ADDIS ABABA UNIVERSITY
COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

STUDY ON THE EFFECT OF ACETIC ACID SPRAY ON ESCHERICHIA COLI LOAD AND MEAT QUALITY AT AN EXPORT ABATTOIR, MODJO, ETHIOPIA.

BY

AMSALU WUDIE

THESIS SUBMITTED TO THE SCHOOL OF GRADUATES, ADDIS ABABA UNIVERSITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN VETERINARY PUBLIC HEALTH

\

JUNE, 2012
DEBRE ZEIT/BISHOFTU, ETHIOPIA

STUDY ON THE EFFECT OF ACETIC ACID SPRAY ON *ESCHERICHIA COLI* LOAD AND MEAT QUALITY AT AN EXPORT ABATTOIR, MODJO, ETHIOPIA.

BY

AMSALU WUDIE

BOARD OF EXAMINERS	SIGNATURE
1. Prof.Tesfu Kassa	_____
2. Dr.Kalay Belhu	_____
3. Ato Tesfaye Lema	_____

ACADEMIC ADVISOR:	Signature
Girma Zewude (DVM, PhD)	_____

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGMENT

I am highly indebted to Dr Girma Zewde, my advisor, for his critical comments and guidance in preparing this thesis. I am also grateful for Dr Tesfaye Ali and Dr.Tariku Jibat for their support through providing literatures and advices.

My cordial gratitude goes to W/ro Alem Mengistu owner of an export abattoir for creating conducive environment in order to accomplish this thesis being on job. I would like to convey my gratitude to Tsige Birhan, Tigist Adugna, Dr Fetia and all staffs of the Export Abattoir.

I would like to express my special thanks to staffs of NVI, particularly Dr Gelagay Ayelet, Dr Shiferaw Jemberu and W/ro Selam Tariku for their assistance in laboratory work. My last but not list thanks go to all my families for their support throughout my life.

ABBREVIATIONS

CAC	Codex Alimentarius Commission
CCP	Critical Control Point
CDC	Center for Disease Control and Prevention
CFU	Colony Forming Unit
CL	Critical Limit
EFSA	European Food Safety Authority
ESISO	Ethiopian Standard International Standard Organization
EU	European Union
FDA	Food and Drug Authority
FSMS	Food Safety Management System
GHP	Good Hygienic Practice
GMP	Good Manufacturing Practice
GRAS	Generally Recognized As Safe
HACCP	Hazard Analysis and Critical Control Points
HELMEX	Hashim Ethiopian Livestock and meat export abattoir
HIV/AIDS	Human Immune deficiency Virus/ Acquired Immunodeficiency Syndromes
HPB	Health Product and Food Branches
IMVIC	Indole,Methyl red,voges-proskauer reaction and Citrate utilization tests
ISO	International Standard Organization
MENA	Middle East and North Africa
MOA	Ministry Of Agriculture
MOH	Ministry of Health
MR	Methyl Red
NACMCF	National Advisory Committee on Microbiological Criteria for Foods
NASA	National Aeronautic and Space Agency
OPRPs	Operational prerequisite programs
PCA	Plate Count Agar
pH	Power of Hydrogen
PPM	Part Per Million
PRPs	Prerequisite Programs
QSAE	Quality Standard Authority of Ethiopia

RVF	Rift Valley Fever
SPSS	Statistical Package for Social Sciences
SSOP	Standard Sanitary Operating Procedures
STEC	Shiga Toxin *E. coli*
UNIDO	United Nations Industrial Development Organization
USA	United States of America
USDA/ERS	United States Department of Agriculture Economic Research Service
VP	*V*oges-Proskauer
VTEC	Vero Toxin *E. coli*
WHO	World Health Organization

ABSTRACT

The study was conducted to determine the effect of acetic acid (2.5%) spray on *E. coli* load in goat carcasses slaughtered in an export abattoir in Modjo, Ethiopia. A total of 144 swabbed samples were taken from 24 carcasses. Forty eight swabs were taken from front leg and hind leg areas before acetic acid spray, immediately after spray and after 24 hrs of chilling at 2±1^{o}C. Following incubation of the samples at 37 ^{o}C for 48 hrs, loads of *E. coli* was visually counted in CFU/cm^2. A portable hand pH meter was used to measure pH of carcass at 15 minutes after slaughter and at 24 hrs chilling at 2±1°C. Color changes of acetic acid sprayed carcass were monitored subjectively after 24 hrs of chilling at 2±1°C. The log mean of *E. coli* count before acetic acids spray, immediately after spray and after chilling were 2.53log$_{10}$ CUF/cm^2, 1.35log$_{10}$ CUF/cm^2 and 1.97log$_{10}$CUF/cm^2, respectively. The number of *E. coli* counts before acetic acid spray was higher in samples from front leg than hind leg area. Paired t-test comparison for means of *E. coli* counts before and after acetic acid spray showed significant difference ($p<0.05$). The log mean of *E. coli* counts for sprayed and chilled carcasses were higher when compared with sprayed carcasses; this variation was statistically significant ($P< 0.05$). Relatively lower pH were measured in sprayed chilled carcasses (with mean pH=5.77) than non-sprayed chilled carcasses (Mean pH=5.98). Mean pH of for non-sprayed chilled carcass and sprayed chilled carcasses were compared using a paired t-test; statistically, the result were significantly different ($p<0.05$). Goat carcasses after spray and chilling showed less darkness this was not appreciable in magnitude. It could be suggested that 2.5 % acetic acid spray with written sanitation standard operating procedures, with implementation of food safety management systems/hazard analysis and critical control points, reduce *E.coli* load, lowers the pH and reduces the darkness of carcasses.

Key words*: Acetic acid, Color, E. coli, Export abattoir, Goat carcass, pH.*

1 INTRODUCTION

Gradual increase in world population and change in lifestyles has resulted in demands for quality oriented foods of animal origin. Meanwhile, the number of incidences of food poisoning cases is increasing throughout the world and many of these outbreaks have been associated with red meats and poultry (Goksoy *et al.,* 2000). The global incidence of food born disease is difficult to estimate but it has been reported that in 2005 alone 1.8 million people died from diarrheal diseases in industrial countries. The percentage of the population suffering from food borne diseases each year has been reported to be up to 30%. Even though the Center for Disease Control and Prevention (CDC) estimated 76 million illnesses with 325,000 hospitalizations and 5,000 deaths occur each year in the US, still there are many limitations in the availability of data on the incidence and impact of food borne illness (Scoti and Stevenson, 2006). Worldwide, Campylobacter, Salmonella and Shigatoxin-producing *E. coli* (STEC) are among the most important bacterial food-borne pathogens. In US; medical costs, productivity losses, and costs of premature deaths for diseases caused by food borne pathogens (*E.coli* 0157,Shiga-toxin producing *E.coli, Campylobacter, Listeria monocytogenes* and *Salmonella*) estimated $6.9 billion per year (USDA/ERS, 2000). According to a recent estimation, food-borne illnesses cost the U.S. $152 billion in health-related expenses each year (Loretz, 2010). In the European Union (EU), 190,566 confirmed human cases of campylobacteriosis, 131,468 cases of samonellosis and 3,159 STEC infections were reported in the year 2008 (EFSA, 2010).

The source of contamination for carcasses are feces, paunch contents and hide or skin (Lahr, 1996). Additional sources for contamination are cross contamination in the slaughter process such as processing tools and equipment, structural components of the facility, water, human contact and carcass-to-carcass contact. Fortunately, the majority of micro floras transferred to carcass surfaces, while aesthetically undesirable are non-pathogenic (IFT, 2000). Microbial contamination of meat starts with settling of microorganisms to the carcass surface from where they penetrate into deeper layers of the meat. Food animals are naturally contaminated with a variety of potential pathogens, meat processors has been applied many microbiological control methods during the slaughter and processing of the meat. Even if the existing approaches to food safety management system has given safe food supply in some countries, estimates of the

morbidity due to food borne illness clearly showed that the existing approaches still inadequate. Thus, reducing the primal surface contamination and avoiding or limiting the microbial growth helps to extend the shelf life of meat. Several intervention strategies have been developed to reduce the level of bacteria on carcass surfaces such as washing and sanitizing with chilled water, hot water, chlorinated water, food grade acids and salts, alone and in combination (Dubal *et al.*, 2004).

Topical spray washes with lactic or acetic acid solutions are widely employed in the meat industry as a post harvest intervention to reduce meat bacterial load. Bacterial load reduction after acid washing has been suggested to result from several factors, including the immediate decontamination (via the physical dislocation) of bacteria from meat surfaces; the bactericidal combination of acid concentration and application temperature and from residual inhibitory effect that may initially be bactericidal due to lowered pH on the meat surface for a short time following an acid wash (Carpenter *et al.,* 2011). Based on studies, lactic acid reduces the counts of naturally occurring *Enterobacteriaceae* on beef carcasses, cuts and trimmings to a variable degree. These reductions were usually significantly higher compared to untreated or water treated controls. While, the prevalence of *Salmonella* and/or Shiga-toxin producing/Verotoxin-producing *Escherichia coli* (STEC/VTEC) after spraying lactic acid on carcasses, beef cuts and trimmings to varying degrees depending on study design and contamination level. Usually reductions were higher on carcasses compared to meat cuts and trimmings (Loretz, 2010). The implementation of a HACCP system has forced meat producers to study their production process and find, monitor and control the critical points (Bolder, 1997).Organic acids are legally allowed as a surface (including meat) decontaminant in the USA; the US Department of Agriculture permits the use of lactic acid for pre-evisceration rinsing of carcasses (Smulders, 1987). Since the treatments of beef carcass with lactic acid are expected to leave small amounts of residual on the surface of the beef carcasses, trimmings or cuts; exposure to the expected low level deriving to use of lactic acid in such treatments. Provided that the use of lactic acid comply European Union specifications for food additives; the endogen city of such substance avoids the concern of food safety (EFSA, 2010).

Subsurface myoglobin plays a role in product appearance, since discoloration is often referred to as the amount of surface area covered by metmyoglobin. Factors for metmyoglobin formation are oxygen partial pressure, temperature, pH, meats reducing activity, and in some cases microbial growth. Antimicrobials have been investigated as intervention treatments to extend shelf life and control pathogens routinely by evaluating microbial growth; however, researchers pay less attention to the effect of antimicrobials on color. Ideally, antimicrobial technologies should minimize microbial growth while either not affecting or improving product color. Stivarius *et al.* (2001) reported that 1% ozonated water followed by 5% acetic acid spray decreased redness, likely because the acetic acid promoted pigment oxidation by reducing muscle pH .The color of fresh meat during retail display is of prime importance in consumer acceptability.

Ethiopian huge livestock resources and high demand of meat in Middle East and North African (MENA) countries consequences the establishment of modern export abattoirs in Ethiopia and hence, currently there are eight operating export abattoirs in the country and more standard export abattoirs are under construction in different parts of the country. According to the report of Ethiopian Meat and Dairy Technology Institute (EMDTI), the annual export potential of the meat of Ethiopia is estimated 72,000 metric tons of meat valued at USD 136 million; however, the country only secured 34 million USD by exporting 10,000 metric tons of fresh meat by the year 2010/2011 (EMDTI, 2011). Despite the crucial role of the sector in countries economy, there are no standard decontamination technologies and food safety management systems are not getting the desired attention in controlling invisible hazards such as pathogenic bacteria. There are organisms like total coli forms, fecal coli forms and *E. coli* which their presence in the given product are indicators of the possible presence of pathogens. Based on customer feedback or anecdotal evidence, personnel of many abattoirs in Ethiopia exporting small ruminant carcasses to markets in the Middle East have stated that shelf-life is shorter for animals from highland than lowland areas. Furthermore, it is claimed that the problem of early darkening of carcasses of highland animals exists for both sheep and goats, without a noticeable difference in magnitude (Abebe *et al.*, 2010). Recently, customers were claimed the problem of early darkening of carcasses of highland sheep and goats. To avoid such problems some export abattoirs came to a decision to use acetic acid as antimicrobial treatment and request the Ministry of Agriculture for an approval. Starting from 2011, Ministry of Agriculture of Ethiopia has approved abattoirs to

use acetic acid spray on carcasses. All export abattoirs working in Modjo, Ethiopia are using 2%-3% acetic acid staring 2011 after getting approval for use from Ministry of Agriculture. Their intention of using acetic acid spray on carcass is mainly for the purpose of improving early darkening of the carcass. Personnels working in these abattoirs believe that after spraying acetic acid, the color of carcass has improved (personal Communication).However, the effect of acetic acid spray has not been assessed in none of the export abattoirs.

Therefore, this study was conducted with the following objectives:

- To determine the effect of 2.5 % acetic acid spray on *E. coli* load.
- To determine the combined effect of acetic acid spray and chilling on *E. coli* load.
- To determine the effect of acetic acids spray on pH and color of the carcass.

2 LITERATURE REVIEW

2.1 Food safety concerns in meat export abattoirs

Highly publicized outbreaks of food borne disease since 1993, primarily caused by bacteria such as *E. coli O157:*H7 and *Listeria monocytogenes*, elicited intense consumer concern about meat safety. In response, regulatory authorities, researchers, and the beef industry initiated efforts to implement food safety management systems to improve microbiological quality. To improve food safety, the USDA Food Safety and Inspection Service (FSIS) began initiating new regulatory requirements during the mid-1990s Meat producers/Packers now must knife-trim carcasses to remove all visible contaminants must comply with written sanitation standard operating procedures (SSOP) must have implemented hazard analysis critical control point (HACCP) systems, and must meet microbiological performance criteria and standards for *Escherichia coli* and *Salmonella* as a means to verify HACCP effectiveness and pathogen reduction. Researchers and meat processors addressed consumer food safety concerns by developing a variety of methods that are now implemented, or are being further developed, to reduce numbers of bacteria on meat and improve microbiological safety (Sofos *et al.,* 1999). Food safety is an important public health issue. Food Safety Management system (FSMS)/ Hazard Analysis and Critical Control Points (HACCP) is a preventive system for assuring the production of safe food products and common-sense application of technical and scientific principles. HACCP is a scientific, rational, and systematic approach used in the identification, assessment and control of hazards during production, processing, manufacturing, storing, packing and use of food to ensure that food is safe when consumed (WHO, 1997). On top of enhancing food safety, HACCP can help regulatory authorities to control the implementation of HACCP and promote international trade by increasing confidence in consumers (Scoti and Stevenson, 2006). Before developing HACCP system, a food business should have effective prerequisite programs which provide the basic environmental and operating conditions that are necessary for the production of safe, wholesome food for human consumption. The HACCP system was started in 1959 when the National Aeronautic and Space Agency (NASA) approached Pillsbury Food Company to produce safe food for astronauts. The system required control over all aspects of food production such as raw material, processing, environmental conditions, personnel, storage

and distribution (Scoti and Stevenson 2006). In 1989 the National Advisory Committee on Microbiological Criteria for Foods (NACMCF), in USA, approved the first major document on HACCP. In 2003, Codex Alimentatrius on food hygiene adopted the latest version of the HACCP guide line document and in 2006. European Union (EU) mandated that all food business to be operated on the principles of HACCP (Scoti and Stevenson, 2006).

