BACTERIAL LOAD AND ISOLATION OF *SALMONELLA* SPECIES FROM CATTLE CARCASSES AT KANO ABATTOIR- KANO STATE, NIGERIA

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FEBRUARY, 2014

ATTESTATION

I hereby attest that the work in this thesis entitled "Bacterial Load and Isolation of *Salmonella* species from cattle carcasses at Kano abattoir, Kano state, Nigeria" was carried out by me in the Department of Community Medicine under the supervision of Dr. M Bello and Dr. E C Okolocha, of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University Zaria.

The information derived from the literature have been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

BA'ABA Ahmed Ibrahim	________________	________________
Name of Student	Signature	Date

CERTIFICATION

This thesis entitled "**BACTERIAL LOAD AND ISOLATION OF *SALMONELLA* SPECIES FROM CATTLE CARCASSES AT KANO ABATTOIR, KANO STATE, NIGERIA" by BA'ABA Ahmed Ibrahim** meets the regulations governing the award of the degree of Master in Public Health of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work was dedicated to my mother Amina Ibrahim Ba'aba and my beloved wife Fatima Baba Idris for their prayers and support throughout the course of my study

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LIST OF ACRONYMS

CFU- Colony forming unit

NFELTP- Nigerian Field Epidemiology and Laboratory Programme

Cm^2 – Centimeter square

'm'- value separating a good result from a marginally acceptable result (values between **m** and **M** are considered to be marginally acceptable)

'**M**' - maximum value for a marginal result (values greater than **M** are unacceptable)

WHO – world Health Organisation

SSA – *Salmonella-Shigella* agar

DNA – Deoxyribonucleic acid

CDC – Centres for Disease Control and Prevention

MRD – Maximum Recovery Diluent

ANOVA – Analysis of Variance

^{o}C – Degree Celsius

MR - Methyl Red

VP – Voges Proskauer

H_2S – Hydrogen sulphide

TSI – Triple Sugar Iron agar

µl – Micro liter

ISO - International Standard Organisation

SUMMARY

The muscle (meat) of a healthy animal is sterile. Contamination may be due to infection within the animal or external contamination during slaughter and handling processes. High bacterial load on carcasses may pose potential risk of meat contamination with Foodborne pathogens.

A cross sectional study was carried out to determine the bacterial load and isolate *Salmonella* species from cattle carcasses slaughtered at Kano abattoir. An eighty cattle carcass was sampled between December 2012 to April, and 2013. Swabs were collected from four positions (neck, shoulder, brisket, and rump) on each carcass. The laboratory procedure was carried out using ISO 6579:2002. Conventional biochemical test as well as Microbact™ 12E was used for bacterial identification. Data was analyzed using Epi info 7 and Microsoft Excel, 2007.

Out of the examined 80 carcasses 41 (51.3%) were from male and 39 (48.8%) from female cattle carcasses. Bacteria but only 50 (62.5%) carcasses sampled yielded growth on Salmonella-Shigella agar contaminated Seventy-nine (98.8%) cattle carcasses sampled. The average colony count ranges from 3.8×10^5 to 2.3×10^6. The mean log cfu/ cm^2 were 4.4±0.77. The study reveals that only 15 (19%) of the total carcasses sampled are contaminated at satisfactory level of less than 5 colonies per plate on average. Among the 50 carcasses sampled that yielded growth on *Salmonella-Shigella* agar only 1 (1.25%) was identified by Microbact™ 12E as *Salmonella Arizonae* with the percentage probability of 76.4%. Other pathogenic bacteria include; *Citrobacter freundii* (4.3%), *Klebseilla oxytoca* (25.5%), *Proteus vulgaris* (66.0%) and *Providentia rettgeri* 4.3%.

The mean log cfu/cm^2 from the findings of this study was within the marginal acceptable limit (3.5-5.0 log cfu/cm^2). The major sources of contamination are multiple contacts with

contaminated tools and operators' hands. Other possible source of contaminants, may due to the unhygienic manner of handling meat in abattoirs, the environment upon which the meat is slaughtered as well the water used in the processing of the meat. The prevalence of *Salmonella* species was (1.25%).

In conclusion, the result of this work reflects poor conditions of carcasses slaughtering and handling, and inadequate hygienic practices at Kano abattoir. Microbial load found in this study shows to be above the satisfactory limit of carcass contamination. *Salmonella* was isolated in this study and four other bacteria were isolated and identified.

It is therefore, recommended that thorough and adequate training on sustainable sanitary and hygiene practice should be given to the abattoir workers periodically as well as implementing strict meat inspection procedures.

Key words: Bacterial load, cattle, carcass, *Salmonella*

CHAPTER ONE - INTRODUCTION

1.1 Background Information

Food-borne pathogens are the leading cause of disease and death in developing countries costing lots of money in medical care and social costs. [1] Changes in eating habits, mass catering, complex and lengthy food supply procedures with increased international movement and poor hygiene practices are major contributing factors. [2] Contaminated raw meat is one of the main sources of food-borne illnesses. [3, 4] The risk of the transmission of zoonotic infections is also associated with contaminated meat.

Meat is considered an important source of proteins, essential amino acids, B complex vitamins and minerals. Due to this rich composition, it offers a highly favorable environment for the growth of pathogenic bacteria. The microbiological contamination of carcasses occurs mainly during processing and manipulation; such as skinning, evisceration, storage and distribution at slaughterhouses and retail establishments**.** [5, 6] Epidemiological reports suggest that meat product is one of the major causes of diarrheal illness which account for 36% of mortality cases in Nigeria. [137]

Food borne Salmonellosis often follows consumption of contaminated animal products, which usually results from infected animals used in food production or from contamination of the carcasses or edible organs .[24, 25] *Salmonella* infection in meat animals, including cattle, swine and sheep, arises from intensive rearing practices and the use of contaminated feeds. [26] Raw poultry and meat products remain the principal source of *Salmonella* in many countries.[135]

Salmonella has been widely reported in cattle [7,8,9,10,11] and infected animals may shed the organism in their faeces without showing any clinical signs of disease. [12] Therefore, cattle may carry this organism undetected into an abattoir at the time of slaughter. In humans, *Salmonella* is one of the most common causes of bacterial gastroenteritis. [13] *Salmonella* infection has been associated with many different food types and the consumption of beef has been associated with a number of outbreaks. [14] Therefore, the presence of *Salmonella* in cattle at slaughter and the consequent cross contamination of edible carcass tissues present a significant food safety hazard. A number of studies have reported outbreak of infections due to consumption of meat that are processed under poor hygiene and in most of the cases, data are loosely based on laboratory isolates which do not reflect the actual ratio of food-borne infections.

However, in different parts of the world a few community-based reports provide evidence of several outbreak caused by *Salmonella*, *Shigella*, *E. coli* and *Listeria* species. [15] In the United State, the Centres for Disease Control and Prevention (CDC) confirm approximately 40,000 human cases of Salmonellosis every year. [16] However there are an estimated 800,000 to 4 million cases of unconfirmed *Salmonella* infections each year with 500 of those cases being fatal.[17]The impact of *Salmonella* is very serious in the food industry, which spent huge amount of money on preventing the contamination of food-grade products and investigating outbreaks of disease[18].

Previous studies conducted in Addis Ababa indicated the occurrence of *Salmonella* in different food animals, meat and meat products. [19, 20,21,22,23] Study conducted in Abakaliki abattoir reveals that out of 300 meat samples analyzed for microbial quality, 5 % were contaminated with *Salmonella typhi*.[24]

1.2 Problem Statement

A number of studies have reported a relationship between live animal/slaughter practices and contamination of carcasses.[28] [139] determined that 5 to 15% of carcass contamination occurs during polishing, 5 to 35% during dressing and splitting, with the remainder as a result of evisceration practices. *Salmonella* is a zoonotic pathogen of food safety concern and has been known to be responsible for several outbreaks that equally lead to death.[29] In a global context; food safety and *Salmonella* are becoming an increasing concern for the global poultry and pork markets. Salmonellosis caused by *Salmonella* serovars has become an important public health problem throughout the world. [30, 31, 32] Salmonellosis is a major cause of bacterial enteric illness in both humans and animals. Even in developed countries where they have potential manpower and finances, the infections of *Salmonella* have still been recognized as a major hazard to humans. [32]

It is estimated that 1.4 million cases of Salmonellosis occur among humans in the United States annually. [33, 36] The zoonoses, which occur most frequently in the industrialized world today, are foodborne infections caused by *Salmonella* and *Campylobacter*. [35] In 2011, a study conducted in Osogbo, Nigeria shows that *Salmonella* was not detected in any of the meat samples except in pork with a prevalence rate of 8%. [37]

1.3 Justification

Study shows that the incidence of human Salmonellosis in Kano is high (13.5%) [165] and no work has been done on the level of *Salmonella* contamination in Kano and because of the poor hygienic practices of butchers in processing and handling of meat from the abattoir to the retail points as well as the increasing incidence of Salmonellosis (typhoid fever) in humans. There is paucity of information on the extent of bacterial load on cattle carcass in Kano abattoir. The

study will help provide information on the bacterial load of the cattle carcasses, and will form the basis for future quality control of beef at Kano abattoir.

1.4 Research questions

What is the level of bacterial contamination in cattle carcasses at Kano abattoir?

Can *Salmonella* species be a contaminant in cattle carcasses at Kano abattoir?

1.5 General and Specific Objectives

1.5.1 General objectives

To determine bacterial load and isolation of *Salmonella* species from cattle carcasses slaughtered at Kano abattoir

1.5.2 Specific objectives

- To determine the level of total aerobic plate counts on cattle carcasses at Kano abattoir
- To determine the acceptable level of bacterial contamination of cattle carcasses slaughtered for consumption at Kano abattoir
- To isolate and identify *Salmonella* species on cattle carcass slaughtered for consumption at Kano abattoir
- To isolate and identify other pathogenic bacteria from cattle carcasses

CHAPTER TWO - LITERATURE REVIEW

2.1 Bacterial Contamination of Meat

It is well documented that contamination of meat with pathogens constitutes a major public health concern. [139] In Nigeria, processing procedures and monitoring of critical points in the meat production are not fully developed. Abattoir has also become a source of infection and pollution, attracting domestic and wild carnivores, and rodents due to inadequate slaughtering and disposal facilities [138]. Apart from incorrect processing procedures, marketing practices of meat consumed by most populace is a major area of concern. [140]

Beef is processed in abattoir, and the design of abattoirs varies from person to person and from state to state including ownership but they are principally a place where livestock are slaughtered.[132] A number of slaughter facilities are found in an abattoir, whether stationary or mobile. These facilities could be a source of contamination to the slaughtering processes. It has been reported that abattoir is not 100% hygienic. [128] Different factors could contribute to the contamination of beef products processed in abattoirs especially during processing and manipulations such as skinning, evisceration, storage and distribution at slaughter houses and retail establishment. [130] In most developing countries, their traditional methods of handling, processing and marketing of meat undermine quality whereas poor sanitation leads to considerable loss of product as well as the risk of food-borne disease. [129] Bacteria which are responsible for food borne diseases contaminate meat directly and indirectly especially from animal excreta at slaughter process. [131] They can also be transferred from beef-contact surfaces, utensils and other slaughtering equipments. [127] The external contamination of meat constitutes a major problem in most developing countries' abattoirs where they are potential sources of infection as microbial surface contamination of carcasses has been repeatedly reported to have a

significant effect on the meat shelf life of beef. Moreover, contaminants may also include pathogens such as *Salmonella*, *Vibrio cholerae*, *Escherichia coli* and *Listeria* species which can contaminate the meat thereby causing severe problem for consumers. [133] Faecal matter is a major source of contamination and could reach carcasses through direct deposition as well as by indirect contact through contaminated and unclean carcasses equipment, surfaces, workers, installations and air. [134] Fortunately, most of the bacterial colonies which have been isolated from beef carcasses are said to be non-pathogenic, except for few human pathogens such as *Salmonella, Campylobacter* and *Listeria* species. which have been isolated in a number of cases. [131]

