

Introduction

Animal resources in the Sudan comprise of sheep, goat, cattle, camel, poultry and wild-game. Establishing a hygienic program for exported mutton is required in order to enable the Sudan facing the international trade parameters. This entails a vital need to improve the slaughter houses and to impose strict hygienic measures to provide healthy and wholesome meat to fulfill the international requirements (International Committee of Microbiological Standards of Foods (ICMSF), 1986; Gracey et al., 1999). Tambool town is one of the famous town in AlGazera State, it is located in eastern part of AlGazera State, near to Rufaa town -35 Kilometers approximately (map). Camel is one of the most fundamental pillars of the national economy and food security for many countries in the world. Camel can provide a substantial amount of high quality meat. The demand for camel meat appears to be increasing due to health reasons, as they produce carcasses with less fat as well as having less cholesterol and relatively high polyunsaturated fatty acids than other meat animals (Knoess, 1977; Mukasa-Mugerwa, 1981; Elgasim et al., 1987; El-Faer et al. 1991; Elgasim and Alkanhal, 1992; Rawdah et al., 1994; Dawood and Alkanhal, 1995).

Meat has been defined as the flesh of animals which are suitable as food (Aberle *et al* 2001). Meat is one of the highly perishable foods because of its high nutritional contents, enzymatic action and the presence of microorganisms (bacteria, yeasts and molds) which may result in oxidative rancidity, discolouration, mouldiness, off flavour, sliminess. The major source of these deteriorative changes being microorganisms, this renders the meat unacceptable and unfit for human consumption (Ajiboye *et al* 2011).

The microbiological contamination of carcasses occurs mainly during processing and manipulation, such as skinning, evisceration, storage and

distribution at slaughter houses and retail establishments (Gill, 1998; Abdalla et al., 2009). Most microbial contaminants of carcasses represent commensal bacteria, some microorganisms such as *Salmonella* spp., *Escherichia coli*O157::H7, and *Listeria monocytogenes* pose a threat to consumer health (Gustavsson and Borch, 1993; Samelis et al., 2001). There were significant increases in total bacterial counts at skinning points than that at washing operations; also, dirty workers hands, clothes and equipments of the slaughterhouse acted as intermediate sources of contamination of meat (Gill, 1998; Gilmour et al., 2004; AbdelSadig, 2006; Abdalla et al., 2009). Ali (2007), recorded high contamination level on flank site and lower contamination level on rump sites during skinning.

In Sudan, hygienic measures to control microbial contamination of meat are unsatisfactorily applied. Storage at refrigerator temperatures is still one of the most effective practices for improving the safety of fresh meat. However, some butcheries still use poor refrigeration, in addition, the retail raw meat in most of butcheries is presented exposed to environmental pollution which might lead to increased bacterial contamination.

Objectives

1. To investigate the microbial contamination of camel raw meat.
2. To identify the main points of contamination of camel carcasses during slaughtering operations.
3. To identify the bacterial *contamination* associated with camel meat in slaughter houses in tambool Town specially *Salmonella* and *E.coli*.

Chapter One

Literature Review

1.1 Sources of Contamination in the Slaughterhouse

Antemortem inspection should remove from slaughter excessively dirty and obviously diseased animals. However, inspection cannot prevent slaughter of stock carrying human pathogens in the intestinal tract or on the hide or fleeces. During slaughter and dressing, head, hide or fleece hocks and viscera are removed. These operations are important. The object is to do this with as little contamination as possible of the exposed sterile carcass tissue and of edible offal. The rumen, lower intestinal tract and the hide and fleece all carry very large numbers of microorganisms. The transfer of contamination through the airborne route is one of the most significant areas of high-care food production (Burfoot *et al.*, 2000). Haines (1933 and Empey and Scott (1939) found that the sources of bacterial contamination of meat are hides, hooves, soil adhering to the hide, intestinal contents, air, water supply, knives, cleavers, saw, hooks, floors and workers. The source of cross contamination exist in the slaughter process, such as processing tools and equipment, structural components of the facility, human contact, and carcass-to carcass contact (IFT, 2002). Thornton (1968) and Ingram (1972) reported that the nature and degree of initial contamination of the carcass surface mainly determined the keeping quality of meat. Prevention of contamination during slaughtering and subsequent processing has, therefore, been Identified as the most important factor in safe guarding the microbiological quality of meat. Camel slaughter operations, such as bleeding, dressing, and evisceration, may expose sterile muscle to microbiological contaminants that are present on the

skin, the digestive tract, and in the environment (Gill and Jones, 1999; Bacon *et al.*, 2000; Abdalla *et al.*, 2009a; Abdalla *et al.*, 2009b). The risk is higher when air is contaminated with eventually foodborne pathogen microorganisms and spores. The risk of contamination derive prior to plant surfaces that includes both product contact and non- product contact surfaces. Airborne contamination should be occurred by indirect contact by means of airborne particles which can be represented by spoilage or pathogen microorganisms (Kang and Frank, 1989).

Frazier (1967) showed that any contaminating bacteria on the knife would soon be found on meat in various parts of the carcass as it's carried by the blood. The contamination of carcasses comes from different sources including: environment and equipments with which meat comes in contact during slaughtering and processing, but hides remain as an important source of contamination. Frazier and Westhoff (1988) reported that the healthy inner flesh of meat contained few or no microorganisms, although microorganisms had been found in lymph nodes, bone marrow and even flesh. They also reported that the important contaminates came from external sources during bleeding, handling, and processing. They pointed out that during bleeding, skinning and cutting the main sources of microorganism's was the exterior of the animal intestinal tract, knives, air, hands and clothes of the workers. During handling, contamination came from cars, boxes and other contaminated meat in chilling storage. During processing contamination came from special equipments (grinders, sausage stuffers and casing) and ingredients in special products (fillers and spices). Main sources of contamination are the slaughtered animals themselves, the staff and the work environment (Belland Hathaway, 1996).