Food borne illnesses are defined as diseases, usually either infectious or toxic in nature, caused by agents that enter the body through the ingestion of food. The global incidence of food born disease is difficult to estimate but it has been reported that in 2005 alone 1.8 million people died from diarrheal diseases in industrial countries. The percentage of the population suffering from food borne diseases each year has been reported to be up to 30%. Even though the Center for Disease Control and Prevention (CDC) estimated 76 million illnesses with 325, 000 hospitalizations and 5,000 deaths occur each year in the US, still there are many limitations in the availability of data on the incidence and impact of food borne illness (Scoti and Stevenson, 2006). In US; medical costs, productivity losses, and costs of premature deaths for diseases caused by food borne pathogens (*E. coli* 0157, *Shiga-toxin* producing *E. coli, Campylobacter*, *Listeria monocytogenes* and Salmonella) estimated $6.9 billion per year (United States Department of Agriculture Economic Research Service (USDA/ERS, 2000). According to a recent estimation, food-borne illnesses cost the U.S. $152 billion in health-related expenses each year (Loretz, 2010). Worldwide, *Campylobacter*, *Salmonella* and Shigatoxin-producing *E. coli* (STEC) are among the most important bacterial food-borne pathogens.

In the European Union (EU),190,566 confirmed human cases of campylobacteriosis,131,468 cases of samonellosis and 3,159 STEC infections were reported in the year 2008 (EFSA, 2010). Food hazards can occur at any stage of the food chain. So, adequate control throughout the food chain is essential. Food safety is related to the presence of food borne hazards in food at the point of consumption. Thus, food safety is ensured through the combined efforts of all the participants in the food chain (QSAE, 2008).

Ethiopian huge livestock resources and high demand of meat in Middle East and North African (MENA) countries consequences the establishment of modern export abattoirs in Ethiopia and

hence, currently there are eight operating export abattoirs in the country and more standard export abattoirs are under construction in different parts of the country. In Ethiopia, the HACCP concept has been introduced at the end of 1990s and beginning of 2000 with the assistance of United Nations Industrial Development Organization (UNIDO). The requirement of the HACCP system in this global market leads the Ethiopian export abattoirs to prepare and develop food safety management/HACCP for their organizations. Among the export abattoirs of Ethiopia, Modjo Modern Export Abattoir is the first HACCP certified abattoir in the country in July 2011; Organic Export Abattoir already certified in ISO 22000; 2005 in 2012, While Luna and Hashim Ethiopian Livestock and Meat Export/HELIMEX implementation/developing phase (Personal communication). In All types of food business, management awareness and commitment are necessary for implementation of an effective HACCP system, since its effectiveness rely upon management and employees having the appropriate HACCP knowledge and skills (WHO, 1997).

2.2 Sources of carcass contaminations in abattoirs

Apparently healthy slaughter animals could be a source of carcass contamination, while they carry dirt, dust, and dung on their hide or skin or feet. This serves as sources of carcass contamination while they carry such contaminants (Dickson and Anderson (1992).Meat is a perishable product, which easily gets spoiled when contaminated with microorganisms. The muscle tissue is free of organisms before slaughter, unless certain bacterial diseases affect the slaughter animals. Contamination of carcass takes place during slaughtering process, bleeding, skinning, and evisceration, washing and chilling (Ellerbrock *et al.* 1993).Microbial contamination of the carcass during the slaughtering process results in spoilage of meat reduced shelf life of meat and public health hazards. Most food borne diseases are related to consumption of meat containing pathogenic micro organisms. It is generally accepted the use of good manufacturing practice (GMPs) in abattoir will result in low microbial load of carcass. The microbial load of meat is directly related to GMP during slaughtering. However even under GMP bacterial contamination of carcass is inherent to slaughtering and expected. Contamination of carcass is uneven due to accidental contact with the meat by contaminant during slaughter process (Assegid, 2008).

2.2.1 Water

The microbiology of natural water is extremely diverse. The number and type of bacteria present will depend on the amounts of organic matter present, the presence of toxic substances, the water's saline content and environmental factor such as PH and temperature (Siragusa, 1995).
The threat to human welfare by contamination of water supply with sewage is a prime concern of every one. Water contaminated with human stool and animal feces serve as a source of contamination. The enteric diseases such as cholera, typhoid fever and bacillary dysentery often results in epidemics where water supplies are not properly protected or treated. Water that contains large number of bacteria may be perfectly safe to drink. Routine examination of water for the presence of intestinal pathogens could be a tedious and difficult if not impossible task. It is much easier to demonstrate the presence of some non pathogenic intestinal types such as *E. coli, Streptococcus foecali*s. Since these organisms are always found in the intestines, and normally are not present in soil or water it can assumed that their presence in water indicates that fecal material has contaminated the water supply. *E. coli* and *Streptococcus foecali*s are classified as good sewage indicators. The characteristics that makes them good indicators of fecal contaminations include they are not normally present water or soil, they are relatively easy to identify and they survive longer in water than enteric pathogen (Siragusa, 1995).

2.2.2 Animals slaughtered

It is obvious that the animals that are slaughtered in an abattoir can be the most important source of contamination. Sick animals can spread their contamination to the meat and other edible by product as well as to workers if strict preliminary measures are not taken to prevent these animals infected with one or more microorganisms (Gracy, 1999). Mud on the hooves and feces on the skin seems to be the most important sources of carcass contamination in slaughter houses. For obvious reason, animal cleanliness is known to affect the likely hood of contamination. Ridell and Koreala (1993) have shown that dungy animal increases carcass bacterial counts of beef in Finland. Slaughter house operators try to manage this problem by slaughtering dungy animals separately by slaughtering after clean animals at slower line speed. Formal regulation to implement this procedure was adopted in Ireland in 1998 (Hennessy, 2003), thus surface hygiene

of live animals is of great importance with respect to *salmonella* and *E coli* contamination. Over loaded intestines present an additional hazard for bacterial contamination of carcass since they are very easily punctured during evisceration (Aganga, 1998). Fecal coli forms can be present in a great number in a freshly slaughter carcass. It's present in meat generally indicated directly or indirectly contamination of fecal origin. The presence of coli forms in great numbers and also indicates improper handling and storage (Yalci *et al.,* 2001). Previous studies on bovine hide and beef carcass have shown that *E. coli* O157: H7 can be transferred to the carcass during hide removal and evisceration operation (MacEvoy *et al.,* 2003).

2.2.3 Meat contact surfaces

Equipments are also another vector for transferring bacteria from surface to meat products.
All machineries and equipment used in an abattoir must be designed and situated as to be easily accessible for cleaning. All equipments used in abattoir must always be kept clean; protected state when not in use. All equipments that have been in contact with fecal or diseased-infected material must be cleaned and sterilized immediately before reuse (Siragusa, 1995). Pathogenic bacteria transfer rates from contact surfaces to food items can be influenced by many factors (Laury *et al.,* 2009).

2.2.4 Meat handlers

Worker hygiene is further critical area. Bell (1997) has shown that hand washing, knife blade sterilization is significant in limiting contamination. Workers hand, workers' apron and foot bridge are three concerns of slaughtering process where they act as a source of *E. coli* O157:H7.The line speed and the care taken by personnel obviously affect the hygiene of carcass. Fast working and less care taken by workers increases the risk of un desirable hygienic accidents such as liberation of the gut content on to the carcass by accidental perforation of the gut with a heavy knife. The operator should not spend more time between carcass in cleaning his tools hand and cloths. It is not line speed in an absolute sense that matters but rather the speed relative the way the line has been designed, the skill of the worker and the nature of the materials

(Hennessy,2003). Personnel can also be a source of transfer of *E. coli* O157:H7 to meat products. If personnel do not wash their hands properly or do not change gloves often, then *E. coli* O157:H7 can be transferred from restrooms or from other uncooked meat products. Food safety training can aid in the reduction of potential occurrences with cross-contamination. Many studies have been conducted to determine what influences the retention of food safety training (Laury *et al*., 2009).

2.3 Carcass decontamination methods

Carcass decontamination refers largely to the use of carcass spray washing systems which are employed to reduce and/or kill bacteria on carcasses. Spray washer must be judged as a single step in the whole process of producing hygienic carcass and not as a single step toward to pathogenic reduction. Application of decontamination process may have an influence on product quality, worker safety, as well as on the environment, and therefore, these criteria should be considered in the treatment selection (Siragusa, 1995). The extent to which carcasses are contaminated with bacteria is influenced by plant design, speed of slaughter and skill of operators but also varies with seasons of the year, type of the animal slaughtered anatomical carcass site and steps in the dressing process. Despite all efforts targeted on the maintenance of good hygiene practice during meat production, prevention of carcass contamination with meat-borne pathogens during slaughter can hardly be warranted. Antimicrobial intervention technologies are therefore gaining interest in order to reduce bacterial contamination levels through implementation of decontamination treatments or antimicrobial procedures for inhibition or retardation of microbial growth. Such interventions should be safe, economic, and feasible in the production process, widely accepted by the consumers and they should not change the organoleptic properties of food. This must be considered because it is a well-know principle of meat hygiene to hold carcasses as dry as possible to limit potential growth of bacteria. Thus, shelf life of the meat can be influenced by the decontamination procedures (Dickson, 1990; Dorsa *et al*., 1998). Efficiency of methods used to reduce number of bacteria on the surface of carcass is influenced by water pressure, temperature, chemicals, present and their concentration, time of exposure and method of application (Sofas *et al.*, 1999). Acceptable decontamination system should not have adverse toxicological or other health effects on workers during their application or on consumers as a

result of their use (Sofas *et al.*, 1999). Even if carcass decontamination technologies are effective, the microbiological status, of resulting product will affected by subsequent handing, exposure to additional contamination, and application of further decontamination or preservation treatments. Nevertheless, carcass decontamination should reduce incidence of pathogens of fecal origin that are mostly introduce in the plant and originating on or in the animals. The followings are description of methods that are currently used or that are being developed for use, to reduce bacterial contamination on carcass (Loretz, 2010).

2.3.1 Physical decontamination treatment

Water

Among the treatments for decontaminating carcasses, rinsing the carcass with water is perhaps the single most effective means to remove at least $1\log_{10}$ APC or specific organism reduction. The fact that water spraying have results in a reduction in APC, like that of spraying with organic acids it is evidence that the physical removal of bacteria from the layer washing. The process of bacterial attachment is complex even if the underlying attachment surface is inert and homogenous like stainless steel. In the case of animal carcass, the number of variables increases greatly and the attachment process become more complicated. The attachment process is thought to be of two stages (Anderson and Marshall, 1979), the first stage being reversible and the second stage more permanent or irreversible. The first stage is largely characterized by a physical interaction between the particle (bacterium) and the surface (carcass).The second stage is thought to be accompanied by the synthesis or action of a general attachment substance (Siragusa, 1995).

Generally, antimicrobial agents, such as organic acids, are apparently effective on those organisms that are contacted by the acid for a long enough period of time to have a lethal or inhibitory response. However, for organisms that are not in effect with the agent for a sufficient period of time, or have become entrapped or protected in the carcass surface, the application of an antimicrobial has no real effect. In the literature, the concentration of organic acids applied are often well below the levels needed to effect any adverse physiological response, much less lethality for species such as *E.coli* (Cutter and Siragusa, 1994a). Therefore, on carcasses

following an antimicrobial spray or hot water, addresses the two steps (i.e. detachment and inactivation) necessary for a carcass decontamination system to be effective (Siragusa, 1995).

Most of the model system used to evaluate the effectiveness of carcass washing system use a very large level of inoculums or rely on endogenous contamination to demonstrate spray washing efficacy. As an example, Barkate *et al.* (1993) sprayed hot water on uninoculated carcasses that had bacterial levels of $\log_{10}$ 2-3.4 APC CFU/cm^2 before treatment. Following treatment with hot water (95^{o}C at source, 82^{o}C on carcass surface) the levels decreased to a mean of $\log_{10}$1.3 APC CFU/cm^2.These results are probably more accurate reflection of commercial carcass washer efficacy than laboratory model generated data with artificially inoculated carcass tissue (Siragusa, 1995).

Chilling

The antibacterial activity of air chilling on red meat carcasses is mainly based on the surface desiccation achieved by high air velocity (Spescha *et al.,* 2006). However, published data indicate that chilling of beef carcasses can result in increases, decreases or no changes in microbiological contamination, dependent on temperature, air speed, humidity, carcass spacing and duration (Nutsch *et al.,* 1997, Gill *et al.,* 1999;. Bacon *et al.,* 2000; Gill and Landers, 2003 Arthur *et al.,* 2004; Corantin *et al.,* 2005; Kinsella *et al.,* 2006). Direct comparison between Studies is often hampered by incomplete information on process parameters. Exact parameter constantly achieving defined bacterial reductions remain to be defined (Bolton *et al.,* 2001).

2.3.2 Biological

Biological interventions such as bacteriocins and bacteriophages show some promise as decontamination treatments. Bacteriocins are proteineous toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strain(s). They are typically considered to be narrow spectrum antibiotics, though this has been debated. In contrast to the currently used antibiotics, bacteriocins are often considered more natural because they are thought to have been present in many of the foods eaten since ancient times. The bacteriocin, Nisin actually has GRAS

(generally Recognized As Safe). Nisin and other bacteriocins produced by lactic acid bacteria have received a great deal of attention because they are produced by bacteria largely considered beneficial to human health and to food production.

Bacteriophages are viruses that infect and multiply in bacteria. For many bacteriophages, release into the environment after replication is accompanied by lysis of the host bacterium (Joerger, 2003). Bacteriophages are increasingly used in the food industry, especially to inactivate *L. monocytogenes* (Greer, 2005). Bacteriophages are generally considered as safe in application and highly host specific (Greer, 2005; Hudson *et al.*, 2005). Yet their use on food commodities is still impaired by factors such as guarantee of a sufficient threshold level or potential resistance development. For beef carcasses, studies on the antibacterial activity of bacteriophages or bacteriocins are so far very limited. In view of bacteriophages, most available data originate from studies examining beef meat and meat products. Cutter and Siragusa (1994a) tested the antibacterial activity of Nisin sprayings on inoculated beef carcass surface parts. Reductions obtained for *B.thermosphacta,Carnobacterium divergens* and *L.innocua* thereby ranged from 1.8-3.5logCFU/cm^2.Under commercial conditions, Nisin treatment of naturally contaminated beef carcasses yielded only marginal reductions (<0.2 orders of magnitude) (Barboza de Martinez *et al.*, 2002). However, the combination of Nisin and lactic acid (1.5%, 25 °C) sprayings reduced aerobic bacteria, coliforms and *E. coli* by 2.0, 2.2 and more than 1.0 log CFU/cm^2 ,respectively (Barboza de Martinez *et al.*, 2002). Compared to single lactic acid spraying, the combination treatment thereby clearly enhancing the reductions obtained (Cutter and Siragusa, 1994b). Nisin was applied to separate beef carcass surface tissues inoculated with approximately 4$\log_{10}$CFU/cm^2 of *Listeria innocua*, *Carnobacterium diverges* or *Brochothrix thermosphacta*. Reduction of 3.3, 3.0 and 3.6$\log_{10}$ CFU/cm^2, respectively were effected after storage of carcasses for 24 h at 5 ^{0}C. The control tissues sprayed with water showed a reduction of less than 1 $\log_{10}$ for each species. The magnitude of the Nisin reduction is largely dependent on the susceptibility of the individual strain and species of target organism. A major step here is to demonstrate the effectiveness of bacteriocins against gram negative pathogens. A measure of the effect of bacteriocin purity in eliminating pathogens would also be valuable. This will require new or different agents with wider bactericidal range or the use of agents that enhance the current

efficacy of bacteriocins. The application of bacteriocinogenic cultures directly to the carcass is another possibility (Siragusa, 1995).

