the nature of the adverse effects associated with consumption of beef contaminated with *Salmonella*. The information gathered were entered in swift Quantitative Microbial risk assessment (sQMRA) model. Finally, the model used to determine the level of risk in terms of number of people who will become ill following consumption of contaminated beef after twelve simulations.

4.4 Exposure Assessment

4.4.1 Case definition

The pathogen of interest was Salmonella species and the targeted product was beef. The population size of Lusaka province was taken to be 2,669,249 in this model according to Central Statistics Office of Zambia (CSO, 2015). A consumption period of one year was defined to assess the number of people who would get ill in this study (i.e., the number of people who would get ill per year).

4.4.2 Consumption data

In this study, a portion size was defined as the amount/size of beef an individual consumes per meal. There was no available beef consumption data in Lusaka province. The study assumed that residents in Lusaka province who were employed consumed beef. According to Labour Force Survey of Zambia, 75% of the 2.67 million Lusaka residents were in formal or informal employment (CSO, 2015). Using the later information, the study therefore logically assumed that 75% of the residents in Lusaka province who were employed consumed beef because of their purchasing power (CSO, 2015; World Bank, 2011). The survey revealed that two portions of beef were served (Lunch and dinner). Hence the number of portions consumed by a population was calculated to be 2,001,937 per consumption period multiplied by 2 servings for lunch and dinner (4,003,874 portions).

4.4.3 Serving portions and consumption patterns

The results of the survey revealed that the average serving portion of beef per serving at a household level was 60 g among low consumers and 83.1 g among medium beef consumers, while that for restaurants (high beef consumers) was 192 g. Most beef at the household level was prepared and consumed well done (91%); 9% was prepared half done; while no (0%) beef was consumed raw. The consumption patterns from the data showed that 60% of respondents consumed beef once every week, 16% consumed once

in every fortnight, 15% consumed beef once a month and 9% consumed beef every day through various forms. At household level, beef was cooked once, but then served in two different periods (2 serving portions-lunch and dinner).

Contamination of raw beef at retail outlets: Literature review showed a wide range of raw beef contamination at retail outlets from 2.42% to 62% (Ahmad *et al.*, 2013; Kumar, Rao and Haribabu, 2014; Mrema, Mpuchane, and Gashe, 2006; Sallam *et al.*, 2014; Tafida *et al.*, 2013; Van *et al.*, 2007; Yang *et al.*, 2010). A similar study in Botswana revealed that retail contamination of beef stood at 20% (Mrema *et al.*, 2006). This study therefore used the data from Botswana because it is a neighboring country with similar experiences in retail beef handling practices like in many other low and middle income countries in Africa (Haileselassie *et al.*, 2013; Mrema *et al.*, 2006).

This study considered only minimum and high concentrations of Salmonella and hence the average concentration of colony forming units (cfu) per gram in a contaminated portion of beef was taken to have a minimum value of 3.36 cfu/g and a maximum value of 12 cfu/g (Ahmad *et al.*, 2013; Ba'aba, 2014; USA-FSIS, 2011).

INPUT PARAMETERS			
Pathogen:		Salmonella	
Food product:		Beef	
Population size:		2669249 million people	
Pop. Characteristics:		Population of Lusaka	
Consumption period:		one year	
Number	para-meter	Question	value
1	N	Portions consumed	4.0E+06
2	M	Portion size in grams	60
3	Sr/+	Prevalence in retail	20%
4	Cr/+	Cfu per gram contaminated product	12.0
5	Scc/r	Portions causing cross. Cont.	45%
6	Fcc	Cfu's from portions to environment	30%
7	Fei	Cfu's from environment to ingestion	9.0%
8	Sprd/cc	Portions prepared done	91%
8	Sprh/cc	Portions prepared half-done	9.0%
8	Sprr/cc	Portions prepared raw	0.0%
9	Fprd	Cfu's surviving when prep. Done	0.0%

9	Fprh	Cfu's surv. When prep. Half-done	20%
9	Fpr	Cfu's surviving when prep. Raw	100%
10	ID50	ID50 (number of cfu's)	9.6E+03
11	Pill/inf	% people infected who get ill	100%
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sQMRA-tool			

