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ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

ESCHERICHIA COLI O157:H7 IN FECES, SKIN AND CARCASSES AS WELL AS TOTAL AND FECAL COLIFORMS ON ARCASSES OF SMALL RUMINANTS AT AN EXPORT ABATTOIR, MOJDO, ETHIOPIA

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A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Veterinary Science in Tropical Veterinary Public Health

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LIST OF ABBREVIATIONS

A/E	Attaching and effacing
A_w	Water activity
BGLB	Brilliant Green Lactose Bile
BHI	Brain heart infusion
BPW-VCC	Buffered peptone water with vancomycin, cefsulodin and cefixime
CCPs	Critical Control Points
CDC	Centers for Disease Control and Prevention
CFU	Colony Forming Units
CT-SMAC	Sorbitol MacConkey Agar with Cefixime and Tellurite
DF	Dilution factor
EAEC	Enteroaggregative *E. coli*
EHEC	Enterohemorrhagic *E. coli*
EIEC	Enteroinvasive *E. coli*
ELISA	Enzyme linked immunosorbent assay
EPEC	Enteropathogenic *E. coli*
ETEC	Enterotoxigenic *E. coli*
GIT	Gastro Intestinal Track
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis Critical Control Point
HC	Hemorrhagic colitis
HUS	Hemolytic uremic syndromes
IFT	Institute of Food Technologists
IMS	Immunomagnetic Separation
IMViC	Indole, Methyl Red,Voges Proskauer and Citrate Utilization
ISO	International Organization for Standardization
LEE	Locus for enterocyte effacement
MIC	Ministry of Agriculture and Rural Development
MoARD	Minimum inhibitory concentration
MPC-S	Magnetic particle concentrator

MPN	Most Probable Number
MRD	Maximum Recovery Diluent
MTSB+n	Modified tryptone soya broth containing novobiocin
MUG	Methyl umbelliferyl- glucuronide
NVI	National Veterinary Institute
OIE	Office for international des epizootics
PBST	Phosphate Buffered Saline with Tween 20
PCR	Polymerase chain reaction
PFGE	Pulsed field gel electrophoresis
SPC	Standard plate count
STEC	Shiga toxin producing *E. coli*
Stx	Shiga toxin
TTP	Thrombotic thrombocytopenic purpura
VRBA	Violet red bile agar

ABSTRACT

The purpose of this study was to find out the presence of association between carcass contamination with *E. coli* O157: H7 and fecal and skin prevalence of *E. coli* O157: H7 and to estimate coliform loads on before and after wash of sheep and goats carcasses at an export abattoir at Modjo, Ethiopia. A total of 711 samples were examined for the presence of *E. coli* O157:H7 from fecal samples, skin swabs, carcass swabs before and after wash from sheep and goats slaughtered between October, 2007 and April, 2008. Water samples were also collected for the isolation of *E. coli* O157:H7 and coliform counts. Total and fecal coliform loads were estimated on same carcasses using Violet Red Bile Agar (VRBA). For the isolation of *E. coli* O157:H7, all samples were enriched with modified Tryptic Soy broth with novobiocin (mTSB+n) and concentrated using immunomagnetic separation (IMS). The bacteria-bead complex was plated onto cefixime-tellurite containing Sorbitol MacConkey agar (CT-SMAC) and presumptive colonies were confirmed by latex agglutination test as recommended by ISO16654, (2001). *E. coli* O157:H7 was isolated from feces (4.7%), skin swabs (8.7%), carcass swabs before wash (8.1%), carcass swabs after wash (8.7%) and water samples (4.2%), respectively. A statistically significant association was found between prevalence of carcass contamination with fecal and skin *E. coli* O157:H7 status. The total coliform counts before and after carcass wash were 3.15 $\log_{10}CFUcm^{-2}$ (SD= 0.77) and 2.97 $\log_{10}CFUcm^{-2}$ (SD = 0.82), respectively. The $\log_{10}$ means of fecal coliforms before and after carcass wash were 2.96 (SD=0.88) and 2.77(SD=0.91) respectively. Statistically significant difference was observed between the means of total coliform counts (P=0.002) and fecal coliform counts (P=0.001) before and after carcass wash. No fecal coliforms were isolated from water samples. PCR analysis identified both *stx1* and *stx2* genes from one *E. coli* O157:H7 isolate collected from goat carcass. The identification of this virulent factor indicates the potential of sheep and goats as sources of *E.coli* O157:H7 for human infection in the country. Control measures to reduce the public health risks arising from *E. coli* O157 in reservoir animals must be addressed at abattoir levels by reducing skin and fecal sources and carcass contaminations at different stage of slaughter operations. Applications of HACCP system are strongly recommended at the abattoirs.

