PREVALENCE OF BACTERIAL SEROVARS CONTAMINATION OF CHICKEN AND BEEF FROM RETAIL AND WHOLESALE MARKETS BY *SALMONELLA* SPP. IN WINDHOEK, NAMIBIA

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ABSTRACT

Salmonella infection in humans is a major public health problem worldwide. There is an increasing concern with this pathogen due to the emergence and spread of antibiotic resistant and potentially more pathogenic strains. Failure to control *Salmonella* in a country presents a potential problem for other countries. To minimise the burden of this pathogen, it is helpful to monitor *Salmonella* serovars distribution in many countries, implement *Salmonella* control measures throughout the food production chain, and monitor the effectiveness of the control measures. This study investigated the prevalence and the serovars distribution of *Salmonella* isolated from chicken and beef samples from retail and wholesale markets in Windhoek, Namibia. Chicken (138) and beef (138) samples were analysed for the presence of *Salmonella* at the Central Veterinary Laboratory in Windhoek. Out of 276 samples of chicken and beef that were analyzed for the presence of *Salmonella,* 7 % (n = 19) were found to be positive. In beef samples, 14 % (n = 19) were found to be positive for *Salmonella.* In chicken samples, 0 % (n = 0) tested positive for *Salmonella.* The prevalence of *Salmonella* in beef was higher; hence there was a significant difference ($p < 0.001$) between the two products. *Salmonella* enterica serovar Fulda was the most prevalent with 58 % (n = 11), followed by *Salmonella* enterica serovar Javiana with 42 % (n = 8). The prevalence rate of *Salmonella* was 56 % (n = 19) in the city centre markets, and maintained a prevalence rate of 0 % in the 10 other localities where samples were taken. This study has helped to bridge the gap in knowledge by establishing the prevalence rate of *Salmonella* in chicken and beef in retail and wholesale markets in Windhoek, Namibia. It is recommended that the

Hazard Analysis Critical Control Points (HACCP) system should be made compulsory to all local food processing establishments, including abattoirs and retail markets in the country.

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ABREVIATIONS

BGA – Brilliant Sugar Iron

BPW – Buffered Peptone Water

CDC – The US Center for Disease Control and Prevention

CVL – Central Veterinary Laboratory

EFSA – European Food Safety Authority

EU – European Union

FAO – Food Agricultural Organization

GFN – Global Foodborne Infections Network

HACCP – Hazard Analysis Critical Control Point

H_2S - Hydrogen Sulfide

ISO - International Standards Organization

KW – Kauffman-White scheme

LPS – Lipopolysaccharide

MKKTn - Mueller Kauffmann Tetrathionate Novobiocin

MoHSS – Ministry of Health and Social Services

NTS - Non Typhoidal Salmonella

RES – Reticuloendothelial System

RNA - Ribonucleic Acid

RSA - Republic of South Africa

RVS - Rappaport Vassiliadis medium with soya

TSI - Triple Sugar Iron

UK - United Kingdom

USA - United States of America

VP - Voges Proskauer

WHO - World Health Organization

XLD - Xylose Lysine Desoxycholate agar

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DEDICATION

I dedicate this research to my late Mother, Miss Grace Namwi Simalumba and late Father, Mr Chrispin Simasiku Simasiku. Mom and Dad thank you very much for the care and support you gave me before you passed away, I love you. Mom and Dad, may your souls continue to rest in eternal peace. May God let this work be a source of inspiration to my son, Mr Dylan Simasiku Simasiku, for his future education accomplishment.

DECLARATION

I, Austin Baepi Simasiku, hereby declare that this study is a true reflection of my own research, and that this work, or part thereof has not been submitted for a degree at any other institution of higher education.

……... ….……

Austin B. Simasiku Date

CHAPTER 1: INTRODUCTION

1.1 Introduction

The purpose of this chapter is to explain the background of *Salmonella* and look into how this bacterium affects people globally, in Africa, and with the focus on Namibia. The chapter will also explain the problem statement, the purpose of the study, the objectives of the study, and the hypotheses of the study. This study could be the first study done on the prevalence of *Salmonella* in chicken and beef in retail markets in Windhoek, Namibia. There is very little knowledge documented on the subject in the Namibian context, hence the importance of this study.

Foodborne diseases are an important cause of morbidity and mortality worldwide and they are among the serious health problems in developing countries (Van Hao, Moutafis, Istivan, Tran, & Coloe, 2007). The worldwide incidence of non-typhoidal salmonellosis is estimated at 1.3 billion cases and 3 million deaths annually (Tassios, Markogiannakis, Vatopoulos, Katsanikou, Velonakis, Kourea-Kremastinou, & Legakis, 1997). Foodborne diseases are one of the serious problems in developing and developed countries (Soltan Dallal, Sharifi Yazdi, Mirzaei, & Kalantar, 2014). *Salmonella* is a bacterium that causes salmonellosis, a disease that is associated with acute enterocolitis, with sudden onset of headache, abdominal pain, diarrhoea, nausea and sometimes vomiting (Heymann, 2008). The infection is commonly associated with the consumption of meat, especially poultry their products, and beef (Nurmi, 2009), although, different studies have demonstrated that the frequencies of chicken and beef contaminations with human pathogens such as *Salmonella*, were not the same (Jorgensen, Bailey, Williams, Henderson, Wareing, Bolton, Frost,

Ward, & Humphrey, 2002; Pui, Wong, Chai, Tunung, Jeyaletchumi, Noor Hidayah, Ubong, Farinazleen, Cheah, & Son, 2011). Due to genetic and environmental diversity, bacterial serotypes are adapted to live in a wide variety of hosts using pathogenic and non-pathogenic means of surviving depending on the environmental conditions (Callaway, Edrington, Byrd, Anderson, & Nisbet, 2008).

There is an increasing concern with this pathogen due to the emergence and spread of antibiotic resistant and potentially more pathogenic strains (Molbak, 2005). Worldwide, *Salmonella* is the most common etiological agent of foodborne diarrheal illnesses (Hendriksen, Vieira, Karlsmose, Lo Fo Wong, Jensen, Wegener, & Aarestrup, 2011; Scallan, Hoekstra, Angulo, Tauxe, Widdowson, Roy, Jones, & Griffin, 2011). Every year, more than 100 million people are afflicted by foodborne and waterborne diseases in the world, especially people with immune system deficiency and malnutrition (Egli, Koster, & Meile, 2002; Todd, 1997). Different species of *Salmonella* are related to foodborne diseases, which can be transmitted through contaminated meat (Soltan Dallal, Sharifi Yazdi, Mirzaei, & Kalantar, 2014).

Epidemiological studies have shown that foods of animal origin are among the most important sources of foodborne diseases (Tauxe, 1997; Petersen & James, 1998). Food products are usually contaminated with pathogens during the production, processing, distributing and retailing in the market (Ono & Yamamoto, 1999). Non-typhoid *Salmonella* species continue to figure prominently in many national epidemiological registries as the leading cause of bacterial foodborne infections, although thermophilic campylobacters have surpassed *salmonella* in many countries

(Pavia & Tauxe, 1991; D'Aoust, 1997; Taylor & Blaser, 1991). The wide spread distribution of *salmonella* in the natural environment and its prevalence in the global food chain raise legitimate concern about the economic and public health consequences attributable to this pathogen (Kapperud, Lassen, & Hasseltvedt, 1998).

According to Scallan *et al*., (2011), human salmonellosis is one of the most frequently occurring foodborne diseases worldwide. Over the past couple of decades the incidence of foodborne salmonellosis has increased considerably in the industrialized world and has reached epidemic proportions in several countries (Rodrigue, Tauxe, & Rowe, 1990). The increase is the result of a combination of factors, including (i) more intensive farming and increased industrialization of all stages of food production, (ii) changes in food handling practices, eating habits and the storage, distribution, and preparation of food, and (iii) more centralized food production and more international trade in food (Baird-Parker, 1994; Baird-Parker, 1994). These changes have brought with them new problems in food hygiene and have greatly facilitated trans-boundary dissemination of *salmonella* as well as other foodborne pathogens (D'Aoust, 1994).

Kapperud, Lassen, & Hasseltvedt (1998) explains that the risk factors for human salmonellosis are likely to varry appreciably across national boundaries in accordance with cultural patterns, climatic factors, husbandry and agricultural practices and implementation of control and preventive measures. These foodborne pathogens are primarily transmitted to humans through the consumption of contaminated foods of animal origin (Shilangale, Giannatale, Chimwamurombe, & Kaaya, 2012). Animals may become infected from other *Salmonella*-infected

animals, directly or through a contaminated environment, including contaminated feed (European Food Safety Authority, 2008). These pathogens may be transmitted to humans through the food chain and cause illness (Crump, Griffin, & Angulo, 2002).

The consumption of contaminated ready to eat foods including beef, have been documented to serve as vehicles for the transmission of several bacterial pathogens and food-borne outbreaks (Borch & Arinder, 2002). Pathogens can survive in the food products, especially in meat, until distributed in the food markets (Knudsen, Sommer, Sorensen, Olsen, & Aabo, 2011). Among the reported outbreaks of salmonellosis with known food vehicles, most were caused by the consumption of contaminated animal products, in which eggs and poultry products are the frequent sources (Smith, Medus, Meyer, Boxrud, Leano, Hedberg, Elfering, Braymen, Bender & Danila, 2008). Surveillance programs that detect *Salmonella* contaminations in a timely manner in the entire food chain (animal feed, living animals, slaughterhouses, retail sector, and restaurants) together with sanitary measures are essential for detecting and preventing human *Salmonella* infections (Bertrand, Dierick, Heylen, De Baere, Pochet, Robesyn, Lokietek, Van Meervenne, Imberechts, De Zutter, & Collard, 2010). Therefore, development of rapid and sensitive methods for the detection and characterization of *Salmonella* may have a significant impact on the disease burden caused by this pathogen (Wattiau, Boland, & Bertrand, 2011).

