KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

COLLEGE OF SCIENCE

MICROBIAL QUALITY OF FRESH BEEF SOLD IN THE BIRIM NORTH DISTRICT OF THE EASTERN REGION OF GHANA

THESIS SUBMITTED TO THE DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY, KNUST IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN ENVIRONMENTAL SCIENCE

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DECLARATION

I declare that this thesis hereby submitted for the M.SC. Environmental Science at the Kwame Nkrumah University of Science and Technology, KNUST had not been previously submitted by me for a degree at any other University.

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DEDICATION

To my lovely wife Emelia Anim and children: Jessica Obenewaa Twum; Emmanuel Twum; Jesse Ernest Twum Senior and Jireh Ernest Twum Junior.

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ABSTRACT

Beef contributes significantly to the daily protein intake of many Ghanaians but can be a source of foodborne illnesses especially under the conditions in which animals are handled, slaughtered, transported and sold on Ghanaian markets. This study assessed the microbiological quality of beef sold in the Birim North District in the Eastern Region of Ghana. Twenty-four (24) fresh beef samples from eight (8) butchers were aseptically collected and analyzed for microbial load using standard microbiological procedures. The samples were subjected to bacteriological analysis such as total viable count (TVC), total *Staphylococcus* count (TSC), total *Salmonella* count (TSC) and total *Escherichia coli* count (TEC). The mean Total Viable Count in colony forming units per gram (cfu/g) ranged between $2.37x10^5$ and $4.23x10^5$. The mean log10 values of total viable count were 5.37, 5.59, 5.58, 5.48, 5.61, 5.62, 5.55 and 5.58 for New Abirem market (East), New Abirem market (West), Noyem Lorry Station, Noyem market, Pankese, Afosu, Akoase and Nkwateng respectively. There was no significant difference between the mean TVC, TSC and TEC counts of all the meat shops ($P<0.05$). The beef samples were contaminated with *Staphylococcus spp, Escherichia coli* and *Salmonella* spp. Prevalence of *Salmonella spp*. in retail beef in the study markets was found to be low (6 %) compared to *Staphylococcus spp* and *Escherichia coli* which recorded 47% each. Hygienic practices of the butchers were also assessed using observation and checklist on items and facilities required for good hygienic practices in the processing, handling and transport of raw meat by butchers. There was poor hygienic standard of meat processing such as dressing of carcasses on filthy floors, use of unsterilized knives and slaughtering equipment in the cutting and processing of meat and inappropriate means of transporting carcasses to sale points. Unhygienic practices and poor handling of beef by butchers in the study area were the major causes of contamination of beef. The presence of *Salmonella* species, *Escherichia coli* and *Staphylococcus* species organisms are of special concern because these could potentially cause food borne intoxication. Therefore, it is important that appropriate hygienic practices are instituted to reduce the potential risk of foodborne pathogens in the study area.

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LIST OF ABBREVIATIONS

AFRSC - Australia Food Regulation Standing Committee

ASNS - American Society for Nutritional Science

ASQC -American Society for Quality Control

BNDA - Birim North District Assembly

CDC - Center for Disease Control and Prevention

CFIA - Canadian Food Inspection Agency

CFSAN - Center for Food Safety and Applied Nutrition

CWFT - Compassion in World Farming Trust

EU - European Union

FAO - Food and Agricultural Organization

FDA - Food and Drug Administration

FSA - Food Standard Agency

FSANZ - Food Standards Australia New Zealand

HACCP - Hazard Analysis of Critical Control Point

ICMSF - International Commission for Microbiological Specification of Foods

IFPRI - International Food Policy Research Institute

IFT - Institute of Food Technologists

ILRI - International Livestock Research Institute

MDH - Minnesota Department of Health

MTU - Meat Technology Update

NACMCF - National Advisory Committee on Microbiological Criteria for Foods

NSWFA - New South Wales Food Authority

PHAC - Public Health Agency of Canada

PHV - Public Health Veterinarian

UN - United Nations

USDA - United State Department of Agriculture

WHO - World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

One excellent source of protein in human diet is meat (Komba *et al.*, 2012). It has long been known for its high nutrients composition hence consumed by many people worldwide. The protein profile of meat has been described as excellent due to the presence of all the essential amino acids required by the body (Collins and Thato, 2011). The protein and vitamins especially vitamin A and B12 in meat is not available in plant sources (Bradeeba and Sivakumaar, 2012).

Majority of the world‟s population depend on meat as a source of food (Collins and Thato, 2011). The increased demand for meat is as a result of increased urbanization, higher disposable income and the human desire for a greater variety in their diets (Sofos, 2008). A large proportion of the people living in towns and urban centers consume beef (Zhao *et al*., 2001). In Ghana for instance about 60,000 people are believed to sell an estimated $100 million worth of food annually on the streets of Accra (Tomlins, 2002) and these individuals rely on butchers for their supply of beef (King *et al.,* 2000). A drastic fall in the consumption of chicken products in recent times has resulted in a sharp increase in demand for beef (Hobbs and Roberts, 1993). Meat market makes an important contribution to the well-being of people but this is not without its health hazards (Tomlins, 2002). There is considerably high food related infections such as diarrhea, typhoid fever and cholera recorded in hospitals and clinics worldwide. In the past people have expressed worry about the role of meat and meat products in food poisoning but available records show that more than 74% of cases of food poisoning worldwide are due to meat dishes (Hobbs and Roberts, 1993). Meat is highly prone to microbial contamination due to its rich source of nutrients which provide a suitable environment for growth of microbes (Steinkraus, 1994). The microbial growth can lead

to meat spoilage and food borne infections in human resulting in economic losses (Komba *et al.*, 2012). Illness due to eating of contaminated food is perhaps the most significant wide spread health problem and an important cause of reduced economic productivity in the world (WHO, 2009). Microbial food poisoning or infections for that matter is a serious public health issue which should be of concern to all (Zhao *et al*., 2001). The widespread distribution of raw meat and meat products which are potential vehicle for transmitting foodborne diseases makes the consequences of meat contamination more serious. Therefore, there is the need for increased implementation of Hazard Analysis of Critical Control Point (HACCP) and consumer food safety education efforts. HACCP refers to any actions and activities that can be undertaken to prevent or eliminate food safety hazard or reduce it to an acceptable level by identifying potential risk areas and putting appropriate measures to avoid contamination (ICMSF, 1988).

Dirty environment and unhygienic food handling influence wide spread of bacterial food poisoning (Burgess *et al.,* 2005; Tutenel *et al*., 2003). Major bacterial pathogens found in meat include *Bacillus cereus, Clostridium botulinum, Clostridium perfringens, Salmonella*, *Escherichia coli* and *Staphylococcus aureus* (Hobbs and Roberts, 1993). Contamination could come from unhygienic slaughtering, handling and processing conditions or from inherent microflora in normal tissues of animals, air and environment (Bell, 1997). Different microbes get introduced at each stage of meat processing after slaughtering, and these tend to contaminate the meat (Ebel *et al.,* 2004 and Sumner *et al*., 2003). Raw beef sold at retail outlets is subjected to a long chain of slaughtering and transportation where each step poses a potential risk of microbial contamination (Gill *et al.*, 2000). Majority of slaughter houses in Ghana particularly in the rural communities and small towns such as those in Birim North District have no HACCP systems in place and cattle slaughtering, handling,

processing and the sale of the meat (beef) is done under unhygienic conditions. The state of health of animals prior to slaughtering can also contribute to the microbial quality of meat from such animals. These conditions coupled with the high ambient temperature, high humidity, lack of portable water and poor handling practices expose meat to microbial contamination and rapid deterioration. There is no available literature on the level of contamination of fresh beef sold in the Birim North District despite generally poor sanitation in the district, and poorly designed slaughtering, processing and transport facilities for handling raw beef.

It is against this background that this study was conducted to map out processing steps; slaughtering, handling and transportation that are likely to introduce microbial contamination and further assessed the microbial quality of fresh beef sold in the Birim North District of Ghana.

1.2 Objectives

The main objective of the study was to assess the microbial quality of fresh beef sold in the Birim North district in the Eastern Region of Ghana.

The specific objectives were to:

1. Determine the bacterial (*Staphylococcus, Escherichia coli, Salmonella* and Total Viable Count) load in beef sold in the Birim North District
2. Map out processing steps; slaughtering, handling and transportation that are likely to introduce microbial contamination in beef.

1.3 Justification of the Study

Slaughterhouses in Ghana are way behind achieving full implementation of HACCP systems and in an environment soaked with filth and insanitary conditions, microbial contamination is inevitable. In order to improve on hygienic conditions in slaughterhouses and enhance food safety, it is important to assess current hygienic practices of butchers and the microbial load of the meat (beef) they sell to the public. The study was important because Birim North District appeared to be a fast growing district in terms of population and economic activities due to the operations of Newmont Gold Mining Company which has brought its attendant influx of people and their negative impacts on the environment. The result of the study would be beneficial in sense that it would help propose recommendations that when implemented could help reduce the potential risk of foodborne intoxications in the district.

1.4 Limitations of the Study

The main limitations of this study were inadequate financial resources for the microbial analysis of samples, the sparse nature of respondents (butchers) in the district and distance to the laboratory as well as reluctance of butchers to give accurate responses for fear of being sanctioned.

1.5 Organization of Thesis

The report is divided into six (6) chapters. Chapter one (1) deals with the introduction, which gives a background of the study. It also highlights on the objectives, justification and limitations of the study. Chapter two (2) covers the review of relevant literature to the study and the synthesis from the literature. The methodology used to undertake the project is also described in chapter three (3).

Detail results and discussions of all the study components are presented in chapter four (4) and five (5) respectively. The conclusions and recommendations from the results and discussions have been presented in chapter six (6).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Meat as Food

Meat is flesh of animal that is eaten as food (Lawrie and Ledward, 2006). Most often meat refers to skeletal muscle and associated fat and other tissues, but it may also describe other edible tissues such as offals (i.e. meat other than meat flesh, including brain, heart, kidney, liver, pancreas, spleen, thymus, tongue and tripe) (Lawrie and Ledward, 2006; FSANZ, 2002). Conversely, meat is sometimes used in a more restrictive sense to refer to the flesh of mammalian species (pigs, cattle, lambs, etc.) raised and prepared for human consumption, to the exclusion of fish and other seafood. Humans have hunted and killed animals for meat since prehistoric times. The advent of civilization allowed the domestication of animals such as chickens, sheep, pigs and cattle, and eventually their use in meat production on an industrial scale (Robert *et al.,* 2000). Meat is produced by killing an animal and cutting flesh out of it. These procedures are called slaughter and butchery respectively. There is ongoing research into producing meat in -vitro that is, outside of animals (McArdle, 2000).

Meat is composed mainly of water and protein, and is usually eaten together with other food. Though it can be eaten raw, it is normally eaten after it has been cooked and seasoned or processed in a variety of ways. Unprocessed meat will spoil within hours or days. Spoilage is caused by the practically unavoidable infection and subsequent decomposition of meat by bacteria and fungi, which are borne by the animal itself, by the people handling the meat, and by their implements (Tutenel *et al.,* 2003). Meat can be broadly classified as "red" or "white" depending on the concentration of myoglobin in muscle fibre. When myoglobin is exposed to oxygen, reddish oxymyoglobin develops, making myoglobin-rich meat appear red. The redness of meat depends

on species, animal age, and fibre type. Red meat contains more narrow muscle fibres that tend to operate over long periods without rest, while white meat contains more broad fibres that tend to work in short fast bursts. The meat of adult mammals such as cows, sheep, goats and horses is generally considered red, while chicken and turkey breast meat is generally considered white (Lawrie *et al,* 2006). The nutritional composition of red meats changes depending on breed, feeding, season and meat cut. However lean red meat shows consistency in high protein content, essential vitamins and minerals, relatively low fat content and moderate in cholesterol (Williams, 2007). Meat is a complete protein food with all the essential amino acids needed for the human body. It is digested slowly, largely because of the presence of fats. Meat consumption varies worldwide, depending on cultural or religion preferences, as well as economic conditions. Vegetarians choose not to eat meat because of ethical, economic, environmental, and religious or health concerns that are associated with meat production and consumption (Sofos, 2008).