The contamination of equipment, material, and workers' hands can spread pathogenic bacteria to non-contaminated carcasses. Food borne diseases often

follow the consumption of contaminated food-stuffs especially from animal products such as meat from infected animals or carcasses contaminated with pathogenic bacteria as *Salmonella* spp., *Staphylococcus aureus*, *Listeria monocytogenes*, *Campylobacter* spp., and *Escherichia coli* O157:H7. The majority of these germs result from contamination occurring at the where conventional veterinary inspection cannot detect the presence of these bacteria on apparently healthy carcasses (Brown *et al.*, 2000; Gill, 2000). Several studies have shown that most of the contaminants were originally of offal origin and that other microbes, originated from soil and water are involved, through the inevitable contact with handlers' skin. These include *Staphylococci*, *Micrococci* and *Pseudomonas* (Nortije *et al.*, 1990).

Hussien (1971) isolated bacterial contaminants fresh meat from the gastrointestinal tract and hides of the slaughtered animals and from the water, halls and air deposits. Lawrie (1979) reported that if a contaminated knife was used or organisms were inadvertently introduced from the skin where the main blood vessels were severed bleeding could lead to contamination of the tissues. Decontaminating floor and other plant surfaces is most important to control under biofilm, the potential for entrapping and protect the microorganisms against disinfectants. Thus airborne transfer of microorganisms is now seen as a significant route for contamination of food products. The shelf life of products is reduced by air borne contamination. Airborne pathogens can cause serious risk for human health. The sources of airborne microorganisms in slaughterhouse are biological aerosols, dust and other viable and not viable particles (Kang and Frank, 1989). Unless properly cleaned, saws, steel-mesh knives and other equipment carry a high bacterial load and can be sources of contamination. Intestinal tract material (rumen and lower intestine) is most likely to be the major source of *E.coli*,

Salmonellae, *C.jejuni*, *Cl. Perfringens* and other *Clostridia* for carcass and offal. The extent and nature of contamination of carcass and offal meat are reflections of the microbial status of the animal as presented for slaughter, and the care and standards of hygiene and sanitation used (ICMSF, 1998). The inner flesh of meats of poultry and fish from healthy animals contain few or no micro-organisms, although they may be present in other parts of the carcasses. Contamination can occur, however, during slaughtering, handling and processing (Albertsen *et al.*, 1957; FAO, 1962; Matyas *et al.*, 1965).

1.2 Slaughter Processes

1.2.1 Skinning

Bacterial contamination includes the normal skin flora as well as organisms from soil and faeces which are on the skin, and includes *Yeasts*, *Bacilli*, *Micrococci*, *Staphylococci*, *Corynebacteria*, *Moraxella*, *Acinetobacter*, *Flavobacteria*, *Enterobacteriaceae*, *E. coli*, *Salmonellae* and *Listeria* species (ICMSF, 1998). Hocks are removed and incisions through the skin are made along inside of the legs, along the neck, sternum and abdomen and around the anus. Knives and operator's fist are used to separate the skin from the underlying hock and skin become heavily contaminated, as do their knives, steels and clothes. *Salmonellae* can often be found on the hands and equipment of these workers (Smeltzer *et al.*, 1980; Stolle, 1981). The incision through the contaminated skin carries microorganisms on-to the carcass tissue. The knife blade and handle and the hands of the operator these are used to free the skin – transfer organisms mechanically onto the carcass. Bacterial numbers are highest on region of the carcass where the initial manual removal of the skin takes place and lowest where skin is mechanically pulled away (Kelly *et al.*, 1980). Cutting the skin around the anus and freeing the anal sphincter and rectal end of the intestine are major source

of carcass contamination with *E. coli* and *salmonellae*, and presumably also with *C. jejuni*. The hide and skin around the tail are often contaminated with faeces. Care taken during this operation is critical in limiting faecally derived contamination. During mechanical slaughter process of camel, the intestine may be occasionally squeezed through cuts in the abdomen, made from the initial knife incision, and the intestine may rupture contaminating the abdomen and chest regions.

1.2.2 Evisceration

As part of the evisceration process, the brisket is cut, the abdomen is opened, and the organs of the thorax and abdomen are removed. Offal's are separated from the viscera and inspected. Care is needed to prevent puncture of the rumen during brisket cutting. The primary goal of effective slaughter is to protect the essentially sterile muscles of the carcass from becoming contaminated by the gastrointestinal (GI) tract. Since many pathogenic microbes originate in the GI tract and can be present on the hide. The GI tract is the major source of microbial contamination. Leakage of ingesta through the esophagus or from the feces through the anus may lead to contamination of the carcass with pathogenic bacteria. *Compylobacter* can occur in bile (Bryner *et al.*, 1972).

The gall bladder and mesenteric and hepatic lymph nodes can be infected with *Salmonellae*. Normally, *Salmonellae* are found in less than 10% of these lymph nodes.

However in cattle and sheep held for some days in contaminated abattoir environments more than 50% of jejunal, caecal and colonic lymph nodes can harbour *salmonellae* (Samuel *et al.*, 1981). Also more than 7500 *Salmonellae/g* of mesenteric, nodes (Samuel *et al.*, 1980). Incision of lymph nodes can contaminate the hands knives of veterinary inspectors and salmonellae can then

spread to edible tissues. Requirements for lymph node incision have been considerably reduced in recent years. Though salmonellae are occasionally present inside livers, significant contamination of the liver surface occurs during evisceration and separation from other viscera, and from the hands and knives of veterinary inspectors livers and offals become contaminated also with *C. jejuni*. General contamination of the heart, liver and diaphragm of camel has been shown to take place during removal from carcass cavity.