2.3.3 Chemicals

Most commercial meat processing plants apply chemical decontaminants via spraying the carcass. Today, decontamination systems using chemical agents are approved by FSIS for use as a component of a HACCP plan if the chemical are recognized as safe by the food and drug administration. This does not create an adulterant situation and can be supported with scientific studies as effective. The most frequently used chemicals are solution of organic acids (1-3%), such as acetic acids which reduces the number of bacteria of carcass tissue (Sofos *et al.,* 1999). Such organic acid are mostly useful as warm (50-60^{o}c) rinse, applied before chilling, especially in combination with preceding treatment using hot water or steam. Potential concerns associated with use of organic acid are selection of resistant bacteria that may accelerate rate of product spoilage, increase undesirable effects on product appearance and speed equipment corrosion. In addition to organic acids, several other chemical solutions have been approved and tested for use in a decontamination system. Such chemicals include common Chlorine and Chlorine dioxide, Sodium hydroxide, Sodium Bisulfate, Sodium chloride etc (Gill, 1998).

Experiments conducted by Delmore (1997) has evaluated effectiveness of decontamination of six beef variety meats with solutions of chlorine, acetic acid, lactic acid or trisodium phosphate hot water (78-80^{0}C) and steam, applied by immersion, spraying and diffusion. Chlorine and application of acid and hot water are among the most effective decontamination treatments. Immersions of meat in lactic acid or acetic acid were effective in reducing in *L.monocytogenes* and *E. coli* O157:H7. Exposure of meat to decontamination treatments also resulted in sub lethal injury of some bacteria. Injured bacteria cells may repair their injury and cause concerns during extended product storage. Highly publicized outbreaks of food borne disease since 1993, primarily caused by bacteria such as *E. coli* O157:H7 and *Listeria monocytogense*, elicited intense consumer concern about meat safety in response, regulating authorities, researchers and meat industries initiated efforts to implement food safety management system to improve microbiological quality.

Those microbiological decontaminations technologies using chemical, dehairing at slaughter spot cleaning of carcass by knife trimming or steam hot water vacuuming and spraying or washing carcass before chilling with water, chemical solution and steam or hot water. Research has demonstrated that such decontamination technologies are most effective when used in combination sequentially as multiple handlers system. Such system improve regulation commercial meat processing plants apply chemical decontamination via spay rinsing cabinets through which carcasses are passed automatically. Today decontamination system using chemical agents are approved by FSIS for use as a component of a HACCP plans. The most frequently used chemical decontaminations are solution of organic acids (1-3%) such as acetic and lactic acids which reduces the number of bacterial on carcass tissue. Potential problems associated with use of organic acids include selection or present of acid resistance bacteria that may accelerate rate of product spoilage, increase undesirable effect on product appearance and spread equipment corrosion (Gill, 1998). The use of chlorinated water to decontaminate has received much attention. Chlorine (50-800 gm/l) spray on beef carcass has not shown significant reduction of *E. coli* (Cutter and Siragusa, 1995). Johnson *et al*. (1978) reported no difference in APC after spraying beef by 200 mg/l of chlorine. Therefore, most researchers conclude chlorine has little or no effect, unless it is sprayed frequently over a prolonged period of time. These results are not surprising considering the infinitely larger organic load contributed by a carcass or tissue section. The applied chlorine very rapidly becomes bound by the organic load and is no longer antimicrobial. With the current climate of concern over the potential of chloramines formation from chlorine, it is problem that chlorine usage will decrease, if not be eliminated altogether. It is problem that chlorine usage such as chlorine dioxide for the purpose of carcass decontamination. Chemical compounds used for the decontamination of beef carcasses comprise a wide variety of substances. The bactericidal activity of most chemicals is mainly based on the disruption of cellular membranes, other cellular constituents and physiological cellular processes. For appraisal of their suitability in beef processing, it must also be considered that the activity of some is counteracted by organic matter; concentrated substances might constitute a health hazard or ecological threat. Some agents show corrosive properties or their stability is limited in solution. In Europe, some years ago no chemicals were approved for the decontamination of beef carcasses (Hugas and Tsigarida, 2008).

Organic acids

An organic acid is an organic compound with acidic properties. The most common organic acids are the carboxylic acids, whose acidity is associated with their carboxyl group –COOH. Sulfonic acids containing the group $–SO_2OH$, are relatively stronger acids. Alcohols, with–OH, can act as acids but they are usually very weak. The relative stability of the conjugate base of the acid determines its acidity. Other groups can also confer acidity, usually weakly: the thiol group –SH, the enol group, and the phenol group. In biological systems, organic compounds containing these groups are generally referred to as organic acids. Organic acids (C_1–C_7) are widely distributed in nature as normal constituents of plants or animal tissues. They are also formed through microbial fermentation of carbohydrates mainly in the large intestine. They are sometimes found in their sodium, potassium, or calcium salts, or even stronger double salts. A few common examples include: Lactic, Acetic acid, Formic acid, Citric acid, Oxalic acid and Uric acid. Simple organic acids like formic or acetic acids are used for oil and gas well stimulation treatments. These organic acids are much less reactive with metals than are strong mineral acids like hydrochloric acid (HCl) or mixtures of HCl and hydrofluoric acid (HF). For this reason, organic acids are used at high temperatures or when long contact times between acid and pipe are needed (Patanen and Mroz, 1999).

Short chain organic acids have been targeted as the most logical agents to spray on carcasses as antimicrobial agents. Organic acids are used in food preservation because of their effects on bacteria. Lactic, citric, formic and propionic acids have all been reported for this purpose. Lactic and acetic acids are inexpensive, environmentally friendly, and naturally occurring. The key basic principle on mode of action of organic acids on bacteria is that non-dissociated (non-ionized) organic acids can penetrate the bacteria cell wall and disrupt the normal physiology of certain types of bacteria that we call *pH-sensitive*, meaning that they cannot tolerate a wide internal and external pH gradient. Those bacteria includes *E.coli*, Salmonella spp, *C. perfringens, Listeria monocytogenes* and Campylobacter species. Upon passive diffusion of organic acids into the bacteria, where the pH is near or above neutrality, the acids will dissociate and lower the bacteria internal pH, leading to situations that will impair or stop the growth of bacteria. On the other hand, the anionic part of the organic acids that cannot escape the bacteria in its dissociated form

will accumulate within the bacteria and disrupt many metabolic functions, leading to osmotic pressure increase, incompatible with the survival of the bacteria. It has been well demonstrated that the state of the organic acids (un dissociated or dissociated) is extremely important to define their capacity to inhibit the growth of bacteria, compared to undissociated acids (Cutter and Sirgusa 1994a).

Lactic acid and its salts sodium lactate and potassium lactate are widely used as antimicrobials in food products, in particular, meat and poultry such as ham and sausages (Dibner and Butin, 2002). Different authors illustrated remarkably similar results from using short chain organic acids. Using lactic and acetic acid mix spray on lamp had reduced of less than 1log APC and 3% lactic acid spray on beef has reduced the load of *E. coli* to 1.1log (Anderson and Marshal, 1979). All these research results give several examples of the reported results from using short chain organic acids antimicrobial agents. These data are summarized to illustrate that, although each experiment was uniquely different in design. The results are remarkable similar counts by 1-2 $\log_{10}$ CFU/cm^2, regardless of the acid type. The antimicrobial effect of short chain fatty acids is largely due to lowering of the pH where the undissociated form of the acid is maintained (Gill and Newton, 1982; Cutter and Sirgusa, 1994a). Acid concentration appears to be an important factor in the magnitude of the immediate pH drop and antimicrobial effect. However, at high acid concentration the effect of these compounds on the product quality (color, flavor) becomes an important consideration (Siragusa, 1995). Organic acids have been targeted as most logical agent to spray on carcasses as antimicrobial agents. Organic acid (1-3%) such as lactic acid and acetic acids are the most frequently used chemical intervention in commercial plants for both beef and lamb dressing plants. Many other organic acids seen; However, have been researched either separately or as mixture for use in chemical washes including formic acid, ascorbic acid, propionic acid and citric acid (Acaff, 2005).

Unfortunately, the corrosive effective on the equipment seems to increase as the temperature rises. There are conflicting reports as to whether be greater bacterial inhibition by acetic acid as compared to lactic or citric acid washes. Lactic acid (2%) was shown to reduce *E. coli* O157:H7 on beef carcass tissues by 3.3Log and 2% acetic acid reduce it by 1.6$\log_{10}$CFU/cm^2 (Ransom *et*

al., 2003). These authors also found that lactic and acetic acid treatment on chicken meat using spray or immersion resulted in 1.1 $\log_{10}$CFU/cm^2 reductions in total bacteria.

Organic acids like lactic, acetic and propionic have been reported to decrease population of *E. coli* and other bacteria when sprayed on sheep and goat carcasses or used as a wash (Raminez *et al.,* 2001, Dubal *et al.,* 2004). The mechanism of actions of organic acids on the microbial cell is not completely understood, but it is hypothesized that it is the undissociated molecules of the acid that is responsible for the antimicrobial activity. There is a lot of variability in the literature in terms of the cited reduction that can be achieved. This is namely due to difference in the concentration of the acids used by different researcher the method of application, and the types of samples tested. There are also some research evidences shows that organic acid may enhance the shelf life of modified atmosphere packaged products, mainly because they increase the lag phase of the micro organisms. In US organic acid are applied as part of a carcass wash pre chilled and can be applied at the level up to 2.5% of solution (USDA/FSIS, 2004).

In addition, lactic acid is approved for use of the beef carcasses, suboptimal and trimming i.e. pre chilling and post chilling offal and variety meats at level up to 5% at temperature not exceeding 55^0C. USDA has specifically approved lactic acid, acetic acid and citric acids as antimicrobial agents in the final wash that is applied to livestock carcasses after trimming and inspect but before chilling (FDA, 2003). Hot carcass surface treated with organic acids often display some dissociation of tissue or fat surfaces. However, as with hot water pasteurization, this often disappears or becomes less evident after chilling. There may be issue with meat surface discoloration and operators may experience the skin/eye irritation when acetic acid is used. Organic acid (acetic acid and lactic acid) have been evaluated as a method of sanitizing beef carcasses in a spray chilling process. The studies showed a significant (up to 3 $\log_{10}$CFU/cm^2) reduction on total aerobic count and pathogenic population (Dickson, 1990). Based on the evaluated studies; organic acids were most frequently used for the decontamination of beef carcasses. Under commercial conditions, acetic and lactic acid mainly yielded bacterial reductions below1.6 orders of magnitude and the results seemed to be influenced by the point of application during slaughter. Higher reductions, mainly in the range from two to three orders of magnitude, were obtained for inoculated carcass surface parts. Basically, organic acids have

considerable potential for acceptance by the industry because they are quite inexpensive and GRAS (Generally Recognized As Safe) (Siragusa, 1995, Calicioglu *et al.,* 2002). In addition, chemicals as e.g. organic acids show some residual bactericidal or bacteriostatic effects (Dickson and Anderson, 1992; Siragusa, 1995; Smulders and Greer, 1998). On the other hand, potential discoloration of carcasses or respiratory and skin irritation of operators might occur when high acid concentrations are used (Bolton *et al.,* 2001). In literature, it is mentioned the possibility use of organic acids to alter the microbial ecology of meat plant environments and potentially that of the beef and this should be considered when selecting food safety technologies for meat (Acuff, 2005). There is also a concern associated with using organic acids in that they may select for the presence of acid resistance bacteria that may accelerate rates of product spoilage increase undesirable effect on products appearance and speed equipment corrosion (Gill, 1998).

Acetic acid

A variety of organic acids applied as a spray or dips for decontamination purposes have been studied extensively and appear to constitute an effective bactericidal or bacteriostatic surface treatment which also effectively prevents the attachment of microorganisms (Dickson and Anderson,1992; Hardin *et al.,* 1995,Bolder, 1997; Huffman, 2002; Pipek *et al.,* 2005b;). Many processors have also implemented 2% lactic acid washes on pre-evisceration carcasses (Joseph *et al.,* 2006). The natural content of lactic acid in meat is approximately about 10 g/kg; it contributes to the flavor of meat and owing to its antimicrobial effects keeping quality. Specifically, acetic acid has been shown to be effective against E. coli O157:H7 and *Salmonella typhimurium*, reducing these pathogens by 0.1 $\log_{10}$ CFU/cm2 to 4.67 $\log_{10}$ CFU/cm2 *for E. coli* O157:H7 and by 0.73 $\log_{10}$ CFU/cm2 to 2.8 log CFU/cm2 for *Salmonella typhimurium*, respectively on carcass tissue surfaces. Moreover, the lactate anion slows down the growth of surviving microbes during storage (Kotula and Thelappurath, 1994, Mead, 1994; Siragusa, 1995; Dincer and Baysa, 2004). Antimicrobial effect of the organic acids is due to reduction of pH below the growth range and metabolic inhibition by the undissociated molecules of the organic acids evaluated, acetic and lactic acids have been most widely accepted as carcass decontamination rinses. Additionally, it has become widely accepted that the effectiveness of

organic acids is best achieved shortly after hide or skin removal when the carcass is still warm (Huffman, 2002).

The implementation of HACCP system has forced meat producers to study their production process and find, monitor and control the critical points (Bolder, 1997). Organic acids are legally allowed to use as a surface decontaminant in the USA. The US Department of Agriculture permits the use of lactic acid for pre-evisceration rinsing of carcasses (Smulders, 1987). Based on various studies lactic acid spray carcass will be of no safety concern provided the substance used complies with the European Union specifications for food additives (EFSA, 2011). Since 2011 the Ethiopian Ministry of Agriculture (MOA) approved the use of acetic acid spray on carcasses and Modjo modern Luna and Organic export abattoir are using acetic acid spray on carcasses (Personal communication).

