بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

Sudan University of Science and Technology

College of Graduate Studies

Detection of Bacterial Contamination at Butcheries in Elfashir –North Darfor State

كشف التلوث الباكتيري في الجزارات في محلية الفاشر بولاية شمال دارفور

A Thesis Submitted to the College of Graduate Studies in Partial Fulfillment of the Requirements for the Degree of Master of Preventive Veterinary Medicine (MPVM)

Candidate:

Tagwa Mohammed Elhassan

B.V.M (2008) College of Nyala University of veterinary Science

Supervisor:

Professor Mohamad Abdalsalam Abdalla

September 2017

قال تعالى :

Dedication

of Mom and Dad This thesis is dedicated to The Soul

To my brothers and my sister.

Acknowledgment

First and foremost, my heartfelt thanks to Allah for giving me strength and will power to complete this task .

My appreciation and gratitude to my supervisor Professor Mohammed Abdalslam Abdallah, for the continuous supervision , and Professor Seham and my lecturers in Sudan University guidance, suggestions, which led to completion of m y MVSC, study .

I am also thankful to my colleagues in laboratory in Elfashir and staff for their kind assistance and guidance .

My appreciation and thanks to the butchers who allowed me to conduct this study.

Lastly, my heat full appreciation and thanks to my friends and my family members for their support and encouragement .

Abstract

The current study was conducted to investigate bacteries encountered in contamination of beef meat in small butchers in Elfashir city in North Darfor State . However forty swabs samples were collected from butcher's hands , scale , surface (tables + cutting boards) and knives. Total viable count (TVC) of contaminating bacteria preformed beside isolation and identification of bacteria . The result revealed that There was no statistically significant difference between the points ($p \leq 0.05$) . The highest contamination level was recorded on the butchery J 7.27 Log10CFU/ cm^2 . The highest contamination level in knives 7.26 Log10CFU/ cm^2 The contaminating bacteria isolated were *E. coli* in scale 45.5% . Microbial contamination can be carried out by bad handling and poor hygiene, but better facilities and processing units with better hygiene make beef meat have concern for suppliers , consumers and public health officials.

المستخلص

قد أجريت الدراسة الحالية للتقصي عن الجراثيم الملوثة التي يمكن العثور عليها في اللحوم المعروضة في المجازر الصغيرة بمدينه الفاشر بولاية شمال دارفور . تم جمع اربعين عينة مسحة من أيدي الجزارين ,الموازين ,الطرابيز ، والسكاكين . وقد تم حساب إجمالي للعد الحي من البكتيريا الملوثة إلى جانب عزل و معرفة البكتيريا الإيشريكية القولونيه .وكشفت نتيجة الدراسة أنه ليس هناك إختلاف إحصائي (ع أصغر من أو يساوي 0.05) بين المواقع التي أخذت منها العينات . وسجل أعلى مستوى للتلوث في المجزرة J 7.27 لوغريثم شكل المستعمرات المتحده . وأعلى نقطة تلوث من السكين 7.26 لوغريثم شكل المستعمرات المتحده. أيضا أعلى نقطة للبكتيريا الملوثة التي تم عزلها الايشريكية القولونيه من الميزان بنسبة 45.5% . المستوى العالي من التلوث الميكروبي كان بسبب .التعامل السيء للحوم وإهمال النظافة الشخصية وضعف اتباع الإرشادات الصحية ولكن تجويد مرافق المجازر ووحدات الذبح والمعالجة مع النظافة تجعل اللحوم ذات أهمية صحية للموردين والمستهلكين ومسؤولي الصحة العامة .

Table of contents

List of tables

Table of figures

INTRODUCTION

Meat , an excellent source of protein in human diet is highly susceptible to microbial contaminations, which can cause its spoilage and food borne infections in human , resulting in economic and health losses (Ahmed et *al* ., 2013) . Meat is considered as an important source of proteins , essential amino acids , B complex vitamins and minerals. Due to this rich composition, it offers a highly favorable environment for the growth of pathogenic bacteria (Gill , 1998).

Meat products are perishable and unless processed, packaged, distributed and stored appropriately can spoil in relatively short time (Lamia , 2015).

Raw meat may harbor many important pathogenic microbes i.e. *Salmonella spp*., *Campylobacter jejuni/coli*, *Yersinia enterocolitica*, *E. coli*, *S. aureus* and, to some extent, *Listeria monocytogenes*, making the meat a risk for human health, as without the proper handling and control of these pathogens, food borne ill-nesses may occur (Ahmad *et al* . , 2013).

Most foodborne bacterial infections cause self however, systemic infection and death can occur, particularly in vulnerable groups such as the elderly, people with diminished immunity or infants and young children(Thi , 2007) .

Food-borne illness is most often linked to bacteria, and less common to viruses, parasites, prions, and molds (Rahuman , 2007) . Bacteria have accounted for more than 70% of deaths associated with food borne transmission (Thi , 2007). Bacteriological quality of meat products strongly influenced by the prevailing hygienic condition during their production and handling(Osama and Ghan , 2011) .

These bacteria arise from contamination in the slaughter house during processing of live animals into meat where the routine veterinary inspection procedures cannot detect presence of bacteria on meat (Paul and Sylvia , 2014)

Raw retail meats have been identified as potential vehicles for transmitting food-borne diseases, and hence the need for increasing implementation of hazard analysis of critical control point (HACCP) and food safety education efforts (Zhao *et a l* . , 2001).

All countries aim at reducing food borne illness, however, most countries have not stated explicitly to what degree they would like to reduce the number of food borne illnesses in their country. Also , they will have different opinions about how they wish to balance costs with the reduction in food borne illnesses ICMSF (2006) .

Objectives:

- To detection of bacterial contamination in beef in small butcheries .

-To isolate *E. coli* in small butcheries in Elfashir city North Darfor State .

CHAPTER ONE

Literature Reviews

1.1Source of contamination

Beef may be the vehicle of foodborne diseases as a result of deficient sanitary conditions during animal slaughter (Hassanien *et al* . , 2006)

Pathogenic micro-organisms are found in the digestive tract of healthy cattle and sheep. These micro-organisms are excreted in the feces and can be found on the hides and fleeces of the live animal. Bacterial contamination of the fleece/hide can then be transferred onto previously sterile meat surfaces during slaughtering and dressing especially when slaughtering performed on the floor with absence of a carcass suspension system with careless evisceration that spreads intestinal content onto the meat surface. Bovine carcasses can be contaminated during the slaughter process through the contact with the animal's skin and hair, limbs, blood, stomach, gut contents, bile and other excretions, facilities, equipment, and hands and worker's clothes (Hassanien *et al* . , 2006) .

The initial microbial population on meat depends on the physiological state of the animal at the moment it is slaughtered and on the level of environmental contamination in the slaughterhouse and areas in which subsequent handling of the mexcoat is performed , including the level of hygiene of employees and the tools and equipment used (Josef , 2013) .

Fecal matter was a major source of contamination and could reached carcasses through direct deposition as well as by in direct contact through contaminated and clean carcasses equipment ,workers installation and air (Abdalla *et al* .,2009) . cattle slaughter operation

,such as bleeding , dressing and evisceration expose sterile muscle to microbiological contaminants that were present on the skin ,the digestive tract and in the environment (Abdalla *et al* . , 2009).

The meat, available at retail outlets comes through a long chain of slaughtering , and transportation , where each step may pose a risk of microbial contamination . The sanitary conditions of abattoirs and its surrounding environment are major factors contributing in bacterial contamination of meat (Ahmad *et al*. ,2013)

There were several genera of bacteria specially associated with the hands and nasal cavities and mouth, the important of there are *Micrococcus and Staphylococcus,* (Bryan, 1978 ; Jay, 1986).

Some of these bacteria may include pathogens. These are the food poisoning microorganisms causing food borne infection and intoxication or spoilage bacteria causing discoloration, bad odors and slime on meat surfaces. If the bacteria on meat include pathogens like *E. coli*, *Salmonella, Staphylococcus* etc. there could be a risk to human health. Members of the gram negative bacteria e.g. *E. coli* are widely distributed in the environment and are the major sources for food contamination. The possible sources of these bacteria are skin of the animal, the equipments used for each operation, clothes and hands of personnel and the physical facilities themselves (Farhana *et al*. , 2015).

Bacterial food borne infections occur when food, that is contaminated with bacteria, is eaten and the bacteria continues to grow in the intestines, setting up an infection which causes illness. *Salmonella, Campylobacter,* hemorrhagic *E.coli* and *Listeria* all cause infections (Kendall , 2012).

Assessment of the hygienic risk a beef slaughtering process should involve enumeration of organism in dicative of fecal contamination,

such as *E.coli,* at specific points in the process and/or cross-contamination during slaughtering operations were demonstrated and the results indicated presence of bacteria of potential public health significance (Abdalla *et al* ., 2009) .

Wahib (2004) showed that any contaminating bacteria on the knife would soon be found on meat in various parts of the carcasses as it is carried by the blood .

Several studies have addressed the sources and potential control measures of bovine meat contamination by Salm*onella* and pathogenic *E. coli* at different stages of the meat chain i.e. primary production (Eugène *et al* . , 2015) Both Salmonella and Shiga toxin–producing E. coli (STEC) have been found to contaminate carcasses at commercial beef processing facilities (Norasak *et al* . , 2014).

Jepsen (1967) noticed that bacteria were carried to the abattoir on skin, hooves and body cavities of animals.

Lamia (2015) isolated bacterial contaminant from fresh meat of the gastro internal tract and hides of the slaughtered animals and the water, halls and deposits.