1.2.3Washing

A usual part of the slaughter process to remove bone dust and other material from trimmed carcass, it will also remove bacteria. Raising the temperature of the wash water above 80°C tends to give a better reduction in carcass contamination, but even then the reduction may be small (Patterson, 1968). When a spray system is used to wash carcasses, there is a marked fall in temperature of the water after it leaves the nozzle. When the temperature of sprayed water at impact on the carcass is 56-63°C, the psychrotrophic population is reduced about 10- fold. At impact temperatures of 65°C, the reduction in the mesophilic load still tends to be variable (1log x 0.2-09). Impact temperatures of 80°C and above appear to be needed to give at least a10-fold reduction in the numbers of Mesophiles on carcasses (Kelly *et al.*, 1981; Abdalla *et al.*, 2009).

The addition of chlorine wash water appears to have only a small effect on reduction of contamination (Kelly *et al.*, 1981). Normally there is not more than five- fold reduction in microbial count. Low concentrations of chlorine (20-30mg/L) give some reduction which is not marked changed with increasing chlorine concentration. Populations of *E. coli* on beef were not significantly

reduced by 800 ppm (Cutter and Siragusa, 1995). Both acetic and lactic acid solution, when applied to carcass surface, reduced bacterial contamination. A 1% solution of lactic acid reduced the mesophilic count on beef, veal and pork carcasses between log₁₀ 0.8 and 1.9 both acetic and lactic acid have a residual effect, reducing the rate of microbial growth on chilled meat. However, acid sprays appear to produce little reduction in *E. coli* and *Salmonella* on meat surface (Brackett *et al.*, 1994).

1.3 Micro-organisms which cause contamination of meat

Frazier (1967) found that meat was an ideal environment and culture medium for the growth of bacteria especially when it is minced. Mohamed (1970) suggested that in meat industry, bacteria is classified according to their temperature requirement into three groups.

1.3.1 Psychrophilic

Which grow comparatively and rapidly at temperatures below 5 °C e.g. *Listeria*, *Pseudomonas* and *Streptococci*. The growth of this type is not slowed down by refrigeration.

1.3.2 Mesophilic

Which grow at temperature between 15 and 40 °C it includes most food poisoning bacteria.

1.3.3 Thermophilic

Which grow at higher temperatures 40°C and above. The predominant organisms on the surface of raw meat are *Brochotrix thermosphacta*, *Lactobacillus* species, *Leuconostoc* species, *Carnobacterium* species, *Pseudomonas* species and *Enterobacteriaceae* (Dainty and Mackey 1992; Borch *et al.*, 1996; in't veld 1996; Jay *et al.*, 2003; Nychas *et al.*, 2008). Rodes

and Fletcher (1966) proved that the psychrophilic and mesophilic types of bacteria were the most important ones. Banwart (1981) reported that the gaseous atmosphere surrounding the food may determine the types of organisms which become dominant. Oxygen favours the growth of aerobes while lack of oxygen will allow facultative anaerobes to dominate.

Hudson and Roberts (1979) reported that the pH of camel carcasses affected in the growth of bacterial count than those from normal pH carcasses. Nickerson and Sinskey (1974) found that *Pseudomonas* and *Acentobacter* caused spoilage of refrigerated meat as they grew at $-3^{\circ}\text{C} - 0^{\circ}\text{C}$. Slantez *et al.*, (1963) suggested that the spoilage of fresh meat was associated with the growth of *Proteus*, *Pseudomonas* and *Escherichia*. In addition to Gram –positive bacteria such as *Bacillus* and *Micrococcus* species, Tsubokura *et al.*, (1973), suggested that the meat, particularly offals, contaminated with *Yersinia* organisms constituted an important source of infection.

Field (1948) isolated 257 strains of *Salmonella dublin*, *Salmonella typhimurium*, *Salmonella derby*, *Salmonella enteritidis* when he examined 554 samples of bile from slaughter camel. Hussein (1975) isolated from fresh meat samples *Staph epidermidis*, *Micrococcus* species, *E. coli*, *Proteus* species, *Aeromonas* species, *Pseudomonas species* and *Achromobacter* species. According to Dolman (1967) meat provides excellent medium for staphylococcal proliferation and if the temperature is warm enough only few hours are needed for the production of the effective amounts of enterotoxin.

1.4 Spoilage of Meat

Food spoilage usually refers to the deterioration of quality in food products due to the growth of contaminating microorganisms, although non-microbial activity, such as the activity of endogenous enzymes, can also contribute to food spoilage. The main defects of spoilage are sensory changes, such as off odors and off-flavours, slime production, texture change, discoloration and gas production. Food spoilage processes determine the shelf life of food products, as the products can only be stored until a maximum unacceptable level of off-odour/off-flavours develops (Borch *et al.*, 1996). The properties of meat that are important in determining shelf life include water binding (or holding) capacity, color, microbial quality, lipid stability, and palatability (Renner and Labadie *et al.*, 1994). Deterioration of quality may include discoloration, off-flavor and off-odor development, nutrient loss, texture changes, pathogenicity, and progression of spoilage factors (Skibsted *et al.*, 1994). Meat is a good support for bacterial growth as shown by the numerous reports dealing with the influence of microorganisms on the storage life of meat products. The main property, which explains rapid microbial growth on meats, is its composition: 75% water and many metabolites such as amino acids, peptides, nucleotides, and (sugars Gill *et al.*, 1982).