Figure 3: sQMRA input parameters for the low beef consumer under the household risk exposure pathway

4.4.4 Kitchen cross-contamination

Due to a lack of literature on Salmonella in beef kitchen cross contamination, Salmonella in chicken kitchen cross contamination was used as a proxy. This is because cross contamination does not differ regardless of the food product where preparation methods are similar (Evers and Chardon, 2010). The percentage of portions that would contaminate the environment such as the hands and kitchen was therefore set at 45% for restaurants and 40% under the household risk exposure pathways (Medeiros, Nascimento, and Robson, 2014). The percentage of cfu on a portion that would contaminate the environment such as hands and kitchen was 30% (Kusumaningrum *et al.*, 2003). The percentage of beef portions that would cross-contaminate the environment such as the hands and household kitchen used in this model was assumed to range from 4 to 32% (12% of dishcloths, 24% of persons' hands, 4% refrigerator door handles, 20% oven door handles, 24% counter-tops and 32% draining boards) (Gorman, Bloomfield, and Adley, 2002), while the percentage of cfu on a beef portion that would contaminate the environment such as the hands and kitchen in household was assumed to be 16.6% (Gorman *et al.*, 2002). In the household and restaurant risk pathways, it was assumed that 9% and 14% of cfu (value ranges from 0.02 to 75%) on a portion would end up being ingested as a result of beef that is prepared half done (Ravishankar, Zhu, and Jaroni, 2010).

4.4.5 Kitchen preparation

From the questionnaire survey on beef preparation, the percentage of doneness on the portion of beef at household kitchen level was; 91% well done, 9% half done and 0% raw, while that at restaurant kitchen level was 84% well done, 16% half done (mostly roasted T-bone) and 0% raw. In the reviewed literature the percentage of beef prepared raw was high at 37% (Bogard *et al.*, 2013) which was not realistic to African cultures like that of Zambia. The percentages of microorganisms surviving on a contaminated portion of beef during preparation in both household and restaurant kitchen were 0%, 20% and 100% when beef was prepared well done, half done and raw respectively (Evers and Chardon, 2010). It was assumed to be zero when well done because of over boiling of meat which is normally practiced in Zambia; and 100% when raw due to poor hygiene practices along the beef value chain in developing countries (Haileselassie *et al.*, 2013). Evers and Chardon (2010) also used 0% in well done and 100% when prepared raw, in their sQMRA model.

4.4.6 Infection and illness

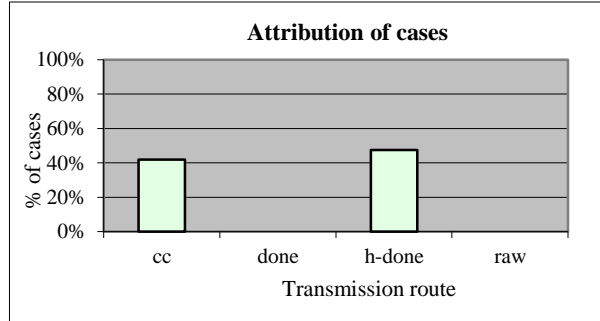
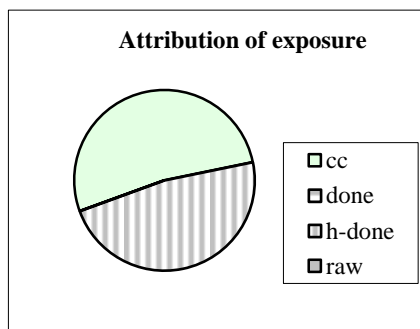
In this study, the dose (number of cfu's) per gram of portion that would cause half of the exposed population to get salmonella infection (ID₅₀) was taken to be a minimum of 9.61×10^3 cfu (9,610) and maximum of 5.0×10^4 (WHO/FAO, 2002; Teunis *et al.*, 2010). The study assumed that 100% of the exposed population would get ill when they ingested such doses of Salmonella (Blaser and Newman, 1982). The infectious dose of Salmonella was assumed to be a minimum of 9.61×10^3 cfu/g and a maximum of 5.0×10^4 cfu/g (Teunis *et al.*, 2010). The average concentration of cfu's per gram in a contaminated portion of raw beef was a minimum of 3.36 cfu/g and maximum 12 cfu/g (Teunis, 1997; USA-FSIS, 2011; WHO/FAO, 2002; Ahmad *et al.*, 2013; Ba'aba, 2014).