2.2 Beef

Beef is the meat from bovines, especially cattle (*Bos primigenius*). Beef can be obtained from cows (adult female cattle), bulls (adult male cattle), heifers (young sexually matured but unmated female cattle) or steers (castrated male cattle). Beef muscle meat can be cut into steaks, roasts or short ribs or can be processed into corned beef and trimmings, minced or used in sausages. The tail, testicles, tongue and the internal organs such as liver, stomach, pancreas brain, heart, and intestines are other parts that are eaten. Beef harvested from steers have more muscle and less fat than that of heifers. Often older cattle with tougher meat are the ones used for beef when they have past their reproductive prime (Raloff Janet, 2003). Twenty-five percent (25%) of meat produced worldwide is beef and it is the third most widely consumed meat in the world after pork and poultry at 38%

and 30% respectively (Raloff Janet 2003). The United States, Brazil, and China are the world's three largest consumers of beef (USDA, 2009). The world's largest exporters of beef are Brazil, India, Australia and the United States in that order (USDA, 2009).

2.3 Meat Consumption and Related Health Issues

The intake of meat varies widely throughout the world (Speedy, 2003). Available records indicate that overall meat consumption is on the rise in the developed nations of the world and that the U.S. remains the highest consumer of total meat (FAO, 2003). Carrie *et al*. (2011) reported that red meat still represents the largest proportion of meat consumed in the U.S. despite a shift toward increased poultry consumption. They further indicated that only a quarter of the meat consumed in U.S. is processed. On per capita basis, the U.S. is the leading meat consumer in the world with 124kg/capita/year higher than the global average of 38kg/capita/year. Africa and South Asia are the least consumers of meat. Their consumption is between 3 and 5 kg/capita/year (Speedy, 2003). The consumption of meat in Ghana is 9.2 kg/capita/year and this is supplemented by a relatively higher intake of fish (26.2 kg/capita/year) (FAO, 2003; ASNS, 2003). On daily basis in the U.S. and other developed countries, meat takes a significant proportion of the normal diet contributing more than 15% energy, 40% protein, and 20% fat (FAO, 2003; Hiza *et al.,* 2008). The demand for meat in developing countries continues to grow as the production and consumption of meat increases with available income (Walker *et al.,* 2005;

Speedy, 2003). There appears to be an emerging trend in dietary requirements where meat has taken the place of cereals and other foods of plant origin though meat selection and consumption vary by education, race, age, and gender (Krebs-Smith, 1998).

Meat in the diet provides an important source of protein and micronutrients such as iron, zinc, and vitamins (Stipanuk, 1999). However, high intake of meat, fats and sugars in diets coupling with sedentary lifestyle have been implicated in the high rate of obesity and diet-related chronic diseases in the world (Mente *et al.,* 2009). There is direct correlation between high meat consumption and high rates of chronic diseases including cardiovascular disease (CVD) and cancer. Cardiovascular diseases (diseases of the heart) are the current leading causes of morbidity and mortality in the U.S. and other westernized countries (WHO, 2009; Melonie Heron, 2010). According to a report by Cross *et al.* (2007), health risks associated with meat consumption may vary depending on the animal the meat is derived from as well as rearing, processing, and preparation methods. Meat cooking and processing techniques such as smoking, curing, salting or addition of chemical preservatives lead to the formation of carcinogenic compounds, such as *N*-nitroso compounds (NOCs), heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs) (Cross *et al*., 2007). The fat content in red meat and dietary cholesterol has been closely linked to chronic diseases (Lichtenstein *et al.,* 2006). A large body of evidence suggests that vegetarians may be at lower risk for CVD, hypertension, diabetes mellitus, obesity, and cancer (Fraser, 2009). In that case, meat should be eaten in moderation and without too much attendant fat so that it can make a valuable contribution to body development and function (Callow, 2009).

2.4 Meat Quality

The term „meat quality" is used to describe a range of attributes of meat. Many factors determine the quality in meat. It includes requirements of food safety and animal welfare. It also includes the sensory appeal of meat such as palatability (visual appearance, smell, firmness, juiciness,

tenderness, and flavor) and perceived healthiness, especially in relation to the amount and type of fat and other fatty components (Aberle *et al.,* 2001). Quality of meat describes how attractive the meat is to consumers. Meat must look good to consumers before satisfying their palate when they decide to buy it. The expectations of the consumer in terms of aroma, tenderness, juiciness, flavor, colour, wholesomeness and nutrition must be met once the meat is bought, cooked, and served, (Aberle *et al.,* 2001; FAO, 2012). Flavour is interwoven with aroma to bring out the sensation the consumer has during eating. Flavour and aroma are perceptions and depend on the ability to smell through the nose and on the sensations of salty, sweet, sour and bitter on the tongue. Meat flavor is affected by type of species, diet, cooking method and method of preservation (e.g. smoked or cured) (FAO, 2012). The source of flavor in meat is the fat. The different flavors among different kind of meat (beef, pork, chicken, turkey, mutton and chevron) come from fatty components. Fat acts as one of precursors of flavor by combining with amino acids from proteins and other components when heated. The aroma and juiciness of meat products can be improved using spices and cooking method. (Dinh Tran Nhat Thu, 2006).

The tenderness depends on textural characteristics, composition of meat, breeds, sex and many other factors. Tenderness of meat is also based on ease of chewing, which is contributed by the fibrous nature of muscle (Gerrard and Grant, 2003). The appearance of meat is the visual meat quality which is based on colour, marbling and water holding capacity. Marbling is small streaks of fat that are found within the muscle and can be seen in the meat cut. Marbling has a beneficial effect on juiciness and flavour of meat. Colour of meat should be normal and uniform when cut through. Another aspect of meat quality is smell. This will differ slightly based on species and breeds. Meat product should have a normal smell without any rancid or strange smelling odour (FAO, 2012).

2. 5 Microorganisms Found in Meat

Microorganisms are minute living creatures found everywhere in nature and in human environments, including our meat supply. They are too small to be seen with the naked eye unless microscope. Microorganisms include bacteria, yeasts, molds and viruses. Some microorganisms are useful for the production of specialty meat products, while others are pathogenic which means they have the ability to cause meat spoilage leading to foodborne illness (Abaidoo and Obiri-Danso, 2008). Therefore meat should be stored in the coldest part of refrigerator or be stored frozen to prevent contamination by microorganisms. Good hygienic practices are extremely important to prevent microbial contamination in meat and other foods in addition to proper handling, cooking and cooling practices (Doyle, 2007).

2.6 Meat Bacteria of Health Concern

The presence of pathogens in our environment is life threatening and poses serious potential health hazards due to their wide range of diversity and complexity. The ability of some of them to survive and or proliferate under refrigeration and in reduced oxygen concentration and for some pathogens, their low numbers do not debar them from causing diseases (IFT, 2004; Abaidoo and Obiri-Danso, 2008). The way and manner in which farm animals are reared

(husbandry practices), slaughtered, processed and transported to the market influence greatly the microbiological condition of carcass meat. When meat is not properly handled, processed and preserved can support growth of a wide range of microorganisms due to its high nutrients content. Contact between hide and carcass allows a multitude of microorganisms to be introduced into the carcass. These contaminating microorganisms are derived from the animal‟s pre-slaughter environment and may be of faecal, soil, water

or feed origin (Bell, 1997). Certainly, high numbers of microorganisms exist in meat animals intestinal tracts and some of these may find their way to the carcass surfaces during slaughter (Bell, 1997). Table1 illustrates the primary source of these carcass microbes from animal‟s pre-slaughter environment. Raw meat have been found to contain high numbers of micro-organisms like *salmonella, Clostridium perfringens, staphylococcus aureus, Yersinia enterocolitica, Listeria monocytogenes Campylobacter jejuni and Eschericia coli.* Some of these are pathogenic and are often incriminated in outbreak of foodborne disease (Bean *et al.,* 1990). In order to cause a disease, pathogens must successfully invade some parts of the body and either produce more of themselves or produce a toxin which interferes with normal body process (Abaidoo and ObiriDanso, 2008).

Table 1: Sources of bacteria of health concern in meat (Church & Wood, 1992)

Organism	**Principal source**
Staphylococcus aureus	Skin, mucous membranes of handlers
Clostridium perfringens	Soil, intestinal tract
Listeria monocytogenes	Soil, water, air or intestinal tract
Enteropathogenic Escherichia coli	Intestinal tract
Yersinia enterocolitica	Intestinal tract
Salmonella spp.	Intestinal tract

Growth of bacteria on meat is dependent on the storage temperature, pH, moisture content, oxygen availability and the general handling of the carcass. Low storage temperatures results in a significant decrease in the rate of microbial growth as well as a reduction in the diversity of the microbial flora. The fairly high moisture content of meat also supports the growth of wide variety of bacteria. The pH of meat which ranges between 5.3 and 6.5 is ideal for microbial proliferation. Several factors such as feeding and handling practices at the time of slaughter affect the pH of meat (NACMCF, 1993). Food borne pathogens contaminate carcasses and causing a major public health problem. Microbial contamination decreases the shelf-life of food and promotes food borne

illness. Outbreaks of food-borne diseases have led to considerable illness and even death. It is reported that every year from 24 to 81 million cases of food-borne illness are recorded in USA, out of which 50% are associated with meat and poultry (Unneveher, 2000; Gravani, 1987). Out of ten (10) pathogens tracked by FoodNet (a reporting system used by public health agencies in United States that captures food-borne illness in over 13% of the population), *Salmonella*, *Campylobacter,* and *Shigella* are responsible for most cases of foodborne illness. The estimated number of cases and mortality rate of food-borne illness caused by these pathogens are high with *Salmonella* causing 31% of food related deaths, followed by *Listeria* (28%), *Campylobacter* (5%), and *Escherichia coli* O157:H7 3% (Mead *et al.,* 1999). It is estimated that 13.8 million cases of foodborne illness are due to known agents. Out of these cases roughly 30% are due to bacteria. Bacteria are the causative agents of 60% of foodborne illness requiring hospitalization (table 2). It is generally accepted in the scientific community that the true incidence of foodborne disease is under reported and that the international impact of foodborne illness is difficult to estimate (Mead *et al.,* 1999). Nevertheless, about 2.1 million children in developing countries die of diarrheal-related illnesses annually. It is suspected that food or water is the vehicle for many of these illnesses (WHO, 2009). Because food is biological in nature and is capable of supplying consumers with nutrients, it is equally capable of supporting the growth of contaminating microorganisms (IFT, 2004).