After slaughter, microbial contamination of carcasses is the consequence of the Processing applied from skinning to conditioning. Processing influences not only the quantity of microorganisms/cm² but also the type of microorganisms present. Spoilage is characterized by any change in a food product that renders it unacceptable to the consumer from a sensory point of view. Microbial numbers are not always related to degree of spoilage, but microbial activity is considered to be of great importance for the manifestation of spoilage (Nychas *et al.*, 1998). The species and population of microorganisms on meat are influenced by animal species, state of health, and handling of live animal ;slaughter practices, plant and

personnel sanitation, and carcass chilling ;fabrication sanitation, type of packaging, storage time, and storage temperature (Nottingham, 1982; Grau,1986). Discoloration, off odors, and slime production are among the deterioration factors caused by bacterial growth (Butler *et al.*, 1953). Gram-positive bacteria are involved in meat spoilage. These include *Micrococcus species*, *Staphylococcus species* *Streptococcus species*, *Lactobacillus species*, *Leuconostoc*, *bacillus species*, *Clostridium species* and *Corynebacterium species* Gram negative bacteria genera reported in cases of meat spoilage included *Pseudomonas*, *Flavobacterium*, *Acinetobacter*, *Klebsiella*, *Salmonella*, *Shigella* and *Proteus* (Gracey and Collins,1992).

1.5 The importance of meat contamination

Fatima (1982) emphasized that pathogenic bacteria found in processed meat which she studied were *Salmonella spp*, *Clostridium perfringens*, *Staphylococcus aureus* and *E.coli*. Gracey (1981) reported that, the organisms responsible for food Poisoning by infection were *Salmonellae*, *Escherichia coli* and *Vibrio parahaemolyticus*. Those responsible for poisoning by toxin production included *Staphylococcus aureus*, *Clostridium perfringens*, *Clostridium botulinum*, *Bacillus cereus* and *Streptococci*. Other bacteria occasionally caused outbreaks of food poisoning, included *Streptococci*, *Proteus*, *Pseudomonas*, *Providencia*, *Citrobacter*, *Aeromonas hydrophilic*, *Yersinia enterocolitica*, *Compylobacter*, *Shigella flexneri*. Hussein (1975) isolated from fresh meat samples; *Staphylococcus epidermidis*, *Micrococcus spp*, *E.coli*, *Proteus spp*, *Aeromonas spp*, *Pseudomonas spp*, and *Achromobacter spp*. no *Salmonella* or coagulase positive staphylococci were isolated. John *et al.*, (1988) reported that *Proteus species* are important in the spoilages of meat, because they grow and

spread readily on moist surface at low temperatures and produce a number of proteases.

According to Holy and Holzopfel (1988) *Pseudomonas* are susceptible to freezing and thawing. Brahmhalt and Anjaria (1993) examined samples of raw meat obtained from shops. They isolate of *E. coli*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Micrococcus luteus*, *Citrobacter freundii*, *Bacillus cerus*, *Streptococcus faecalis*, *Entrobacter aerogenes*, *Proteus mirabilis*, *Bacillus subtilis*, *Aeromonas liquifaciens*, *Proteus vulgaris*, *Klebsiella pneumoniae* and *Pseudomonas deruginosa*. The microbial groups that contaminated fresh beef surface are *Pseudomonas spp*, *Brochothrix*, *Thermosphacta*, *Moraxella spp*, *Lactobacillus spp*, *Flavobacterium spp*, *Vibrio spp*, *Aeromonas spp*, and *Arthobacter* (Gill, 1982). Gracey (1980) stated that the main types of bacteria involved in the spoilage are from the Gram-positive genera *Micrococcus*, *Staphylococcus*, *Streptococcus*, *Lactobacillus*, *Leuconostoc*, *Bacillus*, *Clostridium*, *Corynebacterium* and *Microbacterium*. A total of 71 strain of Gram positive, catalase positive cocci were isolated from 112 abscesses observed during inspection of slaughter animals (sheep, cattle, pigs and goats). Amongst 35 coagulase positive isolate, 30 were classified as *Staph aureus*. Of the coagulase negative isolates 5 were *Staph hominis* and 4 were *Staph xylosus* (Menes *et al.*, 1984). Jay (1986) reported that sausage usually contaminated more varied flora than most other processed meat due to different seasoning agents employed and *Bacillus thermosphacta* was the most predominant spoilage organisms. Most microbial contaminants of carcasses represent commensal bacteria, some microorganisms such as *Salmonella spp.*, *Escherichia coli* O157::H7, and *Listeria monocytogenes* pose a threat to consumer health (Gustavsson and Borch, 1993; Samelis *et al.*, 2001). The members of the genera *Pseudomonas*, *Acinetobacter* and *Moraxella* dominated

the bacterial *content* of unprocessed meat exposed to air at chill temperature (Inter National commission for microbiological specification for food – I .N. C.M.S.F, 1980). Six strains of ureolytic Staphylococci were isolated from rumen of young calves and lambs. Three of them were identified as *Staph xylosus*, *Staph saprophyticus* and *Staph gallinarum* (Laukova and Marounek, 1992) Matthews *et al.* (1989) isolated primarily *Staph xylosus*, *Staph hominis* and *Staph aureus* from bovine origin by using the API *staph*-Trac. The incidence of *Staphylococcus* species in healthy animals was investigated in young and adult individual's cattle, in pigs and in domestic fowl. The samples were taken from Slaughtered animals. *Staph aureus*, *Staph xylosus* and *Staph hominis* were isolated (Shalka, 1991).