INPUT PARAMETERS			
Pathogen:		Salmonella	
Food product:		Beef meat	
Population size:		2669249	
Pop. Characteristics:		Population of Lusaka	
Number	Parameter	Question	Value
1	N	Portions consumed	4.0E+06
2	M	Portion size in grams	83
3	Sr/+	Prevalence in retail	20%
4	Cr/+	Cfu per gram contaminated product	12.0
5	Scc/r	Portions causing cross. Cont.	45%
6	Fcc	Cfu's from portions to environment	30%
7	Fei	Cfu's from environment to ingestion	9.0%
8	Sprd/cc	Portions prepared done	91%
8	Sprh/cc	Portions prepared half-done	9.0%
8	Sprr/cc	Portions prepared raw	0.000%
9	Fprd	Cfu's surviving when prep. Done	0%
9	Fprh	Cfu's surv. When prep. Half-done	20%
9	Fprr	Cfu's surviving when prep. Raw	100%
10	ID50	ID50 (number of cfu's)	9.6E+03
11	Pill/inf	% People infected who get ill	100%

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sQMRA-tool	
EXPOSURE	EFFECT

Figure 4: sQMRA input parameters for the medium beef consumer under the household risk exposure pathway

Figure 5: Model output at 12cfu/g and ID50 at 9.61×10^3 cfu (high probability for low beef consumers under the household risk exposure pathway)



Transmission route	Exposure
Cross contamination	44%
Prepared done	0%
Prepared half-done	56%
Prepared raw	0%

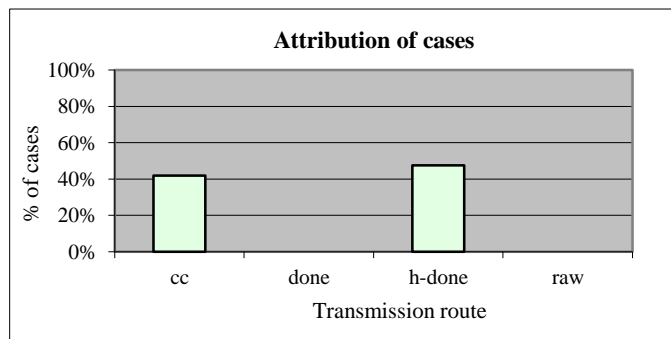
Transmission route	Calculation	Attribution of cases
Cross contamination	$S_{cc}/r = 0\%$	35%
Prepared done	$F_{prd} = 0\%$	0%
Prepared half-done	$F_{prh} = 0\%$	56%
Prepared raw	$F_{prr} = 0\%$	0%

RELATIVE RISK Compared with QMRA *campylobacter* in chicken fillet

Point of comparison	Model output	Reference data	Relative value
Portions consumed	4.0E+06	8.5E+07	4.71%
Contaminated portions (at retail) consumed	8.0E+05	3.3E+07	2.43%
Total number of cfu's before kitchen	5.8E+08	7.0E+10	0.82%
Total number of cfu's after kitchen	1.6E+07	6.1E+06	262%
Number of people ill	1.1E+03	1.2E+04	9.32%