Key words: *E.coli* O157:H7, Coliforms, Sheep, Goat, Carcass contaminations, Abattoir, Modjo

1. INTRODUCTION

Despite the extensive scientific progress and technological developments achieved in recent years in developed countries, microbial food borne illness remains a global concern. A number of diseases are transmitted through food (IFT, 2000). Contamination of meat products with foodborne pathogens and chemical residues has prompted social fears, threatened world trades, economic losses, and has generated challenges and opportunities in food process controls (IFT, 2000).

Specific sources that contribute microbial contamination to animal carcasses and to fresh meat during slaughter and dressing include feces, hide, water, air, intestinal contents, lymph nodes, processing equipment and humans. The types of microorganisms and extent of contamination present on the final product are influenced by sanitation procedures, hygienic practices, application of food safety interventions, type and extent of product handling and processing, and the conditions of storage and distribution (Roberts, 1980; Kiraa *et al.*, 1985; Sofos *et al.*, 1999; Hansson, 2000).

Because food animals are naturally contaminated with a variety of potential pathogens, meat processors apply many microbiological control methods during the slaughter and processing of meat. The existing approach to food safety management may give some countries safe food supply. However, estimates of the morbidity of foodborne illness clearly show that the existing approaches in controling are still inadequate. Microorganisms of concern to meat processors throughout the red meat supply chain include pathogens and spoilage microorganisms. All these may be found in the feces and on the skin of animals presented for slaughter (Reid *et al.*, 2002) and can be transferred to the carcass during skin removal and evisceration (Roberts, 1980; Kiraa *et al.*, 1985; Bell, 1997; Hansson, 2000; IFT, 2000).

According to the report of Ministry of Agriculture and Rural Development (MoARD), Ethiopia secured about 15.5 million USD foreign exchange only from the export of fresh carcass by the year 2006/7(MoARD, 2006/7). Despite the crucial role of this sector in the country's economy there is no meat microbiological standard and meat inspectors are mainly looking for visible

pathological findings such as arthritis and pleuritis, without considering invisible hazards such as pathogenic bacteria that has been receiving more attention during recent years in other part of the world. There are organisms like total coliforms, fecal coliforms and *E. coli* in which their presence in a given product are indicators of the possible presence of pathogens.

Escherichia coli O157:H7 is one of the most important emerging pathogens of humans with worldwide occurrence. This strain is the predominant and most virulent serotype in a pathogenic subset of enterohemorrhagic *E. coli* (EHEC). Its complication results in hemorrhagic colitis (HC), hemolytic uremic syndromes (HUS) and thrombotic thrombocytopenic purpura (TTP) (Fratamico and Smith, 2006). It was recognized in 1982 for the first time and since then infections have been reported from more than 30 countries in 6 continents (Mead and Griffin, 1998). Since 1982, more than 100 outbreaks of *E. coli* O157have been documented in US alone (Elder *et al*., 2000) and more than 75,000 human cases occurs annually. The actual incidence of the disease may be underestimated since the organism is difficult to identify if testing is not done during the early stages of infection (CDC, 2005). Most outbreaks involving large numbers of cases have often been linked to the consumption of undercooked or cross-contaminated cooked meats. Generally, the severity and effect of serotype O157:H7 infections in a given population have a tremendous impact on human health, food industry, and transforming the public's perception about the safety of their food (Mead and Griffin, 1998; CDC, 2005).

Although cattle are the major reservoirs (Chapman *et al.* 1994), sheep and goats are expected to be the second most important source for *E. coli* O157:H7 which is carried in the intestinal tract of animals and excreted in feces (Chapman *et al*., 2001; Battisti *et al*., 2006), but the organism is not associated with disease in these animals (Gansheroff and O'Brien, 2000). This presents a challenge for the detection and control of the pathogen.