Failure to control *Salmonella* in a country presents a potential problem for other countries. To minimise the burden of this pathogen, it is helpful to monitor *Salmonella* serovars distribution in many countries, implement *Salmonella* control

measures throughout the food production chain, and monitor the effectiveness of the control measures (Hendriksen, *et al*., 2011). In January 2000, the World Health Organization (WHO) launched the WHO Global Salm-Surv program, now known as the WHO Global Foodborne Infections Network (GFN), a global effort to build capacity to detect, control, and prevent foodborne and other enteric illnesses from farm to table (Hendriksen, *et al*., 2011). A key objective of GFN is to enhance laboratory-based surveillance worldwide by improving laboratory capacity for serotyping of *Salmonella* (Hendriksen, *et al*., 2011). This objective is facilitated by bench-top training at international courses and workshops.

According to Majowicz., *et al*., (2010), various animals (especially poultry, pigs, cattle, and reptiles) are reservoirs for *Salmonella* species and humans generally become infected by eating undercooked or contaminated food. Although most infections produce mild gastroenteritis, lives threatening disseminated infections are common among elderly and immunocompromised patients. As it has been mentioned at the beginning, there is very little known on the subject, a few studies done on foods of animal origin in Namibia have shown that *Salmonella* is prevalent in the country. Public health facilities do not test stool samples of suspected cases, they only resort to treating them. Nonetheless, *Salmonella* infection is a major public health problem worldwide (Hello, *et al*., 2011).

1.2 Statement of a problem

There is very little, if nothing, done on the prevalence and serovars diversity on chicken and beef in retail and wholesale markets in the Namibian context. Data on

the prevalence of *Salmonella* in retail and wholesale foods in Windhoek, Namibia are or could be unavailable; therefore, this study is needed for documenting its prevalence. It is clear that there is a gap in knowledge on the prevalence and serovars of *Salmonella* spp. isolated from ready to eat foods from retail and wholesale markets in Windhoek, Namibia.

Salmonella is of an important public health concern: according to the World Health Organization (Herikstad, Motarjemi, & Tauxe, 2002), *Salmonella* infection is prevalent worldwide and is one of the major causes of foodborne illnesses in many countries (Iwabuchi, Yamoto, Endo, Ochiai, & Hirai, 2010). The contamination of chicken and beef with *Salmonella* may cause serious public health concerns and found to be the leading cause of estimated hospitalizations and deaths globally, responsible for about 28 percent of deaths and 35 percent hospitalizations (CDC, 2010). The study will help to evaluate and provide answers on the prevalence, and possible population health of *Salmonella* serovars in ready to eat chicken and beef in Windhoek, Namibia.

Although the current global impact of *Salmonella* on public health is not very clear, available data estimates, indicate that there are 93.8 million cases of *Salmonella* infection and 155 000 deaths each year worldwide (Majowicz, *et al*., 2010). With observation, this might be the case in Windhoek, Namibia. However, the lack of research and information on the prevalence of *Salmonella* prevalence and serovars in Windhoek has made it difficult to estimate the potential threat of foodborne illnesses attributable to *Salmonella* in the Capital City of Namibia.

1.3 Purpose of the study

The purpose of the study was to establish the prevalence and serovars diversity of *Salmonella* in chicken and beef from retail and wholesale markets in Windhoek, Namibia.

1.4 Study objectives

The objectives of the study were:

- To determine the prevalence of *Salmonella* spp. isolated from ready to eat chicken and beef from retail and wholesale markets in Windhoek, Namibia.
- To determine the serovars diversity of *Salmonella* spp. isolated from chicken and beef from retail and wholesale markets in Windhoek, Namibia.

1.5 Hypotheses of the study: Two hypotheses were formulated.

1st Hypothesis:

H_O: *Salmonella* serovars are not prevalent in chicken and beef from retail and wholesale markets in Windhoek, Namibia.

H_A: *Salmonella* serovars are prevalent in chicken and beef from retail and wholesale markets in Windhoek, Namibia.

2nd Hypothesis

H_O: Different *Salmonella* serovars are not found in Windhoek, Namibia.

H_A: Different *Salmonella* serovars are found in Windhoek, Namibia.

1.6 Significance of the study

The occurrence of *Salmonella* is now a global challenge in the public health and food production sectors, as the awareness of consumers in regard to food safety is on the increase. In order to solve the problem of Salmonellosis, the prevalence and diversity of serovars of *Salmonella* spp. should be established in any country. This study's findings will help in determining the prevalence and serovars diversity of *Salmonella* in Windhoek, Namibia. Once the prevalence of *Salmonella* is established, appropriate steps can be taken to minimize the presence of these organisms in such ready to eat chicken and beef products.

Information obtained from this study could help increase public health knowledge and awareness of consumers to know how safe the food they consume is. In general, consumers assume that foods bought from retail and wholesale markets are sterile. Furthermore, information obtained from this study could help producers producing for retail and wholesale markets to prevent and control the prevalence of *Salmonella* in their production plants. The information can be used to inform both the Namibia public health and veterinary policy on chicken and beef food products preparation in the food industry and at household level.

1.7 Limitation of the study

The study was limited to retail and wholesale markets selling ready to eat beef and chicken in Windhoek, Namibia. Retail shops that provided services but situated deep in the informal settlements could not be reached as it was not easy and safe to get to such places.

1.8 Chapters layout

The layout of this thesis is as follows:

Chapter 1: Introduction

Chapter 2: Literature Review

Chapter 3: Methodology

Chapter 4: Data analysis and study results

Chapter 5: Discussions, conclusion and recommendations

1.9 Conclusion

This chapter presented the background of *Salmonella* in general and lack of its documentation in the Namibian context. The problem statement was clear, and a gap in documentation of the prevalence and serovars diversity on chicken and beef in retail and wholesale markets in the Namibian context was identified. The study objectives and hypotheses were formulated. Lastly, the layout of the chapters was provided.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

The purpose of this literature review is to explain the burden and the public health importance *Salmonella* globally, in Africa, and Namibia. Most importantly: to also explain Salmonellosis, the origin and history of *Salmonella* as multiple species, the epidemiology and the general characteristics of the bacteria. The morphology, classification, nomenclature, and serotyping of *Salmonella* will also be explained.

2.2 Salmonellosis

Salmonellosis is a major cause of bacterial enteric illness in both humans and animals (Brenner, Villar, Angulo, Tauxe, & Swaminathan, 2000). Infection with *salmonella* in humans and animals primarily causes self-limiting gastro intestinal infections with mild to moderate symptoms, including fever, abdominal cramps, and diarrhoea (Hohmann, 2001). More severe clinical outcomes, including death, may occur in cases of bacteraemia or enteric fever (typhoid), which is often characterized by severe headaches, and high fever but no diarrhoea (Bhan, Bahl, & Bhatnagar, 2005). The symptoms are usually self-limiting and typically and resolve within two to seven days (Foley & Lynne, 2008).

In a small percentage of cases, septicaemia and invasive infections of organs and tissues can occur, leading to diseases such as osteomyelitis, pneumonia, and meningitis (Cohen, Bartlett, & Corey, 1987). People who are very young, very old, or immunocompromised are most susceptible to these severe manifestations of salmonellosis, which typically require antimicrobial therapy (Benenson & Chin, 1995). The number of salmonella human isolates reported to the Centres for Disease

Control has been steadily increasing since 1977, and in 1983 there were over 38,000 salmonella isolates (CDC, 1983). Despite improvements in sanitation and careful monitoring of food processing, large outbreaks of salmonellosis continue to occur when food becomes contaminated (CDC, 1985).

In human disease, the clinical pattern of salmonellosis can be divided into four disease patterns namely enteric fever, gastroenteritis, bacteremia, and other complications of nontyphoidal salmonellosis as well as chronic carrier state (Pui, *et al*., 2011). *Salmonella* Typhi causes typhoid fever where as Paratyphi A, B, and C cause paratyphoid fever with symptoms which are milder and mortality rate that is lower for the later (Pui, *et al*., 2011). Both serotypes are solely human pathogens, infection typically occurs due to ingestion of food or water contaminated with human waste. In recent years, antibiotic resistant strains have been isolated in most endemic areas, particularly Southeast Asia, India, Pakistan and Middle East (Scherer & Miller, 2001).

According to Pui, *et al*., (2011) roughly 10% of patients may relapse, die or encounter serious complications such as typhoid encephalopathy, gastrointestinal bleeding and intestinal perforation patients (Hu & Kopecko, 2003; Parry, 2006). Relapse is the most common occurrence probably due to persisting organisms within reticuloendothelial system (RES). Typhoid encephalopathy, often accompanied by shock, is associated with high mortality. Slight gastrointestinal bleeding can be resolved without blood transfusion but in 1 to 2% of cases can be fatal if a large vessel is involved. Intestinal perforation may present with abdominal pain, rising

pulse and falling blood pressure in those that have the disease. Hence, it is very serious in 1 to 3% of hosipitalized patients (Hu & Kopecko, 2003; Parry, 2006).

Nontyphoidal salmonellosis or enterocolitis is caused by at least 150 *Salmonella* serotypes with *Salmonella* Typhimurium and *Salmonella* Enteritidis being the most common serotypes in the United States (Pui, *et al*., 2011). Infection always occurs via ingestion of water or food contaminated with animal waste rather than human waste. The emergence of multidrug resistant *S.* Typhimurium DT104 has been associated with outbreaks related to beef contamination and resulted in hospitalization rates twice than that of other foodborne salmonellosis (Gray & Fedorka-Cray, 2002; Yousef & Carlstrom, 2003).

Ciprofloxacin is often administered at the first sign of severe gastroenteritis whereas ceftriaxone is given to children with systematic salmonellosis. In production animals like swine, the treatment is usually contraindicated but, when necessary, can be given via injection with several treatment alternatives based on considerations such as withdrawal time. Antibiotic treatment is usually not advised except for rare cases because it can prolong the presence of bacteria in the stool (Gray & Fedorka-Cray, 2002; Yousef & Carlstrom, 2003). About 8% of the untreated cases of salmonellosis result in bacteremia; which is a serious condition in which bacteria enter the blood stream after passing through the intestinal barrier (Scherer & Miller, 2001; Hanes, 2003). It has been associated with highly invasive serotypes like Cholearaesuis or Dublin. Bacteremia caused by *Salmonella* should be taken into account in cases of fever of unknown origin. Patients with bacteremia and other complications should be treated with antibiotics (Scherer & Miller, 2001; Hanes, 2003).