Outbreaks of food borne diseases are caused by foods that are contaminated intrinsically or that become contaminated during harvesting, processing or preparation (Yah *et al* . , 2009).

Food contamination with these pathogens can occur at multiple steps along the food chain, including production, processing, distribution, retail marketing, and handling or preparation. Numerous epidemiological reports have implicated foods of animal origin as the major vehicles associated with illnesses caused by food-borne pathogens (Cuiwei , 2001).

Contaminated raw or undercooked poultry and red meats are particularly important in transmitting these food-borne pathogens. Foods commonly involved include eggs or any egg-based food, salads (such as tuna, chicken, or potato), poultry, beef, pork, processed meats, meat pies, fish, cream desserts and fillings, sandwich fillings, raw sprouts, and milk products. These foods may be contaminated at any of the many points where the food is handled or processed from the time of slaughter or harvest until it is eaten (Kendall , 2012).

Other sources of human infections with *Campylobacter*, *Salmonella*, and STEC include contaminated produce and contact with farm animals and pets. Person-to-person transmission has also been described (Cuiwei , 2001) .

Utensils, equipment's and cutting board surfaces were identified as a major source of contamination in meat processing plant (Rahman , 2007) .

The hands, hair, nose, and mouth harbor microorganism that can transferred to the food during processing, packaging, preparation, and service by touching, breathing coughing, or sneezing (Marriot and Gravani, 2006) .

Jay (2000) suggested that the microorganism, individually and as a group, grow over a very wide range of temperatures, therefore, it is well to consider at this point the temperature growth ranges for organisms of importance in foods as an aid in selecting the proper temperature for the storage of different types of foods. The lowest temperature at which a microorganism has been reported grow is -34^{o}C, somewhere in excess of 100^{o}C, it is customary to place microorganisms into three groups based on their temperature requirement for growth in the following:

- Those organism that grow well at or below 7^{o}C and have their optimum temperature between20° C and 30^{o}C are referred to as psychrophiles.
- Those that are grow well between 20^{o}C and 45^{o}C with optima between 30° C and 40° C are referred to as mesophiles.
- Where those that grow well at and above 45^{o}C with optima between 55^{o}C and 65^{o}C are referred to as thermophiles .

Roders and Fletcher (1966) noted that sychrophilic and mesophilic type of bacteria are the most important. Temperature is seen to be the most important factor influencing spoilage and meat safety (Josef , 2013) .

Gihan (2004) found that the sources of bacterial contamination of meat are hides, hooves, soil adhering to the hide, intestinal contents, air, water supply, knives ,cleavers, saws, hooks, floors and workers .

A wide variety of foods have been implicated in *E. coli* O157 outbreaks. These include apple juice (Nobuyasu *et al* . , 2003) .

Gracey (1985) reported that bacteria associated with meat depend on bacteriology of the soil on which the animals were kept prior to slaughter. The were transferred to the hides and then to the exposed meat. Bacteria .

Wahib (2004) reported that there are different sources of meat contamination for example, invasion of blood vessels by bacteria from the intestine of weakened or ill animals just prior to slaughter. The animal’s digestive tract was claimed to carry dangerous load of bacteria actual contagion with dirty hands, clothing and equipment’s are important factors in the presence of bacteria in frozen meat in chilling storage .

Cattle are regarded as the primary reservoir of *E. coli* O157:H7,with fecal prevalence rates of 2 to 24% being reported (Mcgee *et al* . , 2004).

George and Banwart . (1989) isolated from fresh meat samples, *Staph epidermidis, Micrococcus spp. Escherichia coli, Proteus spp* .

Fatima (1982) isolated *Sallmonella spp., Clostridium perfringes, Staphylococcus* and *E. coli* from processed meat.

Yah et al . (2009) reported that *S .aureus* and *E. coli* contaminated in minced meat and sausage rolls .

The contamination of meat by microbial pathogens can occur at any stage of the meat chain (Eugène *et al* . , 2015) Furthermore, the prevention or mastery of meat contaminations can be carried out at a stage of the chain different from the stages at which the contamination has occurred (Eugène *et al* . , 2015) .

Lawri (1991) reported that if a contaminated knife was used or organisms were in advertently introduced from the skin where the main blood vessels were severed could as source of contamination of tissues.

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* SPP.

According to Dolman (1967) meat provide excellent medium for Staphylococcal Proliferation and if the temperature is warm, enough only few hours it needed for the production of effective amount of enterotoxin.

In Sudan, Salih (1971) isolated from fresh meat samples spoilage bacteria of genera *Micrococcus, Streptococcus, Bacillus, Coli aero*

genes. He also isolated hemolytic and coagulase positive *Staphylococci* from ovine and bovine liver and rumen samples obtained from Omdurman Central Abattoir and isolated *Micrococci* and *Salmonella Doblin* from ovine and bovine offal's.

1.2 The importance e of meat contamination:

Enterohemorrhagic *Escherichia coli* (E H E C) has been recognized as a major food-borne pathogen. Large outbreaks of EHEC infection have occurred throughout the world. One of the largest *E. coli* O157:H7 (one of the serotypes of EHEC) outbreaks associated with food consumption occurred in Sakai City, Japan in 1996. Almost 10,000 people were afflicted in this outbreak (Nobuyasu *et al*., 2003).

Dolman (1967) review that *streptococci* as cause of food poisoning and reported that meat can serve as vehicle.

B.cereus causes food poisoning characterized different syndromes (emetic and diarrhoeal) .

The members of genera *Pseudomonas, Actinobacter and Moraxella* dominated the bacterial content of unprocessed meat exposed to air chill temperature (ICMSF 1980) .

Escherichia coli are bacteria that cause food poisoning. Food infection is the second type of foodborne illness. It is caused by eating food that contain certain types of live bacteria which are present in the food. Once the food is consumed, the bacterial cells themselves continue to grow and illness can result, *Listeria* is a good example of foodborne infection. Each year, thousands of individuals suffer from the discomfort and pain resulting from foodborne illness. True food poisoning or food intoxication caused by eating food that contains a toxin or poison due to bacterial growth in food (Lubnam and Ghadorm , 2012) .

In United states, it has been estimated that seven pathogens found in animal products such as *Escherichia coli* 0157 :H7, *Listeria monocytogenes, Campylobacter jejuni, Clostridium perfringes, Salmonella* spp., *Toxoplasma gondii* and *Staphylococcus aureus* account for approximately 3.3 - 12.3 million cases of food borne illnesses and a record of 3900 deaths each year (Yah *et a l.* , 2009) .

Meat becomes contaminated with clostridia at the slaughterhouse. The sources of this contamination are particles of soil that remain attached to the skin or excrement. Clostridia get onto the surface of carcasses by means of direct or indirect contact. Psychrotrophic clostridia occur in the slaughterhouse environment and on the surface of meat in the form of spores. It is clear from the literature that even a single spore may cause “blown pack spoilage” BPS-type spoilage (Josef , 2013) .

Wahib (2004)examined samples of raw meat obtained from shops, they isolated *E coli, Staphylococcus epidermidis, Staphylococcus aurus, Micrococcus luteus, Citrobacter freundii Bacillus cereus, Sterptococcus faecalis, Enterobactere aerogenes, Proteus mirabilis Bacillus subtilis, Aeromonas liguifaciens, Proteus valgaris, Kelebsilla poneumonae and Pseudomonas deruginosa* .

John *and* Anthony (1974) stated that *Lactobacteriaceae* may be the eventual cause of meat spoilage, under some condition in meat handling, where it enters the product through contamination from plant equipment or handlers of the product.

Lawarie (1991) found that the organisms derived from infected personal or healthy carriers include *Sallmonella SPP., Shigella Spp., Escherichia coli, Bacillus, Proteus, Staphylococcus albus and*

Staphylococcus aurues, Colstridium welchii, bacillus cerues, Bacillus faecal and Streptococcus spp.

Some bacterial species firstly cause change in sugars by oxidation and produce a fluorescent pigment; others pro-duce alkali. The psychrotrophic *Pseudomonas* strains are specific spoilage organisms (SSO) of meat, poultry and fish, detected by analyzing the nitrogenous components producing the volatile compounds (aldehydes, ketones and esters) responsible for the off-flavor produced at the point of spoilage(Abdel-Elaziz ,2015).

Soyiri *et al*. (2008) reported that the butchers of retail beef in Asaiman market –Ghana, which under unhygienic practices and poor handling of beef contaminated with aerobic *mesophiles, Staphlococus aureus Bacilus cereus ,Clostridium perfringens and Escherichia coli* .

Khalid (2004) reported that the contamination of beef in Khartoum slaughter house by the aerobic bacteria from the Gram-positive genera were *Micrococcus sp .*, *Staphylococcus sp. and Bacillus cereus*, while the Gram-negative species was *E.coli*.

At each stage of beef processing after slaughtering, different microbes get introduce and these tend to contaminate the meat (Soyiri *et al*., 2008) .

The microbes cause biochemical and microbiological changes in the meat which may lead to the production of noxious substances resulting in the incidence of illnesses such as cholera, typhoid fever and other fatal diseases. Besides the chemical composition, meat culinary and technological value is determined by its physicochemical properties and one such main indicator is pH (Wajda1 *et al* . , 2004) . The pH of food is critical because at low levels, it favors the growth of moulds and yeasts. In neutral or alkaline pH foods such as meat and meat products,

bacteria are more dominant in spoilage process. The high protein content of meat makes the pH approximately neutral and it leads to a high level of spoilage in the meat and this is further explained by the breakdown of muscle glycogen leading to the production of lactic acid in the muscle fibres (Soyiri *et al*., 2008) .