2.2 Bacterial Contamination of Beef and Beef Products

Effective intervention to reduce contamination of beef carcasses begins with determining potential sources of contamination. Tissues under the hide of healthy cattle are tissues become; [38] consequently usually sterile contaminated during the slaughtering process. Sources of meat contamination during slaughter may be classified as those associated with the animal, processing practices, abattoir facilities and employees. The extent to which potential contamination sources become hazardous to public health depends on management and unpredictable events or factors. Even in the best managed slaughter facilities, contamination may still occur. Fortunately, most bacterial colonies which have been isolated from beef carcasses have been non-pathogenic, although human pathogens such as *Salmonella*, *Campylobacter* and *Listeria* species have been isolated also. [39]

2.2.1 Animal/carcass sources

A major source of microbial contamination on the carcasses of beef is faeces. [40] Faeces, as well as soil adhering to the animal, are carried into the abattoir on the hair, hide, hooves and tail of the

animal. Parasites such as flies, grubs and worms carried by the animal into the plant also can be a source of microbial contamination. Infected body fluids such as urine, milk, blood, mucus, rumen fluid, intestinal fluid and fluid from excised abscesses can be other sources of microbial contamination of carcasses. Contact with lymph nodes, if excised, may also be a source of contamination. [41]

2.3 Evolution of *Salmonella*

It is speculated that the genera of *Escherichia coli* and *Salmonella* diverged from a common ancestor about the time of the emergence of mammals, and emerge as mammalian and avian pathogens through the acquisition of pathogenicity islands and of a virulence plasmid, through variation in lipopolysaccharide antigens, through development of mechanism for flagellar antigen phase shifting, and in other ways. [42] Some writers estimate *Salmonella* diverged from the genus Escherichia 120–160 million years ago.[44] The close DNA relatedness among *Salmonella* serotypes is evidence for their clonal origin, and based on the degree of sequence divergence, it can be estimated that a common ancestor of the genus existed about 25 to 40 million years ago. [43] The causative agent of murine typhoid, (then known as *Bacillus Typhi)* that caused an epidemic typhoid fever-like disease in mice. [45] Recently, *Salmonella* Typhi was identified in ancient skeletal material, thereby incriminating typhoid fever for the plague that devastated Athens in 430-426 B.C. It is hypothesized that accumulation of single mutations, insertions or deletions with the genome of modern-time *Salmonella* Typhi appears to have generated many pseudogenes, suggesting its recent evolutionary origin. [57]

2.4 Genus *Salmonella*

Salmonella organisms are facultative anaerobic gram-- rods within the family of Enterobacteriaceae. [46] Classically, the members of this genus are motile by peritrichous flagella

except *Salmonella* pullorum and *Salmonella* gallinarum, which lack flagella, however, the long standing fact that *Salmonella* pullorum is non motile has been disproved and it has been shown that the motility can be induced under special medium conditions. [47] *Salmonella* grow optimally at 35 ^{o}C to 37 ^{o}C, catabolize a variety of carbohydrates into acid and gas, use citrate as the sole carbon source, and produce H_2S and decarboxylate lysine and ornithine to cadaverine and putrescine respectively. [48] Historically *Salmonella* catabolized glucose and lysine, but failed to metabolize lactose, sucrose and urea, however due to the widespread exchange of genetic elements between compatible bacterial strains in the environment, atypical *Salmonella* biotypes that cannot decarboxylate lysine [52, 53] or that readily use lactose [54, 55, 56] sucrose [50, 51] and urea, have been isolated. They are chemo-organotrophic organisms, having both a respiratory and a fermentative type of metabolism. [49] Many serotypes in the group are closely related to each other by somatic and flagellar antigens and most strains show diphasic variation of the flagellar antigens.

The genus *Salmonella* comprises two species:

1. *Salmonella enterica*, which is divided into six subspecies: *Salmonella enterica* subspecies *Enterica* (I), *Salmonella enterica* subspecies *Salamae* (II), *Salmonella enterica* subspecies *Arizonae* (IIIa), *Salmonella enterica* subspecies *Diarizonae* (IIIb), *Salmonella enterica* subspecies *Houtenae* (IV) *Salmonella enterica* subspecies *Indica* (VI)

2. *Salmonella bongori* (formerly called *Salmonella enterica* subspecies *Bongori* V).

Species and subspecies can be distinguished on the basis of differential characters, and through antigenic formulae, into 2501 serovars. [59] However, a recent report from the Centre for Infectious Disease Research and Policy classifies members of the *Salmonella* species into more

than 2541 serotypes (serovars) according to their somatic (O) and flagellar (H) antigens. [61] The antigenic formulae of *Salmonella* serotypes are defined and maintained by the World Health Organization (WHO) Collaborating Centre for Reference and Research on *Salmonella* at the Pasteur Institute, Paris, France (WHO collaborating centre), and new serotypes are listed in the annual updates of the Kauffmann-White scheme. [58]

2.5 Current nomenclature

Several schemes based on biochemical characteristics, DNA homology, and enzyme electrophoretic patterns have been used for the taxonomic classification of *Salmonella.*[48] *Salmonella* nomenclature is complex, and scientists use different systems to refer to and communicate about this genus. The current usage often combines several nomenclatural systems that inconsistently divide the genus into species, subspecies, subgenera, groups, subgroups, and serotypes (serovars) which causes confusion.[58] The genus *Salmonella* has two systems of nomenclature in circulation. One system proposed by Le Minor and Popoff in the 1980s, which has received a wide acceptance although it does not conform to the rules of bacteriological code, and the other which conforms to the bacteriological code, but used by the minority. The present problem is that two systems of nomenclature are in the use for the members of the genus *Salmonella*. [60]

The nomenclature of the genus *Salmonella* has evolved from the initial one serotype-one species concept proposed by Kauffmann on the basis of the serologic identification of O (somatic) and H (flagellar) antigens. A capsular polysaccharide, the Vi antigen is present on *Salmonella* Typhi and few other serovars of *Salmonella*, including *Salmonella* Dublin. [62] The defining development in *Salmonella* taxonomy occurred in 1973 [62] demonstrated by DNA-DNA hybridization that all serotypes and subgenera I, II and IV of *Salmonella* and all serotypes of “Arizona” were related at

the species level, thus belonging to a single species. The single exception subsequently described is *Salmonella bongori* previously known as subspecies V which by DNA-DNA hybridization is a distinct species. In 1986 the subcommittee of *Enterobacteriaceae* of the International Committee on Systematic Bacteriology at the XIV International Congress of Microbiology unanimously recommended the change of type species of *Salmonella* to *Salmonella enterica*, a name coined by Kauffmann and Edwards in 1952, however the Judicial committee denied with the fact that the *Salmonella* Typhi might be overlooked as it is one of the most important human pathogens. [58]

The current nomenclature used by CDC is based on the recommendations from the WHO collaborating centre and it adequately addresses the concern and requirements of clinical and public health microbiologists.

[63] According to the CDC system, the genus *Salmonella* contains two species, each of which contains multiple serotypes. [58]

2.6 Morphology

*Salmonella*e are Gram--, straight rods not exceeding 1.5 micrometers in width. They are facultative anaerobes usually motile by peritrichous flagella. [66] Most *Salmonella*e form common fimbriae and most of them possess type-1 fimbriae associated with mannose-sensitive adhesive properties. These fimbriae are composed of fimbrillin subunits containing a high proportion of hydrophobic amino-acids. [75] *Salmonella* are routinely classified by serotype on the basis of expression of three surface antigens, the somatic O antigen, the flagella H1 and H2 antigens and the capsular Vi antigen, according to the Kauffmann-White scheme. [80] The absence of flagella may consequently affect complete identification of the serotype; *Salmonella enterica* serovar *Typhimurium* exhibits morphological differences dependent on the peptone constituents of the

culture medium however, in media containing soy-based peptone as the primary nutrient, *Salmonella* displays a normal flagellated morphology. [86]

2.7 Serotyping

Salmonella express flagellar, polysaccharide and capsular antigens which determine strain pathogenicity and therefore variation of these antigens has formed the basis for *Salmonella* serotyping. The Kauffmann-White scheme, first published in 1929, divides *Salmonella* into more than 2500 serotypes according to their antigenic formulae. [89] Today, 57 O antigens and 117 H antigens have been identified and more than 2500 serotypes have been described. Some of the H antigens share common antigen factors. These antigens are clustered in five complexes, the E, G, L, Z4 and 1 complex. *Salmonella* H antigens are expressed in different phases. Most serotypes are diphasic, i.e. they express two flagella antigens, and a minor part are monophasic, i.e. express one flagella antigen. *Salmonella* Gallinarum is the only serotype in the Kauffmann-White scheme that does not express any flagella antigen and is therefore non-motile. [91] *Salmonella* serotyping methods recognize 63 distinct phase 1 flagellar antigenic factors and 37 phase 2 flagellar antigenic factors, although the latter are not always present. Some antigenic factors, denoted by square brackets in formulae, may be present or absent without affecting serotype designation. Serotyping methods are stable, reproducible and have high typability, yet there are several drawbacks, particularly the dependence on availability of antisera, considering the ethics, cost and quality control measures necessary to maintain such a supply. [89]

Salmonella isolates can be differentiated from one another in a wide variety of ways, and the number of *Salmonella* continues to increase. Epidemiologically, it is important to be able to distinguish *Salmonella* isolates, because definitive typing of *Salmonella* isolates may assist in tracing the source of an outbreak and monitoring trends in antimicrobial resistance associated

with a particular type.[90, 93] In addition to the conventional antigen-based serotyping, there are advanced techniques for serotyping currently being used to enhance the tracing of the individual isolates. The following techniques are currently available for serotyping. (1) Conventional method (2) Phage typing (3) Molecular typing a. Restriction digestion based techniques b. Amplification based techniques c. Nucleotide sequencing based techniques

2.7.1 Conventional serotyping

Conventional serotyping of *Salmonella* is the most commonly used method to differentiate strains, which ar e epidemiologically the smallest bacterial unit from which isolates share the same phenotypic and genotypic traits. [93] In most clinical studies, initial serotyping is done using polyvalent O antisera to allow *Salmonella* isolates to be grouped into different O groups designated in capitalized letters. Many *Salmonella* show diphasic production of flagellar antigens and each strain can spontaneously and reversibly vary between these two phases with different sets of H antigens. In phase 1 or the specific phase, the different antigens are designated by small letters, and in phase 2 or the group phase, the antigens first discovered are numbered. In a single cell, usually only one antigen is expressed at a time.[93] Conventional serotyping using the autoagglutination method has some limitations, such as limitations in detection of Vi antigens [92], strains which are not typeable [77, 79]; it only allows detection of a single antigen-antibody reaction at a time, requires well-experienced technologists to perform, consumes relatively high amount of reagents, and takes a longer time. [85, 88]

2.8 *Salmonella*: Disease and Pathogenesis

Salmonella are well-known pathogens, highly adaptive and potentially pathogenic for humans and/or animals. *Salmonella* infections are capable of producing serious infections that are often foodborne and present as gastroenteritis. However, a small percentage of these infections may

become invasive and result in bacteremia and serious extra intestinal disease. [67] The main reservoirs for non-typhoidal *Salmonella* are animals such as poultry, livestock, pets and reptiles. *Salmonella* Typhi and *Salmonella Paratyphi* colonize only in humans, so they can be acquired only from close contact with a person who has typhoid fever, from a chronic carrier, or from water or food contaminated by human feces. [61]