Salmonellosis can be spread by chronic carriers who potentially infect many individuals, especially those who work in food related industries. (Pui, *et al*., 2011). Factors contributing to the chronic carrier state have not been fully explained, on average, nontyphoidal serotypes persist in the gastrointestinal tract from six weeks to three months, depending on the serotypes (Pui, *et al*., 2011). According to Pui, *et al*., (2011), only about 0.1% of nontyphoidal *Salmonella* cases are shed in stool samples for periods exceeding one year. The authors further explains that about 2 to 5% of untreated typhoid infections result in a chronic carrier state. Up to 10% of untreated convalescent typhoid cases will excrete *S.* Typhi in feces for one to three months and between 1 and 4% become chronic carriers excreting the microoganism for more than one year (Scherer & Miller, 2001; Parry, 2006).

2.3 Origin and history of *Salmonella* as multiple species

Salmonella is named after an American bacteriologist, D. E. Salmon, who first isolated *Salmonella Choleraesuis* from porcine intestine in 1884 (Smith, 1894). The organism was originally called "*Bacillus Choleraesuis"* which was subsequently changed to *"Salmonella choleraesuis"* by Lignieres in 1990 (Lin-Hui Su & Cheng-Hsun Chiu, 2007). Kauffmann proposed that each serovars be considered a separate species (Kauffmann, 1966). That is why *Salmonella* serovars identified after 1966 were designated mainly by their antigenic formula and multiple species within the genus *Salmonella* were generally accepted.

However, Lin-Hui Su & Cheng-Hsun Chiu (2007) stresses that some clinically important *Salmonellae* identified before had been given specific names either according to the disease and/or the animal from which the organism was isolated,

such as *S. typhi* and *S. typhimurium*, or by the geographical area where the strain was first isolated, e.g., *S. london* and *S. panama*. These names had been used for a number of years and therefore were adopted without being amended into the new antigenic formula system (Lin-Hui Su & Cheng-Hsun Chiu , 2007).

Due to the complexity of multiple *Salmonella* species, it was proposed that the genus *Salmonella* be subdevided into three species, *S. choleraesuis* (the type species), "*S. thphosa"* (*S. thyphi),* and "*S. Kauffmannii*," with the last containing all the other serovars (Borman, Stuart, & Wheeler, 1944). Later, "*Salmonella enterica*" was proposed by Kauffmann and Edwards to encompass all Salmonellae (Kauffmann & Edwards, 1952). In 1966, a simillar three-species model was proposed, with "*Salmonella enteritidis*" representing all serovars other than *S. typhi* and *S. choleraesuis* (Ewing, 1972). Another proposal in 1970 recommended that Kauffmann's "subgenera" be considered a species, i.e., "*S. kauffmannii"* for "subgenus" I, *S. salamae* for "subgenus" II, *S. arizonae* for "subgenus" III, and *S. houtenae* for "subgenus" IV (Le Minor, Rohde, & Taylor, 1970). Serovars of "*S. Kauffmannii"* would be designated by their species names followed by that of their serovar (e.g. "*S.kauffmanni"* serovar typhi), and serovars of the other three species would be designated their species names followed by their antigenic formulae (Lin-Hui Su & Cheng-Hsun Chiu, 2007).

In 1973, on the basis of DNA-DNA hybridization experiments, it was demonstrated that all *Salmonella* strains should belong to a single species (Crosa, Brenner, Ewing, & Falkow, 1973). In 1982, on the basis of numerical taxonomy and DNA relatedness studies proposed the name "*Salmonella choleraesuis"* for the single *Salmonella*

species and six subspecies were defined (Le Minor, Veron, & Popoff, 1982). These authors also proposed that the name of serovars should be used without italicization or underlining (e.g. *Salmonella choleraesuis* subsp. *choleraesuis* ser. typhimurium). In 1989, a single exception was described: one of the subspecies, *Salmonella choleraesuis* subsp. *Bongori*, was separated from the other subspecies as a unique *Salmonella* species due to differences demonstrated by DNA relatedness studies (Reeves, Evins, Heiba, Plikaytis, & Farmer, 1989).

Because of confusion caused by using "choleraesuis" as a name for both a species and a serovars, in 1986 "*Salmonella enterica"* was proposed again as the type species of *Salmonella* by the subcommittee of *Enterobacteriaceae* of the International Committee on Systematic Bacteriology at the XIV International Congress of Microbiology (Penner, 1988). The proposal was formally made to the Judicial Commission of the International Committee of Systematic Bacteriology by Le Minor & Popoff (1987) of the WHO collaborating center. Lin-Hui Su & Cheng-Hsun Chiu (2007) further explains that the epithet *"entrerica"* was recommended because it has not been used previously for a serovar, they also proposed that the seven subgenera of *Salmonella* be reffered to as subspecies (subspecies I, II, IIIa, IV, V, and VI). Subgenus III was divided into IIIa and IIIb by DNA similarity and phenotypic characteristics. The suggestion was accepted by the Centers for Disease Control and Prevention (CDC) and other experts and laboratories , but denied by the Judicial Commission due to concerns that the status of *Salmonella* serovar Typhi might be overlooked (Ewing, 1986; Old, 1992). *S. choleraesuis* was thus returned as the legitimated type species pending an amended request for an opnion (Wayne,

1991). To comply with this rulling and also to support the proposal by Le Minor & Popoff (1987), Euzeby (1999) made an ammended request to use "*Salmonella enterica*" as the type species of *Salmonella* and reserve the name "*Salmonella typhi*" to reflect its clinical importance.

In 2002, the Judicial Commission carefully discussed the request by Euzeby (1999) and issued an opinion (the Judicial Opinion 80) which finally approved that from January 2005, "*Salmonella enterica*" would replace "*Salmonella choleraesuis*" to become the type species of the genus *Salmonella* (Le Minor & Popoff, 2005). Furthermore, an accompanying commentary was written to help the bacteriologists better interpret both the nomenclatural and taxonomic consequences of Opinion 80 (Tindal, Grimont, Garrity, & Euzeby, 2005). According to the rulling of the Judicial Commission, the genus *Salmonella* consists of two species, "*Salmonella bongori*" and "*Salmonella enterica*". The latter includes six subspecies, "*arizonae*", *"diarizonae", "enterica", "houtenae", "indica", and "salamae"* (Lin-Hui Su & Cheng-Hsun Chiu, 2007). In 2005, a new species, "*Salmonella sub-terranea"* was approved by the Judicial Commission (Shelobolina, Sullivan, O'Neill, Nevin, & Lovley, 2004). Names of some medically important *Salmonella* serovars such as "*Salmonella typhi", "Salmonella typhimurium"* and *"Salmonella enteretidis",* have been used frequently and in 2000 it was proposed that these names be conserved (Euzeby, 1999). However, this proposal was not granted by the Judicial Commission. Furthermore, the proposal to raise "*Salmonella choleraesuis* subsp. *choleraesuis* serovar Paratyphi A" to a new species, *"Salmonella paratyphi",* was not also granted by the Judicial Commission (Ezaki, Amano, Kawamura, & Yabuuchi, 2000).

The antigenic classification system of various *Salmonella* serovars used today has accumulated from many years of studies on antibody interactions with surface antigens of *Salmonella* organisms established by Kauffmann and White almost a century ago (Lin-Hui Su & Cheng-Hsun Chiu, 2007). All antigenic formulae of recognised *Salmonella* serotypes are listed in a document called the Kauffmann-White scheme (Popoff & Le Minor, 2001). The WHO collaborating Center for Reference and Research on *Salmonella* at the Pasteur Institute, Paris, France is responsible for updating the scheme (Lin-Hui Su & Cheng-Hsun Chiu, 2007). Every year newly recognised serovars are reported in the journal Research in Microbiology, in the latest report published in 2004, there were a total of 2,541 serovars in the genus *Salmonella* presented in **Table 1** (Popoff, Bockemuhl, & Gheesling, 2004). Currently, the nomenclature system used at the CDC for the genus *Salmonella* is based on recommendations from the WHO Collaborating Center (Lin-Hui Su & Cheng-Hsun Chiu, 2007).

Table 1 Current Salmonella Nomenclature

Genus (capitalized, italic)	Species (italic)	Subspecies (italic)	Serotypes (capitalized, not italic)	No. of serotypes in each species
Salmonella	*enterica*	*enterica*(or subsp I)	Choleraesuis, Enteritidis, Paratyphi, Typhi, Typhimurium	1504
		salamae(or subsp II)	9,46:z:z39	502
		arizonae(or subsp IIIa)	43:z29:-	95
		diarizonae(or subsp IIIb)	6,7:1,v:1,5,7	333
		houtenae(or subsp IV)	21:m,t:-	72
		indica(or subsp VI)	59:z36:-	13
	bongori subterranea	subspecies V	13,22:z39:-	22

According to the CDC system, the genus *Salmonella* contains two species, *S. enterica*, the type species, and *S. bongori*. A third species *"Salmonella subterranea"* was recognised in 2005, and the CDC may incomporate it in their system in the near future (Lin-Hui Su & Cheng-Hsun Chiu, 2007).

2.4 Epidemiology of *Salmonella*

Typhoid cases are stable with low numbers in developed countries, but nontyphoidal salmonellosis has increased worldwide (Pui, *et al*., 2011). Typhoid fever usually causes mortality in 5 to 30% of typhoid-infected individual in the developing world, the World Health Organization (WHO) estimates 16 to 17 million cases occur annually, resulting in about 600,000 deaths globally (Pui, *et al*., 2011). The mortality rates differ from region to region, but can be as high as 5 to 7% despite the use of

appropriate antibiotic treatment. On the other hand, nontyphoidal cases account for 1.3 billion cases with 3 million deaths, data on salmonellosis are scarce in many countries of Asia, Africa and South and Central America where only one to ten percent of cases are reported (Hu & Kopecko, 2003; Hanes, 2003; Portillo, 2000).