A survey was made on the distribution and isolation of *Staphylococcus species* on the skin of humans and 7 kinds of animals (Pigs, horse, cows, chickens, dogs, laboratory mice and pigeons). *Staph xylosus* and *Staph hominis* were isolated (Nagase *et al.*, 2002). Akatov *et al.* (1983), studied the species characteristics of coagulase- negative *Staphylococci*. They isolated *Staph xylosus* from different animals (cows, sheep, hens etc.). Six strain of ureolytic *Staphylococci* were isolated from rumen of young camel. Three of them were identified as *Staphxylosus*, *Staph saprophyticus* and *Staph gallinarum* (Laukova and Marounek, 1992).

1.6 Hygienic measure adopted in slaughterhouse

Meat inspection was practiced in France as early the year 1162, in Britain in about 1319 in Germany special inspection of pigs were started in 1383, while in USA meat inspection was carried out in 1884 (Ibrahim, 1990) . Dickson (1988) and Hennlich and Verny (1990) emphasized that hygienic measures promote the quality and safety of meat and increase its shelf life. Salih

(1969) proposed that in order to improve the standards of meat hygiene should be revised the laws in the study of animals resources in order to include meat hygiene and regulation. He noted that there is lack of proper training of the various staff members working in the meat inspection services. He suggested that programmes should be formulated to improve their academic and technical abilities, and also suggested the establishment of meat research centre where data pertaining to meat hygiene (Number of slaughtered animals, condemnations and reasons for condemnation throughout the country could be collected and analyzed). Regarding the slaughter houses he suggested that they should be run on sound economical basis and they should be able to make some financial benefits. The main objective of meat hygiene and inspection is to prevent meat spoilage and meat borne infections .The meat hygiene ,inspection and control practices are based on the concept of the transmissibility of diseases through either consumption or handling of meat (Ibrahim, 1990). The effective operations of meat hygiene services are multidisciplinary. They involve the veterinary medicine and engineering professions. The veterinarian is the one who is trained to deal with diseases transmitted through meat (WHO, 1957). According to Thornton (1968) the efficient meat hygiene practices, started in the farm. It should be maintained in the animal collection centers, markets, during transportation of animals for slaughter, in abattoirs, during transport of meat to butcheries and even at the consumer's home. To execute such programs necessary laws and guiding instructions should be laid out vividly and firmly .On the other hand basic knowledge about hygiene and sanitation should be disseminated among people especially those directly concerned with meat hygiene and quality control, i.e.

farmers, butchers and consumers. This knowledge would contribute positively to the understanding of laid out. Policies and to establishment of proper standards .it is also necessary to study and asses the influence of social traditions and religion in the community and also the economic and environmental conditions in a particular area for achieving the goals of meat hygiene programs (Kaplan, 1957; Mann, 1960; Echert *et al.* 1981). The many potential routes of contamination during processing include Contamination from human sources, vermin, or the ingredient materials. Food may be contaminated by each other and by pieces of equipment with which they come into contact. Contaminants may build up in numbers on such equipment and constantly transmit seed organisms into the foods. Disease outbreaks due to commercially processed foods are not uncommon (Cockburn *et al.*, 1962; Riemann, 1969).

1.7 Selection of animal for slaughter

The most important considerations are health, kind of animal expected meat yield, and care of the animal prior to slaughter. Fever, increased breathing rate, and diarrhea. Animals suspected of being unhealthy should be treated by a veterinarian until the animal is returned to a healthy state. It is important to exercise proper care of the animal prior to slaughter, if you expect to obtain high quality meat. Pen the animal in a clean, dry place the day before slaughtering. Restrict the animal from feed 24 hours prior to slaughter, but provide access to water at all times. The slaughter of hot, excited animals increases the risk of sickness, injury, and darker meat; therefore, do not run the animal or wrestle with it. Bruises and whip marks cause bloody spots which must be trimmed out. Prior to the day of slaughter, select the slaughter site, accumulate all equipment, prepare for waste disposal, and, if necessary, arrange with a local processor or

meat market for chilling and cutting the carcass. If you plan to have the carcass chilled and make arrangements concerning the time and day on which the carcass can be accepted, the charges, And specific instructions for chilling, cutting, and wrapping.

However, to minimize the losses resulting from transportation, animals should be rested fed before slaughter to regain physiological normality (Houthuis, 1957; Willson and Payne, 1978). Ibrahim (1989) stated that ante-mortem is of a great value in detection of animals suffering from infectious diseases particularly notifiable diseases and emergency cases. It ensures that food animals released for slaughter are in good state of nutrition, cleanliness and free from signs diseases. Johnston (1990) suggested that faecal contamination of the environment can be restricted by correct disposal of animal and human waste .The use of good husbandry methods and the maintenance of high standards of animal health should be encouraged. Many food poisoning out breaks were traced to the consumption of meat from animals slaughtered while obviously ill but whose carcass and organs showed little noticeable change on post-mortem examinations. According to Houthuis (1957) without ante-mortem inspection no adequate inspection of carcass or meat is possible especially in cases of emergency slaughter of a sick animal. The antemorten inspection should be carried out solely by veterinarians who have had long experience of general clinical practice before taking up that type of work. FAO (1962) suggested that if a food animals is encountered during ante-mortem inspection in a moribund state a blood smear should be taken from the animal and stained with poly-chrome methylene blue and examined for Macfadyean reaction .Such measure is to avoid public health implications.