Table 2: Foodborne disease in the United States, including estimated annual prevalence, (IFT, 2004)

BACTERIA	POTENTIAL FOOD CONTAMINATION	NUMBER OF ILLNESSES	NUMBER OF DEATHS
Bacillus cereus	Meats, milk, vegetables and fish.	27,360	0

Clostridium perfringens	Meat, meat products and gravies.	248,520	7
Salmonella spp.	Raw meats, poultry, eggs, milk and dairy products, fish, shrimp, yeast, coconut, sauces, salad dressings (i.e., homemade items containing unpasteurized eggs and no or insufficient acidification for destroying pathogens.	1,341,873	553
Staphylococcus aureus	Meat and meat products, poultry, egg products, salads (chicken, potato, macaroni), cream-filled bakery products, milk and dairy products.	185,060	2
Yersinia enterocolitica	Meats, oysters, fish and raw milk.	86,731	2
Shigella spp.	Salads (potato, tuna, chicken, macaroni raw vegetables, bakery products (e.g. in stools, tenesmus cream-filled pastries), sandwich fillings, milk and dairy products, poultry.	89,648	14
Campylobacter spp.	Raw chicken, beef, pork, shellfish and raw milk	1,963,141	99

There are three types of bacterial foodborne diseases: intoxications, infections, and toxicoinfections. Foodborne bacterial intoxication is caused by the ingestion of food containing preformed bacterial toxin, such as the toxins produced by *Staphylococcus aureus* and *Clostridium botulinum*, resulting from bacterial growth in the food. Foodborne infection, on the other hand, is caused by ingestion of food containing viable bacteria such as *Salmonella* or *Listeria* which then grow and establish themselves in the host, resulting in illness. Foodborne toxicoinfections result when bacteria present in food, such as *Clostridium perfringens*, are ingested and subsequently produce a toxin in the host. Some pathogens reside in the intestinal tracts of normal healthy animals and in some instances humans. Certain microorganisms are ubiquitous in nature, occurring on soil

and vegetation, in animal wastes, and on animal carcasses. Human skin surfaces and nasal passages harbor staphylococci. Water supplies may contain pathogens when contaminated with fecal matter (IFT, 2004)

2.6.1 Enterobacteriaceae

The family Enterobacteriaceae is a large, heterogeneous group of gram-negative rods whose natural habitat is the intestinal tract of humans and animals. They are also found in soil and plant from where they can contaminate the food chain and cause food-borne gastroenteritis. They are regarded as indicators of faecal contamination when present in foods and are commonly isolated from hooves and hides of cattle. The genera in the family include *Escherichia, Shigella, Salmonella, Yersinia, Enterobacter, Klebsiella, Serratia, Proteus* and others. The Enterobacteriaceae are facultative anaerobes or aerobes, ferment a wide range of carbohydrates, possess a complex antigenic structure, and produce a variety of toxins and other virulence factors (Abaidoo and Obiri-Danso, 2008).

2.6.1.1 *Escherichia coli*

Escherichia coli also known as *E. coli* refers to a large group of bacteria that is commonly found in the intestinal flora of humans and animals. *Escherichia coli* are gram negative, aerobic rod with certain strains that are pathogenic and produce an enterotoxin, but many of its strains are harmless. The bacteria become pathogenic only when they reach tissues outside of their normal intestinal or other less common normal flora sites. Its infections are generally caused by eating contaminated food, drinking contaminated water, or coming into direct contact with someone who is sick or with animals that carry the bacteria. Raw beef can be an important vehicle in the transmission of *E. coli* during slaughtering, processing or from cross-contamination as a result of unsanitary food handling practices. Its presence in meat is usually a result of faecal contamination or when the

intestinal tract is punctured (Doyle and Shoeni, 1987: PHAC, 2014: Abaidoo and Obiri-Danso, 2008).

Symptoms of *E. coli* infection usually start within three to four days after exposure, but the incubation period can be as short as a day or as long as ten days. The disease which is most commonly associated with travelers show a number of varied symptoms that may vary from person to person. However, they often include severe stomach cramps, diarrhea, vomiting and fever. Proper hygiene and safe food handling such as good slaughtering techniques, hygiene during slaughtering and dressing together with prompt adequate cooling are keys to preventing the spread of all foodborne illnesses including *E. coli* (Church and Wood, 1992; PHAC, 2014). It has been reported in Canada that an average of 440 cases of a certain type of *E. coli* infection occur annually in recent years. The figure 1 below shows the incidence rates of *E. coli* from 2003 to 2013. The number of cases of *E. coli* O157 in 2012 was approximately half that reported in 2006. The data continue to show a downward trend (PHAC, 2014).

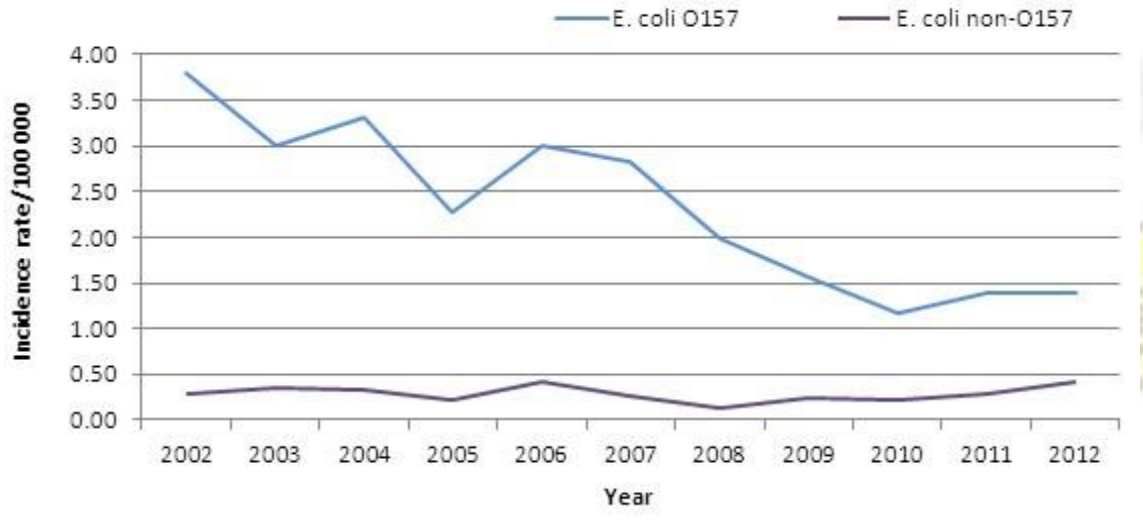

Figure 1: Incidence rate of *E. coli* O157 VTEC and *E. coli* non-O157 in Canada (PHAC, 2014)

2.6.1.2 *Staphylococcus aureus*

For a long time *Staphylococcus aureus* has been known as one of the most important bacteria that causes disease in humans. It is responsible for many skin and soft tissue infections such as abscesses (boils), furuncles, and cellulitis (MDH, 2013). With the right atmosphere for growth and other conditions such as temperature, pH, water activity (aw) and adequate time, contaminating *Staphylococcus aureus* may multiply, and many strains may produce enterotoxins when the population exceeds 10^5 cells/g. An estimated 185,000 cases of foodborne illnesses associated with Staphylococcal food intoxication occurs annually in United States (Mead *et al.,* 1999). More than 50% of healthy individuals carry *Staphylococcus aureus* in the nose and throat, hair and on the skin especially around the hands and fingertips. Coughs and sneezes of individuals with respiratory infections may carry droplet which can easily spread to the environment and food being handled. Therefore any food which requires handling in preparation may easily become contaminated. Infected wounds, lesions and boils of food handlers may also be sources of contamination. However, the two most important sources of contamination to foods are nasal carries and individuals whose arms and hands are inflicted with boils and carbuncles and are permitted to handle foods. *Staphylococcus aureus* also commonly occurs on the skin and hides of animals, and may thus contaminate foods from these animals as a result of cross-contamination during slaughter (Sprenger, 1995; NACMCF, 1993).

Staphylococcal foodborne illness may occur between 30 minutes and 8 hours after ingestion of contaminated food. Common symptoms of staphylococcal intoxication include nausea, vomiting, retching, abdominal cramping, sweating, chills, prostration, weak pulse, shock, shallow respiration, and subnormal body temperature (Sprenger, 1995; IFT, 2004). A number of foods can

support the growth of *Staphylococcus aureus* but food which supports growth best is proteinaceous foods such as meat and meat products, poultry, fish and fish products, milk and dairy products, cream sauces, salads (ham, chicken, potato, etc.), puddings, custards, and creamfilled bakery products (IFT, 2004). The six leading foods, which have been associated with the incidence of Staphylococcal outbreaks, are indicated in Table 3.

Table 3: Leading food sources for staphylococcal gastroenteritis outbreaks in the United States (Bean & Griffin, 1990).

Food source	Number of Outbreaks
Pork	96
Bakery product	26
Beef	22
Turkey	20
Chicken	14
Eggs	9

Institutions such as schools and prisons where food is often prepared in large quantities and held until consumption are often associated with *Staphylococcus aureus* intoxications. Often it is lack of sanitation by workers and improper time-temperature combinations that lead to contamination of the product and growth of the microorganism to levels at which toxin is produced (IFT, 2004). Table 4 indicates the main factors that usually lead to the outbreak of staphylococcal food-borne gastroenteritis. For staphylococcal food poisoning to occur, four things must happen: (1) the food must be contaminated with enterotoxin-producing staphylococci; (2) the food must be capable of supporting the growth of the contaminant; (3) the food must be held at a temperature sufficiently high and for a sufficient period of time to permit sufficient growth to result in the formation of an emetic (vomiting) level of enterotoxin; and (4) the food must be consumed (IFT, 2004).

Table 4: Causes of outbreak of Staphylococcal foodborne gastroenteritis in the United States (Bean & Griffin, 1990).

Causes	Number of Outbreaks
Improper holding temperature	98
Poor personal hygiene	71
Contaminating equipment	43
Inadequate cooking	22
Food from unsafe source	12
Others	24

This pathogen can be controlled by observing proper sanitation at the meat industry, trimming of carcasses to physically remove microorganism, Asepsis, Killing of the microorganism using bactericides and temperature –time control which invariably prevents or delays growth and toxin production (Sprenger, 1995).

2.6.1.3 *Salmonella* species

Salmonella are nonspore-forming, rod-shaped, Gram-negative and predominantly motile enterobacteria with flagella distributed all around the cell body. They are widely spread in nature and are responsible for illnesses such as typhoid fever, paratyphoid fever and food poisoning (Ryan & Ray 2004; Fabrega, and Vila, 2013). Salmonellosis is type of food poisoning caused by *Salmonella* enteric bacteria. For over 100 years, *Salmonella* germs have been known to cause illness. Infections may spread from the intestines to the blood stream, and then to other body sites and can cause death unless the person is treated promptly with antibiotics. The most severely affected people are the elderly, infants, and those with impaired immune systems. (WebMD, 2014: CDC, 2014). Salmonellosis continues to be an important cause of foodborne disease in human

worldwide although total number of cases has remained fairly constant between 1996 and 2002 (CDC, 2003a). Every year, an estimated two to four million cases of foodborne salmonellosis occur in the United States. In the year 2000, it was estimated that 1.3 million cases that occurred cost $2.4 billion in medical costs and lost productivity. Because many milder cases are not diagnosed or reported, the actual number of infections may be greater
(USDA/ERS, 2003).

Salmonellosis is caused by eating food contaminated with *salmonella*, and there are many ways *Salmonella* can enter the food supply to cause illness. Animals may harbor the bacteria making meats, poultry, eggs, and milk often implicated vehicles for salmomnellosis transmission. A number of salmonellosis cases in human are directly linked to human association with animals, both wild and domestic. Foods of animal origin are vehicles for salmonellosis (IFT, 2004). For instance, from 1983 to1987 beef was the major contributor to foodborne diseases from *Salmonella* in the United States (Table 5) (Bean *et al.,* 1990). *Salmonella* was isolated in 19– 54% of cattle carcasses, 1.9% of beef samples at retail and 4.2% of retail chicken samples (Beach *et al.,* 2002; Zhao *et al.,* 2001). The seeming increased incidence of *Salmonella* in slaughter animal is usually associated with transport of animal in dirty vehicles, poor hygiene in the abattoirs and contamination of carcasses by faecal material and intestinal matter. However, according to NACMCF, (1993) the current incidence rates of *Salmonella* on raw beef are rather low (less than 5%). But whether low or high will depend on the conditions of the animal and handling of the animals during slaughter (Hogue *et al.,* 1993). *Salmonella* can also be introduced into the environment particularly soil and water through manure and litter which may persist and contaminate fruits and vegetables on the farm. Cross-contamination in the food service environment or the home during food processing or food handling can also cause salmonellosis.