According to Pui, *et al*., (2011), typhoid fever is endemic throughout Africa and Asia as well as persists in the Middle East, some eastern and southern European countries and central and South America. Pui, *et al*., (2011) again stresses that typhoid incidence in endemic areas is typically low in the fisrt few years of life, peaking in school-aged children and young adults and then falling in middle age.

The most famous outbreak of enteric fever is Typhoid Mary. Marry Mallon, a New York City hired household cook , transmitted typhoid fever to at least 22 individuals causing 3 deaths between 1900 and 1907. After being apprehanded by public health officials in 1907, she was isolated for 3 years. Even though she was released with the stipulation that she never cooks again, she broke the promise and consequently caused at least 25 more cases of typhoid fever at Manhattan maternity hospital when she was employed as a cook in 1915. She was finally isolated until her death in 1938 (Parry, 2006; Scherer & Miller, 2001).

The infectious dose of *Salmonella* depends upon the serovar, bacteria strain, growth condition and host susceptibility. On the other hand, host factors controlling susceptibility to infection include the condition of the intestinal tract, age and underlying illnesses or immune deficiencies. The infectious dose of *Salmonella* is broad varying form 1 to 10^9 cfu/g. However, single food source outbreaks indicate

that as little as 1 to 10 cells can cause salmonellosis with more suceptibility to infection by YOPI groups (Yousef & Carlstrom, 2003; Bhunia, 2008).

Since food animals are the reservoir for most domestically acquired human *Salmonella* infections and transmission from animals to humans occurs through the food supply (Angulo, Jonson, Tauxe, & Cohen, 2000). *Salmonella* serovars have been detected worldwide from various foods, such as eggs, raw meats, vegetables and cheeses (Busani *et al.,* 2005 and Miranda *et al.,* 2009). The widespread distribution of *Salmonella* in the natural environment and its prevalence in the global food chain raises legitimate concern about the economic and public health consequences attributable to this pathogen (Kapperud, Lassen, & Hasseltvedt, 1998).

Egg and egg containing foods are the primary vehicles of *S. Enteritidis* infection, having been implicated in 298 (80%) of the 371 known sources of *S. Enteritidis* outbreaks reported to the Centres for Disease Control and Prevention (CDC) from 1985 through 1999 (Patrick *et al.,* 2004). Over the past couple of decades, the incidence of foodborne salmonellosis has increased considerably in the industrialized world and has reached epidemic proportions in several countries (Rodrigue, Tauxe, & Rowe, 1994).

2.5 Characteristics and Morphology of *Salmonella*

Salmonella is a gram negative rod-shaped, mostly non lactose fermenter, facultative anaerobic, non-spore forming, mesophilic heterotroph, producing acid and gas from glucose, belonging to the family *Enterobacteriaceae* (Mohamed, 2013). Serotypes of *Salmonella* belong to the family *Enterobacteriaceae* and are now considered to

belong to two species, *Salmonella bongori* (formerly subspecies V) and *Salmonella enterica* (Le Minor and Popoff, 1987 and Old, 1992).

According to Liu, *et al*., (2002), *Salmonella* species are very closely related to one another, with their genomic DNA re-association rates estimated to be as higher as 90%. Morphologically, *Salmonella* are gram-negative, straight rods shaped bacteria that measure between 0.7 and 1.5 µm in diameter and 2 to 5 µm in length. They are usually motile with peritrichous flagella and are facultative anaerobes. *Salmonella* ferment mostly glucose with the formation of gas and also reduce nitrate to nitrite. *Salmonella* grow optimally between 35 °C to 37 °C and catabolizes a variety of carbohydrates into acid and gas.

They use citrate as the sole carbon source, and decarboxylate lysine and ornithine to cadaverine and putrescine respectively. They are chemoorganotrophs, obtaining their energy from oxidation and reduction reactions using organic sources. Most *Salmonella* species produce H2S, which can readily be detected by growing them on media containing ferrous sulfate such as triple sugar iron (TSI) where Salmonella is able to produce H2S from thiosulphate. The production of H2S on TSI media is one of the common biochemical characteristics which are widely used when identifying the bacteria.

Historically *Salmonella* was mostly confirmed if it catabolized glucose and lysine but failed to metabolize lactose, sucrose and urea. However, some *Salmonella* have shown to have no reaction on these substances. Most (>99.5%) *Salmonella* isolates from humans are serotypes of *Salmonella enterica* (HPA, 2004). On blood agar, colonies are 2-3 mm in diameter; colonies are generally lactose non-fermenters

(HPA, 2004). *Salmonella* species are motile (with a few exceptions), facultatively anaerobic, produce acid from glucose usually with the production of gas, and are oxidase negative (Le Minor, 1984). Most produce hydrogen sulphide except *Salmonella typhi* and *Salmonella paratyphi A*, which is a weak producer (HPA, 2004).

The usual habitat of different *Salmonella* species and subspecies is the intestines of both cold and warm blooded animals (Brenner, Villar, Angulo, Tauxe, & Swaminathan, 2000). The bacteria can also be found throughout the natural environment. Environmental sources of the organism include water, soil, insects, factory surfaces, kitchen surfaces, animal feces, raw meats, raw poultry and raw sea food. Even though *Salmonella* cannot multiply outside the host digestive tract, the bacteria can live for number of weeks in water and some years in soil if there are favorable conditions such as temperature, pH and humidity (Todar, 2008).

Todar (2008) further explains that *Salmonella* serovars can be found predominantly in one particular host, can be ubiquitous, or can have an unknown habitat. Typhi and Paratyphi A are strictly human serovars that may cause severe diseases which is often associated with the invasion of the bloodstream. Salmonellosis in these cases is transmitted through faecal contamination of water or food. Gallinarum, Abortusovis, and Typhisuis are, respectively, avian, ovine, and porcine *Salmonella* serovars. Such host-adapted serovars cannot grow on minimal medium without growth factors (contrary to the ubiquitous *Salmonella* serovars).

Salmonella bongori and *Salmonella enterica* are the two species that comprise the genus *Salmonella enterica* is further divided into six subspecies, namely, *enterica* (I),

salamae (II), *arizonae* 5 (IIIa), *diarizonae* (IIIb), *houtenae* (IV), and *indica* (VI) (Leader, Frye, Hu, Fedorka-Cray, & Boyle, 2009). *Salmonella enterica* subspecies *enetrica* strains are of the greatest clinical relevance and are typically isolated from humans and warm blooded animals (Leader, Frye, Hu, Fedorka-Cray, & Boyle, 2009). Strains belonging to one of the other five *Salmonella enterica* subspecies and *Salmonella bongori* are associated with environmental or reptilian sources (Brenner, 1998; Grimont & Weill, 2007).

2.6 Classification and nomenclature of *Salmonella*

Classification and nomenclature of *Salmonella* species are confusing, even for the enthusiast (Brenner, Villar, Angulo, Tauxe, & Swaminathan, 2000). Historically *Salmonella* had been named based on the original places of isolation such as *Salmonella* London and *Salmonella* Indiana (Pui, *et al*., 2011). This nomenclature system was replaced by the classification based on the susceptibility of isolates to different selected bacteriophages which is also known as phage typing (Bhunia, 2008).

Serologic classification of *Salmonella* strains based upon properties of various surface polysaccharide (O) and flagellar (H) antigens is the reference method for epidemiologic surveillance (Leader, Frye, Hu, Fedorka-Cray, & Boyle, 2009). This method involves the characterization of over 150 unique O and H antigens to produce an antigenic formula that can be scored using the Kaufmann-White scheme to determine a serovars for an isolate (Bopp, Nancy, Brenner, Fields, & Wells, 2003; Grimont & Weill, 2007).

Currently, serotyping classifies over 2,500 serovars of *Salmonella*, of which over 1,400 belong to *Salmonella enterica* subspecies *enterica* (Bopp, Nancy, Brenner, Fields, & Wells, 2003; CDC, 2005). Although serotyping using the Kauffman-White scheme remains the standard for serovars determination through its longstanding and widespread use, it is not without significant deficiencies (Leader, Frye, Hu, Fedorka-Cray, & Boyle, 2009). Aside from being labor-intensive and expensive, serotyping is also time-consuming to perform, often taking three or more days after receipt of a specimen for a highly trained laboratory technician to produce a result (Leader, Frye, Hu, Fedorka-Cray, & Boyle, 2009).

Phage typing is generally employed when the origin and characteristic of an outbreak must be determined by differentiating the isolates of the same serotype (Pui, *et al*., 2011). More than 200 definitive phage types (DT) have been reported so far, for example, *S.* Typhimurium DT104 designates a particular phage type for Typhimurium isolates (Andrews & Baumler, 2005; Hanes, 2003).

Epidemiologic classification of *Salmonella* is based on the host preferences; the first group includes host-restricted serotypes that infect only humans such as *S. Typhi*, the second group includes host-adapted serotypes which are associated with one host species but can cause disease in other hosts serotypes such as *S.* Pullorum in avian, the third group includes the remaining serotypes (Pui, *et al*., 2011). Typically, *Salmonella* Enteritidis, *Salmonella* Typhimurium and *Salmonella* Heidelberg are the three most frequent serotypes recovered from humans each year (Boyen, *et al*., 2008; Gray & Fedorka-Cray, 2002).

Kauffmann-White scheme classifies *Salmonella* according to three major antigenic determinants composed of flagellar H antigens, somatic O antigens and virulence (V_i) capsular K antigens, this was adopted by the International Association of Microbiologists in 1934 (Pui, *et al*., 2011). Agglutination by antibodies specific for the various O antigens is employed to group *Salmonella* into the 6 serogroups: A, B, C1, C2, D and E. For instance, *S*. Paratyphi A, B, C and *S.* Typhi express O antigens of serogroups A, B, C1, and D respectively (*Pui, et al*., 2011). More than 99% of *Salmonella* strains causing human infections belong to *Salmonella enterica* subspecies enterica. Although not common, cross-reactivity between O antigens of *Salmonella* and other genera of *Enterobacteriaceae* do occur. Therefore, further classification of serotypes is based on the antigenicity of the flagellar H antigens which are highly specific for *Salmonella* (Scherer & Miller, 2001).