While certain serovars of *Salmonella enterica* cause disease in humans and a variety of animals, other serovars are highly restricted to a specific host. *Salmonella* infections range from gastrointestinal infections that are accompanied by inflammation of intestinal epithelia, diarrhea and vomiting, to typhoid fever, a life threatening infection. [68] The outcome of *Salmonella* infections is determined by the host and the status of the bacterium. Whereas, age, genetic and environmental factors mainly determine the status of the host, the status of the bacterium is determined by so-called virulence factors. [87]

Serotypes adapted to man, such as *Salmonella Typhi* and *Salmonella Paratyphi*, usually cause severe diseases in humans as a septicaemic typhoidic syndrome (enteric fever). These serotypes are not usually pathogenic to animals. Serotypes that are highly adapted to animal hosts, such as *Salmonella Gallinarum* (poultry) or *Salmonella Abortus-ovis* (sheep), usually produce very mild symptoms in man. However, *Salmonella Choleraesuis* which has the pig as a primary host also causes severe systemic illness. In the same way, *Salmonella Dublin*, which has a preference for bovines, is primarily responsible for the systemic form of Salmonellosis. In young calves this disease causes high mortality, and in adult cattle it results in fever, reduced milk yield, diarrhea, abortion, and occasionally death. Ubiquitous serotypes, such as *Salmonella Enteritidis* or *Salmonella Typhimurium*, which affect both man and animals, generally cause gastrointestinal infections usually less severe than enteric fever. However, they also have the capacity to produce

typhoid-like infections in mice and in humans, or asymptomatic intestinal colonization in chickens. [84] *Salmonella* avoid host defense in the stomach and reach the intestines, [61] and the bacteria interact with the non-phagocytic cells such as the epithelial cells of the intestinal mucosa. [68] They adhere to the intestinal epithelial cells by adhesive structures (fimbriae) that promote binding and invade epithelial cells to provoke gastroenteritis. The organisms have virulence factors such as virulence-plasmids, toxins, fimbriae and flagella that help in establishing an infection. [87]

The mechanism of pathogenesis has been described in the following steps

a) *Bacterial mediated endocytosis:* A highly coordinated series of interactions between proteins released by *Salmonella*e and proteins of the host cell causes host cellular surface membrane ruffling and engulfment of bacteria in cellular vacuoles.

b) *Neutrophil recruitment and migration: Salmonellae* associated with gastroenteritis induce a secretory response in intestinal epithelium and initiate recruitment and transmigration of neutrophils into the intestinal lumen.

c) *Epithelial cell cytokine secretion:* In tissue culture models of *Salmonella Enteritidis,* translocation of SPI-1 proteins into intestinal epithelial cells leads to synthesis and polarized secretion of inflammatory mediators and neutrophil chemoattractants.

d) *Fluid and electrolyte secretion:* Several translocated SPI-1 proteins contribute to intestinal inflammation and fluid secretion. Intestinal inflammation probably contributes to fluid secretion and diarrhea by disrupting the epithelial barrier and increasing water flux by an exudative mechanism. Innate immune system activation also contributes to intestinal inflammation.

e) *Systemic infection*: *Salmonella* Typhi invades macrophages and the migration of infected macrophages to reticuloendothelial organs via the lymphatic system and blood produces systemic illness with less diarrhea.

2.8.1 Salmonellosis in humans

With respect to human disease, *Salmonella* serotypes can be divided into three groups that cause distinctive clinical syndromes, typhoid fever, bacteremia and enteritis. [45] The non-typhoid *Salmonella* serotypes can cause protean manifestations in humans, including acute gastroenteritis, bacteremia, and extraintestinal localized infections involving many organs. [65] Within *Salmonella enterica* subspecies I (*Salmonella enterica* subspecies *entericae*), the most common O-antigen serogroups are A, B, C1, C2, D and E. Strains within these serogroups cause approximately 99% of *Salmonella* infections in humans and warm-blooded animals. Serotypes in other subspecies are usually isolated from cold-blooded animals and the environment but rarely from humans. [84]

Following ingestion of contaminated food or water, the pathogenesis of both typhoid and *Salmonella* enteritis begins with the intestinal phase, while only typhoid progresses to a systemic phase. [94] Transmission of this disease within the human population is generally a result of poor sanitation of water and food supplies in developing nations. The broad host-range *Salmonella* serovars are prevalent within warm-blooded animal populations that make up the human food supply, and bacterial transmission generally results from consumption of raw or undercooked food products.[95]

The vast majority of *Salmonella* infections are transmitted from animals to humans through food and occasionally from person to person through the fecal-oral route. In general, *Salmonella* cause

one or more of four broad clinical syndromes such as gastro-enteritis, enteric fever, septicemia with associated focal lesions, and asymptomatic long-term carriage.

2.8.2 Salmonellosis in animals

Salmonella serotypes have a broad host range [78], prevalent in the warm-blooded animal population, [95] including rodents, [76] snakes, [81] and free living terrestrial and aquatic turtles, [83] and the pathogenicity of *Salmonella* serovars is known to be specific for animal species. [69] Some serotypes are highly adapted to animal hosts, such as *Salmonella Gallinarum* in poultry and *Salmonella Abortus-ovi*s in sheep. Many nontyphoidal *Salmonella* strains such as *Salmonella Typhimurium* and *Salmonella Enteritidis* infect a wide range of animal host including poultry, cattle and pigs.[73] These serotypes generally cause self limiting gastrointestinal infections usually less severe than enteric fever in humans. However, they also have the capacity to produce typhoid-like infections in mice and in humans or asymptomatic intestinal colonization in chickens[84]. At the abattoir, the initial source of contamination is the carrier animal. Transmission at the abattoir occurs by direct contact between carrier and non-carrier animals and also by exposure to contaminated environment. It has been suggested that stress associated with transportation, overcrowding and feed withdrawal experienced by animals before slaughter increases shedding of *Salmonella*.[26, 148]

During slaughtering operations these carrier animals are able to contaminate the area, the equipment and personnel, and eventually the final products.[149, 150]

2.9 *Salmonella*: A Public Health Perspective

Salmonellosis is an important global public health problem causing substantial morbidity, and thus also has a significant economic impact. Although most infections cause mild to moderate self-limited disease, serious infections leading to deaths do occur. [96] In spite of the improvement

in hygiene, food processing, education of food handlers and information to the consumers, foodborne diseases still dominate as the most important public health problem in most countries [100]. Many foods, particularly those of animal origin, have been identified as vehicles for transmission of these pathogens to human beings and spreading them to the processing and kitchen environment. [82] In developed countries food is recognized as the most frequently implicated vehicle of transmission and causes heavy financial burden on health care systems. [71] In the United States alone, an estimated 1.4 million non-typhoidal *Salmonella* infections, resulting in 168 000 visits to physicians, 15 000 hospitalizations and 580 deaths occur annually and the total cost associated with *Salmonella* is estimated at US$ 3 billion annually. [101] Apart from the foodborne infections, the other major epidemiological development in Salmonellosis is the emergence of multiple-antibiotic resistant *Salmonella,* particularly in the developing countries**.** [74]

2.10 Global Overview

In many countries incidence of human *Salmonella* infection has increased drastically over the years. The two most commonly isolated serotypes of concern and mostly implicated in disease outbreaks are *Salmonella enterica* serotype *Typhimurium* and *Salmonella enterica* serotype *EnteritidiSalmonella* [98, 65, 99] Besides the importance of this micro-organism for public health, another aspect is the cost generated by human Salmonellosis. During 1999, the cost linked to foodborne Salmonellosis ranged between 560 million and 2.8 billion € in Europe, where *Salmonella* was estimated to be responsible for nearly 166 000 cases. [97] It is reported that the rate of Salmonellosis in the United States is between 15 to 20 cases per 100 000 people and approximately 10% of the Salmonellosis cases are caused by consumption of poultry meat**.** [102] The *Salmonella* species is one of the eight micro-organisms in the European Union Zoonoses

Monitoring Directive, which shows it is a disease considered to have a high impact on human health in the Union.[96] The Enter-net surveillance program reported *Salmonella enterica* serotypes *Enteritidis* and *Typhimurium*, the most predominant organisms identified by the participating countries making up over 80% of all isolates during the period of 1998-2003. It also reported that for all *Salmonella* the general trend is declining with a reduction of 35.3% in 2003 over 1998. [103]

2.9.1 Status in developing countries

Ensuring consumer health concerns by greater involvement of the health sector, development of Codex standards, guidelines and incorporation of the work of the Commission into the national legislation to promote food safety and fair trading practices are reflected in the priorities of the Codex Alimentarius Commission in the developing countries. [109] The Food and Agriculture Organization of the United Nations and the World Health Organization jointly state that "illness due to contaminated food was perhaps the most widespread health problem in the contemporary world," and "an important cause of reduced economic productivity".[107] With the increasing population in the developing world, there is an increasing demand for meat and meat products which will force the present resource driven system of livestock production to a demand driven system [115] which will increase the disease transmission risks. There is a multifactorial risk of foodborne hazards in the developing countries due to poor sanitation and inadequate access to potable water. [106]

Beef products have always topped the incidence of Salmonellosis in many developing countries including India, Egypt, Brazil and Zimbabwe [106] and is the most seriously perceived food risks in chicken meat, even in the developed countries. [114]

The reported prevalence of *Salmonella* in cattle carcasses in South Asian countries varies from country to country. Studies in northern Thailand revealed 57% prevalence in chicken meat at the market during 2002-2003, [110] 14.5% prevalence in Kathmandu, Nepal[108] and 42.63% prevalence in Ho chi Minh city, Vietnam. [104] Sero-prevalence of poultry *Salmonella* in Bangladesh has been reported to be 23.46%. [112] Not much literature has been available on the prevalence of *Salmonella* in cattle carcasses from developing countries, few researches reports negligible [113] to as low as 5%, [111] to a prevalence of 69%. [105] However, the overall annual incidence of foodborne Salmonellosis in India is nearly 6 per 1000 inhabitants. [106]

2.9.2 Salmonella in food animals in Nigeria

Epidemiological reports suggest that meat product is one of the major causes of diarrheal illness which account for 36% of mortality cases in Nigeria.[140] Number of studies in Nigeria shows low prevalence of *Salmonella* species in food animal. The prevalence of 3.5 % of *Salmonella* species was observed from balangu (both spiced and unspiced meat). [141]

2.9.3 Salmonella in Kano

Few studies show bacterial contamination of carcass in Kano. Fresh beef was found to be more contaminated by bacteria (E coli). [142,143] There is paucity of literature about the presence or isolation of *Salmonella* in Kano.