The *salmonella* bacteria may survive and contaminate foods that are not properly cooked. It is therefore common to have cross-contamination of foods after cooking. Food handlers may transfer *salmonella* from raw products to cook or other uncontaminated foods as a result of unsanitary practices such as poor hygiene (IFT, 2004).

Table 5: Food sources known for salmonellosis outbreaks in the United States, (Bean and Griffin, 1990)

Rank	Food sources	Outbreaks	Percentage
1	Beef	77	9.7
2	Turkey	36	4.5
3	Chicken	30	3.8
4	Ice cream	28	3.5
5	Pork	25	3.2
6	Dairy products	22	2.8
7	Eggs	16	2.0
8	Bakery products	12	1.5
9	Mexican foods	10	1.3
10	Fruits and vegetables	9	1.1

Symptoms of salmonellosis include diarrhea, abdominal cramps, vomiting, and fever, which develop 12 to 72 hours after infection, and the illness generally last from one to seven days (FDA/ CFSAN, 2003b). Due to these serious side effects of Salmonella poisoning and potential fatalities, it is hoped that its presence in food will usually be negative (FDA/ CFSAN, 1999). A number of steps may be taken to reduce the incidence of *Salmonella* contamination of foods. The most common method of eliminating *Salmonella* from food products is heating. *Salmonella* is sensitive to heat and ordinary cooking is sufficient to kill it in high-moisture foods. The pathogen can also be controlled in meat by hygiene during slaughtering and dressing in addition to prompt adequate cooling (IFT, 2004; Church and Wood, 1992).

2.7 Sources of Microbial Contamination of Beef Carcasses

In general, carcass contamination by pathogens is related to a number of activities that occur during pre-slaughtering, slaughtering and post slaughtering operations. The slaughter stock themselves have long been recognized as a major source of carcasses contamination. The hide, gastrointestinal and respiratory tracts of slaughtered animals are the main sources of carcass contamination where potentially pathogenic and spoilage bacteria reside (Sofos *et al.,* 1999a). Slaughtering, dressing and evisceration processes of the beef carcass have also been identified as probable introduction points of major contamination (Anon 1995). When there is contact between carcass and hide a mixture of micro organisms can be introduced onto the carcass.

These contaminating microorganisms are derived from the animal‟s pre-slaughter environment and may be of faecal, soil, water or feed origin (Bell, 1997). Sanitation situation in abattoirs, physical structures, personnel and their equipment also constitute a significant source of contamination. Strict attention to hand-washing practices and the wearing of gloves will minimize the risk from personnel (Meat Technology Update, 2010).

2.7.1 Slaughter Stock

A significant source of carcasses contamination results from the animals themselves (Aberle *et al.,* 2001). The hides, skins, faecal material, hoves and hairs of cattle are major sources of microorganisms. Contamination from hide‟s surface has been found to range from 3.53 to 12.5 log10 cfu/cm² (MTU, 2010). Hayes (1985), found bacteria counts of 10^5 per cm^2 on the hides of cattle. Microbial counts and prevalence of foodborne pathogens on hides is greater than intestinal contents or faeces and is probably due to the high proportion of other organic and inorganic material in faeces contributing a dilution effect on the concentration of microorganisms. Neither normal work practices by trained staff nor the hygiene risk posed by the gut contents can compare to that posed by the microbiological load on the skin of the animal. Generally, carcass from animal with wet hide contains more coliform count. Carcass contamination is significantly lower following contact with clean hides than following contact with faecally soiled hide that had been washed prior to slaughter (MTU, 2010). According to Bell (1997), dressed carcasses can be contaminated with faeces when there is a direct contact with the faeces or as a result of contact with surfaces that have themselves been in contact with faeces such as hides. The report further indicated that in respect of microbial contamination of carcasses, it is the area over which the contamination is spread that gives the most influence on the count obtained but not the weight of faeces. Many micro organisms notably pathogens such as *Salmonella*, *Campylobacter, E. coli* O157:H7 and others are found in the intestinal biota of livestock and poultry (PHV, 2011). There is a risk that intestinal contents may contaminate carcases during evisceration if practices are poor, or if the gut is ruptured. It is therefore recommended that animals are fasted prior to slaughter to reduce the gut volume and reduce the risk of spillage of intestinal content during dressing. However, the fasting times are varied depending on marketing and transport conditions. Prolonged or interrupted fasting may increase the number

of pathogenic bacteria carried by animals and deposited into the lairage and slaughterhouse environment. In cattle, a period of feed withdrawal can cause a rise in rumen pH, which may favour the survival of *Salmonella* and promote a slow rise in faecal *E. coli* content over a 24–48 h period (MTU, 2010). The environmental conditions to which the animals have been exposed to influences the extent of carcass contamination. These environmental conditions include climate, geographic location, husbandry condition, method of transportation, holding condition and animal feed (Cray *et al.,* 1998). Dispatched animals should not have full paunches during transportation to avoid the spread of faecal contamination. Any undue stress caused to the animals during transport or at the lairage can lead to increased spread of pathogens from infected animals to uninfected animals (Church and Wood, 1992: Cray *et al.,* 1998). The sanitary conditions at the lairage and the length of time the animal spends at the lairage are also important as lack of proper care at the lairage can lead to heavy soiling of the animals‟ hides (Sofo *et al.,* 1999).

2.7.2 Slaughterhouse and Equipment

A slaughterhouse or an abattoir is a facility where animals are killed and processed into meat products. In developed countries where there are large abattoir facilities, slaughtering is carried out in fully mechanized lines and carcasses move on a conveyor system from station to station until the slaughter process is completed. In many developing countries however, adequate slaughter facilities are not available. In Ghana for example, majority of the butchers use knives and machete as the main slaughtering equipment (FAO, 1985; Adzitey *et al.,* 2011). At rural or local level slaughtering is often either carried out under a tree or in deteriorated and outdated slaughter units without any waste treatment facilities. This often results in health hazards through contamination of the meat during slaughter operations and of the surrounding land and water through uncontrolled release of waste and effluents. Adequate and regular supply of potable water as well as adequate facilities for treatment, lairage and disposal of liquid and solid waste is important in modern abattoir. The design of the facility should effectively restrict entry of pests,

such as files, rodents, bird, cats, and dogs which contaminate meat with microorganisms by transferring microorganisms from one source to the next or from their droppings (FAO, 1985; ICMSF, 1988).

Severe hygienic problems in the slaughtering of cattle in many places stem from the difficulty in handling these heavy carcasses where there is inadequate or no slaughter equipment available. Essential for the hygienic handling of carcasses and meat is equipment for hoisting the carcasses, when slaughtered. In traditional slaughtering, where carcasses are placed with the back on the ground and the hide serving as protection of the meat surfaces from direct contact to the ground, heavy bacterial loads on the meat through cross contamination cannot be avoided. A wellorganized cleaning, disinfection and sanitation programmes for rooms, machines and equipment is very important to achieve a hygienic standard. Process hygiene, personal hygiene, cleaning and sanitation must be carried out simultaneously to guarantee complete hygienic standard (FAO, 1985). Improper cleaning of equipment has been implicated in outbreaks of foodborne diseases and it is therefore apparent that cleaning and disinfecting processes should be fully enforced and must comply with standard regulations such as Standard Operating Procedures (SOPs) (Gill *et al.,* 1999). For example in the year 1999 Samelis and Metaxopoulos reported that the processing environment are more implicated as a source of *Listeria monocytogenes* than live animals or carcasses. A chief source of *E. coli* deposited on meat during the deboning process appears to be the detritus in equipment which was not removed during daily cleaning (Gill and McGinnis, 2000).

2.7.3 Carcass Dressing and Processing

The way and manner animals are treated prior to slaughter has impact on their meat quality. Long period of stress before slaughter such as a prolonged period of fighting during transport and/or

lairage leads to exhaustion. The sugars are used up so that less is available to be broken down and less lactic acid is produced. The reduced acidity leads to an abnormal muscle condition which darken carcass. The low acidity also favours rapid bacterial growth resulting in meat spoilage. To prevent animals from fighting, animals not reared together must not be put together during transport and lairage. Overloading and under loading during transport of animals should be avoided as overloading causes stress and bruising due to crushing and under loading results in animals being thrown around and falling more than necessary (FAO, 1991).

Animals for slaughter must be clean and should not be slaughtered in full glare of other stock. Basic equipment needed for the slaughtering operation such as stunning gun, knives and matchet may all act as sources of contamination during slaughter (Lawrie 1998; FAO, 1991). The hide of animal contains large numbers of bacteria especially when it is dirty (MTU, 2010). This will result in the knife becoming contaminated when it cuts through the skin. Bacteria then enter the blood stream and spread through the body. Therefore it is important to sterilize the equipment at 82 °C in between cuts of different animals. Inability to sterilize knives and equipment regularly will result in bacteria being transferred from the hide to the carcass and from carcass to carcass (MTU, 2010; Hechelmann, 1995b).

Animals must be stunned by a humane method prior to slaughter. Stunning if properly done makes animals temporarily unconscious so that they will not feel pain during sticking. This reduces struggling of the animal and makes it less hazardous for the operator, and also promotes effective bleeding. Whilst stunning has been embraced by the animal welfare activist as a way of promoting animal welfare, some religious bodies such as Muslims and Jews disagree with stunning on religious grounds. Their religion forbids consumption of meat which was not killed by bleeding and it is difficult to guarantee that animals will not die after being stunned by any particular method

(FAO, 1991). It has been reported that slaughter routine in many parts of the world is sometimes dictated by religious beliefs and local customs (Payne, 1990). In Ghana for example, stunning of animals prior to sticking is not practiced due to the total dominance of Muslims in the slaughtering, butchering and meat business. The butchers perceive that stunning animals before slaughter is against their religion and slaughter requirements. They therefore do not have much knowledge about the stunning of animals and the benefits on the quality and shelf life of meat (Adzitey *et al.,* 2011).

Sticking, severing the major arteries of the neck should immediately follow stunning. The animal is shackled and hanged before the arteries and veins are severed to allow for proper bleeding. The objectives of bleeding are to kill the animal with minimal damage to the carcass and to remove as quickly as possible much blood from carcass. Blood is an ideal medium for bacterial growth. The time between stunning and bleeding should be short to allow natural pumping out of blood as the heart continues beating. The animals should be hoisted to facilitate bleeding and decrease the risk of contamination of the carcasses (FAO, 1991; Hechelmann, 1995b).

The brisket is sawn down the middle. The carcass is then raised to the half-hoist position and when hide removal is complete the abdominal cavity is cut carefully along the middle line. The carcass is then fully hoisted to hang clear of the floor so that the viscera fall out under their own weight. Care must be taken in all operations not to puncture the viscera. All viscera must be identified with the carcass until the veterinary inspection has been passed (FAO, 1991). The main hygiene principle in processing is that clean and unclean operations are efficiently separated. Evisceration can be carried out with minimal contamination of the carcass provided the intestinal tract is not ruptured or punctured (Lawrie, 1998). Tying off the esophagus and enclosing the rectum to prevent

leakage of ingesta and faeces respectively are critical preliminary operations in controlling contaminations during evisceration. Proper evisceration demands that gastrointestinal tract is secured at both esophageal and anal ends and is removed intact (Bell, 1997). Contamination can occur during carcass dressing from the workers, the equipment and from the animal being processed. In most cases, the deep tissues of healthy livestock at the time of slaughter are bacteriologically sterile and contamination is introduced onto the meat surfaces during the dressing process (MTU, 2010).