Although discoloration is often referred to as the amount of surface area covered by metamyoglobin, subsurface myoglobins also play a role in product appearance. This is because metmyoglobin beneath the surface (located between superficial oxymyoglobin and interior deoxymyoglobin) gradually thickens and moves towards the surface. Metmyoglobin formation depends on numerous factors including oxygen partial pressure, temperature, pH, meat reducing activity and in some cases microbial growth. Although antimicrobials have been investigated as intervention treatments to extend shelf life and control pathogens, researchers often evaluate only microbial growth and pay less attention to the effects of antimicrobials on color. Ideally, antimicrobial technologies should minimize microbial growth while either not affecting or improving product color. Stivarius *et al.* (2001) reported that 1% ozonated water followed by 5% acetic acid spray decreased redness, likely because the acetic acid promoted pigment oxidation by reducing muscle pH. The color of fresh meat during retail display is of prime importance in consumer acceptability. Acceptable fresh meat color is often short lived and a greater or lesser degree of surface discoloration is inevitable.Since consumer purchasing decisions depend on color. Discolored meat considered as spoiled by consumers even microbial loads low and the product is perfectly edible (Mancini and Hunt 2005). Currently, many options are available for instrumental color analysis. For example, several types of instruments (colorimeters and spectrophotometers) are available. Steaming and spraying with lactic acid increased slightly the

lightness of meat surface. Coordinates of (redness) decrease slightly after decontamination treatment (Pipek *et al.,* 2005)

.

2.4 Major bacterial contaminations of carcass

Microorganisms of relevance with regard to meat hygiene include bacteria, parasites, fungus and viruses. Therefore, the focus of meat plant internal hygiene measures is mainly on bacteria, while fungus and virus plays minor role but disinfection measures must also target them. The incidence of parasites should normally pose no major problem in meat which has passed meat inspection or if efficient internal pest control programs are in place on measures are in place. The impact of food poisoning bacterial, depending on the species of microorganisms, is either as a food borne infection or food borne intoxification.

The pathogenic bacteria of most concern include E.coli O157:H7, Salmonella sp.,Listeria monocytogenes,Campylobacter, Clostridium botulinum, Clostridium perfringens, Staphylococcus aureus, Aeromonas hydrophila and Bacillus cereus. According to Kotula and Kotula (2000), the bacteria of most concern for meat spoilage include Pseudomonas, Acinetobacter/Moraxella, Aeromonas, Alteromonas putrefaciens, Lactobacillus and Brochothrix thermosphacta. From food safety point of view, in order to estimate sanitary quality of a given food item, the classic approach is based on the search for not only pathogenic microorganisms but also indicator organisms. The major indicator organisms are belonging to the *Enterobacteriaceae a*nd whose presences indicate possible pathogens and fecal contamination of human and or animal origin (Leclercq *et al.,* 2002). Currently, total coliforms, fecal coliforms and *E. coli* are used as indicators, but in different applications. Microbial indicators are employed more often to assess food safety and sanitation than quality (Feng et al., 2002).

2.4.1 Enterobacteriaceae

Bacteria belonging to the family *Enterobacteriaceae* cause the major gastrointestinal microbial diseases of domestic animals. Most members of this family are gram negative, medium size rods, peritrichate of flagella, if motile; facultative anaerobic and ferment rather than oxidize glucose;

reduce nitrate and able to grow on non-enriched media. However, there are few species of the members that have exceptional properties (Feng *et al.*, 2002; Quinn *et al.*, 2002).

Enterobacteriaceae can be divided in to three groups based on their pathogencity for animals. The first group is those with uncertain significance for animals, which include species from the first genera of the family. The second group is major pathogens of animals such as *Salmonella spp., E. coli* and three of the *Yersinai spp* and the third is opportunistic pathogens that are known occasionally to cause infection.The latter includes species within the genera *Kleibsella, Enterobacter, Proteus, Serratia, Edwardseilla, Citrobacter, Morganella,* and *Shigella.* The members of the *Enterobacteriaceae* are geographically widespread and many are distributed throughout the environment in soil, water, on pla nts as well as in the intestine of animals and humans. However, a few species occupy a limited ecological niche (Quinn *et al.*, 2002; Schaffnen and Smith, 2004).

2.4.2 *E. coli* as indicator of fecal contamination of meat.

An indicator refers to a single or group of organisms or alternatively, a metabolite product whose presence in food or the environment at a given level is indicative of potential quality or safety problem. Microbiological indicators are used in place of direct testing for a pathogen largely because they are easier to work with (IFT, 2000). Extensive studies have been done to determine the total microbial load of fresh meat in abattoir along the slaughter line. The first fecal indicator was *E. coli*. When the concept of fecal indicators is applied to food safety, some additional criteria are stressed. However, the inclusion of *Klebsiella spp in* the working definition of fecal coli forms diminished the quality of this group with fecal contamination. As a result, *E. coli* has reemerged as an indicator, partly facilitated by the introduction of newer methods that can rapidly identify *E. coil*. Since *E. coil* is more indicative of fecal pollution than other genera, it is often desirable to determine its incidence in a coliform population. The growth and survival characteristics of *E. coli* are broadly comparable to many pathogenic *Enterobacteriacea* and pathogenic *E. coil*. Therefore, increases in the number of *E. coil* during chilling, storage and distribution suggest that products has been subjected to condition, which would also growth of these pathogens (Feng *et al.*, 2002; Schaffner and Smith, 2004).

Review of studies shows that total microbial load *c*oliform and *E. coli* counts are influenced by a number of factors including cleanliness of the animal, post slaughter wash season ,dressing methods, sampling techniques incubation temperature and duration .Higher aerobic plate count was recorded in carcasses coming from dirty wooly lamb (Biss and Hathaway, 1995; Biss and Hathaway, 1996).

Characteristics of *E. coli*

E. coli is a gram negative rod (bacillus) in the family *Enterobacteriaceae*. Most *E. coli* species are normal commensal found in the intestinal tract. *E. coli* are bacteria that can survive in an environment with or without air (facultative anaerobes) and depending on the environment may or may not produce thin hair like strictures that allow the bacteria to move and to attach the cell of animals or human. Those bacteria live in the intestine of the people and animals worldwide. Most of the *E. coli* are normal's inhabitants of the small interne and colon and do not cause disease (non-pathogenic). Never the less those non-pathogenic *E. coli* can cause disease if they spread out side of the urinary tract (where they cause bladder or kidney infection or in to the blood stream (sepsis). Other *E. coli* can cause poisoning or diarrhea even though they usually remain within the intestine by producing toxin or intestine inflammation. Most strains of *E. coli* 0157:H7 possess several characteristics in common. It has no ability to grow well if at all at a temperature of greater than 44.5^0C, inability to ferment sorbitol within 24 hours, inability to produce B-glucoron. Unlike most food borne pathogen, *E.coli* O157:H7 uniquely tolerant to acid environment. Studies using acetic, citric and lactic acid at a concentration of up to 1.5% as organic acids sprays on beef revealed that *E. coli* 0157:H7 were not appreciably affected by any of the treatment. Early survey of an antibiotic resistance revealed that *E. coli* O157:H7 were sensitive to most antibiotics. However, recent studies have shown increase resistance to antibiotics. Studies on the thermal sensitivity of *E. coli* O57:H7 in grounded beef have revealed that the pathogen has no unusual resistance to heat. Proper heating of foods of animal origin i.e heating food to an internal temperature of at least 68.3^0C as an important control point to ensure inactivation of *E. coli* 0157:H7 (Quinn *et al.,* 2002).

Colony characteristics

Isolation of *E. coli* in a pure growth from carefully taken samples such as cervical swaps mastitis milk samples and mid stream urine is sufficient for the diagnosis of opportunistic infection caused by *E. coli*. Pathogenic strain of *E. coli* is quite often hemolytic. As they are among lactose fermenters, the colonies on the MacConkey agar are bright pink. In Eosin methylene blue (EMB) agar, *E. coli* colonies have metallic shine (Quinn *et al.,* 2002).

2.4.3 Determination of bacterial number

The examination of foods for the presence, type and number of microorganisms and /or their products is basic to food microbiology. Bacterial numbers can be enumerated as viable count or total (viable and non viable) count in a given samples. Viable bacteria are assumption is made that one well spaced bacterial cell gives rise to one colony. Therefore, bacterial colonies, rather than bacterial cells are counted in most of these methods. It is more commonly used in diagnostic and food hygiene procedures. In other hand, total counts will enumerate both viable and non-viable bacterial cells. In spite of the importance of microbiological counts in food microbiology, none of the methods permits the determination of exact numbers of microorganism in food products. Therefore, although some methods of analysis are better than others, every method has certain inherit limitations associated to its use (Jay, 2000; Quinn *et al.*, 2002).

These methods are classified in to viable counting methods and total (viable and non viable) counting methods. The viable counting methods include: spread plate method, pour plate method, miles-misra technique, filtration methods, most probable number (MPN) and the total (viable and non viable) counting methods including breed direct method, counting chamber method and the turbidity standards (Quinn *et al.,* 2002).

2.4.4 Bacteria attachment

Bacteria must attach to meat surface to remain intact and cause infection. Bacteria attachment to meat has been described as a two-stage process. The first stage involves a reversible adsorption

by the bacteria to the carcass surface. Then, in the second stage the attachment becomes irreversible. Attachment to issue causes bacteria to become trapped in the meat tissue fibrils. The irreversible bindings of *E. coil* O157:H7 to the carcass surface though cross contamination from the hide, and time, the numbers of bacteria that be removed from the surface dramatically decrease (McEvoy *et al.,* 2003).

2.4.5 Sample taking methods on carcass surfaces

For a given sampling situation, the same sampling technique should be used each time to ensure that results are comparable. Sample from carcass surface can be collected in two different methods: destructive and non-destructive methods. An example of destructive method is template excision methods as the non-destructive method includes wet and dry swab, sponge sampling and gauze tampon methods. Secondly, it damages the carcass, which may be unacceptable commercially and laborious. Because of this meat industry personnel prefer swabbing- based method for assessing carcass hygiene (ISO 17604, 2003 and Byrne *et al.,* 2005).

Of the non-destructive sampling methods, swab sampling is the oldest and most widely used method for the microbiological examination of surfaces. In this case moistened swab will be used to rub the carcass surface with pressure at the selected sites by placing a sterile temple first then the swab will be place or the same bottle and stored at refrigerated temperature until plated (Jay, 2000). Exact knowledge of the diagnostic values that is the sensitivity, the specificity, the precision and the predictive value of the classical sampling methods is not available in most literature (ISO 17604, 2003).

2.4.6 Public health impacts of *E. coli*

Most confirmed human *E. coli* O157:H7 out breaks have been associated with the consumption of undercooked grounded beef and less frequently unpasteurized milk, Hence cattle have been the focus of many studies to determine their involvement in transmitting the pathogen. Based on disease symptoms characteristics and on effect on certain cell cultures and serological grouping, there are four to six groups Enterohemorrhagic *E. coli* (EHEC) Entero toxogenic *E. coli* (ETEC),

Enteropathogenic *E. coli* (EPEC), Enteroinvasive *E. coli* (EIEC) and Entroaggregative *E. coli* (EAEC) .The most notorious type of pathogenic *E. coli* known as *E. coli* O157; H7. The name refers to the chemical composition found on the surface of the bacterium. This strain was identified in 1982 following an outbreak of diarrhea resulting from the eating of under cooked beef. The over 700 serotypes are identifying by small antigenic changes in their surface "0" antigens (lipopolysaccharides or molecules bacterial surface of gram negative bacteria. For example, *E. coli* O157:H7 or *E. coli* O55 strains. These zero types are identified by immunological test. *E. coli* strains are further distinguished by "H" protein antigenss (different types of flagella that make the bacteria motile). The O157; H7 *E. coli* strains belongs to a group of bacteria known as Shega toxin production *E. coli* (STEC).They referred to as Verocytotoxic *E. coli* (VCEC) or Enterohaemorrhagic *E. coli* (EHEC)(www.cdc.gov/ecoli).

Researchers suggest that only a few number of *E. coli* O157: H7 are needed to cause infection. Ingestion of about (10-100 organisms) in needed instead of thousands to million needed for infection by other *E. coli* serotypes. Infection is aided by adhesive receptor spili or fimbrae (that attach the bacteria to human intestinal cell. The most important problems caused by the bacteria are due to two Shiga toxins termed as Stx1 and Stx2 and also termed as Vero toxins. Toxins are chemicals that are produced by bacteria and that damage human cells. These toxins produced by another related bacterium. *Shigella spps* that can damage and kill intestinal cells and occasionally cause anemia, damage to platelet, specially the kidney(www.cdc.gov/ecoli).

The initial symptoms of *E. coli O157; H7* infection usually appears about 3 to 5 day after a person ingests the bacteria. The symptoms include nausea, vomiting, stomach cramps and bloody diarrhea with mild fever. *E. coli* O157: H7 is a major health problem. It is estimated to cause infection in more than 70,000 people per year in USA. The US Center for Disease control and prevention (CDC) suggests *E. coli* O157:H7 is responsible for the majority of *E. coli* out breaks in US.The diagnoses of *E. coli* O157:H7 infection begins with an accurate history, physical examination and analyses of a sample of stool from the patient. A presumptive diagnoses is frequently made if the patient has symptoms of blood diarrhea and history of being exposed to a person, food or liquid known to be a source of *E. coli* O157; H7 outbreak. Because of other bacteria for example *Shigella* and *Salmonella* can give patients similar initial symptoms. A

definite diagnosis is based on culture of *E. coli* O157:H7 from the patients sample of stool on special culturing plates that then tested with antiserum (antibodies) that react only with *E. coli* O157:H7 (www.cdc.gov/ecoli).

2.5 The impact of microbial contamination on carcass

Meat hygiene serves to minimize the impact of undesirable microorganism and chemical residues on meat. Control of microbial contamination is the responsibility of meat plants. Bacteria that cause food borne infection must first multiply to a high infections numbers in rich protein foods such as meat and have to be ingested by consumers. They cause sickness through microbial metabolic substances like toxic substance released by the living micro organisms inside the human digestive tract. The most known examples of food borne infection are those caused by Salmonella bacteria. It is estimated that 10^{5} of Salmonella bacteria /g of food are needed the ingested food to cause Salmonellosis. On cases of a recently emerged very pathogenic form of the normally harmless *E. coli* bacteria (Entero-pathogenic form most type of O 157; H7 residing in fecal martial. on skin of animals) only a few hundred bacteria per gram of food can cause severe illness with gastro-intestinal symptoms, fever and even death. Micro organisms causing food borne intoxifications produce and release the poison during their multiplication in the food. Upon ingestion by consumers of such food, which was heavily intoxicated outside the human body, severe gastro intestinal food poisoning symptoms (vomiting, diarrhea, abdominal pain fever) occur (CAC, 2005).

Food borne intoxifications are frequently caused by staphylococcus. These bacteria are present in purulent wounds and frequently in the respiratory tract of healthy people. When they get in to meat, which is not sufficiently refrigerated, they multiply rapidly and produce toxins, which cause severe gastrointestinal symptoms only a few hours after ingestion by consumers. Another bacterium, *Cl. botulinium*, in the absence of oxygen. The canned food or deep lagers of raw fermented ham, is capable of producing one of the strongest toxins known. Intoxication if not treated immediately, can be fatal to consumers. Bacteria are the most common food poisoning microorganisms a part from bacteria, fungus can also play a role in the incidence of food poisoning (CAC, 2005).