In brief, O antigens are lipopolysaccharide (LPS) on the outer bacterial membrane. They are heat stable, resistant to alcohol and dilute acids. H antigens are heat-labile proteins associated with the peritrichous flagella and can be expressed in one of two phages. The phase 1 H antigens are specific and associated with the immunological identity of the particular serovars whereas phase 2 antigens are non-specific antigens containing different antigenic subunit proteins which can be shared by many serovars (Pui, *et al*., 2011). K antigens which are heat-sensitive carbohydrates are produced by *Salmonella* serovars that express a surface-bound polysaccharide capsular antigen (Hu & Kopecko, 2003; Yousef & Carlstrom, 2003).

Bacteria can be classified based on phylogeny; a phylogenetic tree can be derived from the comparison with 16S rRNA or other genes sequences. There are 2463

Salmonella serotypes which are now placed under 2 species due to the difference in 16S rRNA sequence analysis: *Salmonella enterica* (2443 serotypes) and *Salmonella bongori* (20 serotypes). The system is currently used World Health Organization (WHO) Collaborating Centre, Centre's for Disease Control and Prevention (CDC) and some other organizations (Pui, *et al*., 2011).

Salmonella enterica is further divided into six subspecies, which are designated by roman numerals. *Salmonella enterica* subspecies I is mainly isolated from warm blooded animals and accounts for more than 99% of clinical isolates whereas remaining subspecies and *S. bongori* are mainly isolated from cold blooded and account for less than 1% of clinical isolates. As an example, the Kauffmann species *Salmonella Typhimurium* is now designated as *Salmonella enterica* subspecies I serotype Typhimurium. Under the modern nomenclature system, the subspecies information is often omitted and the culture is called *S. enterica* serotype Typhimurium and subsequent appearance, it is written as *S.* Typhimurium. This system of nomenclature is used nowadays to bring uniformity in reporting (Bhunia, 2008; Andrews & Baumler, 2005; Parry, 2006).

2.7 *Salmonella* serotyping

Salmonella serotyping is a method which has proven to be very useful in distinguishing isolates of the different species of *Salmonella*, predominantly for public health purposes, e.g., surveillance and outbreak investigations. Epidemiologically, it is increasingly becoming important to be able to distinguish *Salmonella* isolates. This is because definitive typing of *Salmonella* may assist in

tracing the source of an outbreak and monitoring trends in cases associated with a particular type of *Salmonella* (Yan, *et al*., 2003).

Epidemiological typing of bacterial strains can be carried out by a variety of techniques, including serotyping, biotyping, bacteriophage and bacteriocin typing. Traditionally, conventional antigen based serotyping technique has been widely used for typing bacteria. However, phage typing and other advanced molecular techniques are currently being used for *Salmonella* serotyping and subtying. Comparing conventional method and advanced molecular techniques, the later are thought to be rapid and inexpensive. However, according to Hunter & Gaston (1988), the choice of which typing method is the most efficient is usually based on a number of factors such as typability, reproducibility and discrimination.

2.7.1 Typing of *Salmonella* by phenotypic methods

2.7.1.1 Serotyping by agglutination (Kauffmann-White-Le Minor scheme)

Salmonella serotyping is based on the Kauffmann-White-Le Minor (KW) scheme which is a modification of the original scheme from the 1930s (Grimont & Weill, 2007; Guibourdenche, *et al*., 2010). Serotyping is based on the agglutination of the bacteria with specific sera to identify variants of the somatic (O) and flagellar (H) antigens (Wattiau, Boland, & Bertrand, 2011). These antigens are highly variable, with 64 O and 114 H variants identified (Grimont & Weill, 2007; McQuiston, Fields, Tauxe, & Logsdon, 2008). The O antigen is the saccharidic component of the lipopolysaccharide (LPS) exposed on the bacterial surface (Reeves, *et al*., 1996; Schnaitman & Klena, 1993).

Its reactivity toward specific antisera forms the basis of the *Salmonella* serotyping scheme (Grimont & Weill, 2007). Several O antigens may be expressed together at the surface of a single cell, in contrast, although most salmonellae possess two different copies of the gene encoding the flagellar protein at a time (McQuiston, Fields, Tauxe, & Logsdon, 2008). Most isolates are therefore termed diphasic (phase I and phase II, also called H1 and H2) with respect to the flagellar antigens (Wattiau, Boland, & Bertrand, 2011). Monophasic *Salmonella* are not rare; triphasic and quadriphasic subtypes are exceptional (Burnens, Stanley, Sechter, & Nicolet, 1996). Although only one H antigen is expressed at a time, both H1 and H2 can usually be detected in pure cultures because the two phases are expressed by discrete bacterial populations in the same culture or colony (Wattiau, Boland, & Bertrand, 2011).

However, if one H phase is undetectable, a method called "phase inversion" is used, which consists of inhibiting the dominant phase by a specific antiserum on a special medium which will favour the growth of the bacterial population expressing the other H antigen (Grimont & Weill, 2007). By conversion, the antigens identified in a given strain are reported in a so-called antigenic formula in which the subspecies number followed by the O, H1, and H2 antigens separated by colons is reported (Wattiau, Boland, & Bertrand, 2011). The slide agglutination test is an early typing technique reflecting the technology available at the time, it is exclusively based on phenotypic characteristics (Wattiau, Boland, & Bertrand, 2011). False-positive reactions may occur as a result of weak, nonspecific agglutination (Schrader, FernandezCastro, Cheung, Crandall, & Abbot, 2008).

Autoagglutination and loss of antigen expression, such as that observed with rough, nonmotile, and mucoid strains, may occasionally lead to strain untypeability, but these strains typically have little epidemiological significance (Wattiau, Boland, & Bertrand, 2011). The method is intended neither to provide a sensitive fingerprint (e.g. for tracing during outbreaks) nor to define phyletic relationships. It requires the use of over 150 specific antisera and carefully trained personnel. It is still defined as the reference method and is commonly as an initial screening, followed by molecular subtyping to identify outbreak-related strains (Wattiau, Boland, & Bertrand, 2011).

2.7.1.2 Serotyping by antibody microarrays

Development of a serotyping assay based on SuperEpoxy microarray slides spotted with antibodies and fitting the Kauffman White Scheme was developed by (Cai, Lu, Muckle, Prescott, & Chen, 2005). Identification of 20 commonly isolated and clinically important serovars, representing 80 to 90% of the total *Salmonella* isolates collected in Canada, was shown to be successful. In this assay, the antibody-antigen reactions are conducted on a microvolume scale on slides following fluorescent labelling of the investigated *Salmonella* strain. Detection is carried out with a common fluorescence scanner. The main advantages of antibody microarray-based serotyping over traditional serotyping are reduced analysis time, standardised agglutination detection, and simultaneous detection of the O and H antigens, for which phase inversion step can be skipped (Wattiau, Boland, & Bertrand, 2011).

2.7.1.3 Other phenotypic methods

Phage typing is used to discriminate between *Salmonella* strains belonging to the same serovars (Wattiau, Boland, & Bertrand, 2011). Phage types are assigned on the

basis of the ability of a given phage to lyse the investigated strain (Anderson & Williams, 1956). Initially, it was designed for *Salmonella* serovars Typhi, *Salmonella* serovars Paratyphi A, and *Salmonella* serovars Enteritidis and a few other serovars (Ward, de Sa, & Rowe, 1987; Hickman-Brenner, Stubbs, & Fammer III, 1991). The advantage of phage typing resides in the simplicity of its implementation, which requires only basic laboratory equipment. Ambiguous lysis reactions are common drawbacks, and careful coordination between reference laboratories is required in order to ensure reproducibility of the assay (Wattiau, Boland, & Bertrand, 2011). The method is also limited by the number of available phages.

2.8 Conclusion

This literature review focused on salmonellosis as a major cause of bacterial enteric illness in both humans and animals, the origin and history of *Salmonella* as multiple species; it also looked into aspects of the epidemiology of the bacteria. It further explained the characteristics and the morphology, classification and nomenclature, and serotyping of *Salmonella*.

CHAPTER 3: METHODOLOGY

3.1 Introduction

The purpose of this chapter is to describe and justify the research design that was used in this study. The study population, sample and sampling procedure, and data analysis will be explained.

3.1 Research design

The research was a cross-sectional, descriptive quantitative study design to estimate prevalence and frequency distribution of serovars of *Salmonella* in chicken and beef from retail markets in Windhoek, Namibia. One in which the investigator primarily uses postpositivist claims for developing knowledge (i.e., cause and effect thinking, reduction to specific variables and hypotheses and questions, use of measurement and observation, and the test of theories), employs strategies of inquiry such as experiments and surveys, and collects data on predetermined instruments that yield statistical data (Creswell, 2003). In quantitative research, relationships between variables are quantified.

3.2 Population

The target population is the collection of individuals about whom we want to draw conclusions or inferences (Enarson, Kennedy, & Miller, 2004). The target population of this research is defined as ready to chicken and beef in selected retail and markets in 2015.

3.3 Samples

Samples used in this study were from chicken and beef from retail and wholesale markets in Windhoek, Namibia.

3.3.1 Sample size

A convenient sampling technique was used to select the samples that were used for the study, for example, every third pack of chicken and beef on the shelf depending on sample size was selected. A minimum of 138 samples in each group was calculated using the following formula:

$$n = \frac{Z^2 pq}{d^2}$$

Where:

n = minimum sample size

Z = 1.96

P = Expected frequency

d = level of precision

q = 1 – p

Calculated as follows:

$$n = \frac{Z^2 pq}{d^2}$$

$$= \frac{(1.96)^2\ 0.1 * 0.9}{(0.05)^2}$$

= 138

3.3.2 Sampling plan

The study investigated the prevalence of *Salmonella* in ready to eat chicken and beef from retail and wholesale markets. In retail and wholesale shops, samples that were presented for *Salmonella* analysis were selected randomly based on the producers, 138 chicken and 138 beef samples were purchased at 17 retail stores in Windhoek. Since this study was a laboratory based research, the isolation and identification of *Salmonella* contamination was done on all chicken and beef samples randomly selected from retail and wholesale shops at the Central Veterinary Laboratory, Windhoek, Namibia. A convenient sampling method was used whereby all the *Salmonella* isolates were included as samples until the minimum sample size for the study was reached.