Adzitey *et al.,* (2011) reported that majority of butchers in Ghana dress their beef carcasses with unclean water on the bare floor in the abattoir and or unclean slaughter slabs which are always smeared with blood, rumen contents and other waste from previous dressing. These practices increase the risk of carcass contamination. The primary object of carcass washing is to remove visible soiling and blood stains and to improve appearance after chilling. It is worth mentioning that washing is no substitute for good hygienic practices during slaughter and dressing since it is likely to spread bacteria rather than reduce total numbers. The objective of carcass dressing is to remove all damaged or contaminated parts and to standardize the presentation of carcasses prior to weighing. Veterinary inspection of carcasses and offal by qualified personnel follows after carcass dressing and washing. In the event of serious signs of disease or damage the entire carcass and offal may be regarded as condemned and must not enter the food chain. No diseased part should be removed by anybody until they have been seen by the inspector otherwise they may mask a general condition which should result in the whole carcass being condemned (FAO, 1991).

2.7.4 Personnel

It is essential for workers in the meat industry to have good health. The human body is a receptacle for numerous pathogenic microorganisms. These microorganisms may be transferred to the meat/food with the risk of causing disease to the consumers. Among the major causes of food contamination are the working practices of food handlers and disease-causing microorganisms present in or on the food handler‟s body (Gordon-Davis, 1998; FAO, 1985). Human beings shed about 1 x 10^3–1 x 10^4 viable micro-organisms per minute and an estimated one in every fifty food handlers sheds around 10^9 pathogens per gram of faeces without showing any symptoms of the related illness (Frazier and Westhoff, 1988; Forsythe, 2000). As much as 10^7 counts of pathogenic microorganisms are present in the fingernails of people handling food due to poor personal hygiene practices such as negligence to wash hands after visiting the washroom (Forsythe, 2000). Genera of bacteria originating from infected food handlers include *Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp., Bacillus cereus,* faecal *streptococci,* and *Shigella spp.* (Lawrie, 1998). Hands of human are the main agents for cross contamination of food within the food handling establishment and usually a true reflection of their environment and habits

(Gordon-Davis, 1998; Jay, 1996). The hands of food handlers are heavily loaded with Staphylococcus micro-organisms due to contact with saliva and other body fluids during spitting, coughing and sneezing. Therefore food handlers should wear gloves while handling food, and must wash hands regularly with soap and hot running water to reduce the microbiological load on hands (Desmarchelier *et al.,* 1999; MTU, 2010). It is ideal to use disposable paper towels for wiping of hands due to its single usage which can help eliminate the possibility of cross contamination (Hobbs & Roberts, 1993). Overalls, hairnets, boots and aprons should at all times be worn by meat handlers. The purpose of wearing overalls is to protect both the food product and the meat handler

from cross contamination (CFIA, 1990; Van Zyl, 1995). Human hair and beards are normally heavily contaminated with bacteria and to prevent contamination of food a hair or beard covering in the abattoir is a necessary part of the working clothes. The clothing of slaughterhouse workers must be clean. The purpose is not to protect the worker against contamination but to protect the meat/food against contamination. Working clothes should be free of loose adornments (buttons, sequins etc.). During work, jewellery, wrist-watches etc. are prohibited as these objects may be sources for contamination and make hand-washing difficult (FAO, 1985).

It has been reported that butchers in the northern parts of Ghana and Ashaiman do not observe adequate hygiene. Personnel at the abattoir do not use and/or wear clean aprons, clothing, boots, mesh gloves and hair cap during meat processing. The poor hygienic conditions during slaughtering and marketing of meats contributed in the isolation of various pathogens in beef, mutton and chevon sold in various markets of the region. Maintaining clean hands, wearing clean protective clothing to cover both body and hair, and using thoroughly cleaned and regularly sterilized slaughtering knives and equipments are requirements for good hygienic practices and production of high quality meat (Sulley, 2006; Soyiri *et al.,* 2008; Adzitey, Teye and Dinko, 2011). Food handlers when engaged in food handling operation must not spit, smoke or use tobacco or similar preparations in areas in which food is handled. During smoking the fingers that handle the cigarette/ tobacco come into contact with the lips and saliva which are potential sources of micro-organisms. These may subsequently be transferred from the hands to the food. Smoking also induces coughing, thus transferring aerosols containing micro-organisms to the food (NSWFA; Burton, 1996; Gordon-Davis, 1998). Training and education of food handlers regarding the basic concepts and requirements of personal and general hygiene can play an integral part in ensuring a safe product to the consumer (Adams & Moss, 1997). Therefore some form of induction training

with regular updating and refresher courses on the risks associated with contamination of food by microorganisms and how it can be avoided will be necessary for the food handler (Nel *et al.,* 2003). Food handlers must undergo periodic medical examinations to ascertain their general health conditions (Ziady *et al.,* 1997).

2.7.5 Dispatch and Transport

During transport of carcass meat unprotected or poorly wrapped and/or packaged meat may be exposed to microbiological agents from the environment or through cross-contamination from other food. Vehicle for transporting meat that is not properly cleaned or maintained may also give rise to chemical hazards (FSA, 2008). Vehicles for transporting meat and carcasses should be considered as an extension of the refrigerated storage. The temperature of meat before loading and during transport should be at or near 0°C. Uninsulated vans and open trucks are not suitable transport for meat particularly in hot climates. This is because in open trucks the meat is exposed to dust and attack from insects. Meat carcasses must be transported under conditions that maintain their wholesomeness (FAO, 1991). The meat carrying compartment of meat transport vehicle, the equipment to be used in the meat carrying compartment and the equipment to be used for the loading of meat and meat product should not be a source of contamination of meat and meat product. They must be cleaned with portable water and disinfected before and after commencement of work each day (AFRSC, 2007)

Poor quality wrapping materials can tear and expose meat to contamination whilst inappropriate wrapping materials may contain chemicals that can taint food. Wrapping materials are to be stored in such a manner that they are not exposed to a risk of contamination. Wrapping and packaging material that can be re-used should be easy to clean and where necessary to disinfect (FSA, 2008).

Live animals, inedible material and condemned material are not transported in meat transport vehicle. Carcasses, portions or red offal may not be transported in the same loading space just as exposed carcasses or meat may not be transported in the same loading space as cartonned products unless in such a way that the packaging material and the manner of packing cannot be a source of contamination for the meat. Persons transporting meat and meat products must practice good personal hygiene (AFRSC, 2007).

The situation in Ghana looks different as Adzitey *et al.,* (2011) and Abuska (2006) reported that the popular means of transporting raw beef carcass from the abattoir to sale points in the Bawku Municipality and Garu-Tempane District respectively are through motor bikes, bicycles, push trucks, basins on butcher‟s head, and on the hands and shoulders of butchers. These transport materials are always smeared with blood from previous use and instead of preventing contaminations they serve as potential sources of contaminants.

2.8 Microbial Control

Animal products including fresh meat are easily contaminated with microorganisms if not properly handled, processed and preserved (Sofos, 1994; Sofos *et al.,* 1999). Contamination with spoilage microorganisms may lead to product and economic losses, while presence of pathogens or their toxins may be the cause of many foodborne diseases that may lead to loss of human life (Sofos, 1994). By limiting the microbial growth, we can considerably prolong the shelf life of carcasses and improve food safety. The presence of pathogens in fresh meat can be reduced or eliminated through animal cleanliness, sanitation and hygienic practices, carcass dehairing and carcass decontamination (Sofos et al., 1999; James *et al.,* 2000). The initial three (animal cleanliness,

sanitation, hygienic practices) are preventive methods most recommended for microbial control and the principle on which the HACCP system is based (ICMSF, 1988; NACMCF, 1993).

2.8.1 Animal Cleaning

In this approach the contamination on the animal‟s external parts is reduced by washing the hide of the animal before slaughter and dressing so as not to further contaminate the carcass (Sofos *et al.,* 1999). Animal washing before slaughter has variable influence on carcass contamination. Factors such as climate, type of animal, and availability of facilities may affect the application of the procedure (Sofos and Smith, 1998a).

2.8.2 Sanitation and Hygiene

Cleaning, disinfecting, insects and rodents control at the abattoir are essential in reducing contamination of meat carcasses. Common chemical disinfectants that are recommended for use include chlorine-containing compounds, aldehydes, quaternary ammonium compounds and oxygen-releasing substances. For cleaning and sanitation to be efficient there should be easy and practical access to all contaminated areas and equipment. Personnel must be regularly instructed and trained in cleaning and sanitation methods. Where cleaning and disinfecting are impossible, there will be a very high level of permanent contamination of the facility (FAO, 1985).

Adequate personal hygiene gives impetus to the overall cleaning process. If sanitation is good and there is poor personal hygiene, microorganisms can be transferred from unwashed hands to well-cleaned surfaces before processing starts and subsequently to meat. Good sanitation, process hygiene and personal hygiene if carried out together in an optimal manner, they will guarantee a complete hygienic standard (FAO, 1985).

2.8.3 Chemical Dehairing

Scientific studies have shown variable results. The process was first applied in an experiment in a commercial beef slaughtering operation to chemically dehair cattle before slaughter and processing. It was reported that the chemical combination used (10% sodium sulphide, water washes, and 3% hydrogen peroxide) did not significantly reduce the naturally occurring bacterial load (total aerobic bacteria and *E. coli*) on carcasses (Schnell *et al.,* 1995). Castillo *et al.,* (1998) used a similar chemical dehairing process but on small hide pieces (not applied to full carcasses) under controlled laboratory conditions, and found a significant (5 log) reductions in numbers of inoculated *E. coli* O157:H7, *Salmonella spp. Coliforms*, and *L. monocytogenes* present in the hide. Chemical dehairing together with other interventions contribute to a reduction in incidence of hide-to-carcass contamination with pathogens such as *E. coli* O157:H7 (Nou *et al.,* 2003). Removing dirt, feces, and hair in a separate room and before hide removal should decrease the risk of transferring pathogens to surfaces of beef carcasses. However, the implementation of chemical dehairing comes with its own challenges; high capital investment, waste management and residual effect of chemical on carcasses.

2.8.4 Carcass Decontamination

Decontamination treatments involve the application of a chemical substance or several other interventions to animal carcasses during the slaughter process to reduce contamination by microbes. Decontamination treatments are not substitutes for good hygiene practices and can only be considered if a substance is shown to be safe and effective. Decontamination processes are effective in reducing contamination on carcasses (Sofos and Smith, 1998a). Carcass

decontamination processes are based on a number of variables including immersion, flooding, cascading, deluging, rinsing, or spray-washing with water or chemical solutions. In general, it is believed that carcass decontamination interventions such as chemical decontamination, thermal decontamination, ionizing radiation, hydrostatic pressure, electric fields, pulsed light, sonication, microwaves and decontamination with multiple processes contribute to the production of carcasses with lower levels of contamination and that reduced incidence of enteric pathogens (Sofos *et al.,* 1999; Belk, 2005).