2.6 Overview of meat export in Ethiopia

Ethiopia being an agricultural country is bestowed with immense agricultural potentials it ranges first in Africa and tenth in the world in number of cattle (CSA, 2004). It has high livestock population with diverse genotype. Recent data indicate that there are 52 million cattle (25 breeds), 63 million sheep (13 breeds) and goats (15 breeds) and 2.5 million camels (4 breeds). These huge livestock resources and high demand of meat in Middle East and North African (MENA) countries results establishment of modern export abattoirs in Ethiopia. Currently eight export abattoirs are functional in Ethiopia. The annual export potential of the country is estimated at 72,000 metric tons of meat valued at USD 136 million. As a response for the meat export opportunities in MENA countries, In 2009/2010 Ethiopia earned 124.5 million USD from meat and live animal export. Total earnings from meat was raised by 23.1% and it reached 34 million USD in 2010, with the highest meat export of the country being recorded in the same year (10,182 metric tons of meat) to different destinations. In general, all the export abattoirs in the country lack the required sanitation standards to produce quality and safe hygienic export meat and by products, and in other words, the value chain lacks, efficient transport system for slaughter animal, efficient cold chain system and high levels of sanitation procedures. Apart from this, almost all abattoirs do not have physical separations between clean and dirty lines; hot water system is not available. As a result, meat exported from the country has a very short shelf life. The shelf life of products at times is very short and it gets spoiled before it is distributed in the recipient country (EMDTI, 2011).

2.7 Meat safety concerns in Ethiopia

Food safety plays a significant role in the national economic and health development by safe guarding the health of the nation enhancing tourism, national and international trade. Countries with well established food safety assurance system could export and trade their products without any barriers and become competitive in the global trade. The issue of food safety is primarily a public health issue and to obtain safe food is a basic human right. In recent years these concern has increasingly become the issue of developing and less developed countries specifically with an increase in the number of vulnerable group of the society, immune compromised patients

including people living with HIV / AIDS. Food safety system in developing countries in general and Africa in particular is weak, unable to protect human health and as a result of stringent food safety laws of developed nations are unable to export its potential raw and processed food .These countries are not only losing the economic benefits, they should get through export meat but also over stretching the national health services as a result of food borne illness. The meat inspection proclamations and regulations provide for the control of slaughter houses and establishment and ensuring safety of meat and meat products. Health and health related in dictators of the Ministry of Health (MOH) published in 2004 shows that among the ten leading causes of outpatient visits to health institution are all forms of diarrhea which are directly related to food.

3 MATERIALS AND METHODS

3.1 Study area and animals

An export abattoir in Modjo town is the area used to conduct the study from October 2011 to April 2012. Modjo is a town found in central Ethiopia, located in the East Shewa Zone of Oromia Regional State at distance of 70 Kms South East of Addis Ababa. The latitude and longitude of the town is 8°39′N and 39°5′E, respectively, with an altitude of 1778 meters above sea level. The average minimum and maximum temperature are 18^{o}C and 28^{o}C, respectively and has an experience of bimodal rain fall pattern in which the main rainy season occurs between June and September and short rainy season from March to May .The average annual rain fall is of 800 mm (ILRI, 2005). The study was conducted at one of the export standard abattoirs in Modjo At this export abattoir 500-1500 sheep and goats are slaughtered every day depending on the demand from customers, availability of supply of animals and air cargo space but for ethical reason the name of the export abattoir is not mentioned.

Ethiopian indigenous goat types which originated from pastoral (low land) areas including Borena, Awash Metahara, Arbaminch, Jinka, Messo, Bable, Bati (Wollo) were used in the abattoir as slaughter animal. These goats were considered to have been maintained under traditional management condition i.e. normal feeding and watering regime. Only male adult animals were slaughtered based on dentition (ESGPIP, 2008).From these goats' apparently healthy goats that were rested in the lairage for 24 hrs were used as study animal.

3.2 Study Design

A total of 24 goat carcasses were selected randomly from a standard commercial slaughtering procedure to determine the load of *E coli.* From these carcasses, 144 swabbed samples were taken; forty eight samples were from front and hind legs before acetic acid spray (controlled). Similar samples were taken from the same carcass immediately after 30 minutes of spray(treatments)) and after chilling 24 hrs at 2±1°C. Each sample was labeled for easy identification.

E. coli counts were converted to $\log_{10}$ CFU/cm^2 before data analysis in order to normalize the data.

Goat carcasses were sprayed with acetic acid solutions (2.5%) for 10 seconds using low-pressure hand held sprayers. The samples from the front and hind leg were taken to determine *E. coli* load on the two sites. Swabbing at the time of sampling was done at the area of 50 cm^2 that are delineated by sterile aluminum template (10 mm X 5mm).The pH of the carcasses was determined twice with a hand HANNA pH meter. The initial pH measurements of the carcasses were determined 15 min after dressing before spraying with 2.5% acetic acid solution. Second pH was measured after chilling the carcass at 2±1°C for 24 hours.

Figure 1; Acetic acid spray using low-pressure hand held sprayers.

3.2.1 Swabbing

t was first soaked in 10 ml of peptone water in a test tube and rubbed first horizontally and then vertically several times on the sampling site within the metal template. On completion of rubbing process, the swabs were then put into sterile test tube filled with 10 ml of 0.1% sterile peptone water and transported using an insulated ice box at 4°C to the Microbiology Laboratory of National Veterinary institute (NVI), Debre Zeit/Bishoftu.

3.2.2 Bacteriological sample processing

Up on arrival at the laboratory the swabs, samples were processed in the laboratory within an hour of collection. However, non processed samples were stored at 4^{o}c only for less than 24 hours; For culturing, the upper shaft of the swab was cut by sterilized scissor and discarded while leaving the cotton swabs back in to the 10 ml of 0.1% sterile peptone water in test tube Using sterile pipette, after thorough agitation of the swabs, starting from the higher concentration rate, 1 ml was poured to sterilized Petri dishes previously filled with 15 ml sterilized MacConkey agar by partly lifting its cover. The sample and the media were mixed by moving the Petri dishes in a circular motion and were left on a table until solidified. The inoculated media were incubated at 37°C for 48 h. To check sterility of the media two sample free Petri dishes were incubated under the same condition.

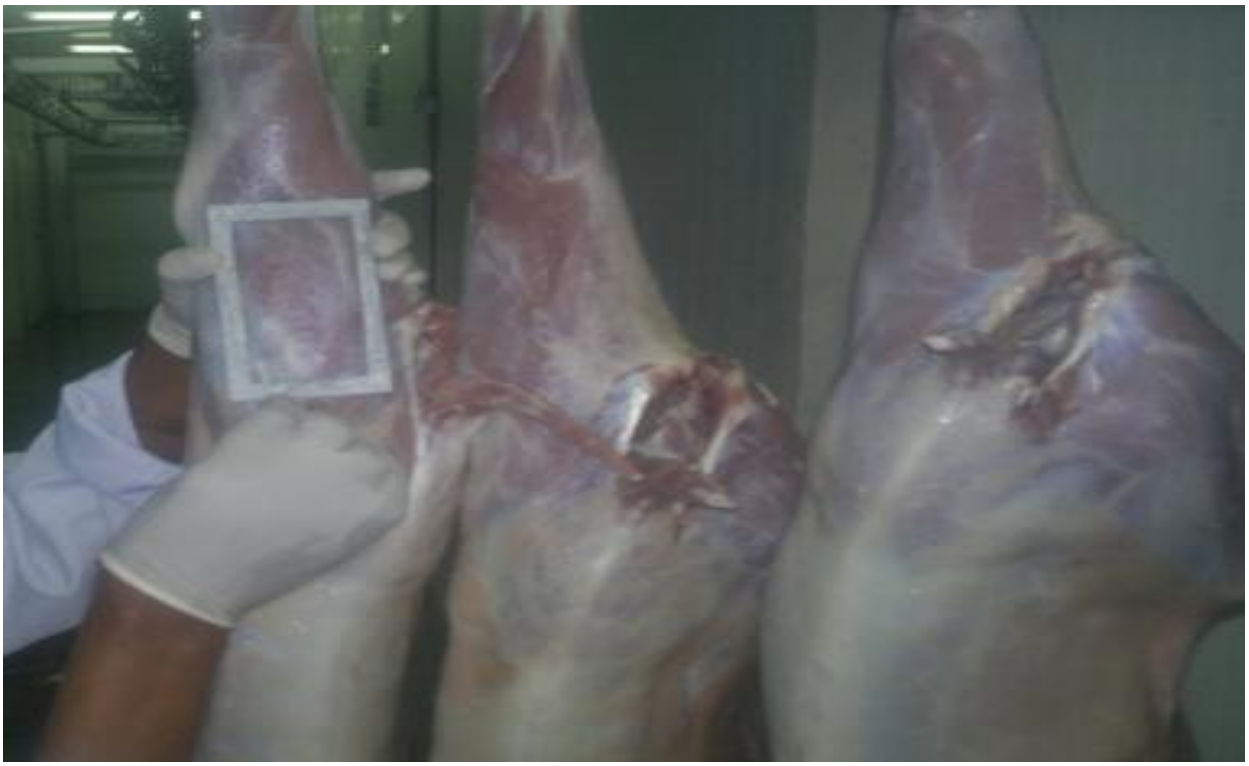


Figure 2: swabbing a sample site in sterile aluminum template (10 mm X 5 mm).

Figure 3; Sample processing, mixing sample with media in a sterile condition at NVI Lab.

Preparation of Decimal dilutions

Preparation of decimal dilutions, identifications and enumerations of *E. coli* was done following the method described in Feng *et al*. (2002) and HPB method (2001). A series of sterile test tubes were filled with 9 ml of peptone water labeled as 10^{-1}, 10^{-2}, 10^{-3} etc depending on the expected bacterial load. Further dilutions were prepared using the same technique until the dilution rate of required. Using sterile pipette, after thorough agitation of the swabs collected from the abattoir with vortex agitator for seven seconds, 1ml of the aliquot was transferred from properly homogenized initial test samples in to test tubes labeled as 10^{-1} .This again was thoroughly agitated, and using new sterilized pipette from $10^{-1.}$test tubes, 1ml was transferred to the test tube labeled $10^{-2.}$.Then again after thoroughly agitation with new sterile pipette, 1ml was transferred from 10^{-2} in to test tubes labeled $10^{-3.}$

Preparation of MacConkey agar

Preparation of MacConkey agar (CM7 0109,OXOID ltd, Basing Stoke ,Hampshire ,England) was carried out according to the direction of the manufacturer.Briefly,51.5 gm of MacConkey agar was suspended in 1 liter distilled water and was boiled to dissolve completely. This was dispensed in to 15 ml test tube and sterilized using autoclave at 121^{o}c for 15 minutes. Following this, the media was cooled in water bath to temperature of 46^{o}C.

Biochemical Tests

In the differentiation of *E. coli,* from other related species, Indole, Methylred, Voges-proskauer reaction and Citrate utilization tests (IMVICTest) were done on presumptive *E. coli* subcultured on Broth agar. For this purpose Indole test were carried out using SIM media (BBL) and Methylred and Voges Proskeauter reaction were performed with MR-VP broth (TM324Titan Biotech Limited, India).Simon's Citrate agar (Difco USA) was used for citrate utilization. The test reagents utilized for the tests include Kovac's reagent for Idole test,Methyl red for methyl red test, and VP-1and VP-2 chemicals for Voges-Proskauer reaction (annex -1).Interpretation of results particularly for citrate utilization test was based on the change observed on the media

utilized. *E. coli* is in dole positive, MR positive, VP negative, and Simmons citrate negative. Interpretation of biochemical test results was accomplished according to (Peng *et al*., 2001 and Prescott, 2002).

Determining *E coli* load

Following the IMVIC test test to confirm the grown colony, the total count of *E. coli* was determined according to US Bacteriological Analytical Manual (Feng *et al.*, 2001 and ISO, 17604, 2005,). After48 hrs of incubation, colonies with bright pink color and with flat or elevated surface and complete white edges were counted visually. The numbers of colonies grown and counted were then multiplied by the level of dilution. Finally; the total number of *E .coli* per ml of sample culture was obtained.

3.2.3 Effect of acetic acids spray on pH and color measures

A portable hand calibrated HANNA pH meter was used to measure the pH of goat carcass 15 minutes after slaughter and after 24 hrs chilling at 2±1°C. Carcass pH measurements were done for non-sprayed non-chilled carcass, non-sprayed chilled carcass (control) and sprayed chilled carcass (treatment). After the treatment, carcass color changes were monitored subjectively after 24 hrs chilling at 2±1°C.

3.3 Data analysis

Data were encoded into Microsoft Excel. The database was transferred to SPSS 15.0 for windows version. After normalizing the data by using the $\log_{10,}$ descriptive statistics such as means and standard deviations were performed. The means of sample specific *E. coli* counts were compared using t-test at 95% confidence interval.

4 RESULTS

4.1 Mean of total *E. coli*

The *E.coli* load of 144 samples before spray, immediately after spray, after chilling for 24 hrs at 2±1°C and after 48 hrs of incubation, was determined. The log mean of *E. coli* count before acetic acids (2.5%) spray, 30 minutes after application of acetic acids (2.5%) and after 24 hrs chilling at 2±1°C were 2.53 $\log_{10}$ CFU/cm^2, 1.35 $\log_{10}$ CFU/cm^2 and 1.97 $\log_{10}$ CFU/cm^2 respectively. The respective maximum values were 4.38 $\log_{10}$CUF/cm^2, 3.12 $\log_{10}$CUF/cm^{2}and 3.87 $\log_{10}$CUF/cm^2 (Table1).

Table 1: The summary of descriptive statistics for the total *Escherichia coli* count for different treatments.

Sample Type	Sample size	Mean $\log_{10}$CUF/cm^2	95% CI for mean $\log_{10}$CUF/cm^2	SD	Min	Max
TECBS	48	2.53	2.15-2.91	1.31	0.00	4.38
TEAS	48	1.35	1.04-1.65	1.06	0.00	3.12
TECASC	48	1.97	1.64-2.30	1.12	-0.04	3.87

TECBS= Total E. coli count before spray, TECAS =Total E. coli after spray, TECASC= Total E. coli after spray and chilling.

The log mean of *E. coli* count before acetic acid spray for samples from front leg area were 2.84 $\log_{10}$CFU/cm^2 (SD= 0.87); whereas, from the hind leg area were 2.25 $\log_{10}$CFU/cm^2. The number of *E. coli* counts before acetic acid spray was higher for samples from front leg area (Table2).

Table 2: The summary of descriptive statistics for the *Escherichia coli* count for different treatments at the two sampling site.