2.10 *Salmonella* in Beef and Beef Products

Health and economic impact while it is recognized that the prevalence of foodborne illnesses in developing countries is considerable, in most countries there is limited data through which the incidence of particular diseases and trends can be assessed over time. [106] The growing movement of people, live animals, and food products across borders; rapid urbanization in developing

countries; increasing numbers of immune - compromised people; changes in food handling and consumption; and the emergence of new or antibiotic-resistant pathogens all contribute to increasing food safety risks. [123] Food safety requirements in export markets have a profound impact on the way that supply chains for agricultural and food products in developing countries operate. Food safety regulations and standards are increasingly influencing the ability of developing countries to access markets for agricultural and food products, particularly in industrialized countries. [120]

Food safety is of particular concern in developing countries not only because of the high prevalence of the foodborne illness and other hazards associated with food, but also because of the considerable economic and social cost that, in turn, reflects prevailing levels of economic development. The majority of trade-limiting factors in developing countries relate to economics, poor infrastructure and lagging skills; food safety is still mainly the responsibility of the consumers. Improving food safety along western standards, however, may carry considerable costs and price food out of reach of the poor. [124]

The Center for Disease Control and Prevention has estimated that *Salmonella* infections were responsible for 1.4 million annual illnesses, resulting in nearly 600 deaths in 2003 in United States.[16] The proportion of illnesses attributed to *Salmonella*-contaminated meat and poultry is unknown. More severe cases of Salmonellosis tend to occur in the very old, the very young, and the immuno-compromised. The estimated annual costs in dollars in 1998 and 2003 of medical care and lost productivity due to food-borne *Salmonella* infections in the United States were $2.3 and $2.9 billion. [119,117]

All together 150 165 cases of human Salmonellosis have been reported in 14 member states of the European Union in the year 2000, [117] and 192 703 cases in 2004. [126] In the year 2000, 16 983 laboratory confirmed cases of Salmonellosis were reported in the United Kingdom, most commonly associated with the consumption of chicken and undercooked egg dishes. [125] In the year 2001, China recorded that 17.9% of the total food poisoning was caused by *Salmonella* species. [118] The trend in Salmonellosis in Australia has been increasing over the time, both in the number of cases recorded as well as in rate per population. [122]

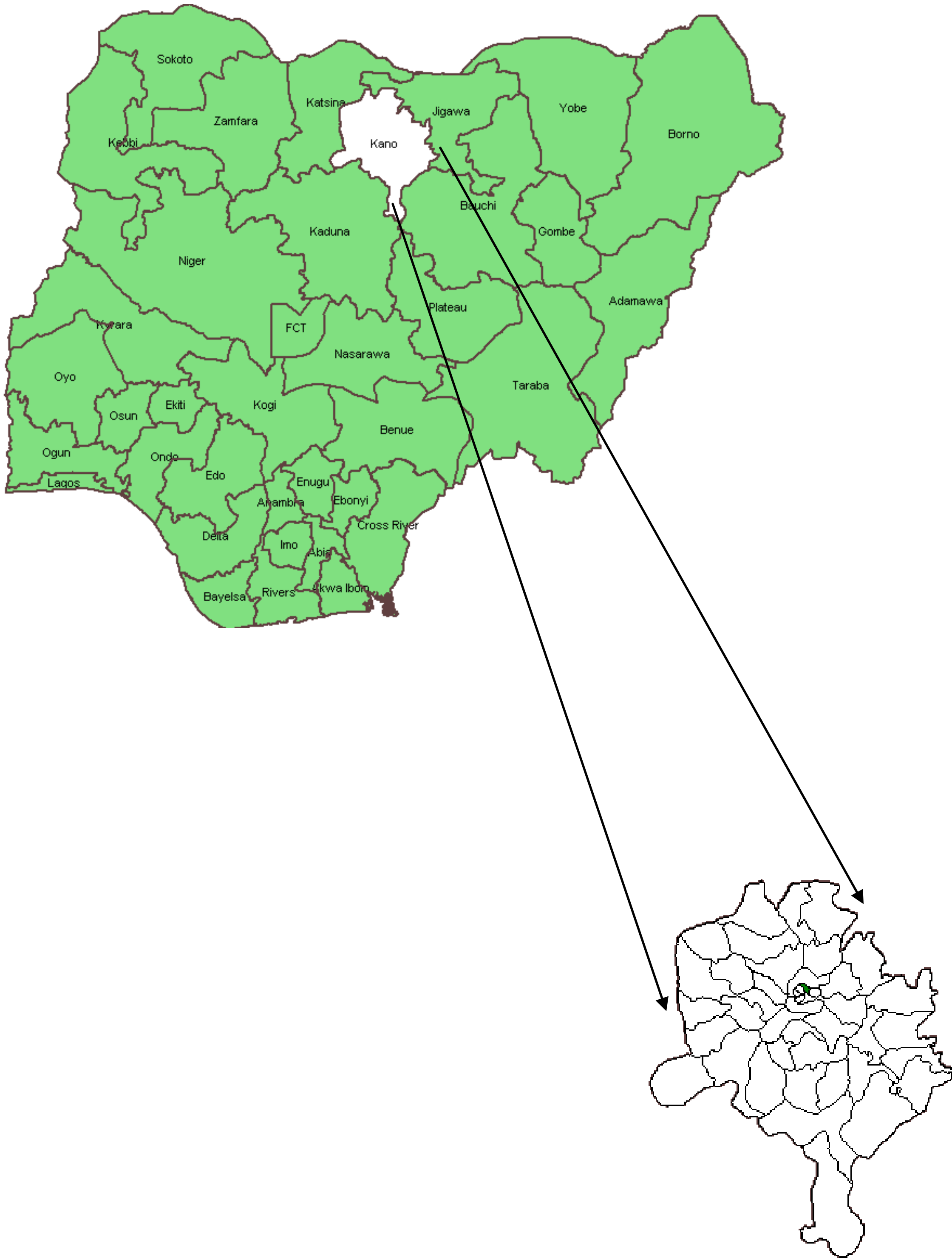
Apart from the impact on the health of the individuals, economic losses on international and national trade due to *Salmonella* in the poultry products and product recalls have direct economic and public perception effects on the processing industries. The recalls also have an effect on the demand of the product and affect a move towards non-meat products. In the United States, although the incidence has decreased, *Salmonella* accounts for 5% of USDA recalls. [121]

CHAPTER THREE - METHODOLOGY

3.1 Study Area

Kano abattoir was established 43 years ago and is a privately owned abattoir. It is located close to the ancient city gate of Mazugal (Kofar Mazugal) in Fagge Local Government area of Kano State. It is manage by a manager and on daily basis, approximately 500 people work in the abattoir. Livestock slaughtered in the abattoir are source from within and outside the country. They include different livestock markets within Kano state and neighboring state of Jigawa, Katsina, Kaduna, Bauchi, and Yobe and from other states like Borno and Gombe states. Outside the country, livestock are sourced from Chad, Niger, and Cameroun Republics. Livestock slaughtered in this abattoir include; cattle, sheep, goat and camel.

Veterinarians and other meat inspection staff are posted to work in the abattoir by Kano state Ministry of Agriculture and Natural Resources



Source: Health Mapper WHO, Version 4.3, 2008

Figure 3.1: Map of Nigeria showing Kano state and that of Kano state highlighting Fagge LGA

3.2 Study Design

Cross-sectional study

3.3 Study Period

December 2012 to April 2013

3.4 Study Population

The population under study is cattle slaughtered in Kano abattoir

3.4.1 Inclusion criteria

All cattle of both sex without distinction to breed and age slaughtered in Kano abattoir was enrolled in to the study.

3.4.2 Exclusion criteria

- Other livestock slaughtered in Kano abattoir other than cattle and fetuses was not enrolled in to the study.
- Cattle not slaughtered within the abattoir

3.5 Sample Size

Sample size required for this study was determined depending on the expected prevalence of *Salmonella*[140] and desired absolute precision.[27]

$$n = \frac{Z_{\frac{\alpha}{2}}^{2} p(1-p)}{d^{2}}$$

Zα= 1.96

$P=0.05$[140]

$Q\ (1-p) = 0.95$

$d=0.05\ (5\%)$

$$n = \frac{1.96^2 \times 0.05 \times 0.95}{0.05^2} = 73$$

3.6 Sampling Method

3.6.1 Collection of samples

Collection of samples

Ten carcasses were selected during each visit using systematic random sampling. The first slaughtered cattle was chosen, and then followed by the tenth and the twentieth cattle and so on. Twenty carcasses were sampled per week making the total of 4 weeks sampling period.

Carcass swabs

Swabs were moistened prior to the collection of samples using sterile maximum recovery diluent (MRD) using 0.1 % peptone and 0.85% NaCl for a minimum of five seconds. [147]

The swabs were taken from the sample sites by swabbing vertically, horizontally, and diagonally for not less than 20 seconds using a sterile $100cm^2$ square template and putting as much pressure as possible.[147]

Sampling was carried out after the completion of carcass dressing. Samples were taken half way through the slaughter day, and to ensure that they are representative of the factory throughput, the day on which sampling is carried out was altered each week.[147]

Sites of sample collection

The four individual samples from each sample site were pooled: neck, brisket, flank and rump samples are pooled[147] and placed in a sterile container containing 5 mls of sterile diluent.

Sample maintenance, storage and transport

The sample container was stored between 0-4°C (using a cool box) and then transported to Bacterial zoonoses Laboratory of the Department Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria within 8hours of sample collection

3.7 Overall Number of Sample

Eighty (80) samples were taken from carcass swabs for detecting contamination and swabs were taken after evisceration.

3.7.1 Sample preparation

3.8 Laboratory Procedures for Isolation and Identification of *Salmonella*:

In this study, we followed the protocol of ISO 6579:2002 (Microbiology of food and animal feeding stuffs - horizontal method for the detection of *Salmonella* species.) to detect *Salmonella* species.

3.8.1 Plating and counting

One (1ml) suspension of the inoculated physiological saline was appropriately diluted using 10-fold serial dilution. Four labeled (1-4) sterile tubes were arranged serially on a test tube rack.

One mls of the sample was pipetted in to 9mls of sterile physiological saline. 0.1ml of the last tube was spread on the plates and incubated at 37°C for 24 hours.

The dilution factor of 10^{-5} was used on the plates. The colony was counted after incubation and result expresses as cfu/cm².

Laboratory results are recorded as colony forming units (cfu/cm^2) for each pooled carcass sample (container) using the following formular.

$$cfu/cm^2 = \frac{Avereage\ cfu\ per\ plate\ \times a\ (Volume\ of\ original\ suspension}{b(Total\ surface\ area\ [100cm^2 \times 4] \times Dilution\ factor[10^{-5}]}$$

The logarithm of each result was obtained by taking the logarithm to base 10 ($\log_{10}$) of cfu/cm^2 for each pooled carcass sample.

3.9 Plating and Identification

After 6-8 hours incubation of the serial dilutions, aliquots were plated out on to solid culture media as described below;

One mile of the samples was inoculated in to sterile 5ml of Selenite broth and incubated at 37°C for 18-24 hours. A loop of the overnight culture broth was inoculated on SSA and incubated at 37°C for 18-24 hours. The suspected colony was inoculated on nutrient agar slant and incubated at 37°C for 18-24 hours. The inoculated nutrient agar slant was stored in the refrigerator for further identifications

3.10 Biochemical Identifications of Isolates

The stored isolates on nutrient agar slants was inoculated on Citrate agar, TSA, urea, SIM, MR and VP, and incubated at 37°C for 24hrs

3.10.1 Microbact (Oxoid)

One to three isolated of the 24 hours culture was picked and emulsified in 2.5ml sterile peptone water and incubated at 37°C for 4 hours.[163] The wells of the individual substrate sets were exposed by cutting the end tag of the sealing strip and slowly peeling its back. The plate was placed on a holding tray and using a sterile Pasteur pipette 4 drops (100µl) of the bacterial suspension was added to the wells.[163] Using a sterile pipette a drop of sterile mineral oil was added to wells 1, 2 and 3. The inoculated row was resealed with the adhesive seal and specimen identification number was written on the end of the tag with marker pen. This was then incubated at 37°C for 18-24hours.[163]

After 18-24 hours incubation, to well 8 (Indole production) 2 drops of Indole (Kovacs) reagent was added and evaluated within 2 minutes of the addition of the reagent. To well 10, (Vogues-Proskauer reaction), one drop each of VPI reagent and VPII reagent was added and evaluated within 15-30 minutes the addition of the reagents. To well 12 (Tryptophan Deaminase, TDA) 1 drop of TDA reagent and was evaluated immediately.[163]

Reactions were evaluated as + or - by comparing them with the color chart. The results were recorded under the appropriate heading on the report form.