Chemical solutions that have been used for the decontamination of meat include organic acids, chlorine and chlorine dioxide, trisodium phosphate, hydrogen peroxide and sodium hydroxide. Others are ozone, sodium bisulfate, sodium chloride, acidified sodium chlorite, nisin, potassium sorbate, cetylpyridinium and chloride (Sofos and Smith, 1998a; Belk, 2005). Organic acids solutions such as acetic and lactic at 50-55 ºC have been found to reduce bacterial counts on carcasses in the United States and Canada (Castillo *et al*., 1998b; Smulders *et al.,* 1986). Bacterial counts were also reduced with hydrogen peroxide and ozonated water (Gorman *et al.,* 1995a; Reagan *et al.,* 1996). Several factors including safety, efficacy, product quality, adaptability and cost affect the approval and acceptance of these chemicals for decontamination purposes (Sofos and Smith, 1998a). Exposure of meat carcasses to hot water (>70 ºC) is effective in controlling pathogenic bacteria, including *Salmonella, Y. enterocolitica, E. coli* O157:H7 and *L. monocytogenes* (Castillo *et al.,* 1998b; Gorman *et al.,* 1995a; Smith, 1992). Reagan *et al.,* (1996) reported that washing beef carcass with hot water at high pressure and temperature reduced bacterial counts significantly better than knife-trimming. Another form of thermal decontamination involves exposure of carcasses to pressurized steam (Davidson *et al.,* 1985). Using two or more processes may yield synergistic or additive decontaminating effects (Sofos and Smith, 1998a). For

instance increased water temperatures (50-55 ºC) enhance the effect of acid solutions and lactic acid rinse after hot water washing is more effective than their use in the opposite order (Cutter *et al.,* 1997; Castillo *et al.,* 1998b).

2.9 Synthesis from the Literature Review

Beef is the third most consumed meat in the world contributing greatly to the daily protein intake of human. Despite this, beef provides an ideal nutrition source for micro-organisms that can cause food-borne diseases. Extremely high numbers of micro-organisms (pathogenic and nonpathogenic) are found in meat animals hide and intestinal tracts, and some of these find their way to the carcass surfaces during slaughter, dressing and transport. This transfer may be caused by direct contact or through cross-contamination by slaughterhouse staff, equipment, surfaces, water or aerosols.

By eliminating or limiting the microbial growth, we can considerably prolong the shelf life of carcasses and improve food safety. This requires full implementation of preventive and decontamination programs. In developed countries there are large abattoir facilities and better enforcement of legal regulations on the hygienic standards of handling, processing and transporting meat. In most developing countries however, standard and hygienic methods of handling and processing meats are compromised even though they are enshrined in the country‟s rules and regulations on production and harvesting meat.

In Ghana, particularly small towns including the Birim North Districts most abattoirs and meat retail points operate under poor hygienic conditions. Vehicles for transporting meat are of substandard and poorly cleaned, and the meat itself is poorly packaged/ wrapped. Meat is sold in

open markets on tables that are not well maintained or cleaned after work. This exposes the meat to a number of microorganisms some of which may be pathogenic or non-pathogenic.

In order to ascertain this, the study was conducted to assess the microbial quality of beef sold in Birim North District of Ghana and to identify the possible sources of contamination.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 The Study Area

The Birim North District is in the Eastern Region of Ghana and the capital is New Abirem. The Birim North District was carved out of the former Birim District in 1987 as part of the Ghana Government‟s decentralization programme to promote effective decentralized governance and speed up the development of the area. It is bordered by Kwahu West Municipal to the north, the Asante Akyem South to the west, Birim South Municipality and Akyemansa district to the south and Atiwa and Kwaebibirem districts to the east (Figure 2). The district has a very strategic location especially its capital New Abirem as it is situated among major commercial towns such as Nkawkaw, Oda and Kade. The district is mostly undulating and mountainous in nature. The district is drained mainly by the Pra River. The tributaries of the River Pra include the Nwi, Mamang, Adechensu, Sukrang and Afosu. These rivers and streams have high volumes of water, which is a very good potential in boosting agricultural production in the district. The district lies within the Semi-deciduous forest belt of Ghana comprising tall trees with evergreen undergrowth that experiences substantial amounts of precipitation. The annual amount of rainfall received in the district is between 150 cm and 200 cm. Temperatures range between an average minimum of 25.2 degrees Celsius and a maximum of 27.9 degrees Celsius. The district has a relative humidity of about 55-59 percent throughout the year (http://birimnorth.ghanadistricts.gov.gh). The district is home to forest reserves such as the Ajenua Bepo Forest Reserve. The forest contains large species of economic trees.

The Birim North District like any other District in Ghana is grappling with basic environmental challenges such as drains, sewage systems and waste management. High percentage of the population still lacks access to basic sanitation facilities like toilets, a situation which has resulted in people openly defecating into gutters and water bodies. The major sources of water supply in the District are boreholes, rainwater, wells, public standpipe and surface water (rivers and streams). These sources of drinking water are insufficient to cover the growing demand in the District. Distance to portable water point and the inability to pay water fees compel some households to resort to river and stream water which can lead to water-borne diseases. Two major types of latrines (private and public latrines) are found in the Birim North District. Many households do not have healthy family latrines and as result of that resort to open defecation and toilets that do not comply with hygiene standards. This condition is favourable to the proliferation of diseases. The most common liquid and solid waste discharge system is dumping on a piece of wet land, pouring-out in the streets, compound yards or into streams and rivers. These practices are favourable for the contamination of the environment and spread of disease (BNDA, 2008).

Figure 2: Map of Birim North District (Ghana Statistical, 2010 Population and Housing Census)

3.2 Observation and Checklist

The study methodology also employed visual inspection and observations mainly to determine the processing steps that were likely to introduce microbial contamination in raw beef and also to have a fair idea about the general hygienic practices of butchers. The field observation was guided by a checklist on items and facilities required for good hygienic practices in the handling of raw beef by butchers. Main items on the checklist included conditions of the animal before slaughter, slaughter house facilities and general hygiene conditions, processing practices, personnel, equipment, transportation and sales point in make shift structures.

3.3 Samples Collection

Samples of fresh beef were taken from eight (8) butchers/beef vendors from six (6) communities who supply fresh beef in the District. The meat shops selected were at New Abirem, Afosu, Noyem, Akoase, Pankese and Nkwateng (figure 2). The communities were selected based on the availability of the meat shop and the population density. Freshly cut beefsteaks from the fore or hind limb areas were sampled. Eight samples each weighing 100 g were aseptically collected in sterile polythene pouches, sealed and transported on ice to the KNUST Microbiological Laboratory for microbiological analysis within some few hours of collection. This exercise was repeated weekly for three weeks in April 2014. A total of twenty-four (24) fresh beef samples were used.

3.4 Chemical Reagents

The agars used were products of OXOID Laboratories, Basingstoke Hampshire, England. They included Plate Count Agar used for the isolation of total viable count; Mac Conkey Agar for the estimation of

Escherichia coli; *Salmonella-Shigella* Agar, peptone water and selenite broth for the isolation of *Salmonella;* Mannitol Salt Agar for isolation of *staphylococcus*.

3.4.1 Preparation of Plate Count Agar

Plate Count Agar (Nutrient agar) was prepared by suspending 23.5 grams in 1000 ml (1 liter) distilled water and heated to boil to dissolve completely. It was sterilized at 121°C for 15 minutes in sealed bottle. The sterilized agar was left to cool at 50°C before pouring into sterile Petri plates.

3.4.2 Preparation of Mac Conkey Agar

The medium was prepared according to the method of OXOID. MacConkey Agar powder (52 g) was suspended in 1 L of purified water and mixed thoroughly. The solution was heated with frequent agitation and boiled to completely dissolve the powder. It was sterilized at 121°C for 15 minutes. Sterilized agar was left to cool at about 50 °C before plating.

3.4.3 Preparation of Mannitol Salt Agar

Agar powder (111 g) was suspended in 1 liter of distilled water and brought to boil to dissolve completely. It was sterilized by autoclaving at 121°C for 15 minutes.

3.4.4 Preparation of *Salmonella Shigella* Agar (Ss Agar)

Agar powder (63g) was suspended in 1 liter of distilled water and boiled with frequent agitation to dissolve the agar. It was allowed to cool at about 50°C before pouring into sterile Petri dish.

3.5 Meat Sample Preparation

Ten grams (10 g) of the beef sample was weighed and aseptically taken into a sterile jar containing 90 ml sterile normal diluents. It was homogenized with a pulsifier for 15 seconds and a 1 ml aliquot of homogenate was transferred to a test tube containing 9 ml sterile distilled water to make 10^{-1} dilution and shaken well with vortex mixer. Serial dilutions up to 10^{-4} were prepared for the microbiological analysis.

3.6 Microbiological Analysis

The procedures described below were used to test for presence of microorganisms in beef. Colonies on selected plates were counted using a colony counter. The morphological characteristics of colony such as colour, shape and size were examined to facilitate grouping and identification.

3.6.1 Total Viable Count (TVC)

Total Viable Counts were isolated and enumerated by pour plate method and grown on Plate Count Agar (PCA). Serial dilutions of up to 10^{-4} were prepared by diluting 10 g of the sample into 90 ml of sterilized distilled water. One milliliter (1ml) aliquots from each of the dilutions were inoculated into Petri dishes with already prepared PCA. The contents were swirled clockwise and anticlockwise to thoroughly mix the agar with the inoculums. The plates were then inverted and incubated at 35 ºC for 24 hours. After incubation all white spot or spread were counted and recorded as total viable count using the colony counter.

3.6.2 Enumeration of *Staphylococcus species*

Staphylococcus species were isolated and enumerated by pour plate method and grown on Salt Mannitol Agar (SMA). Serial dilutions of 10^{-1} to 10^{-4} were prepared by diluting 10 g of sample into 90 ml of sterilized distilled water. One milliliter aliquots from each of the dilution were inoculated into Petri dishes with already prepared SMA. The inoculum was evenly spread with a sterile bent rod and allowed to dry for 15 minutes at room temperature. The plates were inverted and incubated at 35 ºC for 24 hours. After incubation yellow colonies were counted and recorded as *Staphylococcus* counts using the colony counter.

3.6.3 Enumeration of *Escherichia coli*

Escherichia coli were isolated and enumerated by pour plate method and grown on MacConkey agar. Serial dilutions 10^{-1} to 10^{-4} were prepared by diluting 10 g of beef sample into 90 ml sterilized distilled water. One milliliter aliquots from each of the dilution were inoculated into Petri dishes with already prepared MacConkey agar. The plates were then incubated at 35ºC for 24 hours. After incubation *Escherichia coli* pink colonies were counted and recorded as *E. coli* counts using the colony counter.

3.6.4 Enumeration of *Salmonella*

Prepared 10 ml of manufactured formula of Buffered peptone water (BPW), Oxoid CM009 (containing peptone 10.0; sodium chloride 5.0; pH 7.2 ± 0.2 at 25 ºC) was in a universal bottle and serial dilution of samples added to it. It was incubated at 37 ºC for 24 hours. Then 0.1 ml of the sample from the BPW was placed in a 10 ml of serenite broth in universal bottle and incubated at 44 ºC for 48 hours. *Salmonella- Shigella* agar (SS agar) was added and incubated for 48 hours at

37 ºC. Cream colonies with black centers on the SS agar indicated the presence of *Salmonella*. Confirmation was done by inoculating single cream colonies with black centers by stabbing in the TSI and incubated at 44 ºC for 24 to 48 hours. Yellow butt, red slant with or without blacking confirmed the presence of *Salmonella*.

3.7 Statistical Analysis

The raw data obtained from the microbiological examination of the carcasses were transferred to Microsoft Excel 2007 spreadsheet. The counts were then transformed to $\log_{10}$ values to make them normally distributed. One–way ANOVA in Microsoft Excel 2007 was used to see the significance ($P<0.05$) of the results of the microbial species.