1.3Pathogen associated with meat

1.3.1.Gram positive cocci

1.3.1.1. *Micrococcus spp*

Organisms that fit current description of micrococci are commonly encountered in routine laboratories either as environmental contaminat-ion or as commensals from normal skin and only occasionally from infections. The difficulty is recognized when these colony distinct required (Barrow and Feltham, 1993).

1.3.1.2. *Staphylococci*

Staphylococci are Gram-positive bacteria, with diameters of 0.5 – 1.5 μm and characterized by individual cocci, which divide in more than one plane to form grape-like clusters. To date, there are 32 species and eight sub-species in the genus *Staphylococcus*, many of which preferentially coloniese the human body (Harris *et al* . , 2004) .

Saphylococcus aureu cell could be eliminated from the food but the toxin remains stable under rigorous heating .contamination is mainly associated with improper and extensive manual handling and/or improper storage temperature of the food (Dora , 2011) .

1.3.1.2. Gram positive rods

1.3.1.2.1.*Bacillus cereus :*

Bacillus cereus is a group of ubiquitous facultative anaerobic spore forming Gram-positive rods commonly found in soil. The spores frequently contaminate a variety of foods, including produce, meat, eggs, and dairy products. Foodborne illnesses associated with toxins produced by *B. cereus* can result in self-limiting diarrhea or vomiting. Plate enumeration methods recommended by recognized food authorities to detect the presence of *B. cereus* in potentially contaminated food products do not inhibit other Gram-positive competitive bacteria (Tallen *et al.*,2012).

Bacillus cereus Lives in soil, water, and the gastrointestinal tract of humans and animals .

Bacillus cereus spores represent a huge advantage for the organism, allowing attachment, as well as survival of heattreatment or other procedures which remove species of vegetative bacteria which could otherwise outgrow *B. cereus.* Strain differences in spore characteristics, such as hydrophobicity, exosporium and appendages, have been shown to significantly affect the ability of the spore to adhere to surfaces such as food processing lines (Lotte *et al*., 2008) ,

1.3.2. Enterobacteriaceae:-

1.3.2.1. *Salmonella* spp:

Salmonella is a Gram-negative rod, facultative anaerobic, it grows at temperatures range between $5^{\circ}C - 46^{\circ}C$, *Salmonella* is found in the environments and in the gastrointestinal tract of animals, it was reported in all farm animals, but most frequently in poultry. In red meat animals *Salmonella* are most frequent in pigs, followed by cattle, infected cattle may not show symptoms but it can excrete the organisms in their faeces , which cause continuation of water, environment and other animals and meat. Raw meats are important sources of *Salmonella* and *Clostridium*

perfringens, which are often incriminated in outbreaks of food-borne disease Micro Organisms in Foods *Salmonella* is a worldwide issue in public health sector. People most at risk for serious complications due to *Salmonella* food poisoning include older adults, pregnant women, infants, children, and people who have compromised immune systems. Salmonellosis is manifested clinically in all hosts by one of three major syndromes, per acute systemic infection, an acute enteritis or a chronic enteritis (Nesa , 2011) .

1.3.2.2.*E. coli* :-

Escherichia coli was first isolated in 1885 from children's feces by the German bacteriologist Theodor *Escherich* . It is a normal commensally organism of the gastrointestinal tract of human beings and, although, generally harmless, it can cause a number of infections such as Gram-negative sepsis, urinary tract infection, pneumonia in immune compromised patients and meningitis (Deisingh and Thompson , 2014) .

E. coli is a Gram-negative rod, facultative v anaerobe, and generally motile organism. *E. coli* strains are non-pathogenic and exist harmlessly in the intestinal tract of humans and animals. Pathogenic *E. coli* strains cause a variety of diseases including gastroenteritis, dysentery, haemolytic uremic syndrome, urinary tract infection, septicemia, pneumonia, and meningitis. However, the major concern in recent years has been the increasing numbers of outbreaks of enter hemorrhagic *E. coli,* due to consumption of contaminated meat, fruits, and vegetables (Bhandare *et al* . , 2008) .

Many food industries use *E.coli* and Total Coliform bacteria as indicators for the presence of pathogenic microbes (Faroog , 2016) .

Several strategies that aid the reduction of *E.coli* O157:H7 carcass contamination have been introduced during the slaughter process, such as steam pasteurization and organic acid washes (Mcgee *et al* . ,2004) .

These bacteria are mostly transmitted through undercooked minced beef and meat products, which have a relatively short shelf life; therefore, rapid detection in these particular foods is required (Seran *et al* . , 2012) .

E.coli O157:H7 has been isolated from the feces or gastrointestinal tract of cattle, sheep, horses, pigs, turkeys, dogs, and a variety of wild animal species; however, epidemiologic studies have found that cattle manure is the source of most human *E. coli* O157:H7 infections. *E. coli* O157:H7 has also been isolated from bodies of water (e.g., ponds, streams), wells, and water troughs and has been found to survive for months in manure and water trough sediments (Risk Assessment of the Public Health Impact of *Escherichia coli* O157:H7 in Ground Beef)

According to Gansheroff and O'Brien , (2000) . *E.coli* O157:H7

- Infection

Asymptomatic (weaned calves ,adult cattle)

- Sheding

Highest in warmer months Duration of shedding per animal :~several weeks ~2 months Quantity of organisms shed : ~10^2->10^6 cfu per g feces .

In general, *E. coli* O157:H7 is deposited on the surface of beef carcasses during the slaughtering process. After chilling the carcasses are cut into larger portions for sale as bone-in or boneless beef cuts (e.g., round, loin, rib, chuck). During this process the larger portions are trimmed of excess fat and other tissue. The trimmings commonly are used in the manufacture of raw ground beef and a wide variety of ready-to eat products (e.g., sausages). The same process of trimming meat and

fat occurs at other steps along the food chain with much of it being used for ground beef. Wherever it may occur along the food chain the process of trimming and subsequent grinding distributes the pathogen throughout the ground meat . The most common scenario leading to illness has involved undercooking, survival of the pathogen and subsequent infection, particularly among the more susceptible consumers. Cross-contamination in kitchens and food service establishments from beef to other ready-to-eat foods also has occurred (FAO/WHO,2006).

1.3.2.3. *Listeria monocytogens* :-

L. monocytogenes is the only important human pathogen ,is a Gram-positive , facultative anaerobic, catalase - positive, oxidase - negative, non-sporeformer listeriosis, the disease caused by *Listeria monocytogenes* was primarily of veterinary concern, where it was associated with abortions and encephaliti0s in sheep and cattle (Kendall , 2012) .

1.3.2.4.*Pseudomonas* spp

Pseudomonas spp. present everywhere and are isolated from a multiplicity of sources including drinking water, domestic and wild animals, human beings, plants, and also from a variety of foods. These Gram-negative bacteria are non-fermentative rods, aerobic, oxides-positive and motile with polar flagella . The genus *Pseudomonas* is the most heterogeneous and ecologically significant group of known bacteria, and includes Gram-negative motile aerobic rods that are widespread throughout nature and characterized by elevated metabolic versatility The nutritional requirements of *Pseudomonas* spp. are very simple, and the genus is found in natural habitats like soil, fresh water, marine environments etc., but it has also been isolated from clinical

instruments, aseptic solutions, cosmetics and medical products. Some species are important medically (Laura and Mauro , 2007) .

Table (1):- control measure applies to the critical limit of the cooking

The following table 1-1 further illustrates this concept. Note that the only unique control measure applies to the critical limit of the cooking step for each of the products (Other food safety hazards and control measures may exist: Managing Food Safety 2006).

control measure applies to the critical limit of the cooking:-

Preparation for Same Day Service		
Example Products	Meatloaf	Chicken
Example Biological Hazards	*Salmonella*	*Salmonella*
	E. coli O157:H7	*Campylobacter*
	Clostridium perfringens	*Clostridium perfringens*
	Bacillus cereus	*Bacillus cereus*
	Various fecal-oral route pathogens	Various fecal-oral route pathogens
Example Control Measures (there may be others)	Cooking at 155 °F for 15 seconds	Cooking at 155 °F for 15 seconds
	Hot Holding at 135 °F or above OR Time Control for 4 hours or less	Hot Holding at 135 °F or above OR Time Control for 4 hours or less
	Refrigeration 41 °F or below	Refrigeration 41 °F or below
	No bare hand contact with RTE food, proper hand washing, exclusion/restriction of ill employees	No bare hand contact with RTE food, proper hand washing, exclusion/restriction of ill employees