Results were recorded in forms containing the substrate that were tested. Twelve (12) substrates were tested; lysine, Ornithine, Hydrogen sulphide, Glucose, Mannitol, Xylose, o-Nitrophenyl β-D-galacotopyranoside (ONPG), Indole, Urease, Voges Proskauer, Citrate, and TDA. Three substrate form one group with each substrate assigned a number; when substrate gave a + result, the corresponding number for that group was summed up and recorded. . The results of the consecutive 3 wells were added and 4 digit codes was then obtained which was fed in to the

computer identification software; this immediately gave the probable identity of the organism tested in percentage.[163]

3.10.2 Interpretation

An octal coding system has been adopted for Microbact™. Each group of three reactions produces a single digit of code. Using the result obtained, the indices of the positive reactions are circled. The sum of these indices in each group of three reactions forms the code number. This code is entered in to the computer package.[163]

3.11 Statistical Analysis

All petrifilm count will be converted in $\log_{10}$ cfu/cm^2 and the mean log was calculated as the logarithm of the arithmetic average of all individual carcass counts in cfu/cm^2 for all carcasses sampled

Descriptive statistics was used to express the mean $\text{Log}_{\mathbf{10}}$ and standard deviation of the bacteria load. ANOVA was used to determine mean difference of Log cfu/cm^2 by week of sample collection.

3.12 Ethical Issues

Permission was sort from the Kano state Ministry of Agriculture and Natural resources and informed consent was obtained from the butchers. Confidentiality was maintained throughout the study.

3.13 Limitation

The study was only limited to Kano main abattoir and other species of food animals were not part of this study.

CHAPTER FOUR - RESULTS

This study shows that 250 to 300 cattle were slaughtered per day in Kano abattoir during the period of the study. The 80 cattle carcasses were sampled. Out of the examined 80 carcasses 41 (51.3%) were from male cattle carcasses and 39 (48.8%) from female beef carcasses beef carcasses. Seventy nine (98.8%) cattle carcasses sampled were contaminated by bacteria but only 50 (62.5%) carcasses sampled yield growth on *Salmonella-Shigella* agar.

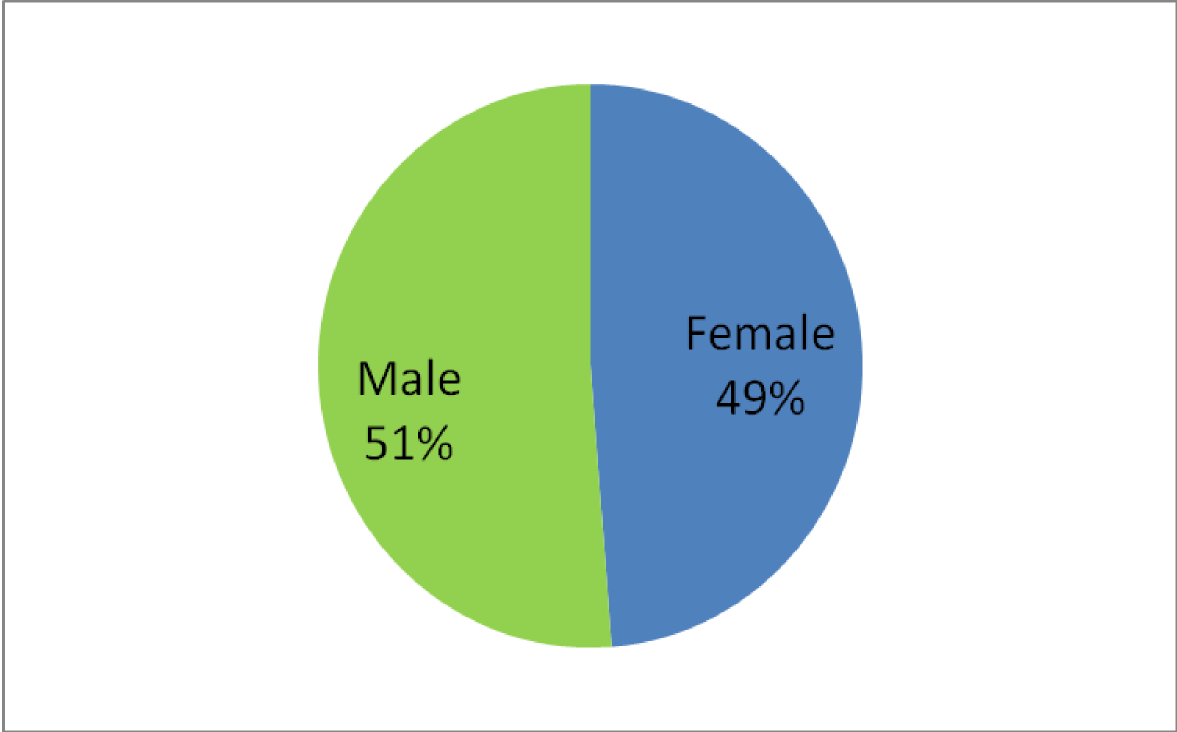
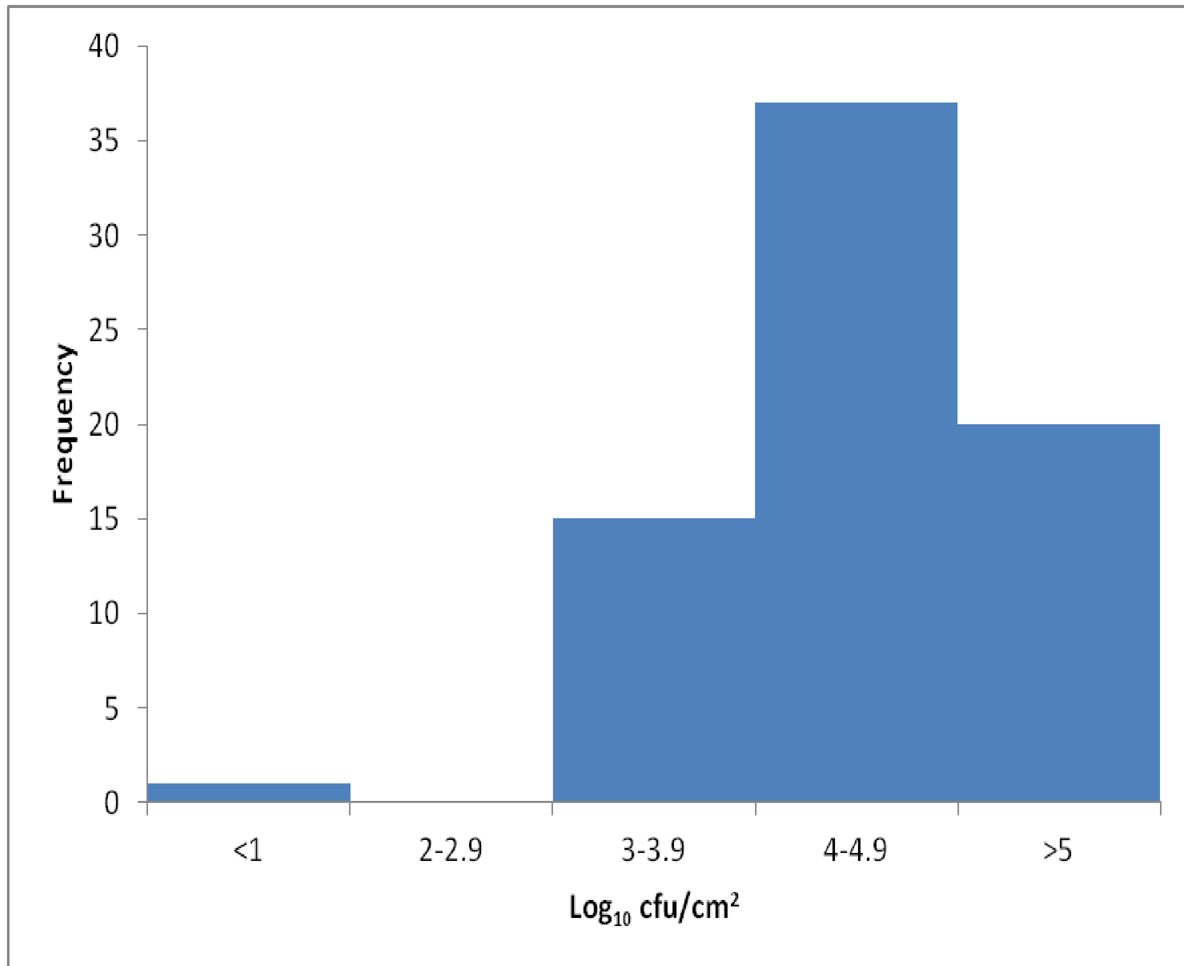


Figure: 4.1 showing the distribution of carcasses sampled by sex of the cattle at Kano abattoir

4.1Determination of Total Aerobic Plate Counts

Examination of the aerobic microbial loads on cattle carcass at slaughter in the morning showed that the average colony count for the pooled carcass swabs ranges from 3.8×10^5 to $2.3\times10^{6.}$ The Log CFU/cm^2 of the carcass swab ranges from 3.1 to 5.4 and the mean CFU/cm^2 were 4.4±0.77. Sex of the carcass shows no statistical significance with the Log CFU/cm^2 ($P > 0.05$).

The distribution of CFU/cm^2 by week indicates that the highest value (5.35 CFU/cm^2) was during week 4 of cattle carcasses swab collection while the lowest (3.10 CFU/cm^2) was during weeks 2 and 3. Weeks of sample collection show statistical significance difference ($P<0.05$) in the Log CFU/cm^2.



Mean=4.43 Std dev=0.77

Figure: 4.2 Histogram showing the distribution of Total Aerobic count data from cattle carcass at Kano abattoir between December, 2012 and April, 2013.

4.2 Determination Of Acceptable Level Of Carcass Contamination

Table: 4.1 Average number of colony per plate from cattle carcass at Kano abattoir between December, 2012 and April, 2013.

Average number of colony per plate n=79	**Frequency**	**Percent**	**Interpretation**
<5	15	19.0	Satisfactory
5-25	21	26.6	Further investigation required
>25	43	54.4	Very immediate action necessary
Total	**79**	**100.0**	

*Satisfactory means the minimum acceptable limit at which cattle carcasses is contaminated by bacteria

Process control chart was use to describe the satisfactory nature of carcass processing. In this study the mean Log cfu/cm^2 by week of sample collection is within this acceptable limit (4.4±0.77 Log cfu/cm^2) as shown in figure: 3 below. Log cfu/cm^2 of ≤3.5 was considered satisfactory level of carcass contamination (‘m’) and Log cfu/cm^2 of ≥5.0 was considered unsatisfactory (‘M’) while the values between m and M were considered marginal acceptable. Only 5 (6.3%) of the total carcasses sampled had value ≤3.5 Log cfu/cm^2 which are below the mean value while 19 (23.8%) had value ≥5.0 Log cfu/cm^2 which is above the mean. Even though most values are within the marginal acceptable limit, corrective actions are required.