3.4 Procedure

The study was conducted on chicken and beef from local retail and wholesale markets by analysing ready to eat chicken and beef in Windhoek, Namibia. The sample of chicken and beef products was collected from the selected retail and wholesale, and transported to the laboratory, for salmonella testing. *Salmonella spp.* isolated in 2015 was used to determine the prevalence and serovars of *Salmonella* spp. in Windhoek, Namibia. A total number of 276 samples, 138 chicken and 138 beef samples were purchased at 17 retail stores in Windhoek. The analysis was done to determine the prevalence, identifying the existing serovars of *Salmonella* in retail and wholesale markets in Windhoek, Namibia.

3.4.1 Salmonella isolation

Salmonella serovars was isolated from samples that were found to have been contaminated. The isolation procedure was performed at the Central Veterinary Laboratory (CVL) in August 2015, in Windhoek, Namibia. The methods for the isolation of *Salmonella* spp. was performed in accordance with the ISO (International Standards Organization) 6579:2002 for microbiology of food and animal feed stuffs horizontal method for the detection of *Salmonella* spp. The procedure has four main stages; pre-enrichment, enrichment, plating and confirmation. The flow diagram for the procedure is shown **Figure 1** on page 51. The isolation procedure for *Salmonella* was performed at the Central Veterinary Laboratory, Windhoek, Namibia. Serotyping investigations were performed at the ARC-Onderstepoort Veterinary Institute, Republic of South Africa.

3.4.1.1 Non-selective pre-enrichment

Twenty five grams of Meat or chicken sample was weighed and transferred into a sterile stomacher bag. About 225 ml of buffered peptone water (BPW) (Merck, Darmstadt, Germany) was added into the sample. The sample was then homogenized with a stomacher machine for about 2 minutes, followed by incubation at 37 °C ± 1 °C for approximately 18 to 24 hours.

3.4.1.2 Selective enrichment

After the incubation time, samples from the pre-enrichment broth were mixed and then inoculated into a selective media. Approximately 0.1 ml of the pre-enrichment broth was transferred into a tube containing 10 ml of Rappaport-Vassiliadis medium with soya (RVS) broth (Scharlau Chemie S.A. Barcelona, Spain). Another 10 ml of

the pre-enrichment broth was transferred into a tube containing 100 ml Selenite cystine broth (Merck, Darmstadt, Germany). Selenite cystine broth was used instead of Mueller Kauffmann Tetrathionate Novobiocin (MKTTn) broth (Merck, Darmstadt, Germany). The inoculated RVS broth (Scharlau Chemie S.A. Barcelona, Spain) was incubated at about 41.5 °C ±1 °C for approximately 18 to 24 hours, whereas inoculated Selenite cystine broth was incubated at 37 °C ±1 °C for approximately 18 to 24 hours.

3.4.1.3 Culture and identification

After incubation, a loop full of the enriched cultures of RVS broth and selenite cysteine broth was streaked separately onto two selective agar plates: Xylose Lysine Desoxycholate (XLD) (Merck, Wadeville, South Africa) and Brilliant Green Agar (BGA) (Scharlau Chemie S.A. Barcelona, Spain). These plates were incubated in an inverted position at 37 °C ±1 °C for 18 to 24 h. After incubation, the black and pink colonies with or without black centre on XLD agar and the colourless or opaque white colonies surrounded by pink or red zone on BGA were identified as suspected *Salmonella*. Those colonies were selected and sub-cultured on nutrient agar (Merck, Wadeville, South Africa) and incubated at 37 °C ±1 °C for approximately 18 to 24 hours.

3.4.1.4 Biochemical confirmation

Two or more colonies of typical suspicious *Salmonella* were selected from the Nutrient agar using a sterile inoculating wire loop. The selected colonies were used to perform biochemical confirmation tests. The summary of typical biochemical

reactions for *Salmonella* is presented in **Table 2**. The following biochemical tests were performed:

Triple sugar iron agar (TSI agar)

The TSI agar slant was inoculated by streaking slant and stabbing the butt with pure culture of typical suspicious Salmonella from the Nutrient agar. After inoculation, the TSI agar was incubated at 37 °C ±1 for approximately 18 to 24 hours. The inoculated tubes were caped loosely to maintain aerobic conditions while incubating in order to prevent excessive Hydrogen Sulphide (H_2S) production.

For the interpretation of the TSI results, typical *Salmonella* cultures display alkaline (red) slants and acid (yellow) butts with gas formation (bubbles) and (in about 90% of the cases) formation of hydrogen sulphide (blackening of the agar). When lactose-positive *Salmonella* is isolated, the TSI agar slant is yellow. The preliminary confirmation of *Salmonella* cultures was not based on the results of the TSI agar test only.

Urea agar test

The urea agar slant surface was inoculated by streaking the agar slope surface and stabbing the butt with pure culture of typical suspicious *Salmonella* from the Nutrient agar. The Urea agar slants were then incubated at 37 °C ±1 °C for approximately 18 to 24 hours, followed by results interpretation. The positive reaction showed splitting of urea which liberated ammonia, with changes of the colour from phenol red to rose pink, and later to deep cerise (moderate red). The reaction is often apparent after 2 to 4 hours. For a negative reaction, the colour of the Urea media remained unchanged.

$_{L}$-Lysine decarboxylation medium

Typical suspicious *Salmonella* from the Nutrient agar was inoculated in the $_{L}$-Lysine decarboxylation medium just below the surface of the liquid medium. The medium was then incubated at 37 °C ±1 °C for approximately 18 to 24 hours. Turbidity and a purple colour after incubation indicated a positive reaction. A yellow colour indicated a negative reaction.

Detection of β-galactosidase

A loop-full of the suspected colony of *Salmonella* from the Nutrient agar was inoculated in a tube containing about 0.25 ml of the saline solution. One drop of toluene was added and the tube was shaken to mix the solution. The tube was put in a water bath set at 37°C ±1°C for approximately 5 minutes. About 0.25 ml of the reagent was added for detection of β-galactosidase and mixed with the solution. The tube was replaced in the water bath and was left for 24±3 hours, being examined at intervals. A yellow colour indicated a positive reaction. The reaction was often apparent after 20 minutes.

Medium for Voges-Proskauer (VP) reaction

A loop-full of the suspected colony of Salmonella from the Nutrient agar was suspended in a sterile tube containing 3 ml of the Voges-Proskauer (VP) medium and incubated at 37 °C ±1 °C for 24 ±3 hours. After incubation, two drops of creatine solution was added followed by 3 drops of the ethanol solution of 1-naphthol and then 2 drops of potassium hydroxide solution. The solution was shaken after each addition of the reagent. The formation of pink to bright red colour within 15 minutes indicated a positive reaction.

Table 2 Summary of typical biochemical reactions for *Salmonella*

Test or substrate	Positive	Negative	*Salmonella* reaction
Glucose (TSI)	Yellow butt	Red butt	+
Lysine decarboxylase	Purple	Yellow	+
H_2S (TSI)	Blackening	No blackening	+
Urease	Purple-red	No colour change	-
Lysine decarboxylase broth	Purple	Yellow	+
Indole test	Red colour	Yellow colour	-
Voges-Proskauer (VP) test	Pink-to-red colour	No colour change	-
β-galactosidase	Yellow	No colour change	-

3.4.1.5 Serological Confirmation

Elimination of auto-agglutinatable strains

This is a procedure that was done to eliminate auto-agglutinable microbial strains. One drop of saline solution was placed onto a clean glass slide. A portion of the colony to be tested was dispersed in the drop in order to obtain a homogeneous and turbid suspension. The slide was then gently shaken for approximately 30 to 60 seconds and the result was observed in contrast to a dark background. If the bacteria are clumped, the strain was considered auto agglutinable and was not submitted to the serological tests as the detection of the antigens is impossible.

Examination for O- and H-antigens

One drop of the anti-O serum (Merck, Marburg, Germany) or anti-H serum (Merck, Marburg, Germany) was placed onto a clean glass slide. A portion of the non-auto

agglutinating pure colony to be tested was dispersed in the drop in order to obtain a homogeneous and turbid suspension. The slide was then gently shaken for approximately 30 to 60 seconds and the result was observed in contrast to a dark background. If agglutination occurred, the reaction was considered to be positive for the respective antigen.

3.4.1.6 Interpretation of biochemical and serological reactions

All reactions for both biochemical and serological reactions for the presence of *Salmonella* were interpreted according to the ISO 6579: 2002 for Microbiology of food and animal feeding stuffs horizontal method for the detection of Salmonella. The summary for the interpretation is presented in **Table 3**.

Table 3 Interpretation of biochemical and serological confirmatory tests

Biochemical reactions	Auto-agglutination	Serological reactions	Interpretation
Typical	No	O-or-H antigen positive	Strain *Salmonella*
Typical	No	All reaction negative	
Typical	Yes	Auto-agglutination	May be *Salmonella*
None typical	No/No	O-or-H antigen positive	Not considered to be *Salmonella*