Type of sample	No of sample	Mean log 10 CFU/cm^2	SD	Min.	Max.
ECBSF	24	2.84	0.87	1.97	3.71
ECBSH	24	2.25	1.60	0.53	3.82
ECASF	24	1.57	1.00	0.57	2. .57
ECASH	24	1.13	1.10	0.03	2.33
ECASCF	24	2.06	1.04	1.03	3.09
ECASCH	24	1.89	1.20	1.69	3.09

ECBSF= E. coli count before spray of front leg area, ECBSH= E. coli count before spray hind leg area, ECASF= E. coli count after spray front leg area, ECASH= E. coli count after spray hind leg area, ECASCF= E. coli count after spray and chilling Front leg area, ECASCH=E. coli count after spray and chilling hind leg area.

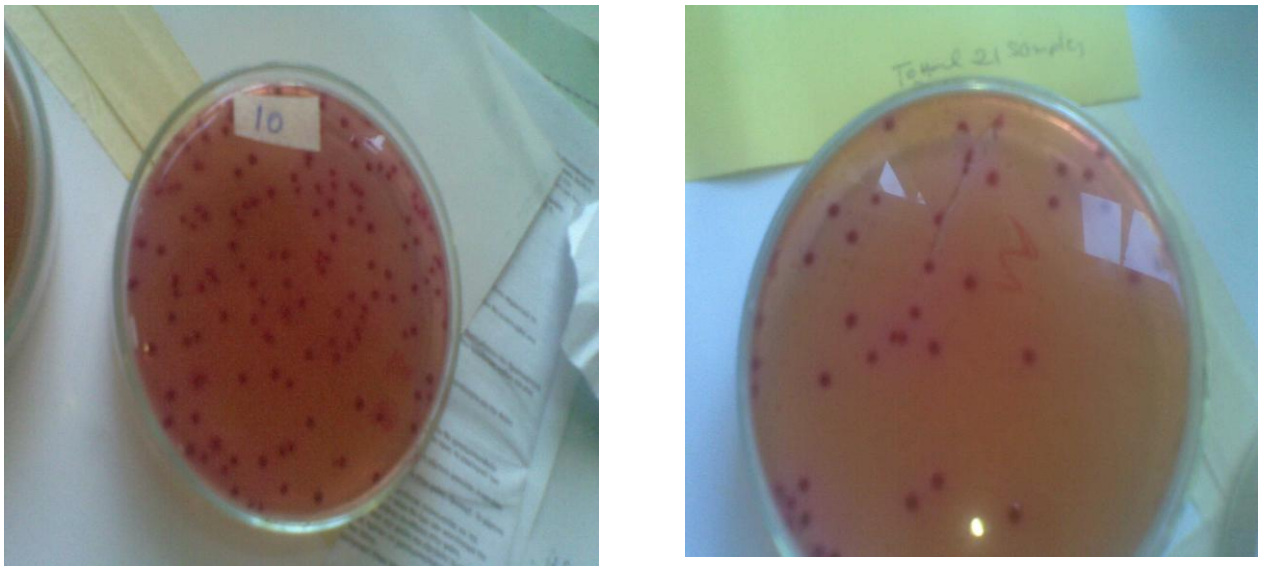
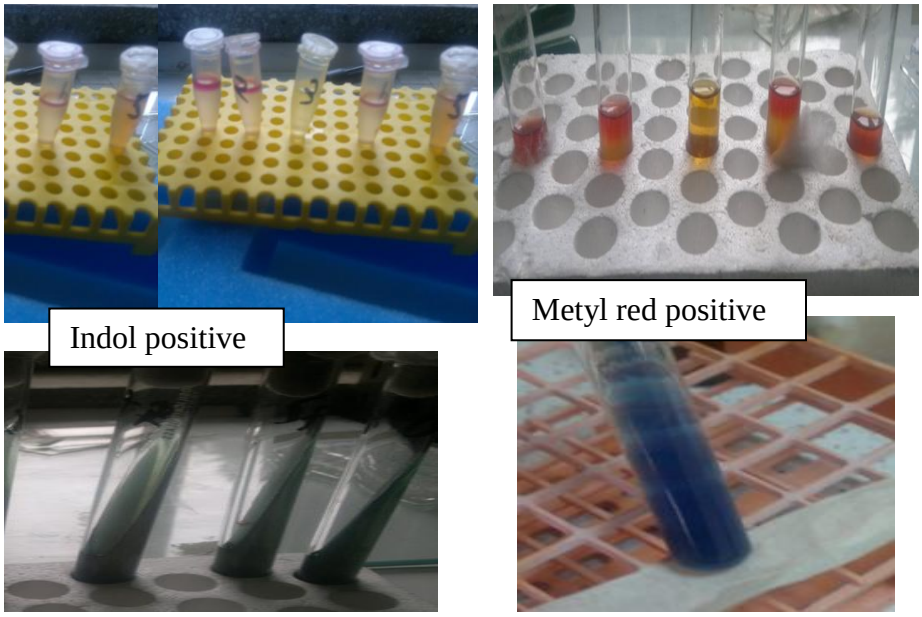


Figure 4: E. coli grow on MacConkey media from samples before acetic acid spray (left) and after spray (right).



Citrate negative Citrate positive control

Figure 5:IMVIC test test to confirm the grown colony

4.2 Comparison of the means of *Escherichia.coli* count before and after acetic acid spray

Paired t-test statistical analysis for mean of *E. coli* counts before and after acetic acid spray showed significant difference ($p<0.05$) (Table 3). The paired t-test on the mean of *E. coli* count before spray between the two sampling sites were statistically significant ($p<0.05$) (Table 3).

Table 3: Paired comparison of the mean difference of in total Escherichia coli count before and after acetic acid spray.

Type of sample	sample size	*Mean difference	SD	95%.CI for the mean	t-test	P-value
ECBS	48	2.53	1.31	2.15-2.91	13.39	0.00
ECAS	48	1.35	1.07	1.04-1.66	8.77	0.00
ECASC	48	1.97	1.12	1.65-2.30	12.19	0.00

**=$Log_{10}CFU/cm^2$ value ECBS = E. coli count before spray, ECAS= E. coli count after spray and ECASC = E. coli count after spray and chilling, SD= Standard deviation of the mean difference, CI= Confidence level of the mean difference*.

4.2 The combined eeffect of acetic acid spray and chilling on *Escherichia coli* load

The $log_{10}CUF/cm^2$ mean of *E. coli* count of carcasses sprayed acetic acid and chilled at 2±1°C were high compared with the $log_{10}CUF/cm^2$ mean of carcasses immediately after spray (picture5).The paired t-test on the difference in *E. coli* count between the two treatments were statistically significant ($p<0.05$) (Table 3).

4.3 Effect of acetic acid on pH and color of carcass

4.3.1 Effect of acetic acid spray on pH

. The mean pH value of goat carcasses at 15 minutes after slaughter in this study was 6.38. Relatively lower pH were obtained in sprayed chilled carcasses (with mean pH=5.77) than in non-sprayed chilled carcasses (Mean pH=5.98)(Table 4).

Table 4. PH value of the fresh goat carcass before, spray and chilling after only chilling and after chilling and sprayed.

Type of sample	Number of samples	PH Mean	SD	SE
PHNSC	24	6.38	0.42	0.09
PHNSCC	24	5.98	0.15	0.03
PHSCC	24	5.77	0.09	0.02

PHNSC = pH for non-sprayed, PHNSCC = pH for non-sprayed chilled carcass, PHSCC= pH for sprayed chilled carcasses.

The mean pH of non-sprayed chilled carcass and sprayed chilled carcasses were compared using a paired t-test; statistically, the result were significantly different ($p<0.05$).

Table 5. Paired comparison of the mean of pH value before and after acetic acid spray after chilling.

Type of sample	Sample size	Mean of difference	SD	95% CI for the mean	t-test	P value
PHNSNC	24	6.38	0.40	6.20-6.56	73.78	
PHNSCC	24	5.97	0.10	5.98 -6.04	306.60	0.23
PHSCC	24	5.77	0.00	5.73-5.81	196.00	0.01

PHNSNC = pH for non-sprayed &non-chilled carcass, PHNSCC = pH for non-sprayed chilled carcass, PHSCC= pH for sprayed chilled carcasses, SD= Standard deviation, CI= Confidence level of the mean difference.

4.3.2 Effect of acetic acid spray on color change

Non-sprayed chilled and sprayed chilled carcasses were visually and subjectively monitored for color changes. According to this subjective observation sprayed goat carcasses after chilling showed less darkness.

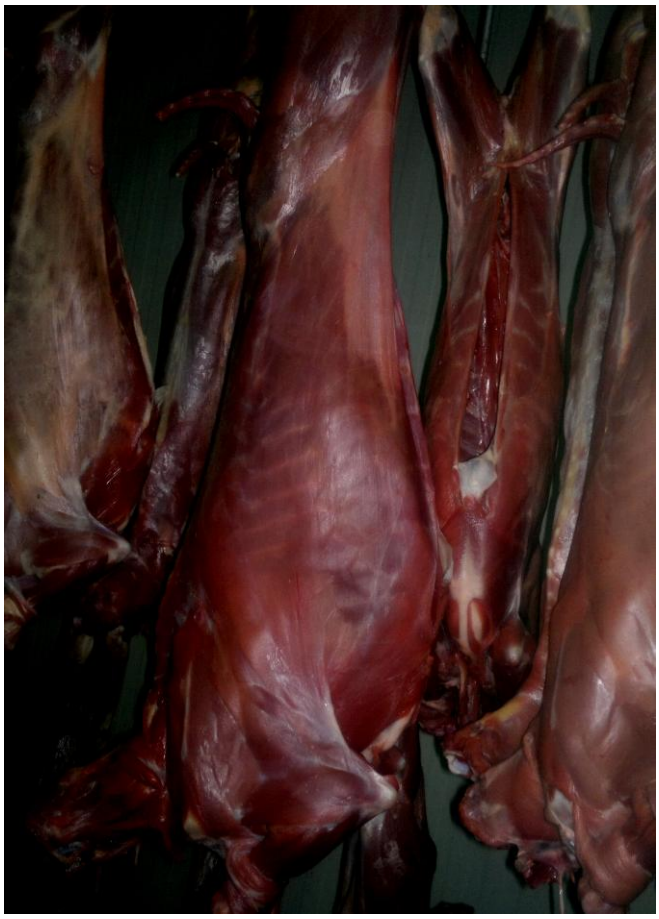


Figure 6: Photo of non acetic acid sprayed chilled carcasses

Figure 7: Photo of acetic acid sprayed chilled carcasses

5 DISCUSSION

In the current study, spraying of goat carcasses with 2.5% acetic acid (treatment) significantly (P<.0.05)) reduced total *E. coli* count by 1.18 $\log_{10}$ CFU/cm^2 compared with carcasses before spray (control) with acid (Table 1). Carcasses may become contaminated by fecal material, pouch content and when it comes in contact with skins during slaughtering operation. Additionally, carcasses may get contaminated during dressing operations as a result of direct contact with workers hand, knives, other equipments and structural facilities. High total *E .coli* count indicates poor hygienic practice in the slaughter house as it is indicator of fecal contamination .This E. *coli* count (2.2 $\log_{10}$ CUF/cm^2 -2.9 $\log_{10}$ CUF/cm^2) obtained in goat carcass samples before spray in this study was comparable with previous work done in Ethiopia. Mengistu (2007) reported mean value *E.coli* count ranging from 2.4 $\log_{10}$CUF/cm^2 -2.9 log10 CUF /cm^2 at different abattoir. Similarly, Assegid (2008) reported *E.coli* mean value ranging from 1.7 log $_{10}$CUF/cm^{2-} 2.8 $\log_{10}$ CUF/cm^2.

The $\log_{10}$CFU/cm^2 mean of *E. coli* count before 2.5% acetic acid spray from samples from the front leg area and hind leg area were 2.8 and 2.3 log $_{10}$ CFU/$cm^{2,}$ respectively (Table 2).The number of *E. coli* count before acetic acid spray on front leg area was high. The differences in *E. coli* count between these two sampling sites were statistically significant (p<0.05). This difference may be due to variations on distribution of contaminants. The lower part of the goat carcass (front leg area) is more exposed for various contaminants such as water used to wash fecal materials may be splashed from the floor. The rump region of the carcass was the most contaminated area with fecal organisms than the other sites, usually associated with the skinning process and the presence of more fecal and dirt matter prior to slaughter (Gill *et al.,* 1996). Contamination of carcass may be occurred from the gut, skin, equipment, personnel and splashes of water from the floor during cleaning and slaughtering process (McEvoy *et al.,* 2003; Assegid, 2008).

Relatively low number of total *E. coli* count were obtained from carcasses sprayed with acetic acids (mean 1.35$\log_{10}$CFU/cm^2) than carcasses before sprayed (mean 2.53logCFU/cm^2). This result was comparable with previous works reported in other countries. Decontamination with organic acid solution reduces the number and prevalence of food borne pathogens and microbial load of meat (Huftman, 2002). The reduction in total *E. coli* count in acetic acids spray carcasses indicates the effectiveness of acetic acid spray as decontaminant.

A variety of organic acids applied as a spray or dips for decontamination purpose have been studied extensively and appear to constitute an effective bactericidal or bacteriostatic surface treatment which also effectively prevents the attachment microorganisms (Dickson and Anderson, 1992; Hardin *et al*., 1995; Bolder, 1997; Huffman, 2002; Pipek *et al.,* 2006). Moreover, the study conducted to demonstrate effectiveness of lactic acid (2%) on sheep carcass by spraying after 30 minutes applications and after 24 hrs chilling at 2 ± 1^0C showed a 2.06logCUF/cm^2 *E. coli* reduction. The antimicrobial effect of the organic acids is due to reduction of pH below the growth range and metabolic inhibition by the undissociated molecules (Beyaz and Tayar, 2010). Acetic acid has been shown to be effective against *E. coli* O157:H7 by reducing the pathogen by 0.1log CFU/cm^2 to 4. 67 lo9g CFU/cm^2 (Joseph *et al.*, 2006) and 2% acetic acid reduce load of *E. coli* by 1.6log (Ransom *et al.*, 2003). Thus, acetic acid is capable to reduce the pH of the carcass surface, and makes it difficult for microbes to survive.

In this study, *E.coli* count was found to be higher in goat carcasses before spray with 2.5% acetic acid, followed by chilling (combined effect of acetic acid spray and chilling) compared with the *E.coli* count of carcasses that have been sprayed with 2.5% acetic acid before chilling. Compared with the immediate effect of acetic acid spray on *E.coli* count, the antibacterial activity of the combined effect of spraying and chilling was low. This increase in *E.Coli* was statistically significant ($P< 0.05$). This increase may be due to cross contamination from workers hand, facilities and apron during chilling. According to Spescha *et al.* (2006) the antibacterial activity of air chilling on red meat carcasses is mainly based on the surface desiccation achieved by high air velocity. However, chilling of beef carcass may increase, decrease or no changes in microbiological contamination depending on temperature, air speed, humidity, carcass spacing

and duration (Nutsch *et al.,* 1997; Gill *et al.,* 1999; Bacon *et al.,* 2000; Arthur *et al.,* 2004; Corantin *et al.,* 2005; Kinsella *et al.,* 2006).