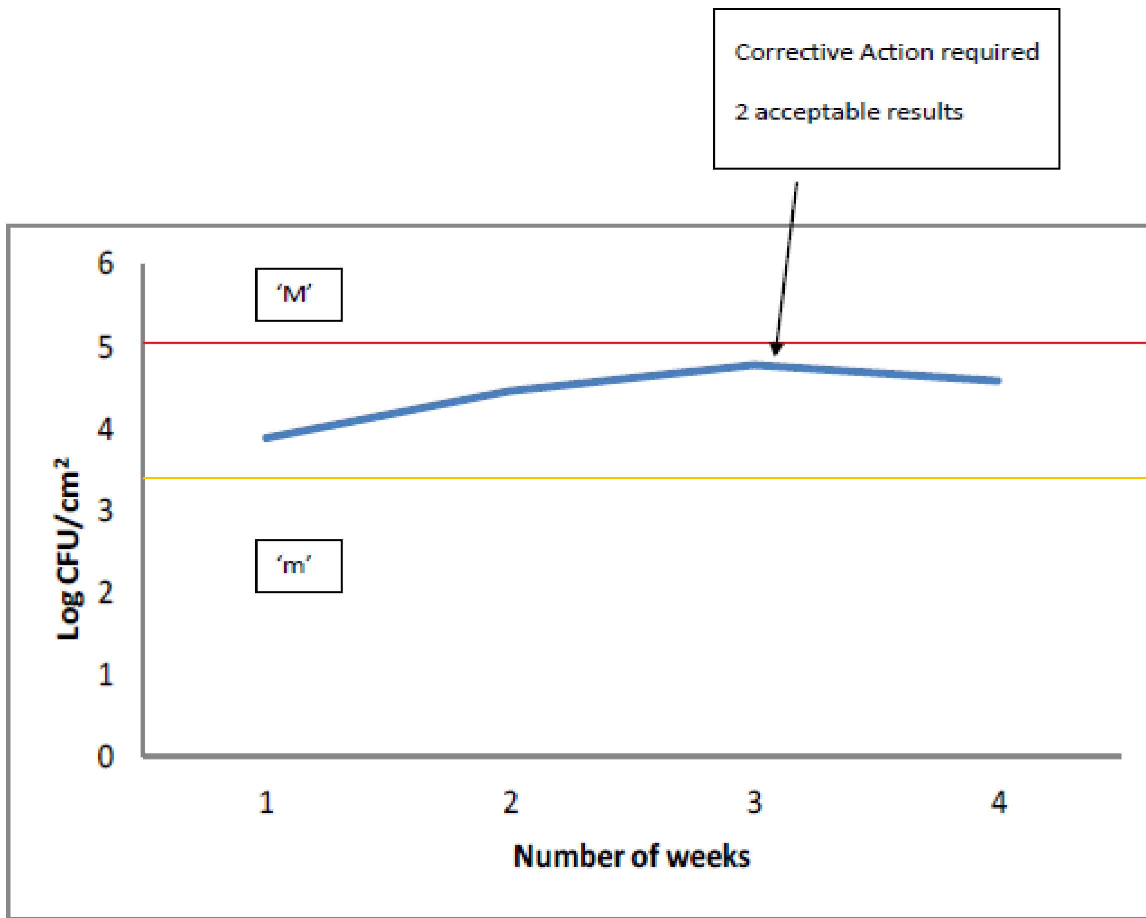


Figure 4.3: Process Control Chart A: Aerobic Colony counts- mean log values of colony forming units (cfu/cm2) by week of sample collection of cattle carcasses at Kano abattoir

*'**m'** is a defined value separating a good result from a marginally acceptable result (values between **m** and **M** are considered to be marginally acceptable)

*'**M'** is the maximum value for a marginal result (values greater than **M** are unacceptable)

4.3 Isolation of *Salmonella* Species

Among the 50 carcasses sampled that yield growth on *Salmonella-Shigella* agar 3 (3.75%) samples were identified as *Salmonella* using conventional biochemical test but only 1 (1.25%) was identified as *Salmonella Arizonae* by Microbact^{TM} 12E. The percentage probability that *Salmonella* is a contaminant of cattle carcass(s) at Kano abattoir was 76.4%.

4.4 Other Pathogenic Bacteria Isolated

Four other pathogenic bacteria were isolated are: *Citrobacter freundii, Klebseilla oxytoca, Proteus vulgaris* and *Providentia rettgeri* as summarized in table: 4.3 below:

Table 4.2: Bacteria isolated on cattle carcass slaughtered for consumption in Kano abattoir n=47

Organism Identified	**Frequency**	**Percent**	**Percentage probability**
Citrobacter freundii	2	4.3	92.8
Klebsiella oxytoca	12	25.5	98.3
Proteus vulgaris	31	66.0	79.1-96.9
Providentia rettgeri	2	4.3	77.8
Total	**47**	**100**	

CHAPTER FIVE - DISCUSSION

Meat is the most perishable of all important food since it contain sufficient nutrient needed to support the growth of microorganisms. [145] This study shows that 80 cattle carcasses were sampled, out of which 79 (%) were contaminated by bacteria. The finding of this study revealed that cattle carcasses in Kano abattoir are contaminated with pathogenic Gram – negative bacteria.

The result of this study reveals that there was significant difference in mean total aerobic plate count ($P<0.05$) by week of sample collection and the maximum Log cfu/cm^2 was 5.4 while the minimum was 3.1. The mean Log cfu/cm^2 was 4.4 similar to the findings from three slaughtering plants in Canada that reported 3.8, 4.2 and 4.6 Log cfu/cm^2 [156] but does not corroborate with the findings in Zaria that record 7.6.[151] The mean Log cfu/cm^2 from the findings of this study was within the marginal acceptable limit (3.5-5.0 Log cfu/cm^2). Two results within the marginal acceptable limit are required before corrective action could be instituted but one result above the acceptable limit (>5.0 Log cfu/cm^2) require corrective actions.

The corrective action required include evaluation of cattle cleanliness, improving working procedure/instructions, retraining review of cleaning/disinfection materials and maintenance/cleaning equipment, improve supervision.

The possible source of contaminants may be due to the unhygienic manner of handling meat in abattoirs, the environment upon which the meat is slaughtered as well the water.[166] used in the processing of the meat. This also implies that these meats are viable source of various diseases. Some of these diseases including Salmonellosis could spread and acquire epidemic status which poses serious health hazards, since improper handling and improper hygiene might lead to the contamination of fresh meats and this might eventually affects the health of the consumers. [146]

As observed in the course of this study, the method of slaughtering of animals is responsible for the microbial contamination.

The major sources of contamination may be multiple contacts with contaminated tools and operators' hands. [149] have noted that greatest reductions in microbiological contamination on carcasses were achieved by adoption of dressing procedures that minimized hand contact with the carcass during carcass handling. The presence of germs on the carcass explained also by contact with floor, bad evisceration practices, often resulting in rupture of the gastrointestinal tract, and especially hide-to-carcass contamination transfer.

Salmonella is among the most common agents of Foodborne illness in humans and have been isolated from beef and dairy cattle at all stages of production, but fecal shedding may be intermittent and difficult to detect, however the organism appears to be fairly spread throughout bovine population.[152]

The prevalence of *Salmonella* (1.25%) in this study was observed to be similar to the findings from cattle rectal swab (1.5%) in Zaria[154] but lower than 8.4% in raw beef [155] 6%[153], 5% in raw beef in Abakaliki[143] and 2.43% in retail beef and related meat products in Zaria. [151] *Salmonella Arizonae* was the specie isolated and the percentage probability of isolating this bacterium was 76.45%.

The true incidence of Salmonellosis is difficult to evaluate because lack of an epidemiological system in place, which is particularly true in developing countries.[165] *Salmonella arizonae* is mainly isolated from cold-blooded animals or the environment but rarely from mammalian sources (including humans).[170] Bovine species is particularly susceptible to *Salmonella* Infection. [167] The presence of *Salmonella arizonae* on cattle carcasses could be explained by an external

contamination (cold-blooded animals or environment), as a result of very poor hygienic conditions at Kano abattoir. The significance of *Salmonella arizonae* subspecies serotypes for human beings has not been clarified. However, any serovar must be regarded as potentially pathogenic for the consumers.[168] In France, it has been reported that serovars of *Salmonella arizonae* subspecies were isolated from human sources.[169]

Microbact™ 12E test kit confirmed 1 out of 3 *Salmonella* suspects identified by conventional biochemical tests carried out. The use of Microbact™ kit for the identification of *Enterobacteriaceae* was observed to be sensitive; less labour intensive, rapid, and convenient method of identification of *Salmonella* over the conventional biochemical method of identification.

Other pathogenic bacteria isolated were of *Enterobacteriaceae* family. The bacteria isolated are *Citrobacter freundii* (4.3%), *Klebseilla oxytoca* (25.5%), *Proteus vulgaris* (66.0%) and *Providentia rettgeri*4.3%. The percentage probability for the isolation of C*itrobacter freundii* was 92.8%, *Klebseilla oxytoca* 98.3% and *Providentia rettgeri* 77.8% while *Proteus vulgaris* ranges from 79.1% -96.9%. The presence of these organisms on carcasses could be attributed to the fact that carcasses contain an abundance of all nutrients required for the growth of bacteria in adequate quantity. Microorganisms isolated from fresh meat samples in this study have been earlier found in foods, environment and other places, and their pattern is similar to previous reports. [140,141142,157,158,159,160] Most of the organisms found in this study are those commonly found in soil and water.

In addition to C*itrobacter freundii* and *Proteus vulgaris* isolated in this study, [156] isolated *Klebsiella pneumoniae*, *Enterobacter species*, *Pseudomonas aeruginosa*, and *E. coli*. The presence of higher number of pathogenic *K. pneumoniae*, *Salmonella* species and *E. coli* among

others, encountered in fresh meat from conventional beef is alarming. The presence of these organisms in meat foods should receive particular attention, because their presence indicate public health hazard and give warning signal for the possible occurrence of food borne intoxication. [161]

CHAPTER SIX - CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

In conclusion, our results reflect poor conditions of carcasses slaughtering and handling, and inadequate hygienic practices at Kano abattoir. Microbial load found in this study are found to be above the satisfactory limit of carcass contamination. These contamination levels limit the opportunities of meat preservation and therefore their commercial life; as a result, they accentuate economic risks by loss of foodstuffs, and health risks to public health by food borne diseases.

The findings of this study may have implication on *Salmonella* species and can serve as a basis for future quality control of beef at Kano abattoir. Other pathogenic bacteria were isolated and identified were also members of the family *Enterobacteriaceae.* Furthermore, food hygiene and handling practice remains a major challenge in

6.2 Recommendations

It is therefore recommended that;

1. Thorough and adequate training on sustainable sanitary and hygiene practice should be given to the abattoir workers. This should include;

- Periodic continuing education programs
- Maintenance of proper hygiene within the abattoir and the environment, target areas for sanitization include: infrastructures and facilities contained therein, equipment, surrounding areas, abattoir workers and visitors
- Periodic Sanitary-hygienic evaluation of abattoirs and slaughterhouses
- Enforcement of existing health and hygiene regulations.

2. All facilities in the Kano Abattoir should be overhauled and up graded; at the same time ensuring strict compliance to proper sanitation
3. Public health education program on the importance of ethical abattoir practices for the abattoir personnel especially the butchers should be organized at least once a year.
4. The general public should be made to understand the techniques of detecting diseased meat especially the organ meat
5. Practice of WHO basic hygiene principles, which cover food safety procedures from the farm of origin, to ante-mortem and post-mortem inspection, to handling until the food is consumed.
6. The scientific community should join regulatory authorities to spread awareness about basic hygiene principles.