EXPOSURE

EFFECT



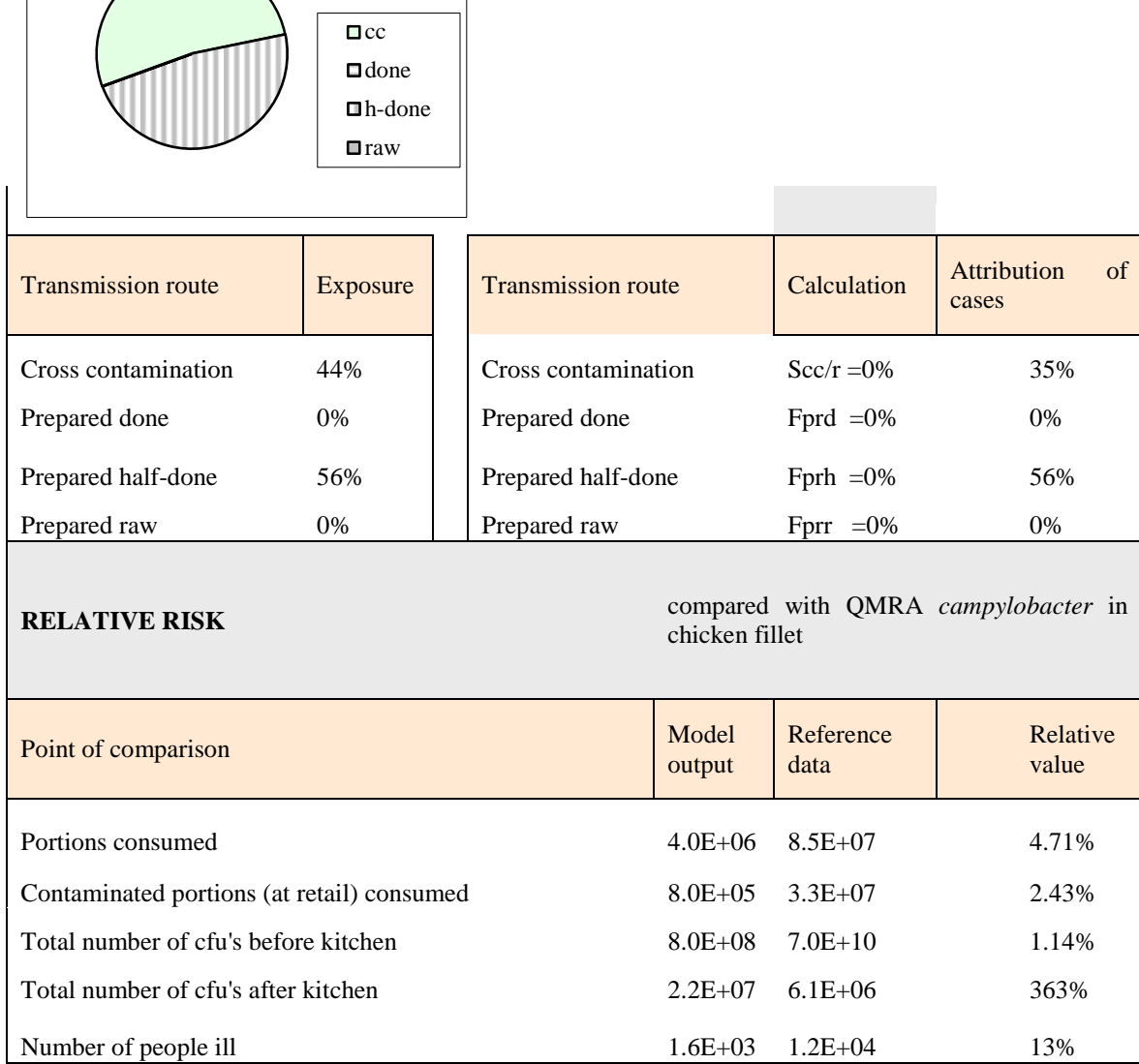
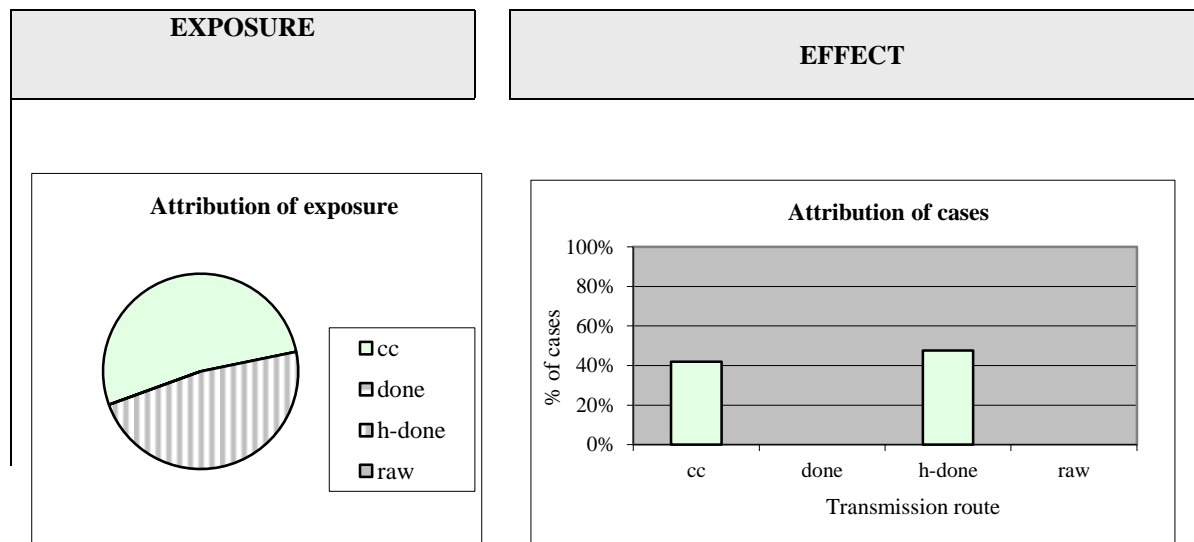