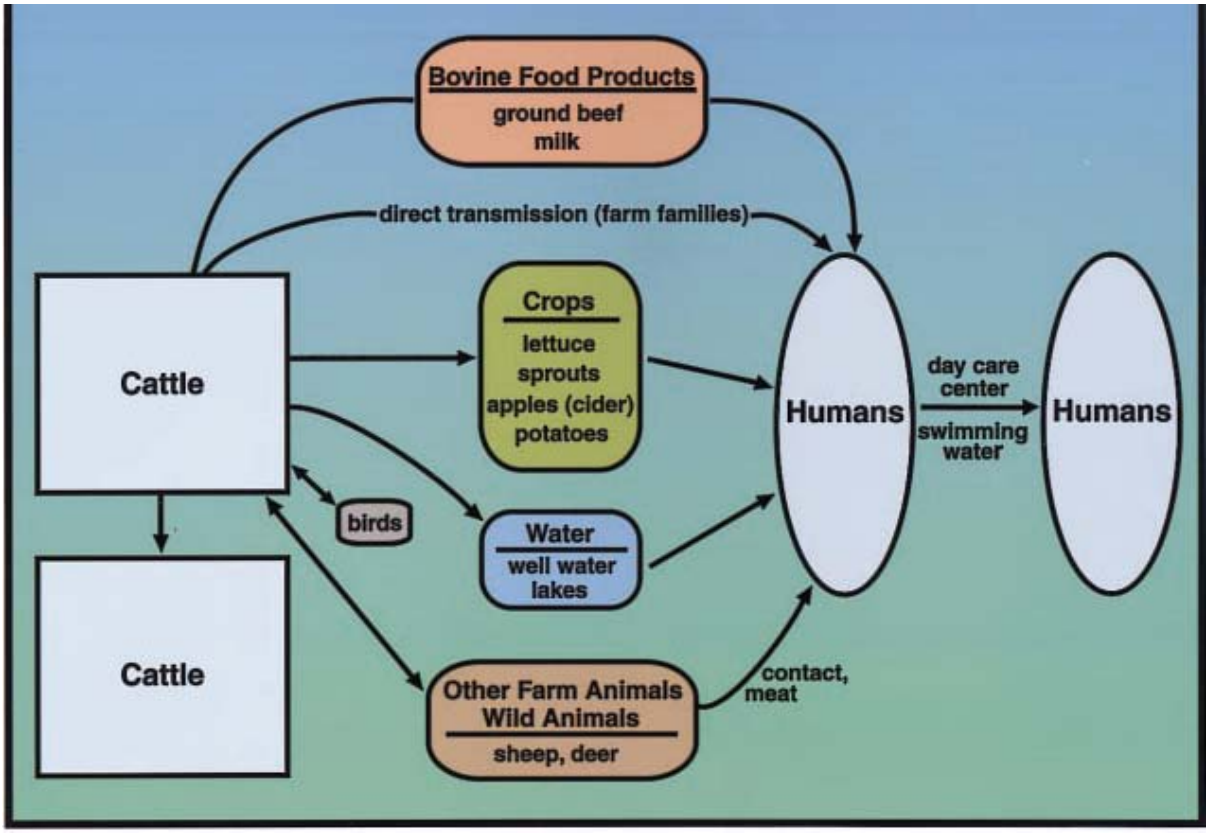

Figure 1(Gansheroff and O'Brien, 2000) **:-**

1.4 Model for transmission of *E. coli* O157:H7 from cattle to humans. :--

The figure above represented data from numerous studies and depicts examples of the major classes of foods and other sources of *E. coli* O157:H7 infection that have been reported. The contamination of crops and water sources is associated with the use of manure in fertilizer or with potential fecal contamination from nearby cattle. The sources of human infection with *E. coli* O157:H7 were identified first by epidemiological methods. In some cases, *E. coli* O157:H7 was isolated from the suspected food or other source; in many of these cases, including outbreaks associated with ground meat, PFGE or phage typing provided additional confirmation that the bacteria isolated from

the patient and the suspected food or other source were the same strain. PFGE typing has also been useful in linking geographically separated outbreaks to a common source of contaminated meat. The finding of *E. coli* O157:H7 in birds, deer, and other animals has led to speculation that these organisms may also be vehicles for O157:H7 transmission (Gansheroff and O'Brien, 2000). *E. coli* is typically part of the fecal bacterial community in cattle and can acquire antibiotic-resistance traits quickly through horizontal gene transfer (Rehema *et al* . , 2016) .

Food can become contaminated with foodborne bacteria mainly

Animals — manure or saliva, or disease microorganisms within the animals. For example, if meat contains harmful bacteria, and it is not thoroughly cooked to kill the bacteria, foodborne illness may result once the food is eaten.

Soil — contaminated by animal droppings, which can be transferred to the crops that can be eaten and also by normal soil residents.

Water — contaminated by animal droppings, which can be transferred to humans when the water is consumed or sprayed on crops.

Humans — from infected hands that touch the food can be eaten (Sciene and our food supply)

1.5.Cross-contamination:

According to Elen *et al* . (2012) , 25% of foodborne outbreaks are closely associated with cross-contamination events involving deficient hygiene practices, contaminated equipment, contamination via food handlers, processing, or inadequate storage. Identifying sources of infection frequently proves complicated as accurate data is often difficult to obtain from governments, industries, and also incomplete investigations.

Cross-contamination is one of the most common causes of food poisoning. It happens when harmful bacteria are spread onto food from either other food sources, such as raw meat or soiled vegetables (known as direct cross-contamination) or from surfaces, hands or equipment that have been contaminated (known as indirect cross-contamination)(Food A standards Agency) .

Sources of superficial contamination of carcases were quantitatively estimated by Amal (1995) as follows:

1. Direct on the skin of animals , approximately 33%.
2. Pollution in abattoir atmosphere , approximately 5%.
3. The visceral content in normal condition , approximately 3%.
4. Transport and storage , approximately 50% or more.
5. Halving , quartering and packing of carcases, approximately 2%.
6. Miscellaneous , utensils , personnel … etc , approximately 3%.

There are general standard methods for determination of the foodborne pathogens and mycotoxins in food products including conventional methods such as culture and microscopic methods, chemical and biological methods (immunological, molecular genetic methods, gel diffusion), there are also more rapid methods which were recently developed including physical methods (biosensors, impedance, microcalorimetry, flow cytometry, biosys instrument) and bioassays(Yeni *et al.,* 2013) .

1.6 Prevention and control:-

Require a multidisciplinary approach in animal and plant production as well as risk-based approaches along the entire food supply chain. These include the application of Good Agricultural Practices (GAP), Good Manufacturing Practices (GMP), Good Hygiene Practices (GHP)

and Hazard Analysis Critical Control Point (HACCP) from the farm to the consumer Preventing *E coli* in Food. The control of pathogenic *E. coli* and *Salmonella* infections on cattle farms includes the treatment of all carriers and infected subjects but also limiting the spread and severity of the disease. When the infection is identified early in the herd and few animals are affected, their isolation is an important measure to consider. Furthermore, faecal dejections from infected animals should be managed in a manner to avoid contamination of feed, water or livestock equipments. The treatment of *E. coli* and *Salmonella* infections in cattle herds has been thoroughly reviewed by (Eugène *et al* .,2015).

According to FAO/WHO (2003) official or officially-recognized programmers for specified zoonotic agents should include measures to:

- Control and eradicate their presence in animal populations, or subsets of populations .
- Prevent the introduction of new zoonotic agents;
- Provide monitoring and surveillance systems that establish baseline data and guide a risk-based approach to control of such hazards in meat .
- Control movement of animals between primary production units, and to abattoirs, where trade animal populations are under quarantine .

1.7.Meat hygiene and safety:-

Veterinary involvement in food safety activities throughout the food chain may encompass food safety, zoonoses and animal health. Risk management activities in these areas will contribute in various

ways to reduce food-borne risks to human health by preventing eliminating or controlling hazards transmitted by food (OIE , 2002) .

Prevention of foodborne infections is based on: pathogen-free food production, hazard control in food processing, surveillance for foodborne illness, and safe food handling by consumers and food-service workers (Lubnam and Ghadam , 2012) . Practices developed by professionals to ensure the safety of food production and processing are based primarily on knowledge of microbiology (Lubnam and Ghadam , 2012) .

The microbiological safety of food is achieved by as far as possible ensuring the absence of pathogenic microorganisms and by all means preventing their multiplication (Okonko *et al* . , 2008) .

When contamination occurs in any stage of food supply chain and it cannot be eliminated before the produce item becomes available for consumption, detecting the contaminated products appears as the best option in order to prevent any public health issue. Because the fresh produce items have a short shelf life and they are consumed instantly, sampling and detection of contaminated products must be considerably rapid and reliable. Many outbreaks caused by contaminated fresh produce items reveal the urgent need of using rapid methods in national and international surveillance systems. More recently, due to the errors and delays in detection of the source of the outbreak, the *E.coli* O104:H4 outbreak in Europe 2011 became the biggest foodborne outbreak occurred in Europe (Yeni *et a l.* , 2013) .

The Hazard Analysis Critical Control Point (HACCP) concept is used to identify microbiological vulnerable points in the food production process and processing, to determine the most appropriate methods of control to be applied, usually such methods as improved

handling techniques, monitoring of temperature and more intensive supervision(Okonko *et al* . , 2008) .

Heemskerk (2005) reviewed the recent literature on the slaughter process and came to the conclusion that improvements on the hygienic could only be obtained by intervention at several places in the slaughter process at the same time. The high food safety requirements are met by the eat sensitivity of these cultural methods. However, these techniques with their well-established, standardized and broadly accepted protocols are time-consuming, tedious, labour-intensive and often expensive (Velusamy *et al* . , 2010) .

Since the rules of how to avoid food poisoning are known, the problem in mass catering is enforcement which must include not only proper equipment and installations but also training of personnel(Lubnam and Ghadam , 2012).

The food and Agricultural Organization of the United Nations (FAO) and the World Health Organization (WHO) state that illness due to contaminated food is perhaps the most widespread health problem in the contemporary world and an important cause of reduced economic productivity (Okonko *et al* . , 2008) .

1.8. History of HACCP

HACCP is basically a preventative system, designed to correct problems before they affect the safety of food products. It is prevent problems by making sure that controls are applied at any point in a food production system where hazardous or critical situations could occur (Gihan , 2004) .