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APPENDICES

Appendix: 1 Summary of *Salmonella* Nomenclature

***Salmonella* species and subspecies**	**Number of serovars within subspecies**	**Usual habitat**
SALMONELLA enteric subsp Entericea **(I)**	1,454	Worm blooded animals
SALMONELLA enteric subsp salamae **(II)**	489	Cold blooded and the environment
SALMONELLA enteric subsp Arizonae **(IIIa)**	94	Cold blooded and the environment
SALMONELLA enteric subsp Diarizonae **(IIIb)**	324	Cold blooded and the environment
SALMONELLA enteric subsp Hauteriae **(IV)**	70	Cold blooded and the environment
SALMONELLA enteric subsp Indica **(VI)**	12	Cold blooded and the environment
SALMONELLA bongori	20	Cold blooded and the environment

Appendix: 2 conventional biochemical test results of the samples that yielded growth on SSA

Sample ID	TSI	Citrate	Urea	Sulphide	Indole	Motility	Methyl red	Voges-Proskauer
C01	Acid/Acids+H_2S	+	+	+	-	Motile	+	-
C02	Acid/Acid+Gas+H_2S	+	+	+	-	Motile	+	-
C03	Acid/Acid+Gas	+	+	+	+	Motile	+	-
C06	Acid/Acid+H_2S	+	+	+	+	Motile	+	-
C08	Acid/Acid+Gas+H_2S	+	+	+	+	Motile	+	-
C09	Alk/Acid+Gas+H_2S	+	+	+	-	Motile	+	-
C10	Acid/Acid+Gas+H_2S	+	+	+	+	Motile	+	-
C11	Alk/Acid+Gas+H_2S	+	±	+	-	Motile	+	-
C12	Acid/Acid+Gas+H_2S	+	+	+	+	Motile	+	-
C13	Alk/Acid+Gas+H_2S	+	±	+	-	Motile	+	-
C14	Acid/Acid+Gas+H_2S	+	+	+	-	Motile	+	-
C15	Alk/Acid+Gas+H_2S	+	±	+	-	Motile	+	-
C16	Alk/Acid+Gas+H_2S	+	±	+	-	Motile	+	-
C18	Acid/Acid+H_2S	+	±	+	+	Motile	+	-
C19	Alk/Acid+Gas+H_2S	+	±	+	-	Motile	+	-
C20	Alk/Acid+Gas+H_2S	+	±	+	-	Motile	+	-
C22	Acid/Acid+Gas+H_2S	+	+	+	-	Motile	+	-
C23	Acid/Acid+Gas+H_2S	+	+	+	-	Motile	+	-
C24	Acid/Acid+H_2S	+	+	+	+	Motile	+	-
C25	Acid/Acid+Gas+H_2S	+	+	+	-	Motile	+	-
C51	Alk/Acid+H_2S	-	+	+	+	Motile	+	-
C52	Alk/Acid+H_2S	+	-	+	-	Motile	+	-
C53	Acid/Acid+H_2S	-	+	+	+	Motile	+	-
C54	Alk/Acid+Gas	+	-	-	-	Motile	+	-
C55	Alk/Acid+H_2S	+	-	+	-	Motile	+	-
C56	Alk/Acid+H_2S	+	-	+	-	Motile	-	-
C57	Acid/Acid+H_2S	+	+	+	-	Motile	+	-
C58	Acid/Acid+Gas	+	-	+	-	Motile	+	-
C59	Alk/Acid+H_2S	+	-	-	+	Non motile	+	-
C60	Alk/Acid+H_2S	-	-	+	+	Motile	+	-
C61	Alk/Acid+H_2S	+	-	+	-	Motile	+	-
C62	Alk/Acid+H_2S	+	-	+	-	Motile	+	-
C63	Alk/Acid+H_2S	-	+	+	-	Motile	+	-
C64	Alk/Acid+H_2S	+	+	+	-	Motile	+	-
C65	Alk/Alk+H_2S	+	-	-	+	Motile	+	-
C66	Alk/Acid+Gas	+	-	-	-	Motile	+	-

C67	Alk/Acid+Gas	+	-	-	-	Motile	+	-
C68	Alk/Acid+Gas	+	+	+	-	Non motile	+	-
C69	Alk/Acid	+	-	+	-	Motile	-	-
C70	Alk/Alk+Gas	-	+	+	-	Motile	+	-
C71	Alk/Acid+Gas+H_2S	+	-	+	+	Motile	+	-
C72	Alk/Acid+Gas	+	-	-	-	Motile	+	-
C73	Alk/Alk	-	+	+	-	Motile	+	-
C74	Alk/Acid	-	+	+	-	Motile	+	-
C75	Alk/Acid+Gas	-	+	+	-	Motile	+	-
C76	Alk/Acid+H_2S	+	-	+	+	Motile	+	-
C77	Acid/Acid+H_2S	+	-	-	-	Motile	+	-
C78	Alk/Acid+H_2S	+	-	+	+	Motile	-	-
C79	Acid/Acid+H_2S	-	+	+	+	Motile	+	-
C80	Acid/Acid+H_2S	-	+	-	+	Motile	+	-

Appendix 3: Table shows sample identity, week of sample collection, sex of the carcass, reaction of sample on SSA and the Organism identified by Microbact 12E

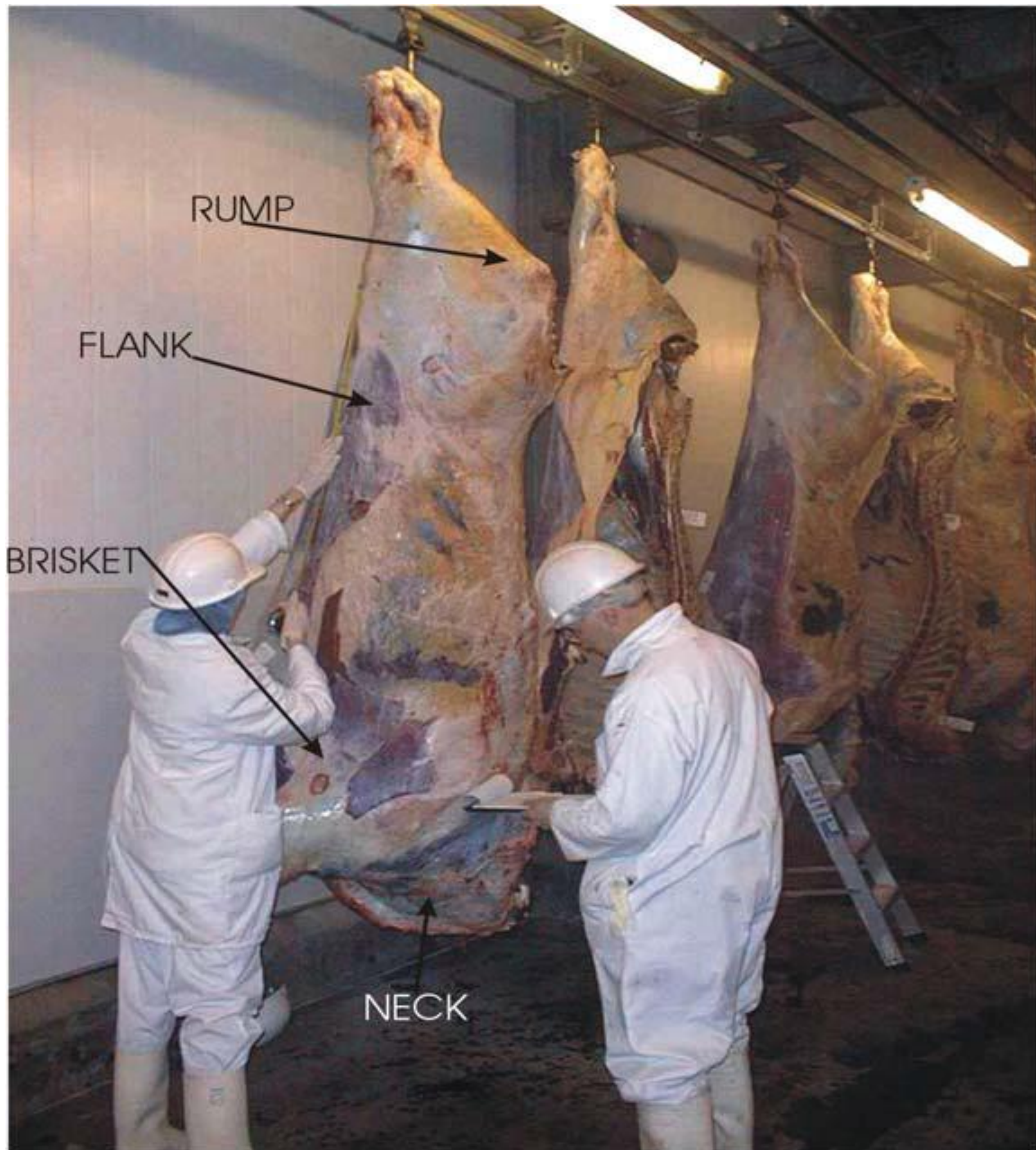
S/No	Sample ID	Week	Sex	Log_{10} cfu/cm²	Isolates on SSA	Organism Identified
1	C01	1	F	3.57	Positive	*Proteus vulgaris*
2	C02	1	F	4.05	Positive	*Proteus vulgaris*
3	C03	1	F	3.7	Positive	*Proteus vulgaris*
4	C04	1	M	5.23	NG	
5	C05	1	F	0	NG	
6	C06	1	F	3.94	Positive	*Proteus vulgaris*
7	C07	1	M	4.64	NG	
8	C08	1	M	3.57	Positive	*Proteus vulgaris*
9	C09	1	M	4.1	Positive	*Proteus vulgaris*
10	C10	1	M	3.94	Positive	*Providentia rettgeri*
11	C11	1	M	4.38	Positive	*Klebsiella oxytoca*
12	C12	1	M	3.8	Positive	*Proteus vulgaris*
13	C13	1	F	5.17	Positive	*Proteus vulgaris*
14	C14	1	F	3.4	Positive	*Proteus vulgaris*
15	C15	1	M	4.18	Positive	*Proteus vulgaris*
16	C16	1	F	4.83	Positive	*Klebsiella oxytoca*
17	C17	1	M	4.67	NG	
18	C18	1	F	3.7	Positive	*Citrobacter freundii*
19	C19	1	F	3.1	Positive	*Klebsiella oxytoca*
20	C20	1	F	3.88	Positive	*Salmonella arizonea*
21	C21	2	F	4.1	NG	
22	C22	2	F	4.14	Positive	*Proteus vulgaris*
23	C23	2	F	5.1	Positive	*Klebsiella oxytoca*
24	C24	2	M	3.8	Positive	*Proteus vulgaris*
25	C25	2	F	3.8	Positive	*Proteus vulgaris*
26	C26	2	F	4.14	NG	
27	C27	2	F	4.53	NG	
28	C28	2	M	4.77	NG	
29	C29	2	F	3.57	NG	
30	C30	2	M	5.3	NG	
31	C31	2	M	5.13	NG	
32	C32	2	M	3.1	NG	
33	C33	2	F	5.35	NG	