Figure 6: Model output at 12cfu/g and ID50 at 9.61x10³ cfu (high probability for medium/high beef consumers under the household risk exposure pathway)



Transmission route		Exposure	Transmission route		Calculation	Attribution of cases
Cross contamination		52%	Cross contamination	Sc/r = 0%		42%
Prepared done		0%	Prepared done	Fprd = 0%		0%
Prepared half-done		48%	Prepared half-done	Fprh = 0%		47%
Prepared raw		0%	Prepared raw	Fpr = 0%		0%
RELATIVE RISK Compared with QMRA <i>campylobacter</i> in chicken fillet						
Point of comparison			Model output	Reference data	Relative value	
Portions consumed			2.0E+06	8.5E+07	2.36%	
Contaminated portions (at retail) consumed			4.0E+05	3.3E+07	1.21%	
Total number of cfu's before kitchen			9.2E+08	7.0E+10	1.32%	
Total number of cfu's after kitchen			4.4E+07	6.1E+06	728%	
Number of people ill			3.2E+03	1.2E+04	26%	

Figure 7: Model output at 12cfu/g and ID50 at 9.61x10³ cfu under the restaurant risk exposure pathway

4.5 Risk characterization

A total of 12 simulations which included eight from the household risk pathway (4 for the low beef consumers, 4 for medium beef consumers) and 4 for the restaurant (high beef consumers) risk exposure pathway, were run. Each run produced a summary of the input parameters (Fig. 3) and the output model results for the highest risk of developing salmonellosis among the low beef consumers (Fig. 4) and medium beef consumers (Fig.

5) in a household risk pathway and high beef consumers in a restaurant risk pathway (Fig. 6). Table 1 (risk characterization) summarises the results of all the outputs of the 12 simulations. Of the 4 case scenarios for the low beef consumers (through the household risk pathway), scenario 3 recorded the highest risk with 1100 out of a population of 2,001,937 people developing salmonellosis through the consumption of Salmonella contaminated beef, representing a probability of 0.04%. Among the medium beef consumers through the household risk pathway, 1600 out of a population of 2,001,937 people risked developing salmonellosis through consumption of salmonella contaminated beef, representing a probability of 0.05%. Among the heavy consumers of beef (through the restaurant) risk pathway, 3200 out of a population of 2,001,937 people risked developing salmonellosis through consumption of salmonella contaminated beef, representing a probability of 0.16%.

Table 1: Summary of the outputs of 12 simulations under household and restaurant risk exposure pathways.