The mean pH value of goat carcasses at 15 minutes after slaughter in this study was 6.38. Relatively lower pH was obtained in sprayed chilled carcass (Mean pH= 5.77) compared with non-sprayed chilled carcass (Mean pH= 5.98). A paired t-test comparison indicates, statistically significant ($p<0.05$) difference between pH values for non-sprayed chilled and sprayed chilled carcasses (Table 5).Twenty four hours after slaughter the pH value of the meat gradually decreased from 7.0 to between 5.0 and 6.0 due muscle cells disintegration. The pH value of goat carcass 24 hrs after of slaughter is 6.3 (MOA, 2010). Similarly, Abebe *et al.* (2010) reported that the pH of goat carcass at 15 minutes after slaughter and at 24 hrs post slaughter after chilling were recorded 6.54 and 5.83 respectively. Whereas, Smulders *et al.* (1992) reported that muscular pH decreased from 7.0 to drop approximately to 5.3 - 5.8 upon slaughter. This implies that spray of acetic acid on carcass was capable of reducing the pH of the carcass surface by remarkable magnitude, making it difficult for microbes to survive, resulting reduction in the number of *E. coli*. Thus, acetic acid spray is effective to lowers the pH level of the meat that can be inferred to use acetic acid as decontaminant.

Goat carcasses that were chilled after spray acetic acid showed less darkness than non sprayed chilled carcass after 24 hrs. Darkening of carcasses of highland animals exists for both sheep and goats, without a noticeable difference in magnitude (Abebe *et al.,* 2010). According to Stivarius *et al.* (2001), the main cause of fresh meat discoloration is accumulation of metamyoglobin at the lean surface. This MetMb production is high at pH values above 5.8 but acetic acid spray in this study drops the mean pH to 5.77 which may decrease formation of MetMb and decrease discoloration.Thus acetic acid spray may contribute a lot by reducing the microbial load to reduce the formation of MetMb and darkening of meat. Personnel working in this abattoir believe that after spraying acetic acid early darkening of carcass has been decreased (personal communication). Stivarius *et al.* (2001) reported that 1% ozonated water followed by 5% acetic acid spray decreased redness, oxymyoglobin content, and color stability during display, likely the acetic acid may promoted pigment oxidation by reducing muscle pH (Mancini and Hunt, 2005).

6 CONCLUSIONS AND RECOMMENDATIONS

In the current study, spraying of goat carcasses with 2.5% acetic acid significantly reduced *E. coli* count significantly compared with carcasses not sprayed. This significant reduction indicated effectiveness of acetic acid (2.5%) as decontaminant. In addition, spraying of goat carcass with acetic acid reduces the pH and darkness. Therefore, meat export abattoirs should improve the safety and the quality of meat and meat by products through using acetic acid. All Export abattoirs should implement Food Safety Management System/Hazard Analysis and Critical control Points incorporating acetic acid spray as microbial decontaminant.

Based on the above conclusions, the following recommendations are forwarded:

- Abattoirs need to be aware of presence of high level of contamination in carcasses and must comply with written sanitation standard operating procedures (SSOP) and, have to implement Food Safety Management System/Hazard Analysis Critical Control Point
- The decontaminant effect of acetic acid and other organic acids with various concentrations, temperature, chilling duration and other factors affecting its efficacy should be further validated and verified under Ethiopian conditions.
- Further detailed research on the effect of acetic acid on color of meat specially using instrumental color scanner should be undertaken.

7 REFERENCE

Abebe, G., Kannan, G. and A. Goetsch, L. (2010): Effects of small ruminant species and origin and length of rest and feeding period on harvest measurements in Ethiopia. *African Journal of Agricultural Research*. **5**: 834-847.

Acuff, G. R. (2005): Chemical decontamination strategies for meat: In Improving the Safety of Fresh Meat. Wood head Publishing Limited. CRC Press, New York. Pp 351-363.

Aganga, A.O. (1998): Guide line of prevention and control of Salmonellosis in meat industry. Concerning mechanical tenderization and packing of meat. *Proceedings of Meat Industry Reciprocal Conference.* Pp. 137–140.

Anderson, M.E and Marshal, R.T. (1990): Reducing microbial food populations on beef tissues: concentration and temperature of lactic acid. *Journal of Food Safety*. **10:** 181-190.

Arthur, T.M., Bosilevac, J.M., Nou, X., Shackelford, S.D., Wheeler, T.L., Kent, M.P., Jaroni, D., Pauling, B., Allen, D.M. and Koohmaraie, M. (2004): *E. coli* O157 prevalence and enumeration of aerobic bacteria, *Enterobacteriaceae,* and *E. coli* O157 at various steps in commercial beef processing plants. *Journal of Food Protection,* **67:** 658-665.

Assegid, S. (2008): Study on *E. coli* count in fresh sheep and goat export carcasses in three export abattoirs. MSc thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia.

Bacon, R.T., Belk, K.E., Sofos, J.N., Clayton, R.P., Reagan, J.O. and Smith, G.C. (2000): Microbial populations on animal hides and beef carcasses at different stages of slaughter in plants employing multiple-sequential interventions for decontamination. *Journal of Food Protection,* **63:**1080-1086.

Barboza de Martinez, Y., Ferrer, K. and Salas, E.M. (2002): Combined effects of lactic acid and Nisin solution in reducing levels of microbiological contamination in red meat carcasses. *Journal of Food Protection,* **65:** 1780-1783.

Barkate, M..L, Acuff, G.R., Lucia, L.M., and Hale, D.S. (1993): Hot water decontamination of beef carcasses for reduction of initial bacterial numbers. *Meat Science, **35*:**397-401.

Bell, R.G. and Hathaway, S.C. (1996): The hygienic efficiency of conventional and inverted lamb dressing system. *Journal of Applied Bacteriology,* **81**: 225-234.

Bell, R. (1997): Distribution and sources of microbial contamination on beef carcasses. *Journal of Applied Microbiology*, **82**: 292-300.

Beyaz, D. and Tayar, M. (2010): The Effect of Lactic Acid Spray Application on the Microbiological Quality of Sheep Carcasses. *Journal of Animal and Veterinary Advances*, **9:** 1858-1863.

Biss, M.E. and Hathaway, S.C. (1996): Microbiological contamination of ovine carcasses associated with the presence of wool and fecal material. *Journal of Applied Bacteriology.* **81**: 594-600.

Biss, M.E. and Hathaway, S.C. (1995): Microbiological and visible contamination of lamb carcasses according to pre slaughter presentation status: implication for HACCP. *Journal of Food Protocol*, **58**: 776-783.

Bolder, N.M..(1997): Decontamination of meat and poultry carcasses. *Trends Food Science Technology*, **8:**221-227.

Bolton, D.J., Doherty, A.M., and Sheridan, J.J. (2001): Beef HACCP: Intervention and non-intervention systems. *International Journal of Food Microbiology*, **66**: 119-129.

Brul, S.; Coote, P. (1999): "Preservative agents in foods, mode of action and microbial resistance mechanisms". *International Journal of Food Microbiology*, **50**: 1–17.

Byrne,B.Dummea,G.Lyngh,l and Bolton ,D.(2005):Microbiological Carcass Sampling Method to achieve compliance with 2001/471/EC and New Hygiene Regulation. *Research of Microbiology*,**156**:104-106

CAC (Codex Alementarius commission)(2005):The code of Hygienic Practice For Meat.Pp1-52

Calicioglu, M., Kaspar, C.W., Buege, D.R. and Luchansky, J.B. (2002): Effectiveness of spraying with lactic acid in decontaminating inoculated *Escherichia coli* O157:H7 and indigenous *Escherichia coli* biotype I on beef. *Journal of Food Protection,* **65:** 26-32.

Carpenter, C.E., Smith, J.V., Broadbent, J.R. (2011): Efficacy of washing meat surfaces with 2% levulinic, acetic, or lactic acid for pathogen decontamination and residual growth inhibition. *Meat Science*. **88**: 256–260.

CDC.govAboutColi.http://www.cdc.gov/ecoli/aceessed in 20/04/2012

Corantin, H., Quessy, S., Gaucher, M.L., Lessard,L.,Leblanc, D., and Houde, A. (2005): Effectiveness of steam pasteurization in controlling microbiological hazards of cull cow

carcasses in a commercial plant. *The Canadian Journal of Veterinary Research,* **69:** 200-207.

CSA (Central Statistical Agency) (2004): Federal Democratic Republic of Ethiopia. Central Statistical Agency.Agricultural Sample Survey.VolumeII.Reporton Livestock and Livestock Characteristics (Private Peasant holdings) Statistical Bulletin 302. CSA, Addis Ababa, Ethiopia.

Cutter, C.N. and Siragusa, G.R. (1994a): Decontamination of beef carcass tissue with Nisin using a pilot scale model carcass washer. *Food Microbiology,* **11**: 481-489.

Cutter, C.N. and Siragusa, G.R. (1994b): Efficacy of organic acids against *Escherichia coli* O157:H7 attached to beef carcass tissue using a pilot scale model carcass washer. *Journal of Food Protection, **57***: 97-103.

Cutter, C.N and Siragusa, G.R. (1995): Application of chlorine to reduce populations of *E. coli* on beef. *Journal of Food Safety*, **15**:67-75.

Delmore, G. L.R., Sofos, J.N., Reagan, J.O, and Smith, G.C. (1997): Hot-water rinsing and growth on beef plate under extended storage after washing and sanitizing. *Journal of Food Protection*, **42**:398–392.

Dibner, J.J. and Butin, P. (2002):"Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism". *Journal of Applied Poultry Research.* **11** : 453–463.

Dickson, J.S. (1990). Surface moisture and osmotic stress as factors that affect the sanitizing of beef tissue surfaces. *Journal of Food Protection,* **53***:* 674-679.

Dickson, J.S. and Anderson, M.E. (1992): Microbiological decontamination of food animal carcasses by washing and sanitizing systems: A review. *Journal of Food Protection, **55***:133-140.

Dincer, A.H. and Baysa, T. (2004): Decontamination techniques of pathogen bacteria in meat and poultry. Critical Review on Microbiology, **30:** 197-204.

Dorsa, W.J., Cutter, C.N., and Siragusa, G.R. (1998): Long-term effect of alkaline, organic acid, or hot water washes on the microbial profile of refrigerated beef contaminated with bacterial pathogens after washing. *Journal of Food Protection, **61**:* 300-306.

Dubal, Z. B., Paturkar, A.M., Waskar, V. S., Zende, R.J., Latha, C., Rawool, D.B. and Kadam, M.M. (2004): Effect of food grade organic acids on inoculated *S. aureus, L. monocytogenes, E. coli* and *S.Typhimurium* in sheep/goat meat stored at refrigeration temperature. Meat Science, **66**: 817-821.

EFSA (European Food Safety Authority) (2010): Scientific opinion: Scientific Opinion on the evaluation of the safety and efficacy of lactic acid for the removal of microbial surface contamination of beef carcasses, cuts and trimmings. *European Food Safety Authority Journal*, **9**: 2317.

Ellerbrock, L.I., Wegner, J.F. and Arndt, G. (1993): Does spray washing of lamb carcasses alters bacterial surface contamination. *Journal Food Protocol*, **56**: 432-436.

EMDTI (Ethiopian Meat and Dairy Technology Institute) (2011): Overview of Ethiopian Livestock sector**.** Presentation to OIE delegation, EMDTI, Bishoftu/Debre Zeit.

ESGPIP (Ethiopian sheep and goat productivity improvement program) (2008): Sheep and goat production hand book for Ethiopia. Pp 43-44.

FDA (Food and drug Authority) (2003): Code of Federal Regulations. Title 21, Government Printing Office, USA.

Feng, P., Weagant, S. and Grant, M. (2002): Bacteriological analytical manual. 8th edition : US Food and Drug Administration centre for food safety and applied nutrition, Washington D.C, USA, Pp 24-26.

Gill, C.O. (1979): A review: Intrinsic bacteria in meat. *Journal of Applied Bacteriology*, **47**:367-378.

Gill, C.O. (1980): Total and intramuscular bacterial populations of carcasses and cuts. *Proceeding of 33rd Annual Reciprocal Meat Conference*, **33**: 47-53.

Gill, C.O and Newton, K.G.(1982): Effect of lactic acid concentration on growth gram negative psychrotrohs from a meat works. *Applied and Environmental Microbiology*, **43**:284-288.

Gill, C. O, Badoni M. and Jones T.(1996):Hygienic effect of Trimming and washing Operation in a beef Carcass Dressing Process. *Journal of Food Protection* .**59**:666-669.

Gill, C. O. (1998): Microbiological contamination of meat during slaughter and butchering of cattle, sheep and pigs. In: The Microbiology of Meat and Poultry_Blackie Academic & Professional, London. Pp118-157.

Gill, C.O., Bryant, J. and Bedard, D. (1999): The effects of hot water pasteurizing treatments on the appearances and microbiological conditions of beef carcass sides. *Food Microbiology*, **16:** 281- 289.

Gill, C.O. and Landers, C. (2003): Microbiological effects of carcass decontaminating treatments at four beef packing plants. *Meat Science,* **65:** 1005-1011.

Goksoy, E.O., James C. and Corry, J.E.L. (2000): The effect of short-time microwave exposures on inoculated pathogens on chicken and the shelf-life of uninoculated chicken meat. *Journal Food Engineering*, **45**: 153-160.

Gracy, J.F., Collius, D.S. and Haly, R.J. (1999): *E. coli* O157:H7. *Meat hygiene*. 10th Ed. W.W.Saunders Company LTD. London Pp: 332-387.

Greer, G.G. (2005): Bacteriophages control of food borne bacteria. *Journal of Food Protection*, **68:** 1102-1111.

Hardin, M.D., Acuff, G.R. and Lucia, L.M. (1995): Comparison of methods for decontamination from carcass surfaces. *Journal of Food Protocol.* **64**:1344-1345.

Hennessy, D.A. (2003): Slaughter house rules: human error, food safety and uniformity in meat packing. Working paper 03-WP 346, Center for Agricultural and Rural Development, Iowa state university, Ames, Iowa, Pp. 1-3.

HPB (Health Product and Food Branches) Methods (2001): Determination of *coliforms* in food using Violet Red Bile Agar Canadian Food Inspection Agency, Ottawa, Canada, Pp1-7.

Hudson, J.A., Billington, C., Carey-Smith, G. and Greening, G. (2005): Bacteriophages as biocontrol agents in food. *Journal of Food Protection*, **68:** 426-437.

Huffman, R.D. (2002): Current and future technologies for the decontamination of carcass and fresh meat. Meat Science, **62**: 285-294.

Hugas, M. and Tsigarida, E. (2008): Pros and cons of carcass decontamination: The role of the European Food Safety Authority. *Meat Science,* **78**: 43-52.

IFT (Institute of Food Technologists) (2000): Expert Report on Emerging Microbiological Food Safety Issues, Implications for Control in the 21st Century. Institute of Food Technologists, Chicago, IL. Pp 1-32.