34	C34	2	F	4.88	NG	
35	C35	2	M	4.8	NG	
36	C36	2	F	3.57	NG	
37	C37	2	F	5.02	NG	
38	C38	2	F	5.18	NG	
39	C39	2	F	4.77	NG	
40	C40	2	F	4.24	NG	
41	C41	3	M	3.1	NG	
42	C42	3	M	5.11	NG	
43	C43	3	M	4.27	NG	
44	C44	3	F	3.7	NG	
45	C45	3	M	4.59	NG	
46	C46	3	F	5.03	NG	
47	C47	3	F	4.88	NG	
48	C48	3	F	5.2	NG	
49	C49	3	F	5.24	NG	
50	C50	3	M	5.25	NG	
51	C51	3	F	5	Positive	*Proteus vulgaris*
52	C52	3	M	4.81	Positive	*Proteus vulgaris*
53	C53	3	M	5.27	Positive	*Providentia rettgeri*
54	C54	3	M	4.8	Positive	*Klebsiella oxytoca*
55	C55	3	M	5.14	Positive	*Proteus vulgaris*
56	C56	3	M	4.99	Positive	*Proteus vulgaris*
57	C57	3	M	4.88	Positive	*Proteus vulgaris*
58	C58	3	F	5.01	Positive	*Proteus vulgaris*
59	C59	3	M	4.38	Positive	*Klebsiella oxytoca*
60	C60	3	F	4.94	Positive	*Klebsiella oxytoca*
61	C61	4	M	4.49	Positive	*Citrobacter freundii*
62	C62	4	F	4.1	Positive	*Klebsiella oxytoca*
63	C63	4	M	4.94	Positive	*Salmonella arizonea*
64	C64	4	M	5.03	Positive	*Klebsiella oxytoca*
65	C65	4	F	4	Positive	*Proteus vulgaris*
66	C66	4	F	4.7	Positive	*Klebsiella oxytoca*
67	C67	4	M	4.27	Positive	*Proteus vulgaris*
68	C68	4	M	4.88	Positive	*Proteus vulgaris*
69	C69	4	M	4.3	Positive	*Proteus vulgaris*

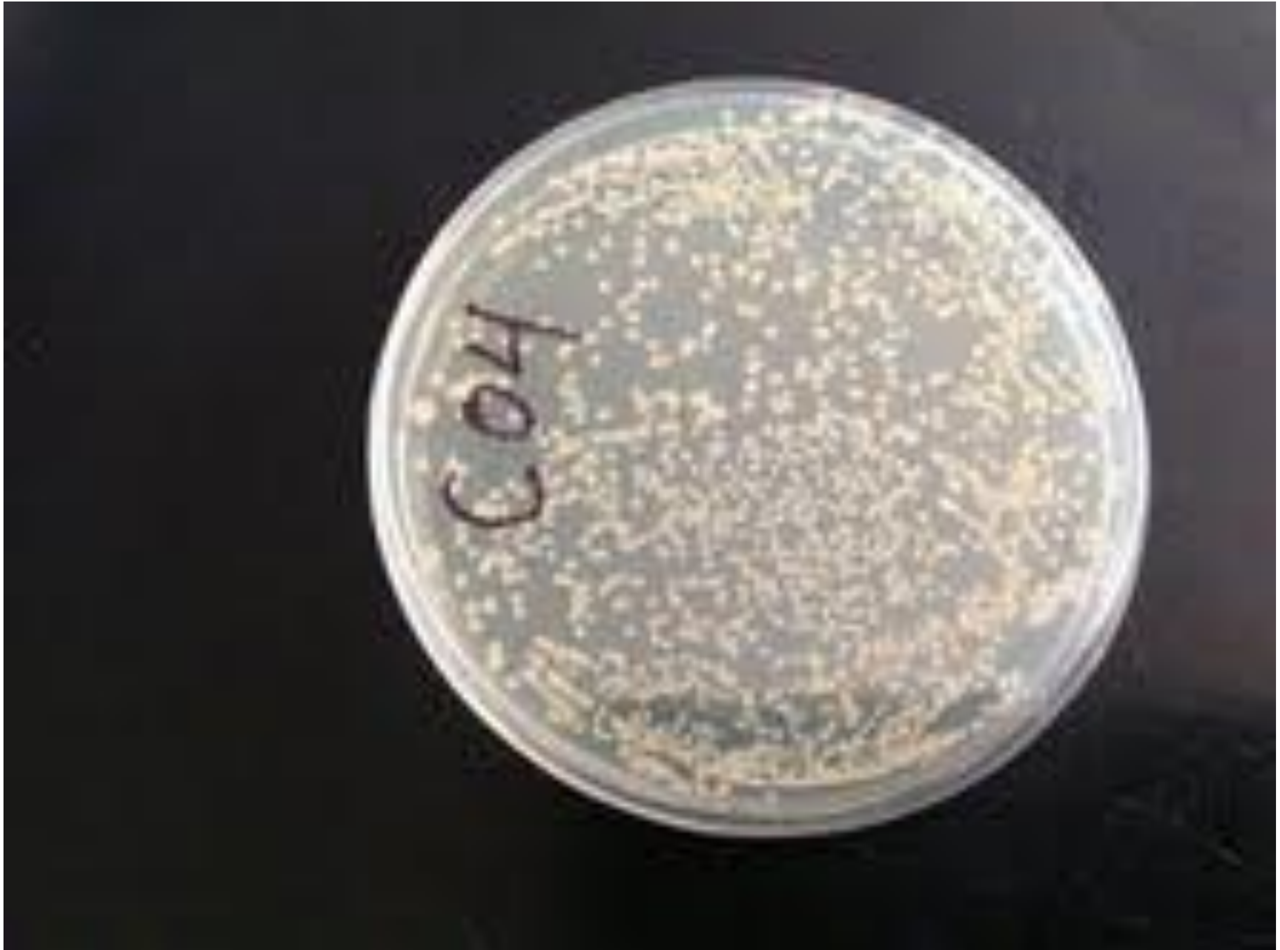
70	C70	4	F	4.95	Positive	*Klebsiella oxytoca*
71	C71	4	F	4.95	Positive	*Proteus vulgaris*
72	C72	4	F	4.68	Positive	*Proteus vulgaris*
73	C73	4	M	4.24	Positive	*Klebsiella oxytoca*
74	C74	4	M	4.1	Positive	*Proteus vulgaris*
75	C75	4	M	4.4	Positive	*Proteus vulgaris*
76	C76	4	M	4.78	Positive	*Proteus vulgaris*
77	C77	4	M	5.1	Positive	*Klebsiella oxytoca*
78	C78	4	M	4.1	Positive	*Proteus vulgaris*
79	C79	4	M	5.07	Positive	*Proteus vulgaris*
80	C80	4	M	4.56	Positive	*Proteus vulgaris*

Appendix: 4 Showing Triple sugar iron agar reaction in relation to organisms identified

	Organisms identified					
TSI	*Citrobacter freundii*	*Klebsiella oxytoca*	*Proteus vulgaris*	*Providentia rettgeri*	*Salmonella arizonae*	TOTAL
Acid/Acid+Gas	0	0	2	0	0	2
Acid/Acid+Gas+ H2S	0	1	6	1	0	8
Acid/Acid+H2S	1	1	5	1	0	8
Acid/Acids+H2S	0	0	1	0	0	1
Alk/Acid	0	0	2	0	0	2
Alk/Acid+Gas	0	2	4	0	0	6
Alk/Acid+Gas+ H2S	0	3	4	0	1	8
Alk/Acid+H2S	1	3	6	0	2	12
Alk/Alk	0	1	0	0	0	1
Alk/Alk+Gas	0	1	0	0	0	1
Alk/Alk+H2S	0	0	1	0	0	1
TOTAL	2	12	31	2	3	50



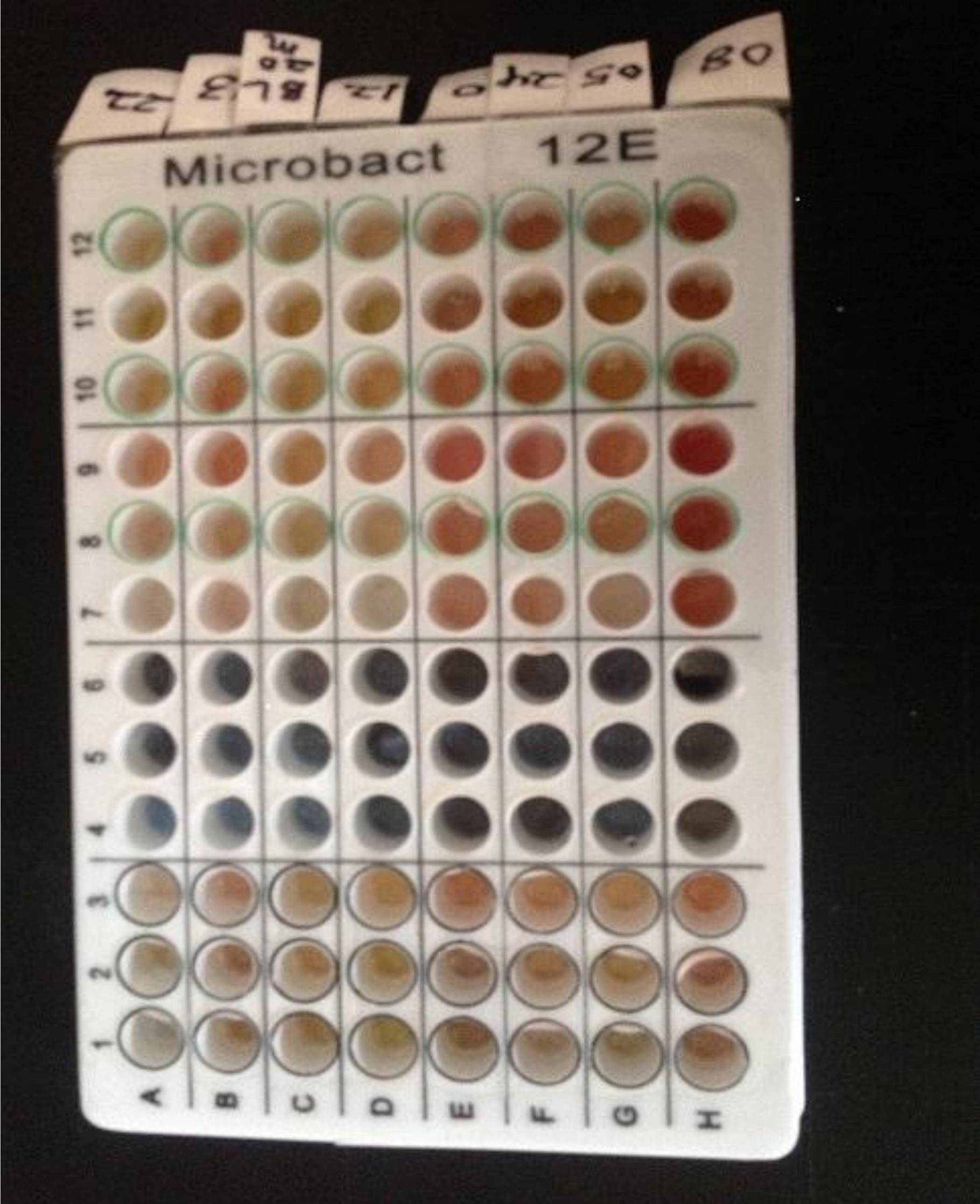
Appendix:5 showing the site of swab collection; (A) Rump (B) Brisket (C) Flank (D) Neck



Appendix:6 Showing the colonial appearance of bacteria on Nutient agar plate

Appendix:7 Showing black colonial morphology of suspected *Salmonella* species on *Salmonella-Shigella agar*

Appendix: 8 Showing black and colourless colonial morphology of suspected *Salmonella* species on *Salmonella-Shigella agar*



Appendix: 9 Microbact™ 12E Microtitre plate before 24 hours incubation

Appendix: 10 Microbact™ 12E Microtitre plate after 24 hours incubation. Observe the colour change

Appendix: 11 Time line of activities

Activity	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	April	May
Writing proposal											
Proposal defense											
Literature review											
Sample collection											
Laboratory analysis											
Data analysis											
Writing thesis											
Internal defense											
External defense											