According to the same reference the meat hygiene starts from the animal being on the farm through its journey till it reaches the consumer as fresh, wholesome, sound and safe meat. In the abattoir, ante –mortem inspection detects diseased or

suspected animals for further detailed examination by the meat inspector. Ante mortem inspection is of a great value, for it aids in the detection of animals suffering from scheduled infectious disease like anthrax, rabies and glanders, which are communicable to man (Thornton, 1968). According to Thornton (1973) There are many diseases of toxic or infectious nature which could not be detected in the carcass and organs after slaughter. Ante-mortem is of special value in cases of septic metritis and septic mastitis, sturdy in sheep and tuberculosis meningitis in young cattle, tetanus and rabies. In all these cases the post –mortem findings are of little diagnostic value but the typical symptoms could be recognized during ante-mortem. Indication of disease detected in the live animal calls for its segregation and detailed examination after slaughtering. Ante –mortem inspection is described as the first line of defense against out breaks of food poisoning.

1.8 Sanitary in the slaughterhouse and hygienic in the meat production

It has been shown by many studies that slaughtering under strict sanitary conditions reduces the bacterial contamination of the carcasses (Hess and Lott 1970; Smulders and Woolthuis 1983; Chandran *et al.*, 1986; Dixon *et al.*, 1991).

According to Schutz (1991) the occurrence of hygienic faults and of a high level of microbiological contamination of carcasses in slaughterhouses are due, not to an absence of hygiene equipment or to failure to use what equipment there is, but rather to faulty slaughter techniques. The spread of pathogen can also be reduced by developing slaughter technique. Especially the technique of removing tonsils from pigs (Christensen and Luthje, 1994) and of enclosing the rectum (Andersen *et al.*, 1991) has reduced the pathogen contamination.

According to Gerats (1990), there is an association between slaughter techniques

and the hygienic practice of workers. Those workers who commit many slaughter mistakes neglect hygienic practices. Grats *et al.* (1981) have found an association between the number of Enterobacteriaceae in pig carcasses and hygiene practices connected with slaughter mistakes during evisceration. The hygiene practice of slaughterhouse workers is regulated in many countries by laws (Anon1990; Schutz, 1991; Anon, 1994). The laws do not always distinguish between critical operation and those that have little effect on the hygiene (Huis in't Veld ;*et al.*,1994). There are many factorial complexity of fresh meat quality and shelf life . The microbial quality of the raw material (carcass), the maintenance of cold chain, sanitary condition of premises, equipments (like saws and mincers) and personnel hands and clothes and general management practices were but a few of factors determining the microbiological quality of the product (Nortje *et al.*, 1990).

According to Gracey (1986) all building in the slaughter house must be vermin-proof and kept free from flies. The surrounding area must be well maintained so that there is no risk to the plant from vermin or insects. Also floor and walls should be of smooth impervious material and the corners must be easily and effectively cleanable. Boyle et al (1990) concluded that waste fluids in slaughter houses can support the growth of *L-monocytogenes* .slaughter house temperature should be as low as possible and cleaning and sanitation should be frequent to minimize contamination of meat with this pathogen. The visceral organs in modern abattoirs kept without contact with the hides , skins and feet and their removal after dressing is completely under hygienic conditions (Gracey,1985). Shuppel *et al.* (1996) suggested that the udder should be removed before skinning and it is generally judged unfit for human consumption. Mousing *et al.* (1997) suggested that there are two reasons for implementing, a visual control system. It decreases cross-contamination (no handling, cutting and

incision) and it reduces inspection costs. The resources released as a result may be allocated to hygiene and surveillance programmes.

1.9 The Hazard Analysis Critical Control Point (HACCP)

Food Safety and Inspection Service, USDA, (1998) emphasized that processing operations were presently required to have sanitation standard operation procedures (SSOP`s) and Functional Hazard Analysis Critical Control Points (HACCP) system, to improve food safety through purchase requirements. Jay (1986) explained that, HACCP was a preventive system of control that included a careful analysis of ingredients products and processes in an effort to determine those components or areas that must be maintained under very strict control to assure that the end product meet the microbiological specifications that had been developed. According to Scarafoni (1967) the dirt and skins of animals contribute to 33% of the pollution, the abattoir atmosphere to 5% , the visceral content 3% , transport and storage elements 50%, having quartering and packing of carcasses3%. The HACCPs can be achieved by the following principles (Brown, 2000).

1.9.1 Conduct a Hazard Analysis

Identify the potential hazards associated with food production at all stages up to the point of consumption, assess the likelihood of occurrence of the hazards and identify the preventive measures necessary for their control.

1.9.2 Determination of the Critical Control Points (CCP)

Identify the procedures and operational steps that can be controlled to eliminate the hazards or minimize the likelihood of their occurrence.

1.9.3 Establishment of Critical Limit(s)

Set target levels and tolerances which must be met to ensure the CCP is under

Control.

1.9.4 Establishment of a System to Monitor Control of the CCPs

1.9.4.1 Establishment of the Corrective Actions

To be taken when monitoring indicates that a particular CCP is not under control.

1.9.4.2 Establishment Procedures

For verification to confirm that HACCP system is working effectively.

1.9.4.3 Establishment of a Documentation System

Establish a documentation system concerning all procedures and records

Appropriate to these principles and their application.

Chapter Two

Materials and Method:

2.1 Area of the Study

The study was conducted in Tamboul town in the east of Gezira State and around 150 kilometer sothren Khartoum town in Butana area which occupies the north-eastern Sudan in area extending over 12000 km^2 representing. It's a geographical zone which less approximately between Latitude 130, 40' and 170, 50' North and Longitude 320, 40' and 360, 00' East. It is bounded by the Main River Nile on its northwestern border, the Blue Nile on its southwestern edge, the Atbara River in the northeast and by the railway connecting Kassala and Sennar on the south. Tambool town is one of the famous towns in AlGazera State, located in eastern part of AlGazera state, near to Rufaa town - 35Kilometers approximately. It contains large number of animal species especially camel (Camelus dromedaries).