CHAPTER FOUR

4.0 RESULTS

4.1 Microbial Loads on Beef Carcasses

The study showed that the maximum total viable count (TVC) (expressed as Log10 cfu/g) was observed at Afosu (5.62) and the minimum at New Abirem market east (5.37). The study also showed that the highest *Staphylococcus aureus* count (TSC) was recorded at Noyem lorry station (5.48) and the minimum at Nkwateng (5.29). The highest *Escherichia coli* count (TEC) was recorded at Afosu (5.38) and the least at New Abirem market east (5.07). There was no significant difference between the mean TVC, TSC and TEC counts of all the meat shops/markets ($P<0.05$). The genera of bacteria isolated showed that *Staphylococcus aureus* and *Escherichia coli* had the highest percentage occurrence of 47% each and *Salmonella* species had the least percentage occurrence of 6%. *Staphylococcus aureus* and *Escherichia coli* appeared in all the samples in all the meat shops but *Salmonella* species was identified only in three samples one each in New Abirem market (east), Noyem market and Akoase.

Table 6: Microbial load of raw beef sold in the Birim North District

COMMUNITY	TVC		TSC		TEC		TSSC	
	Mean bacteria count (cfu/g)	Mean log	Mean bacteria count (cfu/g)	Mean log	Mean bacteria count (cfu/g)	Mean log	Mean bacteria count (cfu/g)	Mean log
New Abirem market (east)	$2.37x10^5$	5.37 [b]	$2.27x10^5$	5.36	$1.16x10^5$	5.07 [b]	$2.31x10^3$	3.36
New Abirem market(west)	$3.92x10^5$	5.59	$2.54x10^5$	5.41	$1.57x10^5$	5.2	0	0
Noyem lorry station	$3.87x10^5$	5.58	$3.02x10^5$	5.48 [a]	$1.81x10^5$	5.26	0	0
Noyem market	$3.35x10^5$	5.48	$2.27x10^5$	5.36	$1.56x10^5$	5.19	$4.01x10^3$	3.6
Pankese	$4.10x10^5$	5.61	$2.70x10^5$	5.43	$1.83x10^5$	5.26	0	0
Afosu	$4.23x10^5$	5.62 [a]	$2.77x10^5$	5.44	$2.39x10^5$	5.38 [a]	0	0
Akoase	$3.72x10^5$	5.55	$2.76x10^5$	5.44	$1.51x10^5$	5.18	$4.12x10^3$	3.61 [a]
Nkwateng	$3.84x10^5$	5.58	$1.99x10^5$	5.29 [b]	$1.54x10^5$	5.19	0	0

TVC- total viable count; TSC- total *staphylococcus* count; TEC- total *Escherichia coli* count

TSSC- *Salmonella* count; a – Maximum; b – Minimum

Table 7: Bacteria identified in raw beef in the Birim North District.

COMMUNITY/MEAT	TYPE OF BACTERIA IDENTIFIED		
	STAPHYLOCOCCUS SPP	*ESCHERICHIA COLI*	*SALMONELLA SPP*
New Abirem market (East)	+	+	+
New Abirem market (West)	+	+	–
Noyem lorry station	+	+	–
Noyem market	+	+	+
Pankese	+	+	–
Afosu	+	+	–
Akoase	+	+	+
Nkwateng	+	+	–
SHOP			

+ Means bacteria is present means bacteria is absent

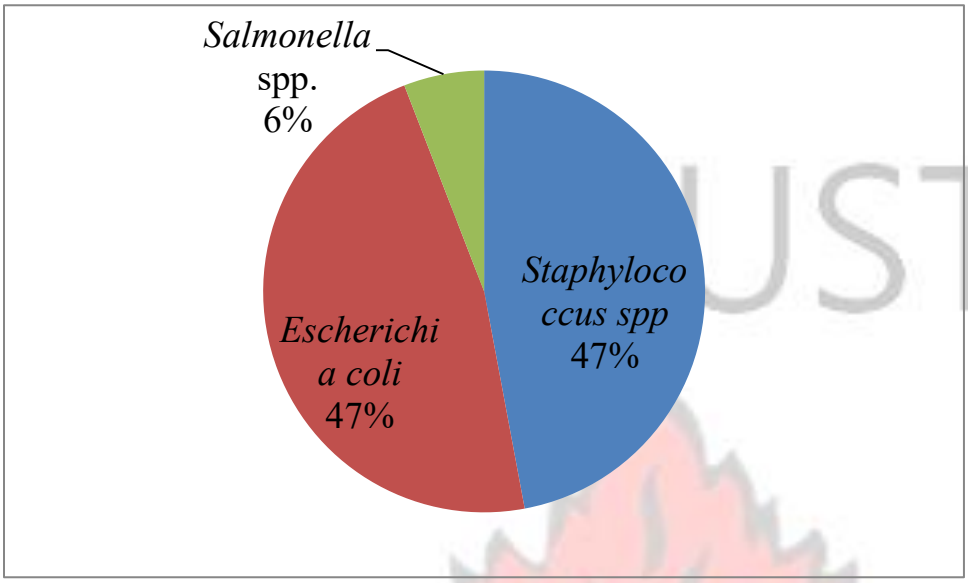

Figure 3: Microorganisms isolated and their percentage occurrence

4.2 Processing Steps that can lead to Contamination of Fresh Beef

4.2.1 Hide of Slaughter Animal

Out of the total animals observed during slaughtering, 72% of the animals had fairly clean hide and the remaining 28% had their hide soiled with droppings and dirt.

4.2.2 Slaughter Inspection

Ante-mortem and post-mortem inspections were carried out by officials of the Environmental Health Unit of the Birim North District Assembly instead of qualified veterinary officer. It was observed that the inspections were not thoroughly done before given approval perhaps the inspectors lacked adequate technical expertise.

4.2.3 Stunning and Bleeding of Slaughter Animals

None (0%) of the animals was stunned prior to bleeding in the study area. However, limbs and head of animals were tied firmly with rope to reduce struggling and ease slaughtering operations. Bleeding was unhygienically done on the ground and ineffective by non- hoisting.

4.2.4 Dressing of Carcasses

All the butchers (100%) dressed their carcasses by singeing with lorry tyre. After singeing, black deposits and singed hairs were scraped off using unsterilized knife or wire mesh. Carcasses were washed with unclean water (turbid water) from a hand dug well. Though, the butchers appeared to be skillful and experienced in the job, some of the carcass visceral components ruptured spilling the intestinal fluids on the meat. The butchers dressed their carcasses on unclean concrete slaughter slab near the slaughterhouse. The slaughter slab was soiled with blood, rumen contents and other waste from previous dressing. It was also observed that after dressing, they leave their meat on the slabs for post-mortem inspection to be carried out before onward transport to the market.

4.2.5 Slaughterhouse Environment

The slaughterhouse building was not used except for keeping some tools like knife and buckets. The study observed high structural defects on the building, inadequate facilities, poor cleaning and sanitary conditions and improper waste disposal. These conditions at the slaughterhouse attracted birds, rodents and insects exuding from the ground unto the meat. In order to achieve safety and quality it is imperative to have adequate cleaning and sanitation systems within the abattoir.

4.2.6 Cleaning of Equipment and Personal Hygiene

Fifty percent (50%) of the butchers cleaned their meat cutting tables and slaughtering equipment by washing with water, sponge and soap or detergent. Thirty seven percent (37%) of the butchers use knife or soft wire mesh to remove dirt on their cutting tables and slaughtering equipment. The remaining 13% apply both cleaning methods. All (100%) of the butchers cleaned their knife and machetes daily after sales. Concerning the cleaning of tables, 63% of the butchers cleaned their tables daily and 37% cleaned their tables at three days intervals. But contrary to the butchers response, a closed observation of tables and equipment appeared that they had not been cleaned for several days. There were blood stains, dirt, and flies having a field-day over the meat.

In table 8, the study observed that 38% of butchers in the study area had water in their service area for washing of hands whilst 25% had water nearby their area of operation. Thirty-eight percent (38%) used soap for washing hands and 25% had some form of towels for wiping hands. Only 25% of the butchers wore aprons over their dress. None of the butchers wore hand gloves and head cover. Seventy-five percent (75%) used nets as screens to protect meat from flies.

Table 8: Hygienic practices of butchers in Birim North District

FACILITIES / PRACTICES	PERCENTAGE OF BUTCHERS
Water for washing of hands in service area	38
Availability of soap for washing hands	38
Towels for wiping hands	25
Use of aprons	25
Head cover	0

Use of nets to prevent flies	75
Use of gloves	0
Smoking	13

With regards to personal hygiene, all the butchers (100%) said they wash and change their clothes every day. They also claimed that they regularly wash their hands with soap and water after any intermittent break. All the butchers have valid health certificate to operate. Thirteen percent (13%) of the butchers at the abattoir smoke cigarette when dressing carcass. For good hygienic practices and production of high quality meat, butchers should maintain clean hands, wear clean protective clothing to cover both their body and hair, and used thoroughly cleaned and regularly sterilized slaughtering knives and equipments.

4.2.7 Transporting Meat to Sale Points

The popular means of transporting carcass from the abattoir to sale points is by the use of taxi. Other means of transport are mini buses (Hyundai H100) and tricycle popularly known as „aboboyaa". All the butchers (100%) use polythene as packaging material. Fifty percent (50%) of the butchers use sacks in addition to the polythene. None of the butchers use meat van to transport his carcasses.

CHAPTER FIVE

DISCUSSION

The results show that raw retail meat is highly susceptible to microbial contamination. Microbial contamination of meat can occur during slaughter, processing and transport. The presence of microbial population in meat is a challenging problem to the meat industry (NACMCF, 1993; Komba *et al.*, 2012). From this study, high microbial counts were enumerated from fresh beef samples which indicated that the beef samples were contaminated. Probable sources of contaminations may include the cutting knives, containers, intestinal contents, water, hides, meat handlers, vehicle for transporting carcasses and the meat processing and selling environments. The study showed that beef sold was contaminated with various genera of bacteria with *Staphylococcus* spp. and *Escherichia coli* being the most abundant (Table 7). The results of this study can be compared with similar studies in Ghana. Soyiri *et al.,* (2008) found various levels and numbers of total bacteria count, *Streptococcus* spp., *Staphylococcus* spp., *Bacillus* spp. and *Escherichia coli* in beef sold in the Ashaiman Municipality of Ghana. Adzitey *et al.,* (2011) also isolated bacteria species (*Escherichia coli, Salmonella* spp., *Staphylococcus* spp. and *Streptococcus* spp.) from raw beef sold in five most popular meat shops in the Tamale Metropolis of Ghana. Microbial contamination of beef samples have also been reported in other parts of the world. In Lahore Pakistan, Ahmad *et al*., (2013) reported high microbial load of *E. coli*, *Staphylococcus aureus* and *Salmonella* in raw meat from abattoirs and retail shops and found no significant differences ($p \leq 0.05$) between the *Escherichia coli* and *Staphylococcus aureus* numbers for the abattoirs and retail outlets of meat.