IN 1961, Codex commission (1961) formed general principles of food hygiene and followed the food chain from primary production through to the consumer, highlighting the key hygiene controls at each stage and

recommending an HACCP approach wherever possible to enhance food safety. These controls are internationally recognized as essential to ensuring the safety and suitability of food for human consumption and international trade.

The H ACCP concept developed by the national aeronautics and space administration (NASA) and Natick Laboratories for use in the aerospace manufacturing. This national approach to process control for food products was developed jointly by the Pillsbury Company, NASA and the U.S army Natick laboratories as an attempt to apply zero defect program to the food processing industry. HACCP was incorporated to guarantee that food use in the US space program would be 100% free of microbial pathogens because it is designed to prevent rather than detect food hazards. HACCP has been identified by the US department of agriculture, food safety and inspection service (FSIS) as a tool to prevent or control food safety hazards during meat and poultry production the developments.

1.9 Good practices and HACCP:

Realising the many shortcomings and lack of food safety assurance provided by traditional inspection and sampling/testing of lots, the concept of Hazard Analysis Critical Control Point (HACCP) was developed in the early 1970s. The HACCP concept has provided great improvements in the production of safe foods. The goal of HACCP is to focus on the hazards in a particular food commodity that are reasonably likely to affect public health if left uncontrolled, and to design food products, processing, commercialization, preparation and use conditions that control those hazards. To be successful, HACCP needs to build on good practices such as good

agricultural practices (GAPs) and good hygienic practices(GHPs), which minimize the occurrence of hazards in the product and the production environment. HACCP involves an assessment of hazards in a particular production sequence and defines steps where control measures that are critical for the safety of a product should be taken. Also, it will state limits, monitoring procedures and corrective actions. However, it is plant/factory specific and does not directly link the effectiveness of such measures to an expected level of health protection, e.g., a reduction in the number of foodborne illnesses occurring in a country (ICMSF ,2006).

CHAPTER TWO

Materials and method

2.1 Area of study

This study was carried out in Elfashir city in North Darfor State in small ten butcheries .

2.2 Sampling metherod

Samples were taken from ten butcheries (A , B ……J) the swabs were taken from four sites of each butchery (knife, scale, butcher hands and surface - table) . The total were 40 swabs then the swabs were transferred in ice box to the microbiology laboratory of minstery of Veterinary Medcine .

2.3 Bacteriology :-

The samples were examined for isolation and identification of *E coli.* Identification was carried out according to the procedure described by Barrow and felthman (1993).

2.3.1 Preparation of Cultural media

These media were prepared accord to Barrow and Feltham (1993**).**

2.3.1.1 Solid culture media

2.3.1.1.1 Nutrient agar

The medium was prepared as described by (Oxoid lab) 25grams of the powder were added to one liter of distilled water and brought to boil to dissolve the powder completely .It is sterilized by autoclaving for 15 minutes at 121° c and 15 pounds per square inch. Then pounred aseptically as 18-20 ml in Petri-dishes.

2.3.1.1.2 Mac Conkey's agar medium

Fifty-two grams of Mac Conkey's agar powder (Oxoid 1982) were added to one liter of distilled water and brought to boiling until dissolved completely. The PH was or adjusted to 7.4 then sterilized by autoclaving at 121^{o}C for 15 minutes. Then it was aseptically distributed in sterile Petri dished as 15-20 ml portion and left to solidify.

2.3.2. Semi-solid media

2.3.2.1 Motility medium

The medium was described by Cruickshank *et al* . (1975) , 10.2% was dissolved in nutrient broth and distributed in sterile test tubes containing Craigie tubes and then the media was autoclaved at 121^{o}c and 15 pounds per square inch.

2.3.2.2 Hugh and Liefson's (o/F)medium

Hug and Liefso's (O/F) medium (Cowan and Steel ,1974) contained peptone (2g), NaCL (5g), KHPO4 (0.3G), agar (3g), distilled water (1000ml) and bromocrysol purple, and 0.2%aqueous solution (15ml). The Solids were dissolved by heating in the water. The pH was adjusted to 7.1, the medium was filtrated. The indicator was added. Sterilization was done by autoclaving for15 minutes and pressure of about 15Ib per square inch. Sterile glucose (1%) was added to the mixture was and they distributed aseptically in ten ml volumes into sterile test tubes with cotton plugs of not more than 16mm diameter.

2.3.3 Liquid cultural media

2.3.3.1 Peptone water

Peotone water was prepared according to Cruikshank *et al* .(1975) ten grams peptone and 5 grams NaCL were dissolved by heating in 1000 ml distilled water. The PH was adjusted to 7.2 and the medium was distributed in test tubes (5ml) and serialized by autoclaving at 115° C for 15minutes under pressure 15Ib per square inch. The stock was preserved in the refrigerator.

2.3.3.2 Nutrient broth

Nutrient broth (Oxoid Lab) contained lab-lemco powder (1g) yeast extract (2g), peptone (5g) and sodium chloride (5g). PH was adjusted to 7.4 approximately. An amount of 13g of the dehydrated medium was added to one liter of distilled water .the reconstituted medium was mixed well then distributed in 5ml amounts and sterilized by autoclaving at for 15 121^{o}c minutes under pressure of 15Ib per square inch.

2.3.3.3. MR-VP medium

MR-VP medium (Oxoid lab) contained peptone (5g), dextrose (5g) and phosphate buffer (5g). The PH was adjusted to 7. One liter distill water was mixed well with 15 gram of the medium .Then distributed in test tubes with cotton plugs and sterilized by autoclaving at 121^{o}c for 15 minutes under pressure 15Ib per square inch.

2.3.3.4 Nitrate broth

Nitrate broth (Cowan and Steel, 1985) contained KNO (1g) and 13 grams of nutrient in 1000 of distilled water. Then the medium was distributed in sterile test tubes with cotton plugs and then sterilized by autoclaving at 121^{o}c for 15 minutes under pressure 15Ib per square inch.

2.3.3.5 Carbohydrates liquid medium

Carbohydrates liquid medium was prepared Cruickshank *et al* . , (1975). The sugars used were glucose, maltose, sucrose, lactose and monitor all percent Andrade s indicator. The medium was distributed in 5ml amount in test tubes with cotton plugs and autoclaved under 10Ib pressure per square inch for 5 minutes.

2.4 Sterilization

2.4.1 Hot air oven

This method was used for sterilization of clean glass containers which were wrapped in paper or put in stainless steel cans, and the temperature was 160^{o}c (Stainer *et al* . 1986) .

2.4.2 Sterilization by red heat

The method was used for sterilization wire loops, straight wire and tissue forceps it was done by holding the object over the flame as near and vertical as possible until it becomes red-hot (Cruickshank *et al.* , 1975).

2.4.3 Sterilization by autoclaving

This method was used for sterilizing of culture media and for materials that could not with stand the dry heat . The temperature was 115°-121° C under 10-15 pounds pressure for 15-20 minutes (Barrow and Feltham, 1993).

2.5 Preparation of the sample

Samples were inoculated onto liquid broth and incubated over night then dilute d with normal saline then , small portion was streaked with a sterile loop on solid media MacConkey's agar and incubated at 37°C for 24 hours for isolation and identification of Gram-negative organisms .

2.6 Examination of culture

cultures on solid media was performed for detection of growth, pigmentation, colonial morphology as well as changes in the media. Plates that showed visible growth were subjected to further bacteriological tests while those that did not show visible growth were incubated for further 48hours and discarded if no growth was detected.

2. Purification of culture

The primary isolates were sub cultured on nutrient agar. The sub culure was repeated several times until pure colonies were obtained.

2.8 Biochemical Tests

2.8.1 Oxidase test

Tetra methyl-pheynlen-diamine dihydro chloride was prepared as 1% aqueous solution. Filter paper of 50x50 millimeter size were impregnated in reagent before and dried at 50^{o}c. A sterile platinum loop was used to spread the isolated colony on oxidase paper. Color change (violet) indicated appositive reaction (Barrow and Feltham , 1993 **) .**

2.8. 2 Hydrogen peroxide

Hydrogen peroxide produced by B.D.H (British Drug House) was diluted to 3% aqueous solution for catalase test.

2.8.3 Kovac's reagent

This reagent was prepared as described by Barrow and Felltham (1993) . Five grams of P-dimethylamino benzaldehyde were dissolved in 75 ml of amylalcohol by warming in water bath. After the mixture was cooled, 25ml of concentrated hydrochloride acid were added. It is used for indol test.

2.8.4 Bromocrysol purple and phenol red indicator

Bromocrysol purple and phenol red indicators were obtained from Britch Drug House. Methyl red was a product of Hopkins and William, it was prepared as 5% solution for use in methyl red test . It was

containing acid fuchsin (5g) distilled water 1000ml and N-NaOH)150-180) ml. The acid fuchin was dissolved in distilled water and 150ml of alkaline solution were added. It was used in sugars test as one percent volume indicator (Cowan and steel ,1974).

2.8.5Catalase test

Using sterile glass rod apart of isolated colony was emulsified in one drop of hydrogen peroxide on a clean slide. Gas bubbles indicated positive reaction (Barrow and Feltham , 1993) .

2.8.6 Motility test

The isolates were studied for motility by Craigie technique (Cruicshank *et al* . , 1975) in which the bacteria was inoculated into a central tube containing semi solid agar placed in test tube using straight wire. After incubation at 37^{o}c for 24 hours, the tubes were examined for migrating of bacteria outside the tube.