Low beef consumers					
Scenario	Portion (g)	cfu/g	ID50	Model output (No. People ill)	Qualitative Risk
1	60	3.36	9,610	320	Medium

2	60	3.36	50,000	62	Low
3	60	12	9,610	1,100	High
4	60	12	50,000	220	Medium
Medium beef consumers					
1	83.1	3.36	9,610	450	Medium
2	83.1	3.36	50,000	86	Low
3	83.1	12	9,610	1,600	High
4	83.1	12	50,000	310	Medium
Restaurant/High risk pathway					
1	192	3.36	9,610	890	Medium
2	192	3.36	50,000	170	Low
3	192	12	9,610	3,200	High
4	192	12	50,000	610	Medium

Quantitative Risk characterization using sQMRA for restaurant/high risk pathway (Table 1)

Scenario 1: 890 people per year in a general population of 2.77 million people of Lusaka Province would develop salmonellosis through consumption of infected beef

Scenario 2: 170 people per year in a general population of 2.77 million people of Lusaka Province would develop salmonellosis through consumption of infected beef

Scenario 3: 3200 people per year in a general population of 2.77 million people of Lusaka Province would develop salmonellosis through consumption of infected beef

Scenario 4: 610 people per year in a general population of 2.77 million people of Lusaka Province would develop salmonellosis through consumption of infected beef

In general, a combination of higher beef contaminations levels and a lower infectious dose and (ID50) would result in more people becoming infected.

Quantitative Risk characterization using sQMRA at Household level (medium beef consumers)

Scenario 1: 450 people per year in a general population of 2.77 million people of Lusaka Province would develop salmonellosis through consumption of infected beef in medium beef consumers.

Scenario 2: 86 people per year in a general population of 2.77 million people of Lusaka Province would develop salmonellosis through consumption of infected beef in medium beef consumers.

Scenario 3: 1600 people per year in a general population of 2.77 million people of Lusaka Province would develop salmonellosis through consumption of infected beef in medium beef consumers.

Scenario 4: 310 people per year in a general population of 2.77 million people of Lusaka Province would develop salmonellosis through consumption of infected beef in medium beef consumers.

Quantitative Risk Characterization Using SQMRA at Household level (low beef consumers)

Scenario 1: 2700 people per year in a general population of 2.77 million people of Lusaka Province would develop salmonellosis through consumption of infected beef in high income families.

Scenario 2: 540 people per year in a general population of 2.77 million people of Lusaka Province would develop salmonellosis through consumption of infected beef in high income families.

Scenario 3: 780 people per year in a general population of 2.77 million people of Lusaka Province would develop salmonellosis through consumption of infected beef in high income families.

Scenario 4: 150 people per year in a general population of 2.77 million people

of Lusaka Province would develop salmonellosis through consumption of infected beef in high income families.

4.6 Uncertainty

Like many risk analysis studies, there were substantial missing data as input parameters in the model. To cover up for these information gaps, a simple survey on the consumption patterns and serving portions of beef in the population was done to get the average serving portions, so as to avoid too much reliance on logical assumptions and use of data from other countries. The pathogen numbers were followed through the food chain, which in this case starts at retail and ends with the number of human cases of illness. It would be more robust to follow the pathogen numbers along the entire value chain (farm to fork at a national level), but this would require more resources. The relative risk was compared to the reference point in the model to avoid overestimation of the results. However, the reference point in the model was for *Campylobacter* in chicken fillet in Netherlands where the model was developed. This however does not have much effect on the model because the epidemiology of these pathogens are more or less the same (Evers & Chardon, 2010) and only serving portions would differ with regard to African countries.