2.2 Method of collection of samples

One hundred fifty swab samples were collected by using sterile swabs from four sites of carcass, namely shoulder, rump, neck and brisket region at

the point of skinning, evisceration and washing and the hand of workers at the moment of skinning, evisceration and washing also the knife of workers at skinning and evisceration . The study was conducted to determine Salmonella contamination of camel carcasses at slaughterhouses in Tambool town in the period from October to November 2014. A total of 150 swab samples were collected for total viable counts (TVCs) from 10 camel carcasses which were randomly selected and sampled from different site. The samples were collected from carcasses after mechanical slaughter of animals.

Skinning was done manually and then the animals were eviscerated after that they were sprayed with tap water and washed thoroughly , then left to dry, and sent to market. The 10 camel carcasses were randomly selected. From each carcass, 4 swab samples were collected from the brisket, shoulder, neck and rump after skinning, after evisceration and after washing respectively. In addition 5 swab samples were collected from the hands of workers after skinning, after evisceration and after washing and 5 swab samples which were collected from the knives surface. The samples were stored in a cooling box and transported to the laboratory, where the microbiological analysis was performed at the same day.

2.3 Method of Sterilization

2.3.1 Dry heat, hot air oven

The method was used for sterilization of clean glass containers which were wrapped in foil or put in stainless steel cans, at a temperature of 160 °C for one hour (Stainer 1986).

2.3.1.1 Flaming

This was used to sterilize the mouth of bottles, cotton plugged tubes and glass slides. It was done by exposing the object to the direct flame for about half to one second.

2.3.2 Moist Heat

2.3.2.1 Autoclaving

This method was used for sterilization of media and materials that couldn't withstand the dry heat. The temperature was 115 °C -121 °C under 10-15 pounds pressure for 15-20 minutes (Barrow and Feltham, 1993).

2.4 Culture Media

Culture media were prepared according to Bridson (2006), unless otherwise specified.

2.4.1 Liquid media

2.4.1.1 Nutrient broth medium

Thirteen grams of the dehydrated medium (Oxoid) were added to 1 litre of distilled water and brought to boiling until dissolved completely. The PH was adjusted to 7.4 ± 0.2 . Distributed into test tubes as 5ml volumes, and then sterilized by autoclaving at 121 °C for 15 minutes.

2.4.2 Solid Media

2.4.2.1 Nutrient agar medium

Twenty eight grams of nutrient agar powder (Oxoid) were added to 1 litre of distilled water and brought to boiling until dissolved completely. The PH was adjusted to 7.4 ± 0.2 . It was then sterilized by autoclaving at 121 °C for 15

minutes. Then it was aseptically distributed in sterile petri dishes as 15-20ml portions and left to solidify.

2.4 Statistical analysis

The data were analyzed using SPSS software (Statistical Package for the Social Sciences, version 16.0, SSPS Inc. and Chicago, IL, USA). All bacterial counts were analysis and ANOVA was performed. Statistical significance was set at a *P-value* of ≤ 0.05 .

Chapter Three

Results

The study revealed a statistically significant difference at *P-value* ($p \leq 0.05$) at the different operational points between the samples tested from slaughterhouse

after skinning, after evisceration and after washing respectively. As shown in Table 1, the TVC revealed the highest contamination level recorded after skinning was from the neck (12×10^3 CFU/ML) while the highest contamination level after evisceration was from knives ($4, 7 \times 10^3$ CFU/ML) But the highest contamination level after washing was from the neck ($3, 1 \times 10^3$ CFU/ML).

Table 1: Total Viable Counts (cfu/ml) from some sites on camel carcasses at Different operational points at Tambool slaughterhouse.

Sites	Operational points			Significance
	After Skinning	After Evisceration	After washing	
Brisket	10×10^3	$5, 4 \times 10^3$	$1, 3 \times 10^3$	*
Shoulder	11×10^3	$1, 4 \times 10^3$	$1, 5 \times 10^3$	*
Neck	12×10^3	$1, 6 \times 10^3$	$3, 1 \times 10^3$	*
Rump	9×10^3	1.2×10^3	$1, 2 \times 10^3$	*
Knives	8×10^3	$4, 7 \times 10^3$	ND	*
Hands of	7×10^3	$3, 1 \times 10^3$	$1, 4 \times 10^3$	*

Workers

* Statistically significant difference at *P-value* ($p \leq 0.05$).

The study revealed a statistically significant difference at *P-value* ($p \leq 0.05$) at the different operational points between the samples tested from slaughterhouse.

Table 2: Summary of the type and parentage of Bacteria isolated from 10 camel carcasses in the Tambool slaughterhouse.

Type of organisms	Number of isolates from sampled carcasses	Relative frequency of isolate (%)
Pseudomonas spp.	23	18,69%
Staphylococcus aureus	20	16,26%
Bacillus spp.	17	13,82%
Klebsiella spp.	15	12,19%
Escherichia coli	13	10,56%
Micrococcus spp.	10	8,13%
Pasteurella	9	7,31%
Proteus spp.	6	4,87%
Staphylococcus spp.	5	4,06%
Streptococcus spp.	3	2,43%
Salmonella spp.	2	1,62%

Total

123

100.00

* Eleven species of bacteria were isolated and the highest average prevalence was *Pseudomonas spp.* 18.69% and the lower average is *Salmonella spp.* 1.62%.