2.8.7 Hugh and Leifson's test

Hugh and Leifson's test or oxidation fermentation test (O/F) was done as shown by Cruickshank *et al* . (1975) . Duplicate tubes of freshly prepared medium were inoculated by stabbing with straight wire. One of the inoculated media was immediately covered with layer of sterile liquid paraffin to a depth of one ml and examined daily for up to 14 days. A colour change from green to yellow in both tubes indicated a fermentative organism but change in the uncovered tube only indicated that the organism was oxidative.

2.8.8 Nitrate reduction test

Nitrate reduction test was carried out as described by Cowan and Steel (1985). Nitrite broth was inoculated and incubated for up to five days. One ml nitrate solution 1 was added followed by one ml of solution 2. A red coloration within five minutes, powdered was added and allowed to stand. Development of red colour indicated that nitrate was present. Absence of red coloration in this case indicated absence of nitrate .

2.8.9 Carbohydrates fermentation tests

Carbohydrates fermentation tests were carried out as described by Cruickshank *et al* . (1975) . 5 carbohydrates media, glucose, lactose,

manitol , sucrose and maltose, were inoculated with peptone water culture by a sterile loop a fermentation reaction was indicated by change of color of the medium to pink .

2.10 Gram Stain

Using a sterile wire loop apart of isolates colonies four primary plots and pure were taken and spread on microscopes slides to make thin smears. They were fixed with heat and placed in staining rack. They were covered by crystal violet for two minutes and washed off by tap water, then decolorized with acetone for few seconds and washed off by tap water, then covered with carbol fuchsine for thirty seconds. Finally, the stained smears were washed and air-dried. Then they were examined under oil immersion lens (100°). The gram positive and negative organisms shape and arrangement of organisms were identified according to (Barrow and Feltham 1993) .

2.11Bacteria Isolation and Identification

For *E. coli* identification, 1 ml of the dilutions were inoculated on Mac Conkey's agar (Oxoid Ltd., Basingstoke, Hampshire, England) and incubated aerobically at 37°C for 24 hrs. Colonies that were suspected to be *E. coli* were isolated and confirmed using gram staining and other biochemical tests .

3.Statistical analysis

The data of bacterial count collected was analyzed by computer program SPSS ,used Cross tabulation and A nova test **.**

CHAPTER THREE

Results

forty Samples were collected from ten butcheries, from four sites (10 butcher hand, 10 knives, 10 surfaces which were cutting board table, and scale) All the butchers displayed meat (beef) mixed with offals openly on tables and wooden logs had no screens which let flies into the butcheries, floors were not clean, knives and other cutting tools were handled carelessly, weighing scales were unclean and all the butcheries lacked hand washing facilities. The butcher men did not wearing protective cloths such as white coat caps and gumboots and the same people handled meat and received money. The butcheries were located along the road side probably for display and marketing purposes but this exposed the meat to dust raised by automobiles. Several butcheries were located next to each other and the butcher men shared weighing scales .

4-1General contamination :-

All of that lead to result below in table (3-2) which explained that their knives had the highest mean of TVC 7.26 , hands come in second place then scales and finally the surfaces had least contamination by bacteria. There was no difference in the mean of bacterial TVC in butchers and points .

4-2 Contamination by *E coli:*

The highest contamination point by *E coli* was scales 45.5%, surfaces 27.3%, hands 18.2% and the last point knives which had 9.1%.

As shown in table 2-3 The contamination at butcheries A , B , C , D, E and F was positive (60 %) .But the rest were negative (40%) .

Table (3-2) Bacterial counts in meats (n=40) at butcheries in EL fashir city in North Darfor

points	Mean	N	Std. Deviation	Minimum	Maximum	% of Total N
hands	7.2453	10	.04308	7.18	7.33	25.0%
knives	7.2663	10	.04435	7.18	7.32	25.0%
surface	7.2278	10	.04655	7.16	7.32	25.0%
scale	7.2333	10	.04323	7.18	7.32	25.0%
Total	7.2432	40	.04514	7.16	7.33	100.0%

Table (3-3) Contamination of Butcheries(n=40) in ELfashir city in North Darfor Sate

Butcheries	Mean	N	E_coli	Std. Deviation	Minimum	Maximum	% of Total N
A	7.2329	4	Yes	.06045	7.18	7.32	10.0%
B	7.2243	4	Yes	.03419	7.18	7.26	10.0%
C	7.2586	4	Yes	.04554	7.21	7.32	10.0%
D	7.2375	4	Yes	.02599	7.21	7.27	10.0%
E	7.2480	4	Yes	.05323	7.20	7.31	10.0%
F	7.2353	4	Yes	.05521	7.19	7.31	10.0%
G	7.2672	4	No	.04529	7.21	7.32	10.0%
H	7.2311	4	No	.03875	7.18	7.27	10.0%

I	7.2180	4	No	.05676	7.16	7.27	10.0%
J	7.2790	4	No	.04072	7.24	7.33	10.0%
Total	7.2432	40	10	.04514	7.16	7.33	100.0%

Acording to microscopic appearance and biochemical properties were identified the bacterial culture as in table 3-4 .

Table (3-4) :- Isolation and identification of *E coli* in meats at Butcheries in ELfashire city in North Darfor State (n =40).

Isolate / Test	E. coli
Gram stain	-ve
Mac conckey	+
DCA	-
Oxidist	-
Motilitry	+
Indole	+
Citrate	-
Urease	-

CHAPTER FOUR

Discussion

Most of the meat contamination is caused by aerobes . These organisms may gain access to the meat from the systems of the living animal or as a results of slaughter contamination (Lawrie , 1991) . Meat contamination is of economic importance because it inverse the meat quality .

Butchers hands ,knives ,surface and scale samples from forty small butcheries yielded marked growth of bacteria. The presence of these organisms on meat could be attributed to the fact that meat contains an abundance of all nutrients required for the growth of bacteria in adequate quantity. It was shown in this study that the bacteria counts in table 3-2 and isolated was *E. coli* in table 3-3 .

The total of forty samples knives had the highest mean of TVC, hand come in second place then scale and finally the surface had least contamination by bacteria. The highest contamination level of the butcheries (J,G,C,E,D,F,A,H,B and I.), But no different mean between contamination point and butchers The highest contamination point by *E coli* was also in scale(45.5%) and the low was in surface (9.1%) .

The results obtained from this study in table 3-2 higher than what is reported by lamia (2015) in butcheries who counting bacteria in meat (5.2 Log10CFU/ cm^2).

This results was also higher to the TVCs (3.0±0.59 to 6.0±0.33 log10 cfu/cm2) reported from sheep carcasses at El-Kadero slaughterhouse by Abdalla *et al*.(2009) .

The high average *E. coli* O157:H7 (6.03±0.03 log10 cfu/cm2) counts were detected from butchers' hands samples from the abattoir.

Whereas the low (5.78±0.00 log10 cfu/cm2) count were detected in abattoir hooks and knives samples (Henok *et al* ., 2015)

The microbiological quality of beef has been investigated in many countries like Pakistan , *E. coli* O157:H7 was not detected in surface swabs (knives, wooden boards, weighing scales, and meat mincers) was taken from 30 individual retail meat outlet markets (Hassan *et al*. 2010) .In Algeria, Nouichi and Hamdi (2009) who found the superficial bacterial contamination levels of 4.48±0.63 log cfu/cm2.

These microorganisms can be opportunistic pathogens of humans and were isolated from human clinical specimens of an outbreak of food poisoning (Carter and Cole, 1990;). Normal flora of different parts of man and animal body, some of them have been associated with many disease problems. They might cause disease in their presence in the animal body or by contamination of food . The contamination of meat by microbial pathogens can occur at any stage of the meat chain (Eugène *et al* .,2015) Furthermore, the prevention or mastery of meat contaminations can be carried out at a stage of the chain different from the stages at which the contamination has occurred (Eugène *et al* ., 2015) .

E coli is one of the pathogenic bacteria related to meat (Food Safety and Inspection Services 2002 .)

the unhygienic practices of meat processing in developing countries results in these meat been contaminated with microorganisms. Meat sellers were also observed busily conversing, coughing, and sneezing, which might result in contamination through introduction of saliva on the meat . Okonko *et al* . (2008) stated that, food can be infected with microorganisms as a result of “coughing” and “sneezing” from those who handled and processed these foods.

These findings are in agreement with the findings of Hussein (1987) who isolated *Bacillus spp* , *Micrococcus spp* and *Escherichia coli* from fresh and refrigerated beef.

E. coli O157:H7 has been isolated from multiple sources on the farm (Mcgee *et al* .,2004). Some studies citing the surface of pens, animal feed, and water in particular as important sources for transmission of the pathogen (Mcgee *et al* .,2004).

contamination of fecal origin occurs during hide and skin removal and evisceration processes (Norasak , 2014).

Another study by Jeffery (2003) revealed that the workers hands and the equipment were the sources of meat contamination and these results are accordance to the present results .

According to Okonko *et al.* (2008) the presence of *E.coli* in food is an indication of faecal contamination the water as source that were utilized in the processing of these food product.

Another study by Jeffery (2003) revealed that the workers hands and the equipment were the sources of meat contamination and these result are accordance to present results . Also Agbeyegbe and Uraih (1982) reported high prevalence rate of *E. coli* in raw meat samples .

Soyiri *et al*. (2008) reported that the butchers of retail beef in Asaiman market –Ghana, which under unhygienic practices and poor handling of beef , contamination with aerobic mesophiles (*Staphylococus aureus* , *Bacilus cereus* , *Clostridium perfringens* , and *Eschericha coli*).