Considering the mean aerobic plate counts of the beef samples (10^5cfu/g), it suggests that none of the beef from the meat shops was unwholesome for human consumption. For beef to be considered unwholesome the count should be 10^7cfu/g and above (>$1.0x10^6$cfu/g) (Adzitey *et al* 2011; ICMSF, 1988). It was also observed that aerobic plate counts for all the samples which ranged from $2.37x10^5$ to $4.23x10^5$ cfu/g were above the Ghana Standards Board requirements of $1.0x10^4$cfu/g but within the International Commission on Microbiological Specification of Food (ICMSF, 1988) (<$1.0x10^6$cfu/g). Nevertheless the presence of *Salmonella* and *Escherichia coli* which are known foodborne pathogens give cause for public health concern (Soyiri *et al.,* 2008). Consumers of beef from meat vendors in the Birim North District must cook meat adequately at high temperatures (75^{o}C and above) before eating. For purposes of food safety, the Ghana Standards Board requires that there should be no pathogen in all ready to eat foods but in this study *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* spp. could not pass the test of a zero cfu/g which the Ghana Standards Board sets for fresh beef (Soyiri *et al.,* 2008).

The samples taken from Afosu recorded the highest *Escherichia coli* count (TEC) (5.38) and the least was at New Abirem market East (5.07). The high rates in counts at Afosu could be attributed to the dusty nature of the Afosu market where meat shops were located. Beef were also displayed on tables with no wire mesh or net protecting beef from flies. The presence of *Escherichia coli* in the meat samples was as a result of contamination with faecal matter which could be from the environment, flies, materials used including water. The hands of the handlers and contents of the intestinal fluid could also be implicated. The environments in which the meat was processed and sold were not hygienically maintained, thus the presence of the *Escherichia coli*.

The study revealed the prevalence of *Salmonella* in raw beef in Birim North District to be low (6 %). Only three samples one each from New Abirem market East, Noyem market and Akoase meat shops recorded *Salmonella* species (Table 7). Its occurrence was lower than that obtained by Adzitey *et al.,* (2011) who attributed the prevalence of *Salmonella* to the poor handling by butchers, storage and environmental conditions. The finding however agrees with National Advisory Committee on Microbiological Criteria for Foods (NACMCF) (1993) who reported that current incidence rates of *Salmonella* on raw beef are generally low (about 5%). Similar results in which little or no isolation of *Salmonella* in carcasses have been recorded in other studies. For instance, Sofos *et al.,* (1999a) detected 3% *Salmonella* from 30 carcasses in the United States, 1.9% of beef samples in retail beef from the greater Washington D.C. area (Zhao *et al.,* 2001) whiles Korsak *et al.,* (1998) did not recover any *Salmonella* from 310 carcasses sampled and analyzed in Belgium. The incidence of *Salmonella* in beef carcasses in the study area could be attributed to poor transport conditions, poor cleaning and sanitary conditions in the abattoirs, puncture of the viscera resulting in spread of infection and an increase in contamination of carcasses by faecal matter and intestinal fluid. Other potential causes include use of contaminated water in abattoirs for carcass washing, unsterilized equipments and exposure of carcasses to flies.

Staphylococcus spp. was isolated from all the samples and this agrees with studies done by other researchers who also found a high prevalence of *Staphylococcus aureus* in raw meats (Ahmad *et al*., 2013; Soyiri *et al.,* 2008; Desmarchelier *et al.,* 1999). In this study the maximum *Staphylococcus spp* count was recorded at Noyem lorry station ($3.02x10^5$cfu/g) and the minimum count was at Nkwateng ($1.99x10^5$cfu/g). Meat shop at Noyem lorry station was located just by the road side with dust around. Beef were displayed in table kiosk and covered with wire mesh or net.

The high prevalence of *Staphylococcus spp.* is an indication of contamination from meat handlers. Poor hygiene amongst personnel in the meat industry and poor sanitation could be the cause. The study did not find any significant difference ($P<0.05$) between the mean TVC, TSC and TEC counts for all the meat shops.

The presence of these pathogens in beef poses potential health hazards in the district because their low numbers do not prevent them from causing diseases (IFT, 2004; Abaidoo and ObiriDanso, 2008). For instance, *Staphylococcus aureus* can multiply and produce many strains with enterotoxins when the population exceeds 10^5cfu/g (Mead *et al.,* 1999). *Staphylococcus aureus* has been implicated for many skin and soft tissue infections such as abscesses (boils), furuncles, and cellulitis (MDH, 2010). Institutions in the Birim North District such as schools where food is often prepared in large quantities and held until consumption should guard against *Staphylococcus aureus* intoxications (IFT, 2004). It is also worth mentioning that microbial contamination of beef can decreases the shelf-life of beef and further promotes food borne illness (Unneveher, 2000; Gravani, 1987). Mead *et al.,* 1999 reported that bacteria (including *Staphylococcus aureus, E. coli and Salmonella*) are the causes of 60% of foodborne illness requiring hospitalization in the United States and about 2.1 million children in developing countries die of diarrheal- related illnesses annually (WHO, 2009).

Poor hygienic practices in food handling translate to unsafe food product for consumers. The findings from this study suggest that majority of butchers did not adhere to good hygienic practices in their business. In the midst of poor personal hygiene, unsterilized tools and equipment, filth and

poor sanitation cross contamination was unavoidable. Fifty percent (50%) of butchers used soap to clean their tables and equipment, 38% had water in their service area for hand washing and 25% had towels for wiping hands. None of the butchers wore hand gloves and head cover. Seventy-five percent (75%) used nets as screens to protect meat from flies. Thirteen percent (13%) of the butchers at the abattoir smoked cigarette when dressing carcass (Table 8). These findings agree with Adzitey *et al.,* (2011) and Sulley (2006) who reported that butchers in the northern parts of Ghana do not observe adequate hygiene. Personnel at the abattoir do not use and/or wear clean aprons, clothing, boots, mesh gloves and hair cap during meat processing and marketing. Consequently, various pathogens were isolated in beef, mutton and chevon sold in various markets of the region. Because the human body is a receptacle for numerous pathogenic microorganisms which may be transferred to the meat/food (Gordon-Davis, 1998; FAO, 1985), it is important for workers in the meat industry to have good health and observe adequate hygiene. As reported by Forsythe, (2000) as much as 10^7 counts of pathogenic microorganisms are present in the fingernails of people handling food due to poor personal hygiene practices. The hands of food handlers are heavily loaded with Staphylococcus micro-organisms due to contact with saliva and other body fluids during spitting, coughing and sneezing. Therefore food handlers should wear gloves while handling food, and must wash hands regularly with soap and hot running water to reduce the microbiological load on hands (Desmarchelier *et al.,* 1999; MTU, 2010). Disposable paper towels such as tissue paper should be used for wiping of hands due to its single usage which can help eliminate the possibility of cross contamination (Hobbs &

Roberts, 1993). Overalls, hairnets, boots and aprons should at all times be worn by meat handlers (CFIA, 1990; Van Zyl, 1995). Human hair and beards are normally heavily contaminated with bacteria and to prevent contamination of food, a hair or beard covering in the abattoir is a necessary part of the working clothes (FAO, 1985). Food handlers when engaged in food handling operation

must not spit, smoke or use tobacco or similar preparations in areas in which food is handled. During smoking the fingers that handle the cigarette/ tobacco come into contact with the lips and saliva which are potential sources of micro- organisms. These may subsequently be transferred from the hands to the food. Smoking also induces coughing, thus transferring aerosols containing micro-organisms to the food (NSWFA; Gordon-Davis, 1998). Insects, birds, flies, rodents and other pests contaminate meat with microorganisms by transferring microorganisms from one source to the next or from their droppings. They should be controlled through proper design of the slaughterhouse that will effectively restrict their entry, proper waste disposal and good sanitation (FAO, 1985; ICMSF, 1988). The means of

transporting carcass from the abattoir to sale points in the study area is not different from those reported by other researchers in the country (Adzitey *et al.,* 2011; Abuska, 2006). Open tracks instead of meat vans were used. Trucks were not properly cleaned and the meat poorly wrapped in polythene bag or sack. Open trucks are not suitable for transporting meat because it exposes the meat to dust and attack from insects. Meat carcasses must be transported under conditions that maintain their wholesomeness (FOA, 1991). Poorly wrapped and/or packaged meat may be exposed to microbiological agents from the environment or through cross-contamination from other food.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

This study to assess the microbial quality of fresh beef sold in Birim North district has shown that beef sold in the Birim North District is contaminated with *Staphylococcus spp., Escherichia coli* and *Salmonella spp*. but the prevalence of *Salmonella* spp. is low. The mean aerobic plate counts on the beef samples were 10^5cfu/g. The general insanitary conditions at the slaughterhouse, meat shops and poor hygienic practices of the butchers were major contributors to the microbial contamination of the beef. The presence of these microorganisms in the raw beef though not above the permissible limit (10^6 cfu/g) is an indication of public health hazard and gives a signal of a possible occurrence of food borne intoxication and infection if not controlled.

It is therefore recommended that fresh beef from the study area be thoroughly cooked before consumption to prevent food poisoning and foodborne diseases. Standard hygienic practices such as HACCP system should be followed at all stages of the meat production chain. This requires training, education and supervision of meat handlers on the basic concepts of personal and general hygiene necessary to improve behavioural changes among butchers and ensuring a safe product to the consumer. Veterinary doctors should inspect the animals before and after slaughtering, before the meat is sold to the general public. Other relevant institutions such as the Birim North District Assembly should improve facilities at the slaughterhouse to a modern standard and also enforce the bye-laws that ensure good hygienic standard at the slaughterhouse and various meat shops in the district. The results presented in this study and the recommendations if implemented can form a basis for improvement of hygiene in the meat industry and ensure meat safety for consumers in the Birim North District. Finally, it is recommended that for a more comprehensive picture on the

microbial load of beef in the Birim North District, further studies be conducted to include other microorganisms such as *Bacillus cereus, Clostridium botulinum, Clostridium perfringens, Yersinia, Listeria, Campylobacter jejuni* and *Klebsiella.*

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APPENDIX

A CHECK LIST TO MAP OUT PROCESSING STEPS THAT ARE LIKELY TO INTRODUCE MICROBIAL CONTAMINATION IN FRESH BEEF

A. Conditions of animals before slaughter

- Hide…….dirty / very dirty/clean/ very clean
- Health……...Sick/healthy/injured

B. Slaughter house facilities and general hygiene conditions:

- Slaughter house environment including killing and dressing area …….clean/dirty/bushy
- Water source and quality …….pipe/well/flowing water/stagnant water/clear/cloudy
- Chemicals for washing and disinfection……present/absent/frequency of use
- Are there proper facilities for holding materials unfit for human consumption prior to dispatch
- Are wastes disposed in covered litter bin or open disposal near the facility?
- Have steps been taken to reduce the presence of insects near the facility?

C. Processing practices

- Are animals stunned properly?

- Evisceration(is there any spillage of rumen and intestinal fluids on the carcass?) …yes/no

  Bleeding (is there any spillage, splatter of blood and body fluid on carcasses?)……yes/no

- Are sticking techniques and bleeding times satisfactory?
- How is the blood handled?
- Washing of carcass……..source of water/ sanitizers
- Does each carcass go through inspection?
- Who does the inspection?

D. Employees/workers/Butchers at abattoir.

- clothing
- hair cover e.g. hat
- aprons
- boots
- gloves

E. Equipment

- Are sterilizes available for sticking knives and other equipment such as tables, cutting boards, sharpeners and scabbards?
- How often are the equipment sterilize?

F. Transport factors

- Type and cleanliness of conveyance material
- What is the general condition of vehicles? Are there signs of mould growth, algae or dried faeces?
- What distance do the carcasses travel from slaughter house to the sales point?
- What is the condition of road?

G. Local butchers Sales point in make shift structures.

- water for hand washing in service area

- Is it running water or water in basin or gallon? Is the water hot or cool.
- Soap for washing hands

- Towels for wiping hands

- Other sanitary facilities (local detergent)

- Used aprons and/or head cover and

- Use of net/screen to protect meat from flies.

H. Any other observation

- Smoking