Chapter Four

Discussion

The level of the TVC is set and agreed to be a criterion for assessing the microbial contamination of carcasses and a useful mean to know the hygienic status of meat (Zweifel and Stephan, 2003). In this study, the TVC ranged from (12×10^3 CFU/ML) to ($1,2 \times 10^3$ CFU/ML) at slaughterhouse. Slaughterhouse had showed TVC above the acceptable value of (10 CFU/ML) set by Decision 2001/471/EC of the EU Commission (Anonymous, 2001). These findings are higher than those reported by Abdalla *et al.*, (2009), who reported a TVC that ranged from ($7,5 \times 10^3$ CFU/ML) to ($0,8 \times 10^3$ CFU/ML) and this could be due to multiple contacts of carcasses with contaminated slaughtering utensils and hands of workers (Nouichi and Hamdi, 2009). Moreover, this study also revealed a statistically significant difference ($P \leq 0.05$) between after skinning, after evisceration and after washing. This finding is similar to what has been found by Gill (1998) who reported bacterial contamination of meat during the different slaughtering operations. The highest level of TVC after skinning was from the neck at the slaughterhouse, (12×10^3 CFU/ML). This could probably be due to that the neck is the first part of the animal to be exposed to the ambient environment. Interestingly the highest level of TVC after evisceration was from the brisket at the slaughterhouse, ($5,4 \times 10^3$ CFU/ML). The possible explanation is that the brisket

gets in contact with the viscera more than any other part of the body. In after wash the highest level of TVC was from the neck at the slaughterhouse, ($3,1 \times 10^3$ CFU/ML) and this could be related to that the carcass is normally washed from up. Another possible explanation to the differences of the points of the highest TVC could be due to multiple contacts of carcasses with contaminated slaughtering utensils and hands of workers (Jeffery, 2003; Nouichi and Hamdi, 2009).

The comparatively high Enterobacteriaceae count in the examined camel samples is an indication of inadequate sanitation during stages of slaughtering, evisceration, washing, transportation, non-cleaned equipment or improper handling. In general, the Enterobacteriaceae were regularly detected on meat surface (Delhalle, et al; 2008).

It was shown in this study that the predominant bacteria isolated were *S.auerus*, *Pseudomonas* spp, *Bacillus* spp. and *E.coli* (Table 2). These micro-organisms can be opportunistic pathogens of humans and were isolated from human clinical specimens of an outbreak of food poisoning (Carter and Cole, 1990; Gracey and Collins, 1994; Holt et al; 1994).

Most microbial contaminants of carcasses represent commensal bacteria, some micro-organisms such as *Salmonella* spp., *Escherichia coli* O157::H7, and *Listeria monocytogenes* pose a threat to consumer health (Gustavsson and Borch, 1993; Samelis et al., 2001). The members of the genera *pseudomonas*, *Acinetobacter* and *Moraxella* dominated the bacterial content of un- processed meat exposed to air at chill temperature (International commission for microbiological specification for food – I.N. C.M.S.F, 1980).

The lowest rates of contamination occurred in critical control points were found to

be in the skinning while the highest rates of contamination occurred on the carcass in the brisket and the lowest contamination occurred in the carcass surface was observed in the rump.

According to Schutz (1991) the occurrence of hygienic faults and of a high level of microbiological contamination of carcasses in slaughterhouses are due, not to an absence of hygiene equipment or to failure to use what equipment there is, but rather to faulty slaughter techniques. The spread of pathogen can also be reduced by developing slaughter technique. Especially the technique of removing tonsils from pigs (Christensen and Luthje, 1994) and of enclosing the rectum (Andersen *et al.*, 1991) has reduced the pathogen contamination.

According to Gerats (1990), there is an association between slaughter technique and the hygienic practice of workers. Those workers who commit many slaughter mistakes neglect hygienic practices. Grats *et al.* (1981) have found an association between the number of enterobacteriaceae in pig carcasses and hygiene practices connected with slaughter mistakes during evisceration.

For long time it was through that it is necessary to ingest 10⁵ or more cells of *Salmonella* per gram of food to cause disease in man. However, studies in recent year found that as low as 3-10 cells / gm cause disease. *Salmonella typhimurium* is more widely distributed than any other serovars, this organism causes severe outbreaks of salmonellosis in all kinds of animals and was frequently the cause of

both sporadic cases and outbreaks of gastroenteritis in man all over the world (ICMSF, 1996).

Involving good sanitary measures during slaughtering processes will lead to the reduction of the amount and/or removal of the microorganisms and other hazards. HACCP should be applied properly during slaughtering operations by using sufficient clean water and safe disinfectants. To make all these, extensive education and training programs for workers should immediately be started. In conclusion, this study revealed that the level of contamination on camel carcasses was much higher than the acceptable value set by the EU Commission.

Conclusion

This study reveals that there was contamination of camel fresh meat in Tambool slaughter house with food spoilage organisms which reduce the quality of meat and pathogenic organisms such as *Salmonella. spp*, *E-coli*, which constitute a public health hazard. Food poisoning bacteria such as *S. aureus* was isolated in most of stages of carcass processing.

Recommendations

1. Each establishment develops and implements written sanitation standard operating procedures (Sanitation SOP's).
2. Regular microbial testing by slaughter establishments to verify the adequacy of the establishments' process controls for the prevention and removal of fecal contamination and associated bacteria.
3. Establishment of pathogen reduction performance standards for *Salmonella* at the slaughterhouse.
4. All meat establishments should develop and implement a system of preventive controls designed to improve the safety of their products, known as HACCP (Hazard Analysis and Critical Control Points).

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