The elimination of contamination sources by practicing good sanitary measures will reduce the occurrence of microorganisms .Appropriate methods should be applied during slaughtering operation using adequate water and disinfection ,such control measures should include

an extensive education programs for proper hygiene and improvement of managements .

The management of microbial risk in beef and other meat products in the event of contamination and growth can present a difficult situation to butchers. Strict hygiene and/or the implementation of decontamination technologies are recommended as a means to assure the safety of meat with respect to food borne pathogens. There is the need for useful data to be generated for assessing food safety issues and analysis. Predictive modelling tools can be employed to determine the survival of some common pathogens in beef and meat products (Soyiri *et al* . , 2008) .

Poor meat hygiene practices in the slaughter houses before and after slaughter would lead to the meat contamination and its products . FAO/ WHO (1962) and Thornton (1968) emphasized that meat hygiene should be observed at all stages of meat production till it reaches the consumer as fresh , sound , wholesome and safe meat (from farm to table) .

The (HACCP) system had not been practiced in slaughter houses in the Sudan before . However the present study was an endeavor to experiment on the feasibility of this system in a slaughter house

Thus to safeguard against the risks of detected microorganisms, there is need to educate and advocate for good manufacturing practices among food processors and food vendors.

Betty and Richard (1994) said that food poisoning / illnesses are entirely preventable by practicing good sanitation and food handling techniques.

This study revealed that the level of contamination on bovine carcasses was much higher. The levels of microbial contamination in Sudanese

abattoirs may reflect the hygiene status of meat production in the developing world.

Conclusion and Recommendations

Conclusion:-

In conclusion ,this study revealed that the level of contamination on bovine carcasses was much higher.But in Sudan a tropical country, with ambient temperatures conductive for the growth of microorganisms , which can rapidly render meat unsafe for human consumption .The general sanitary conditions at the meat shops in addition to poor hygienic practices by the butcher are probable contributors to the microbial contamination on the beef. Ensuring good hygienic standard at the various meat shops .Potential pathogenic bacteria such as *E. coli* were isolated and they could construe a public hazard.

Recommendations:-

1. More advanced training program for meat inspectors and meat handlers is required.
2. Provision of advanced technical equipments.
3. Regular sterilization of tools used in slaughtering, dressing is very important.
4. Use of hygiene program and HACCP in slaughterhouse lead to improve quality of Contamination .

References

Abdalla M.A, . SUliman,S.A, Ahmed ,O.E and Bakhiet ,O.A .(2009) Estimation ofbacterial contamination of indigenous bovine carcasses in Khartoum (Sudan). African Journal of Microbiology Research Vol. 3(12) pp. 882-886.

Abd El-Aziz . (2015) Detection of *Pseudomonas* spp. in Chicken and Fish Sold inMarkets of Assiut City, Egypt Journal of Food Quality and Hazards Control 2 86-89 .

Agbeyegbe JM, Uraih N . (1982). The prevalence of E. coli in meat samples in Benin City, Nigeria. Microbios Lett. 20: 21–28

Ahmad, M.U.D., Sarwar,A., Najeeb,M.I., Nawaz, M., Anjum,A.A., Ali,M.A. and Mansur,M.(2013). Assessment of Microbial lod of raw meat At Abattoirs and Retail outlets . The Journal of Animal & Plant Sciences, 23(3): Page :745-748.

Amal ,A.M.B .(1995). Assessment of Meat Hygiene Statues at Assabaloga Slaughterhouse in Khartoum State, Sudan *thesis* for master. *University of Khartoum* .

Barrow, G. I. and Feltham, R. K. A. (1993). Cowan and steel's Manual for the identification of Medical Bacteria. London: Cambridge university press.

Betty, C.H. and Richard, J.B. (1994): Bacterial causes of food poisoning in food poisoning and hygiene, 8th Ed. Edward Annod. Publishing Limited London PP. 250-25.

.Bhandare, S. G., Sherikarv, A. T., Paturkar, A. M., Bhunia, A. K. (2008).

Food-borne Microbial pathogens. Springer science + business media, LLC.

Bryan, F.L. (1978). Factors that contribute to our breaks of food-borne disease . Journal of Food protection .41; 816-827 carcass at a small abattoir Can.Vet. J. 44: 1 carcasses in a modern Indian abattoir and traditinal.

Carter GR, Cole RJJR (1990). Diagnostic procedures in veterinary bacteriology and mycology. 5lh ed. Academic press Inc . san Diego , California , USA.

Cruickshank, r.; Duguid, G. P.; Marmion, B.P and Swan, R.H.A. (1975) Medical Microbiology, Vo1.2 the practice of medical microbiology 12th edition. London: Churchill Livingstone.

Cowan , S. T and Steel, K . J.(1974). Manual of the identification of medical bacteria .٢nd edition . University press , Cambridge , UK .

Cowan, S. J and steel K.J and (1985). Manual for identification of medical bacteriology. 2nd London: Cambridge University press reprinted

Cuiwei ,Z. , Bellei ,G., Juande ,V., Robert ,S., Emily,Y ., Shaohua,Z., Davidg. W, Davied , W., Andjianghong , M. (2001) .Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella*Serovars in Retail Chicken, Turkey, Pork, and Beef. from the Greater Washington, D.C., Area Applied and Environmental microbiology ,P.5431- 5436.

Deisingh,K.A., and M. Thompson,M.(2014) Strategies for the detection of Escherichia coli O157:H7 in foods Journal of Applied Microbiology

Dolman, C. E. (1967). Epidemiology of meat borne diseases. In meat hygiene Geneva: world health organization.

Dora ,M.(2011). Molecular Monitoring of Meat Spoiling Pseudomonas Species and Analysis Staphylococcal Enterotoxin Expression and Formation.PhD School of Food science.University of Budapest.

Elena, C., Andrés, M.,Rosa , M. G. (2012). Cross-contamination and recontamination by Salmonella in foods: A review. Journal homepage: www.elsevier.com/locate/foodres.

Eugène ,N., Martin, P., Anastase, K.,& Marianne, S . (2015) Risk Factors and Control Measures for Bacterial Contamination in the Bovine Meat Chain: A Review on *Salmonella* and Pathogenic *E.coli*Journal of Food Research; Vol. 4, No. 5

FAO/ WHO . (1962). Second Report of the Joint FAO/WHO Expert Committee on Meat Hygiene . FAO Agricultural Studies ٥٨ Rome : Food and Agriculture Organization of the United Nation.

FAO/WHO . (2003) Report of the 9th session of the codex committee on meat and poultry hygiene. Wellington, New Zealand, 17-23 February 2003, food and Agriculture Organization of the United Nation .Pp.38/45

FAO/WHO .(2006) Development of Practical Risk Management Strategies based on Microbiological Risk Assessment Outputs. Kiel, Germany.

Farhana . J., Mahbub-E-Elahi .A.T.M., and Siddique .A.B. (2015). Bacteriological Quality Assessment of Raw Beef Sold in Sylhet Sada The Agriculturists 13(2): 09-16 (2015) ISSN 2304-7321.

Farooq, A .(2016). Analysis of Raw Meat for Heavy Metals and Bacterial

Contamination and Comparison of Antibiotic Susceptibility of Isolated Bacteria. Proceedings of the Pakistan Academy of Sciences: B. Life and Environmental Sciences 53 (1): 13-20

Fatima, E. M . (1982). Bacteriology of processed meat in Sudan, M.Sc., thesis, university of Khartoum. Food borne pathogens Bacterial agents of food borne illness.

Food Safety and Inspection Service .(2002) Guidance for Minimizing the Risk *Escherichia coli* O157:H7 and *Salmonella* in Beef Slaughter Operations.

***FSA* Food Stander Agency** *E. Coli* O157 Control of Cross-contamination Guidance.

Gansheroff,J.L., and O'Brien,D.A. (2000) Escherichia coli 157:H 7 in beef cattle presented for slaughter in the U.S.: Higher prevalence rates than previously estimated www.pnas.org.

George, J., and Banwart .(1989) Basic food microbiology, An AVI book, Library of congress cataloging in publication data, QR115. B34 88-20837, 576:163.

Gihan, A. E. A .(2004) Quality management of management of meat in a slaughterhouse in Khartoum state .(M.P.V)Thesis University of Khartoum

Gill, C. O. (1998). Microbiological contamination of meat during slaughter and butchering of cattle, sheep and pigs. In: Davies, A. and Board, R. (eds). The microbiology of meat and poultry Blackie academic and professional, London, pp. 118-157

Gracey, J. F. (1985). Thornton's Meat Hygiene. 7th ed. London: Bailliere Tindal.

Harris,G., Foster ,S.J, and Richards,R.J . (2004) Anintroduction to

staphylococcus aureus ,and Techniques for Identifying and quantifing .*Aureus* adheina in relation to adhesion *to* biomaterials LE.uGr.o Hpaearrni s (p a g e s 3 9 - 6 0).

Hassan Ali N, Farooqui A, Khan A, Khan AY, Kazmi SU . (2010) . Microbial contamination of raw meat and its environment in retail shops in Karachi, Pakistan. J Infect Dev Ctries. 4(6):382–8.

Hassanien, A.S.1, Aidaros, H.A.2, Talaat, M.M.3, Nouman, T.M.3, El-Mossalami, E.I. (2006). Contamination of beef carcasses during slaughtering in two Egyptian slaughterhouses https://www.researchgate.net/publication/281592811 .