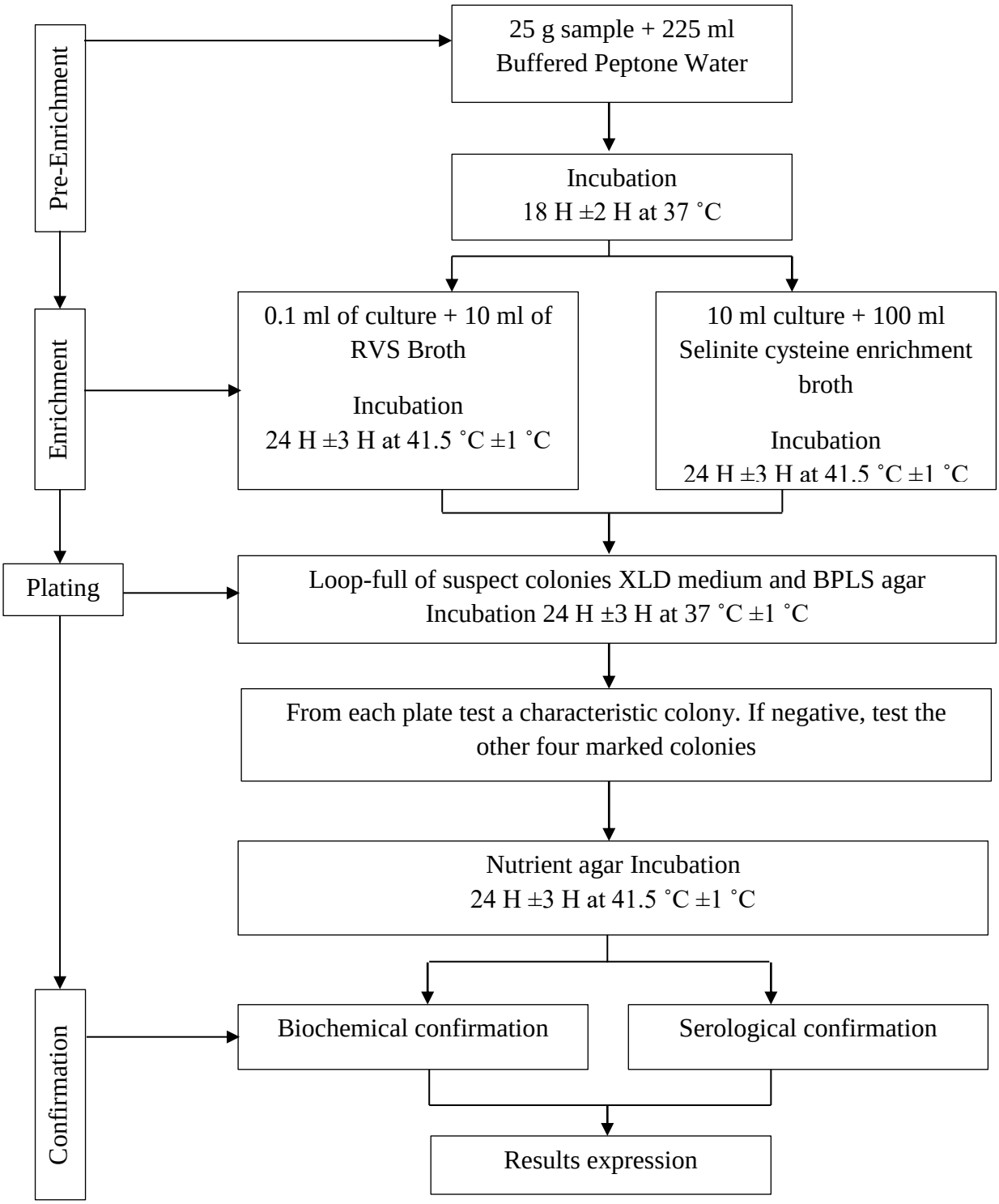

Figure 1 Flow diagram summarizing the procedure for the isolation of *Salmonella* (ISO 6579: 2002)

3.5 Data analysis

Prevalence was evaluated in terms of percent (%) occurrence, the denominator was the total number sampled. Differences between prevalence of *Salmonella* in chicken and beef was analyzed using the Fishers exact test, differences were considered significant if the p-value was less than (<) 0.05. EPI INFO 7, STATA and Microsoft Excel were used to analyze the data; findings were presented as descriptive statistics: tables, graphs and charts were used to display results.

3.6 Conclusion

The methodology chapter covered the research design that was used during the study; the target population and the formula that was used to calculate the minimum sample size. The chapter also covered the sampling plan that was used while collecting data, the procedure or method used at the laboratory is also covered. The chapter also showed how the data was analysed and how it was presented, and the program used in analysing the data.

CHAPTER 4: RESULTS

4.1 Introduction

The purpose and objectives of this study was to establish the prevalence and serovars diversity of *Salmonella* in chicken and beef from selected retail shops in Windhoek, Namibia. Therefore, the results are presented based on the purpose and study objectives. Fisher's exact test will be used to show statistical significance in the prevalence of *Salmonella* between chicken and beef, the results will be presented in **Table 5.** Lastly, the prevalence rate of *Salmonella* per locality will be described, and the results will be presented in **Table 7**.

This study assessed the prevalence and or frequency of contamination of beef and chicken by *Salmonella* serovars in retail markets in Windhoek, Namibia. This is the first documented study on the prevalence of *Salmonella* in beef and chicken, in retail markets in Windhoek, Namibia. The results of this study are presented into four major parts; (1) Prevalence of *Salmonella* in beef. (2) Prevalence of *Salmonella* in Chicken. (3) Prevalence of *Salmonella* in Beef and Chicken using Fisher's exact test to show if there were statistical differences. (4) Distribution of *Salmonella* serovars isolated from chicken and beef and their prevalence presented in **Table 6.**

4.2 Prevalence of *Salmonella* in beef

From a total of 138 samples of beef that were tested for the presence of *Salmonella* at the Central Veterinary Laboratory (CVL), 14 % (n = 19) were found to be positive for *Salmonella.* Only meat samples were collected for analysis.

4.3 Prevalence of *Salmonella* in chicken

A total number of 138 samples of chicken that were tested for the presence of *Salmonella* at the Central Veterinary Laboratory (CVL), 0 % (n = 0) all 138 samples tested negative for *Salmonella.*

4.4 Prevalence of *Salmonella* in chicken and beef

Altogether, a total number of 276 samples of chicken and beef were collected from June 2015 to August 2015. From a total number of 276 chicken and beef samples analyzed at the Central Veterinary Laboratory (CVL) in Windhoek, Namibia, 7 % (n = 19) tested positive for *Salmonella.* The *Salmonella* prevalence rate per individual product is shown in **Table 4** below.

Table 4 The prevalence rate of *Salmonella* per individual product and the general prevalence rate altogether

Product	No of *Salmonella* isolates	% prevalence rate per product	% total prevalence rate (N = 276)
Chicken	0	0 (N = 138)	0
Beef	19	14 (N = 138)	7
Total	**19**	**-**	**7**

The prevalence rate of *Salmonella* in chicken samples (*n* = 138) was 0 %, where as in beef samples (*n* = 138), the prevalence rate was 14 %.

Table 5 Fisher's exact test to determine *Salmonella* contamination levels of significance between chicken and beef

Product	*Salmonella* positive (%)	*Salmonella* negative (%)	Total (%)
Chicken	0 (0.00)	138 (100)	138 (100)
Beef	19 (13.77)	119 (86.23)	138 (100)
Total	19 (6.88)	257 (93.12)	276 (100)
		P < 0.001	

There was a significant difference ($p < 0.001$) of the prevalence of *Salmonella* between chicken and beef samples, since the p – value was less than 0.05.

Table 6 Distribution of *Salmonella* serovars isolated from chicken and beef and the prevalence

Salmonella serovars	No of isolates	Prevalence (%) (N = 19)
Salmonella enterica serovar Javiana	8	42
Salmonella enterica serovar Fulda	11	58
Total	**19**	**100**

Salmonella enterica serovar Fulda is the most prevalent with 58 % (n = 11), followed by *Salmonella* enterica serovar Javiana with 42 % (n = 8).

Table 7 The prevalence rate of *Salmonella* per locality

Locality	**Number of samples examined**	**No. *Salmonella isolates***	**% Prevalence rate per locality**	**% Total prevalence rate (*N* = 276)**
Academia	10	0	0	0
Cimbembacia	10	0	0	0
City Center	34	19	56	7
Katutura	44	0	0	0
Khomasdaal	10	0	0	0
Klein Windhoek	10	0	0	0
Olympia	10	0	0	0
Otjomuise	10	0	0	0
Suiderhof	10	0	0	0
Windhoek North	10	0	0	0
Windhoek West	10	0	0	0
Total	**276**	**19**	**-**	**7**

The prevalence rate of *Salmonella* was 56 % (n = 19) in the city centre, and maintained a prevalence rate of 0 % in the 10 other localities where samples were taken.

4.5 Conclusion

This chapter covered results of the prevalence of *Salmonella* per product, it also went on further to describe the prevalence rate of *Salmonella* in chicken and beef

altogether. The chapter also showed if there were statistical significant differences in the prevalence of *Salmonella* between chicken and beef using the Fisher's exact test. Lastly, the chapter described the prevalence rate of *Salmonella* per locality.

CHAPTER 5: DISCUSSION

5.1 Introduction

The purpose of this chapter is to discuss the results, which will be based on the main objectives of the current study; which are to determine the prevalence of *Salmonella* spp, and to determine the serovars diversity of *Salmonella* spp. isolated from chicken and beef from retail and wholesale markets in Windhoek, Namibia.

The surveillance of *Salmonella* in retail and wholesale foods is essential for the prevention of nontyphoidal salmonellosis, which is one of the most important problems for public health in the world (Humphrey, 2006). According to Humphrey (2006) *Salmonella* frequently enter the food chain, thereby triggering either sporadic cases or outbreaks of human salmonellosis. Livestock and their products are the most significant foods as vehicles of *Salmonella* (FAO & WHO, 2009).

In Namibia, commercial abattoirs are required to test for *Salmonella* as part of their quality control criteria in order to meet the stringent conditions for the export markets such as the European Union (EU) and the Republic of South Africa (RSA). The prevalence of *Salmonella* in beef in this study was investigated, and it was found to be 14 % in beef samples. These results are comparable to other studies that have been carried out in other countries; however, there is very little information on the prevalence of *Salmonella* in Namibia.

A study that was carried out in Italy, found the prevalence rate of *Salmonella* in beef samples from retail markets to be at 1 % (Busani, *et al*., 2005). Another study that was done on beef samples in Japan, found the prevalence rate to be at 0.2 %. Another

study done in the United States of America, found the prevalence rate to be at 45 %, which was higher compared to most studies done in the developed world (Chengappa, Staats, Orberst, Gabbert, & McVey, 1993).

The prevalence rate of *Salmonella* in beef from Windhoek, Namibia was slightly higher compared to what other studies conducted in developed world have demonstrated. This could be because of the poor hygiene practice during processing and handling in such retail shops compared to retail shops in developed countries. On the other hand, it could be due to the fact that beef samples were obtained from non-commercial abattoirs or other sources where the HACCP (Hazard Analysis Critical Control Point) system is not implemented fully. In simple words, HACCP is a safety tool that is used in food production to prevent or reduce the risk of contamination.