CHAPTER FIVE

DISCUSSION

This study was conducted with the aim of assessing the risk of developing salmonellosis through consumption of beef in Lusaka Province of Zambia. The key question was to

find out whether beef sold in Lusaka province posed a risk of Salmonella infection through consumption of meals prepared at home and those consumed in restaurants. In this study, it was observed that the risk of developing salmonellosis as a result of beef consumption was generally low for both exposures from restaurants and in households. The low risk in the current study was attributed to low serving portions per meal, low consumption patterns and preparation methods of beef both in restaurants and in households. The serving portion of beef has the potential to contribute to risk of Salmonella infection in humans. In this study, the average servingportion of beef was 60 g and 83.1 g per meal for low and middle income households and 192 g/meal in restaurants. This contributed to low risks found in this study. The small serving portions could be attributed to the high price of beef on the market and hence most people opted for other livestock products rather than beef. This is in agreement with the previous findings on urban consumption patterns of livestock products in Zambia where consumption patterns of livestock products was influenced by household affluence defined as the low, medium, and high expenditure terciles or income groups (Hichaambwa, 2012). In the same study Hichaambwashowed that within each city, the expenditure shares of livestock products increased from the low to the high income group while it marginally decreased in the case of fish (Hichaambwa, 2012).

In terms of preparation methods, most of the beef consumed in Lusakawas prepared well done through boiling with only few (16%) in restaurants where T-bone was normally prepared half done. Consumption of T-bone contributed to doubling the risk of developing salmonellosis in the current study through the restaurant pathway. Consumption of raw beef was not a common practice in Zambia hence recording 0% and thus further reducing the risk. Although consumption of well cooked beef does not pose a risk of developing salmonellosis, other ways of getting infection with *Salmonella* is cross-contamination in the kitchen which could occur when handling contaminated beef.

Iordache and Tofan (2008) in a study on the cross-contamination of Salmonella enteritidis on sterile and non-sterile meat showed that cross-contamination of *Salmonella* could occur in the kitchen environment (Iordache and Tofan, 2008). In the current study, cross-contamination in the kitchen was one of the contributing factors for risk of

developing salmonellosis. Results showed that much of the risk was contributed by cross contamination at restaurant level compared to other scenarios when concentration of Salmonella in retail beef was 12 cfu/g of beef and infectious dose fifty of (ID50) 9.61×10^3 cfu/g. This was in agreement with the observation by Mughini-Gras *et al.*, (2014) who showed that not using a chopping board for raw meat only (cross-contamination) and consuming raw/undercooked meat were risk factors for infection with Salmonella originating from cattle. In the current study, there were low numbers of predicted cases of salmonellosis at high contamination (12 cfu/g) and high ID50 (5×10^4 cfu/g). This indicated that cooking alone cannot be considered an adequate response to exceptional events of extreme foodborne bacterial pathogen contamination; other factors like cross-contamination could lead to salmonellosis infection even when beef is well cooked (Teunis *et al.*, 2010). In general, the low risk of developing salmonellosis in the current study is in agreement with the observation by Abdunaser *et al.*, (2009) who reported the risk of developing salmonellosis in human per 100 g serving portion of ground beef to be low (ranging from 0 to 2.33×10^{-6}), though it was based on ground beef contrary to the current study which considered beef without specifying whether ground or beef parts. We acknowledge that this model is deterministic and does not allow the variability inherently linked to food-borne diseases to be modelled. However, our model could be a starting platform for further studies on the epidemiology of salmonellosis in Zambia. The model also represents a way of communicating results across regional and cultural/economic borders.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The risk of developing salmonellosis from consumption of contaminated beef is generally very low among the beef consumers in Lusaka. This was attributed to low beef consumption and inadequate cooking methods.

Cooking alone is not enough, retailers and consumers must avoid cross contamination through improved food handling practices.

6.2 Recommendations

More robust studies must be carried out at a national level to come up with data on consumption patterns, general risk and number of people that would actually die from salmonellosis.

Policy makers and stakeholders such as the Food and Nutrition Council of Zambia and meat processors should also come up with mechanism of data collection on consumption patterns of various food items, including beef.

Awareness campaigns should be carried out to encourage the consumers to adequately cook meat to reduce contamination. This is because it has been demonstrated in this study that consuming beef which is well cooked reduces the risk of developing salmonellosis.

Generally, consumers and food handlers have to be educated on the dangers of half cooked beef and the retail contamination levels need to be taken care of so that there is reduction in contamination levels of beef at retail markets.