ILRI (International livestock Research Institute) (2005); Adaa Liben woreda pilot leading site diagnosis and program design ,Addis Ababa,Ethiopia,Pp1-8

ISO (International Organization for Standardization) 17604(2003); Microbiology of Food Animal Feeding Stuff-Carcass Sampling for microbiological analysis ,Pp.2-12

James, C., Thornton, J.A., Ketteringham, L. and James, S.J. (2000): Effect of steam condensation, hot water or chlorinated hot water immersion on bacterial numbers and quality of lamb carcasses. *Journal Food Engineering*, **43**: 219-225.

Jay, J.M. (2001): Modern Food Microbiology. 6th Ed.Aspen Pub. Maryland, USA. Pp 2-50.

Joerger R. D. (2003): Alternatives to Antibiotics: Bacteriocins,Antimicrobial Peptides and Bacteriophages**.** *Poultry Science* ,**82**:640–647

Joseph M.B, Xiangwu, N., Genevieve A.B.G., Terrance, M.A, Mohammad, K. (2006): Treatments Using Hot Water Instead of Lactic Acid Reduce Levels of Aerobic Bacteria and Enterobacteriaceae and Reduce the Prevalence of *E. coli* O157:H7 on Pre evisceration Beef Carcasses. *Journal of Food Protection***, 69**:1808–1813.

Kinsella, K.J., Sheridan, J.J., Rowe, T.A., Butler, F., Delgado, A., Quispe-Ramirez, A., Blair, I.S. and McDowell, D.A. (2006): Impact of a novel spray-chilling system on surface microflora, water activity and weight loss during beef carcass chilling.*Food Microbiology*, **23**: 483-490.

Kotula, K.L. and Kotula, A.W. (2000): Microbial Ecology of Different Types of Food Fresh Red Meats. In: The Microbiological Safety and Quality of Food, Lund, B.M., T.C. Baird-Parker and G.W. Gould (Eds.). Aspen Publishers, Inc., Gaithersburg, MD., Pp: 359-388.

Kotula, K.L. and Thelappurath, R. (1994): Microbiological and sensory attributes of retail cuts of beef treated with acetic and lactic acid solutions. *Journal Food Protocol*, **57**: 665-670.

Lahr, J.A. (1996): Beef carcass microbial contamination post slaughter numbers of bacteria, sources of contamination and variability. Proceedings of 49th Annual Reciprocal Meats Conference, (ARMC'96), Provo, Utah, Chicago, IL, Pp: 132-137.

Laury, A., Echeverry, A. and Brashears, M.(2009): Fate of *E. coli O157:H7* in Meat. Department of Animal and Food Science, Texas Tech University, Lubbock, TX, USA.Pp. 145-160

Leclercq, A., Wanegue, C. and Baylac, P. (2002): Comparison of fecal coliform agar and violet red bile lactose agar for fecal coliform enumeration in foods. *Applied and environmental Microbiology*, **68**: 1631-1638.

Levine, A.S. and Fellers, C.R. (1940): Action of acetic acid on food spoilage microorganisms. *Journal of Bacteriology*, **39**: 499-515.

Loretz, M. (2010): Antibacterial activity of decontamination treatments for cattle hides and beef carcasses, University of Zurich, Vetsuisse Faculty**.** Pp1-50

Mancini, R.A. and Hunt, M.C. (2005): Current research in meat color. *Meat Science*: **71:**100–121.

Marshall, K.M., Niebuhr, S.E., Acuff, G.R., Lucia, L.M. and Dickson, J.S. (2005): Identification of *E. coli* O157:H7 meat processing indicators for fresh meat through comparison of the effects of selected antimicrobial interventions. *Journal of Food Protection*, **68**:2580-2586.

Mc Evoy, J., Doherty, A., Sheriden, J., Thomson-carter, F., Garvey, P., McGuire, L., Blair, I. and McDwell, D. (2003): The prevalence and spead of *E. coli* O157:H7 at a commercial beef abattoir. *Journal of Applied Microbiology*, **95**:256-266.

Mead, G.C. (1994): Microbiological hazards from red meat and their control. *Britain Food Journal***, 96**: 33-36.

Mengistu, T.(2007) :Microbial load on goat carcass slaughter in selected abattoir of East Showa AdminstrativeZone,Ethiopoia,.MScThesis,Faculty of Veterinary MedicineAAU, DebreZeit,Ethiopia .

MOA (Ministry of Agriculture) (2010): Meat Inspection Guide Line. Addis Ababa Ethiopia.Pp38.

MOH (Ministry of Health) (2004): Health and health related indicator's published for Ministry of Health of Ethiopia. Addis Ababa,Ethiopia.

Nutsch, A.L., Phebus, R.K., Riemann, M.J., Schafer, D.E., Boyer, Jr., J.E., Wilson, R.C., Leising, J.D. and Kastner, C.L. (1997): Evaluation of a steam pasteurization process in a commercial beef processing facility. *Journal of Food Protection*, **60:** 485-492.

Patanen, K.H. and Mroz, Z. (1999):"Organic acids for preservation": In Block, S. S. Disinfection, sterilization and preservation (5^{th} Ed.). Philadelphia.

Peng, P., Wegant, S.D. and Grant, M.A. (2001): Enumeration of *E.Coli* and the *coli form* Bacteria.inBacteriologicalAnalyticalManual(BAM).onlinewww.cfsan/fda.gov./ebam/bam /-toc.htm.accessed in 12/03/2012.

Pipek, P., Sikulova, M., Jelenıkova, J. and Izumimoto, M. (2005): Color changes after carcasses decontamination by steam and lactic acid. *Meat Science*, **69**:673–680

Prescott.H.(2002): Laboratory Exercises in Microbiology(5^{th} Ed).The McGraw−Hill Companies.Pp.155-160

QSAE (Quality and Standard Authority of Ethiopian) (2008): FSMS requirement for any organization in the food chain (identical with IS22000:2005), 1st ed. Ethiopian Quality and Standard Autherity.Addis Ababa.Pp.1-30

Quinn, P., Houska, M., Hoke, K., Jelenikova, J., Kyhos, K. and Sikulova, M. (2006): Decontamination of pork carcasses by steam and lactic acid. *Journal of Food Engineering, **74**: 224-231*.

Quinn, P., Carter, M., Markey, B. and carter, G. (2002): *Enterobacteriaceae*. In Clinical Veterinary Microbiology. Wolfe Publication Spain, Pp: 209-236.

Ramirez, A.J., Acuff, G.R., Lucia, L.M. and Savell, J.W. (2001): Lactic acid and trisodium phosphate treatment of lamb breast to reduce bacterial contamination. *Journal of Food Protection,* **64**: 1439-1441.

Ransom, J.R., Belk, K.E., Sofos, J.N., Stopforth, J.D. Scanga, J.A. and Smith, G.C. (2003): Comparison of intervention technologies for reducing *E. coli* O157:H7 on beef cuts and trimmings. *Food Protection Trends*. **23**: 24-34.

Ridell, J. and Koreala, H. (1993): Special treatment during slaughtering in Finland of cattle carrying excessive load on dung: meat hygiene aspects. *Meat Science*, **35**: 223-228.

Schaffnen, D. and Smith, S. (2004): Indicator organisms/microbiogical analysis . *Encyclopaedia of Meat sciences*, Pp:1-6.

Scoti, U.N. and Stevenson K.E. (2006): HACCP: A Systematic Approach to Food Safety, Food Products Association, 4th ed. Washington DC. USA.

Siragusa, G.R. (1995): The effectiveness of carcass decontamination systems for controlling the presence of pathogens on the surfaces of meat animal carcasses. *Journal of Food Safety*, **15**: 229-238.

Smulders, F.J. and Greer, G.G. (1998): Integrating microbial decontamination with organic acids in HACCP programs for muscle foods: prospects and controversies. *International Journal of Food Microbiology*. **44**: 149-169.

Smulders, F.J.M. (1987): Prospective for Microbial Decontamination of Meat and Poultry by Organic Acids with Special Reference to Lactic Acid. In: Elimination of Pathogenic Organisms from Meat and Poultry, Elsevier, New York, Pp: 319-344.

Sofas, J.N., Kochevar, S.L. and Bellinger, G.R. (1999): Source and extent of microbiological contamination of beef carcasses in seven United States slaughtering plants. *Journal of Food protection,***62:** 140-145.

Spescha, C., Stephan, R. and Zweifel, C. (2006): Microbiological contamination of pig carcasses at different stages of slaughter in two European Union-approved abattoirs. *Journal of Food Protection,* **69:**2568-2575

Stivarius, M.R., Pohlman, F.W., McElyea, K.S. and Apple, J.K. (2001): The effects of acetic acid, gluconic acid and trisodium citrate treatment of beef trimmings on microbial, color and odor characteristics of ground beef through simulated retail display. Department of Animal Science, University of Arkansas, Fayetteville, AR 72701, USA.Pp.1-50

USDA/ERS (2000): Economics of food borne disease; estimating the benefits of reducing food. Borne disease access at http//www.usda accessed on 12/03/2012.

USDA/FSIS (2004): Safe and suitable ingredients used in the production of meat and poultry products. FSIS Directive 7120.1. Amendment 6, USDA-FSIS.

WHO (1997): Food Safety Issues: HACCP; introducing the Hazard Analysis and Critical.Pp.1-50

Yalci,S., Nizamlioglu, M. and Gurbuz, U. (2001): Fecal coliform contamination of beef carcasses trimming/washing of beef carcasses to reduce physical and microbiological contamination .Journal food science ,**62**:373-376.

ANNEX

Annex1

Kovac's reagent Preparation

Dissolve 10 g P-dimethyl aminobenzaldehyde (Sigma) in 150 ml alcohol and slowly add 50 ml concentrated hydrochloric acid while constantly stirring the mixture. Finally, pale color was performed and store in brown bottle at refrigerator.

Methyl Red reagents preparation

Dissolve 0.1g methyl red (Mallinckroat) in 300 ml alcohol and finally add 200 ml distilled water. Finally red color was formed and stored in a brown color at refrigerator.

Voges Proskauer) (VP) reagent Preparation

a/ VP-reagent- A; dissolve 5 g Naphithol (Sigma) in small amount of ethyl alcohol and bring to 100 ml in flask. Alcohol should be colorless.Then store in brown bottle in refrigerator

b/ VP-reagent-B:Add less than 100 ml distilled water to 40 g pallets of KOH in cold water bath to prevent overheating and bring to 100 ml .Finally 40 % solution that store in poly ethylene bottle at refrigerator .

Annex 2

Typical formulae of Oxoid CM 10109 MacConkey agar No.2 at pH 7.2 in (g/l) used

Peptone 20

Lactose 10

Bile salt no 2 1.5

Sodium chloride 0.05

Crystal Violet 0.001

Agar 15

CURRICULUM VITAE

A. PERSONAL INFORMATION

1-Name: -	Amsalu Wudie
2- Date of Birth: -	April 10/1978
3-Sex	Male
4- Place of Birth: -	Motta (East Gojjam)
5-Marital Status: -	Single
6-Profession	Veterinarian
7-Occupation	General Manager

B. EDUCATIONAL BACKGROUND

Year	Institution	Award
April2-6,2012	DQS Management Service Plc.	Certificate on Lead Auditor for ISO9001:2008
June 27-29,2011	Praise consultancy	Certificate on Food Safety Management System, Auditing Course
2007-to 2011	Adama University, FBE	Business Management (BA)
January1-5, 2011	Praise consultancy	ISO2200;2005/Food Safety Management System, Development & Implementation course
Sep2008-Dec2008	Dotnet Computer Technology	Certificate on basic computer
Augest,11-29,2008	Addis Ababa University, Faculty of Veterinary Medicine (AAU-FVM)	Certificate on Meat Hygiene &Principles of Food Safety
March7-8,2008	USAID, Praire View A&M Research Foundation, ESGPIP	Certificate on Meat Quality Assessment
March 5-8/2007	USAID, Texas A&M University-LMM	Developing &Implementing HACCP plan for meat industry
October 11,2007	USAID, TexasA&M University-	Certificate on fabrication of

	LMM	Ethiopian Beef export cuts
June 7/12/2004	United Nation Industrial Development Organization	Certificate on Hazard Analyses and Critical control program (HACCP) auditors trainings
March 15 April 8,2004	Addis Ababa University, Faculty of Veterinary Medicine (AAU-FVM)	Certificate on Advanced Veterinary public Health training
1998-2003	Addis Ababa University Faculty of Veterinary Medicine (AAU-FVM)	Doctor of Veterinary Medicine (DVM) Degree
1987-1990	Motta Senior Secondary School	School Leaving Certificate
1985-1986	Sede Junior Secondary School	National Certificate
1982-1984	Keranyo primary School	National Certificate

C. WORK EXPERIENCE

Dec.1 2006-Todate Organic Export Abattoir as Managing Director

January 2003-Nov.30, 2006 HELIMEX Export Abattoir as General Manager

D. RESEARCH WORKS/Technical papers

- Maternal recognition of pregnancy in ruminant (Seminar Paper, 2002).
- Prevalence of Bovine tuberculosis at Nazareth Abattoir (DVM thesis, 2003).
- Ameni, G. and **Wudie,A**. (2003): Preliminary Study on Bovine tuberculosis at Nazareth Municipality abattoir. Bull. *Anim. Hlth.prod.Afr*.**51**: 124-132.
- Ayelet, G., Sori, T**., Wudie, A.** and Gelaye, E. (2007) Prevalence of contagious Caprine Pleuro Pneumonia in the Borena pastoral area of Ethiopia. (M.H. Fahmy, Agriculture and Agro-food Canada, Lennoxville Research center. *Journal of Small Ruminant Research* **(**70); 131-135.
- Jibat, T. Ejeta, G. Asfaw, Y.and **Wudie, A.** (2008) cases of a abattoir condemn from in apparently healthy slaughtered sheep and goats at HELMEX at Abattoir,Debre Zeith, Ethiopia. *Revuede Medecine Veterinaire*(**159**);305-311

- Agga, G.E., Udala, U., Regassa, F. and **Wudie, A**. (2011). Body Measurements of bucks of three goat breeds in Ethiopia and their correlation to breed, age and testicular measurements. *Small ruminant research* **95:** 133-138.
- A study on constraints and opportunities of meat export in Ethiopia for partial fulfillment of the degree bachelor of Art in Business Administration (BA Thesis, 2011).
- The Need for implementation of ISO22000:2005 (FSMS/HACCP in Organic Export Abattoir (Seminar paper Sep.2011)

E. Language Skill

Amharic	Mother Tongue
English	Fluent in all skills

F. Member ship

Ethiopian Veterinarian Association

Present Address; -**Mobile 0911628876**

Tele: 0114333287

Email :amsaluwudie@yahoo.com

SIGNED DECLARATION SHEET

This thesis is my original work, has not been presented for a degree in any other university and that all sources of material used for the thesis have been duly acknowledged

Name ……..
Signature …….
Date of submission ……

This thesis has been submitted for examination with my approval as University advisor

Dr. Girma Zewde (MSc, PhD, Assoc. Professor)……..