However, studies that were conducted in developing countries yielded much higher prevalence rates compared to this present study. For example, a study that was conducted in Algeria found the prevalence rate to be as high as 27 % (Mezali & Hamdi, 2012). Another study done in Ethiopia on the prevalence of *Salmonella* from beef carcasses found the prevalence rate to be 13 % (Dabassa & Bacha, 2012). Another study that was done in Botswana in raw beef sausages found the prevalence rate of *Salmonella* to be 25 % (Samaxa, Matsheka, Mpoloka, & Gashe, 2012). These prevalence rates are way higher compared to this study, it could be because of differences in the sampling methods, handling, hygiene practices and isolation techniques.

5.1 Prevalence of *Salmonella* in chicken

Meat and poultry products are sensitive to microorganism contamination by bacteria, viruses, and parasites (Ndife, Egege, & Komolafe, 2010). The author again explains that poultry have been recognised as an important source of human infection with *Salmonella* ever since they started to be intensively reared and processed on a large scale in order to provide a cheap source of meat. After meat and poultry products become contaminated, they are an excellent environment for growth of bacteria (Ndife, Egege, & Komolafe, 2010). Bacterial contamination and growth is a problem because it may result in foodborne illnesses (Corry, Allen, Hudson, Breslin, & Davies, 2002). *Salmonella*, a major foodborne pathogen found in poultry products which can cause diarrhoea, remains a serious problem for poultry processors (Ndife, Egege, & Komolafe, 2010). In many developing countries, diarrhoea is a common cause of morbidity and ready to eat foods have been implicated in salmonellosis (Bolat, 2002; Michanie, Bryan, Alvarez, & Olivo, 1987).

As it is done for beef, in Namibia, poultry commercial abattoirs are also required to test for *Salmonella* as part of their quality control criteria in order to meet the rigorous conditions for the export markets such as the European Union (EU) and the Republic of South Africa (RSA). The prevalence of *Salmonella* in chicken in this study was investigated, and it was found to be 0 %. These results are comparable to other studies that have been carried out in other countries; however, there is very little information on the prevalence of *Salmonella* in chicken *in* Namibia.

The 0 % prevalence rate that our study demonstrated could be a reflection of good hygiene practice during processing and handling in such retail shops, on the other

hand it could be because of the intervention strategies that are put in place from the feed mill and subsequent controls put in place during the initial breeding, hatching, growing and transportation phases before the processing and preparation of the final product. On the contrary, studies done in Thailand found the prevalence rate of *Salmonella* in raw broiler chicken meat and their products to be as high as 66% (Jerngklinchan, Koowatananukul, Daengprom, & Saitanu, 1994). This is evidently way higher than the one we investigated in this study.

Another study that was done on *Salmonella contamination* in domestic raw chickens in Tama and serovars or drug resistance of isolates found the prevalence rate of *Salmonella* to be 25 % (Kusunoki, *et al*., 2000). A different study done on the prevalence and antimicrobial resistance of *Salmonella* serovars in conventional and organic chickens from Louisiana retail stores found the prevalence rate to be 22 % (Lestari, Han, Wang, & Ge, 2009). Another study done in the United Kingdom found the prevalence rate of *Salmonella* to be 4 % (Meldrum & Wilson, 2007).

The findings of this study were different from those obtained by other authors. The differences in reported isolation rates from different countries could be due to variations in the collection of samples, laboratory techniques, hygiene administration. On the other hand, the differences might simply result dissimilarities in countries. When this study is compared with available information in different parts of the world, it was found that the prevalence of *Salmonella* in chicken in Namibia is lower to that of the developed countries. This could be to the fact that chicken samples were obtained from retail shops where the HACCP system is fully implemented.

5.2 Prevalence of *Salmonella*

The prevalence of *Salmonella* in both chicken and beef was found to be 7 % (n = 276) in this study. Beef evidently had a higher prevalence rate compared to chicken, hence there were significant difference ($p < 0.05$) on the prevalence rate between the two food types. The researchers could not find any clear reasons for these results, but it is well known that the two products are different. It could therefore be due to the fact that the two products were exposed to different processing, handling, packaging, and storage conditions. The prevalence rate altogether was acceptably low as compared to other studies done elsewhere in the world, this could be a reflection of the good hygiene practice during processing, handling and storage conditions at processing plants.

5.3 Distribution of *Salmonella* serovars isolated in beef and the prevalence rate

Salmonella enterica is estimated to cause 1.2 million illnesses each year in the United States and to be the leading cause of hospitalizations and deaths from foodborne disease (Scallan, *et al*., 2011). *Salmonella enterica* subspecies *entetrica* strains are of the greatest clinical relevance and are typically isolated from humans and warm blooded animals (Leader, Frye, Hu, Fedorka-Cray, & Boyle, 2009).

Of the *Salmonella* serovars isolated in this present study, *Salmonella* enterica seovar Fulda could possibly be the most prevalent *Salmonella* serovar in retail and wholesale markets in Windhoek, Namibia. This is because *Salmonella* entetrica Fulda represented (n = 11) 58 % of all *Salomella* isolates in beef. These findings that *Salmonella* enterica serovars Fulda is the most prevalent serovar could mean that the

same serovars is among the top serovars that cause salmonellosis in Windhoek, Namibia.

5.4 Prevalence of *Salmonella* per locality

The prevalence rate of *Salmonella* in both chicken and beef in the city centre was found to be 56 % (n = 19) in the present study, and the rest of the localities were found to have the prevalence rate of 0 %. Surprisingly, in Katutura where the hygiene and handling of meat and meat products was regarded as poor when compared to central city localities, the prevalence rate of *Salmonella* in both chicken and beef was 0 %. Where as in shops that was located around the city centre, the researcher expected the prevalence rate of *Salmonella* to be low; however, the prevalence rate was found to be 56 %.

Hygiene and handling of meat was observed to be very good in such shops, the reseacher could not find a clear explanation as to why the prevalence rate was high and it was only in beef. Since both chicken and beef were exposed to the same conditions, like temperature and packaging, the researcher cannot say it could be due to the hygiene and processing in the shop. However, it could be assumed that it could have resulted from the farm where the retailer sources its beef, or the contamination could also have resulted from the abattoir.

5.5 Conclusion

In conclusion, this chapter discussed the results that the researcher found based on the objectives the study. The prevalence rate of *Salmonella,* and the distribution of *Salmonella* serovars isolated from chicken and beef was discussed.

CHAPTER 6: CONCLUSION AND RECOMENDATIONS

6.1 Conclusion

In conclusion, this study has shown that *Salmonella* spp. are prevalent in retail and wholesale markets in Windhoek, Namibia. In this study, the general prevalence of *Salmonella* in chicken and beef from retail markets was found to be 7 %, which is however lower when compared to many developed and developing countries around the world. This could be due to good hygiene practice during processing and handling in such retail shops. On the other hand it could be because of the intervention strategies that are put in place from the feed mill and subsequent controls put in place during the initial breeding, hatching, growing and transportation phases before the processing and preparation of the final product. This could also be due to effective implementation of the HACCP system in the chicken and beef production plants and retail markets involved in the present study.

Salmonella enterica serovar Fulda was the most prevalent *Salmonella* serovar in retail and wholesale markets in Windhoek, Namibia, resenting (n = 11) 58 % and *Salmonella* enterica Javiana with (n = 8) 48% isolates in beef. These results demonstrate that *Salmonella* enterica serovar Fulda could be among the top serovars that cause salmonellosis in Windhoek, Namibia.

City centre was the only locality that had samples that were contaminated with *Salmonella*, and the rest of the other localities were found to have the prevalence rate of *Salmonella* to be 0 %. Overall, the findings from the present study show that the prevalence rate of *Salmonella* varies from region to region.

6.2 Recommendations

1. This study found out that the rate of prevalence of *Salmonella* in Windhoek, Namibia, is zero percent in chicken, when compared to other studies done elsewhere in the world, prevalence rates of *Salmonella* in chicken is usually higher. The fact that *Salmonella* was isolated in beef suggests that consumers are still exposed to health risks due to *Salmonella* infections. However, the prevalence rates were lower than those reported in other findings elsewhere, this could be due to the fact that that the HACCP system may be the useful tool to minimize the risks. Therefore, it is recommended to the Veterinary Services, Ministry of Health and Social Services, the Municipality of Windhoek, and all relevant stakeholders in the food industry that the HACCP system should be compulsory in all food production establishments in Namibia as a tool to reduce the health risks to consumers. As of now, the HACCP system is only compulsory to the EU export food establishments. In conclusion, the HACCP system should be made compulsory to all local food processing establishments, including abattoirs and retail markets in the country.
2. This investigation showed that *Salmonella* is prevalent in these products from retail markets. Therefore, the researcher recommends further studies to be done on ready to eat foods in retail markets in Windhoek to determine the antimicrobial resistance as well. Failure to control *Salmonella* in a country presents a potential public health problem for other countries. To minimize the burden of this pathogen, it is helpful to monitor *Salmonella* serovars distribution in many countries, implement *Salmonella* control measures throughout the food production chain, and monitor the effectiveness of the control measures.

3. It is recommended to Ministry of Agriculture, Water and Forestry (Epidemiology Division) and the Ministry of Health and Social Services (Epidemiology Division), and the City of Windhoek Municipality to partner and build surveillance programs that would detect *Salmonella* contaminations in a timely manner in the entire food chain together with sanitary measures that are essential for detecting and preventing human *Salmonella* infections.
4. It is very important for any country to know serovars that are prevalent in that country, this is because antibiotic resistant strains and more pathogenic strains are on the increase in the world. The increase in resistant strains can be attributed to the inappropriate use of antimicrobials as therapeutic or prophylactic agents in human and veterinary medicine, it is important to know all this information. The researcher therefore recommend to the Central Veterinary Laboratory, Namibia Institute of Pathology, and other laboratories in the country to expand their capacity and start serotyping *Salmonella* species instead of just isolation. In this case, the cost could have been cut because it could be done locally instead of relying on laboratories from the Republic of South Africa.

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