Heemskerk, W.J.C . (2005) Improving processing hygiene by mechanical removal of faeces before scalding. XVIIth Eur. Symp. on the Quality of Poultry meat and XIth European Symposium on the Quality of Eggs and Egg Products, Doorwerth , the Netherlands 23-26, p37

Henok Ayalew , Amare Berhanu , Berhanu Sibhat and Biresaw Serda . (2015) Microbiological assessment of meat contact surfaces at abattoir and retail houses in Jigjiga town, Somali National Regional State of Ethiopia. Journal of food and Agricultural Science .

Hussein, A . H . (1987). Aerobic bacteria in fresh and refrigerated beef . M V . Sc Thesis , : University of Khartoum .

ICMSF International commission on Microbiological specifications for Food .(1980). Microbial ecology of foods CH. II. Meat and meat products , academic press, New York, London.

ICMSF The International Commission on Microbiological Specifications for Foods.(2006). Modern food microbiology 6th ed. Gaithersburg. M D. Aspen Publishers.

Jay, J. M. (1986). Modern food microbiology. 3rd ed. New Yrok: Van Nostrand Reinhold Company Inc. New York. PP. 679-680.

Jay, J. M. (2000) . Modern food microbiology 6th ed. Gaithersburg, M. D. Aspen Publishers.University of Nvada Las vegas

Jeffery B, Donald AB, Gill CO . (2003). Implementation of validated HACCP system for the control of microbiological contamination of pig carcass at a small abattoir. Can. Vet. J. 44:

Jepsen, A. (1967). Bacteriological examination of manufactured meat products. In meat hygiene. FAO agricultural Studies No. 34. Rome.

John, T. N. and Anathony. J. S. (1974). Microbiology of food and food processing, New Yurok. American Elsevier publishi

Josef, K . (2013) The microbiology of meat spoilage University of Veterinary and Pharmaceutical Sciences Brno Brno. .Department of Meat . Hygien and Technology, Czech Republic,palackeho 61242B M O.

Kendall ,p.(2012). Bacterial Foodborne Illness Ph.D .Colorado State University

Khalid, K. A. (2004). Meat hygiene assessment in Khartoum state.M.V.Sc Khartoum University .

Lemia,W.E.T .(2015) .Detection of bacterial contamination in butcheries at east Nile Kharttoum State.(M.V.P.S) Thesis, Sudan University of Science and Technology.

Laura, F and Mauro, S .(2007) Characterisation of *Pseudomonas* spp. isolated from foods Annals of Microbiology, 57 (1) 39-47

Lawrie, R. A. (1991). Meat science. 5th ed. Tokyo. Pergamon press.

Lotte P. S. A ., Annette F., and Per. E. G. (2008) Fromsoil to gut: *Bacillus cereus* and its food poisoning toxins Department of

Food Safety and Infection Biology, Section for Food Safety, Norwegian School of Veterinary Science, Oslo, Norway .

Lubnam .E., and Ghadam .M.(2012) .Ready to eat meat and poultry pies as asource of potential pathogens assuit city. Assiut Vet. Med. J. Vol. 58 No. 133 .

Marriott, N. G. and Gravani, R. B. (2006). Prinicples of food sanitation, 5th ed. Springer s cience + business media, prepustak an University Indonesia http:/.ui.acid/opac/themes /libriz .

Mcgee .p., Scott .L., Sheridan.J.J., Earley.I.P., and. Leonard . (2004) . Horizontal Transmission of Escherichia coli O157:H7 during Cattle Housing Journal of Food Protection, Vol. 67, No. 12, Pages 2651–2656

Nesa ,M.K., Khan ,M.S.R and Alam ,M .(2011) Isolation, Identification Characterization of *samonell serovars* from diarrhoelc stool samples of human Bangl. J. Vet. Med. 85 – 93.

Nobuyasu, Y., Makoto, S., Mio, Y, and Masao, N .(2003) Rapid Detection of Respiring *Escherichia coli* O157:H7 in Apple Juice, Milk, and Ground Beef by Flow Cytometry. Cytometry Part A 54A:27–35 .

Norasak ,K., Terrance, M. A, Joseph, M. B, John, W. S, Rong, Wang , STEVEN. S, and Tommy,L . (2014). Efficacy of Antimicrobial Compounds on Surface Decontamination of Seven Shiga Toxin–Producing *Escherichia coli* and *Salmonella* Inoculated onto Fresh Beef. Journal of Food Protection.

Nouichi,S. and Hamdi T. M. (2009): Superficial Bacterial Contamination of Ovine and Bovine Carcasses at El-Harrach Slaughterhouse (Algeria). Europ. J. Scientific Res. 38(3): 474-485.

Okonko, Iheanyi, O., Ogunjobi, Adeniyi, A., Fajobi, Enobong ,A., Onoja, B. A., Babalola, Eunice. T., and Adedeji, A O. (2008). Comparative studies and microbial risk assessment of different Ready-to-Eat (RTE) frozen sea-foods processed in Ijora-olopa, Lagos State, Nigeria. African Journal of Biotechnology Vol. 7 (16), pp. 2898-2901.

Osama, A .A. and Gehan, M.A.K . (2011) Effect of Good Manufacturing Practices (GMPs) Application on the Bacteriological Status of Butcher's Area in small scale Meat Processing Plant. Department of Food Hygiene and Control ,Faculty of Veterinary Medicine , cairo University , Cairo , Egyp.

Paul, B., and Sylvia, A. B. (2014) Microbiological Quality of Meat at the Abattoir and Butchery Levels in Kampala City, Uganda. Internet Journal of Food Safety, Vol.16, 2014, p.29-35

Rahman, M. S. (2007). Handbook of food preservation, 2nd ed. Taylor & retail sale in Cote d'ivoire. American . Frances Group. LLC. P. 176 Journal of Food.

Ranad, S. (2009) *Bacillus cereus* spores and cereulide in food-borne illness. Academic Dissertation in Microbiology. Faculty of Agriculture and Forestry of the University of Helsinki, for public criticism Auditorium . .

Rehema M, Catherine L, Bernadetha R, Gaspar M, Murugan S, Douglas R. C

and Joram B . (2016). Detection of microbial surface contamination and antibiotic resistant *Escherichia coli* on beef carcasses in Arusha, Tanzania. African Journal of Microbiology Research.

Roders, A. and Fletcher, D. L. (1966). Principles of industrial microbiology. Pergramon Press. London.

Salih, M. S. E. (1971). Studies on bacteriological quality of fresh meat. Thesis for Ph.. degree, university of Khartoum.

Seran ,T., Ayşegul, E and Şahsene, A.(2012). Prevalence of *Escherichia coli* O157 in red meat and meat products determined by VIDAS ECPT and LightCycler PCR. Turk. J. Vet. Anim. Sci.

Soyiri, I. N. Agbogli, H. K. and Dongdem, J. T. (2008). A pilot microbial ssessment of beef sold in the Ashaiman market, a supurb of Accra, . Ghana. Afr. J. food Agric. Nutr. Dev. 8: 91-103

Stanier, R. X.; Ingraham, J. L.; Eeelis, M. L. and Paintec, P. P. (1986). The microbial world. 5th ed. New Jersey: Prestiee Hall. Technology. 6(9), pp. 835-842.

Slantez, L. W, Chichester, C. O, Gauffin, A. R and Oral, Z. J. (1963). Microbiological quality of food. London, Aca-demic press. science and our food supply www.fda.gov/teachsciencewithfood and www.nsta.org

Tallen,S.M., KriSTin, M. K., Errol a, S., and reginald ,w. B. (2012). Efficient Isolation and Identification of *Bacillus cereus* Group.: Journal

of aoaC InTernaTIonal Vol. 95.

Thi ,T. H. V. (2007) . Detection of Enteric Bacteria in Raw Food Samples from Vietnam and Evaluation of Antibiotic Resistance. A thesis submitted for the degree of Doctor of Philosophy.

Thornton, H . (1968) . Text book of Meat Inspection. °th . Ed . Bailliere, Tindall and Cass ell . London

Velusamy, V., Arshak, K., Korostynska, O., Oliwa, K., Adley, C. (2010) foodborne pathogen detection: in the perspective of biosensors. Biotechnol. Adv. 28, 232e254.

Wahib, I. A. M . (2004). Bacterial Load in Chilled Muttonintended for Export.M.V.Sc Thesis .University of Khartoum.

Wajda1 S, Daszkiewicz1 T and P Matusevičius . (2004) The quality of meat from the carcasses of bulls from crossing Polish black-and-white cows with 103 limousine bulls classified into the different classes in the Europ system . Veterinarija IR Zootechnika IR . T. 27 (49).

Yah C.S., C. Nwinyi O., Chinedu N., and Shalom . (2009). Assessment of acteriological quality of ready to eat food (Meat pie) in Benin City metropolis, Nigeria. African Journal of Microbiology Research Vol. 3(6) pp. 390-395 F.

Yeni ,F., Acar ,S., Polat ,O.G., Soyer ,Y., Alpas,H . (2013). Rapid and standardized methods for detection of foodborne pathogens and mycotoxins on fresh produce.journal homepage: www.elsevier.com/locate/foodcont.

Zhao, C., Ge. , B., De. Villena, j. Sudler, R, Yeh, E, Zhao. S, White, D.G, Wanger D and Meng, J . (2001). Prevalence of Campylobacte SPP,

Escherichia coli and Salmonella serovars in retai chicken, turkey, pork, and beef from the greater Washington, D.C Area.

Appl. Environ. Microbiol. 67:5431-543