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APPENDICES

Appendix 1: The Consent Form

Ihave agreed to take part in this research with a title “Quantitative risk assessment of developing salmonellosis through consumption of beef in Lusaka province, Zambia. I confirm that the study has been adequately explained to me and I understand it to the best of my knowledge.

I attest to participate voluntarily and that I can withdraw at any time without repercussions.

I understand that disguised extracts from my responses may be quoted in the thesis and any subsequent publications.

I agree to provide necessary information needed for this study.

Participant

signature.....Date.....

Name of investigator: Sign.....

Appendix 2: Literature Review Guide

<p><i>Case definition</i></p> <ol style="list-style-type: none"> 1. What is the pathogen of interest? 2. What is the food product of interest? 3. What is the population size? 4. What are the population characteristics? 5. What is the consumption period? 	<p><i>Consumption data</i></p> <ol style="list-style-type: none"> 1. How many portions are consumed in the population per consumption period? 2. What is the average size of one portion? 3. What percentage of the portions is contaminated at retail? 4. What is the average concentration of colony forming units (cfu) per gram in contaminated portions?
<p><i>Kitchens cross contamination</i></p> <ol style="list-style-type: none"> 1. Given contaminated portions, what percentage of the portion will contaminate the environment? E.g. hands and kitchen equipment? 2. Given contaminated portions, what percentage of the cfu's on a portion will contaminate the environment? E.g. hands and kitchen equipment? 3. Given cross contamination, what percentage of cfu's in the environment ends up being ingested? 	<p><i>Kitchen preparation</i></p> <ol style="list-style-type: none"> 1. What percentage of the portions is prepared; Done, Half done, Raw 2. What percentage of cfu's on a portion will survive during preparation? -Done, Half done and Raw <p><i>Infection and illness</i></p> <ol style="list-style-type: none"> 1. At which dose (number of cfu's) per portion will half of the exposed population get infected? 2. What percentage of infected people will get ill?

Appendix 3: Questionnaire used in this study

Section A: Demographic Characteristics

1.0 Name of interviewer

2.0 Date of interview dd/mm/yy

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Respondent

3.0 Gender

(a) Male (b) Female

4.0 Age in years

(a) 15-20 (b) 21-30 (c) 31-40

(d) 41-50 (e) 51-60 (f) Above 60

5.0 Marital status

- (a) Married (b) Single (c) Divorced/ separated
(d) Cohabiting (e) Widowed

6.0 Number of people living in the household:

- (a) 0-2 year
- (b) 3-12 year
- (c) 13-18 years
- (d) Above 18 years
- (e) Others. Specify.....

7.0 Highest level of education

- (a) None (did not attend school) (b) Primary (grade 1-7)
(c) Junior secondary (grade 8-9) (d) Senior secondary (grade10-12)

8.0 Employment status

- (a) Government / salary worker (b) Self employed

- (c) Allowance (remittance) (d) unemployed
(e) Others specify.....

9.0 Location of Household

- (a) Urban (b) Peri-urban (c) Rural
10.0 If urban, is it

- (a) High density (b) Medium density (c) Low density

11.0 Does the household have electricity?

- (a) Yes (b) No

12.0 Household's main source of fuel for cooking

- (a) Electricity (b) Firewood (c) Charcoal
(d) Gas (e) Others specify.....

13.0 Household's cooling facilities

- (a) Refrigerator (b) Freezer (c) Both Refrigerator and Freezer
(d) Others specify.....

Section B: Household Beef Consumption Patterns

14.0 Do you prepare beef in your household?

- (a) Yes (b) no
(b) If no give reasons

15.0 If your answer in 16 was yes, how often to you eat beef in your household??

- (a) Every day (b) Once every two weeks
(c) Once every week (d) Once a month
Others.Specify.....

16.0 How many kilograms of beef have you bought?

17.0 How many meals with beef are normally prepared from the amount of beef bought?

18.0 How is the beef bought consumed?

(a) Raw (b) Half cooked (c) well cooked (d) others specify.....

19.0 Where do you normally buy the beef consumed in your household

(a) Super market (b) local butcheries (c) local market (d) other specify.....