E. coli O157 was reported with a prevalence of 3.8% in Nigeria teaching Hospital as multi drug resistant strain associated with HIV patients (Moses *et al.*, 2006). A recent work on this pathogen in Ethiopia has been reported with prevalence of 4.9% from minced beef and 5.7% from stool samples collected at Bishoftu Hospital (Demisse, 2005). A similar work reported 2.0% prevalence of *E. coli* O157:H7 in goats and 4.0% in sheep meat at abattoirs (Hiko, 2007),

although sampling was taken at one point before chilling. These reports indicated the presence of this hazard and its future impact on the export abattoirs in the country. Because of the ability of this pathogen to affect all human age groups, its low infectious dose, high acid tolerance and its association with foods of animal origin, *E. coli* O157:H7 is currently considered as the most hazardus and emerging foodborn disease (Buchanan and Doyle, 1997).

Recently introduced interventions to control *E. coli* O157:H7 in beef in other countries have had little impact on its incidence in humans. This reflects the importance of other sources such as meat from sheep and goats. Contamination of carcasses with *E. coli* O157:H7 from fecal and skin sources during slaughter is one of the most important risk factor in transmission to human however; no single study has been conducted in fecal and skin samples and subsequent rate of carcass contamination by *E. coli* O157:H7 in abattoirs in Ethiopia. In addition, the country has no microbiological standards in meat and much work has not been done on coliform levels on sheep and goat carcasses.

Thus it is hypothesized that *E. coli* O157:H7 in feces and skin of sheep and goats presented for slaughter has association with carcass contamination. Therefore, the objectives of this study were: -

- To evaluate total and fecal coliform loads from carcasses before and after washing
- To estimate the prevalence of *E. coli* O157:H7 in feces, skin and carcasses from slaughtered sheep and goats and water samples at an export abattoir
- To determine whether association exists between *E. coli* O157:H7 prevalence in feces and skin with contamination of carcass before and after wash in the slaughtering line
- To detect the presence of virulent gene on *E. coli* O157:H7 isolates

2. LITERATURE REVIEW

2.1. *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 sized rods, peritrichate arrangement of flagella, if motile; facultative anaerobic and ferment rather than oxidize glucose; reduce nitrate to nitrite and able to grow on non-enriched media. However, there are few species of the members that have exceptional properties (Feng, 2002; Quinn *et al.*, 2002).

Enterobacteriaceae can be divided in to three groups based on their pathogenecity for animals. The firis group is those with uncertain significance for animals, which include species from 17 genera of the family. The second group is major pathogens of animals such as *Salmonella spp*, *E. coli* and three of the *Yersina spp* and the third is opportunistic pathogens that are known occasionally to cause infections in animals. The later includes species within the genera *Klebsiella, Enterobacter, Proteus, Serratia, Edwardsiella, Citrobacter, Morganella,* and *Shigella*, The members of the *Enterobacteriaceae* are geographically widespread and many are distributed throughout the environment in soil, water, on plants as well as in the intestine of animals and humans. However, a few species occupy a limited ecological niche (Quinn *et al.*, 2002; Schaffner and Smith, 2004).

From food safety point of view, in order to estimate sanitary quality of a given food items, the classic approach are based on the search for not only pathogenic microorganisms but also indicator microorganisms. The major indicator organisms are belongs to the *Enterobacteriaceae* and 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).

2.2. Microbiological indicators

An indicator refers to a single or group of microorganisms, or alternatively, a metabolic product, whose presence in a food or the environment at a given level is indicative of a 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).

As mentioned above indicators can be a specific microorganism such as *E. coli* or a metabolite like lactic acid titration that used to measure sanitation quality. However, using a specific microorganism as an indicator is difficult, as appropriate indicator organisms are difficult to identify. An "ideal" indicator organism has a history of presence in foods at any time that the target pathogen or toxin might be present; is present at concentrations directly related to that of the target pathogen or toxin; is absent from food when the target is not present; has growth rates equivalent to or slightly greater than the pathogen; has rapid, simple, and inexpensive quantitative assays available; has similar resistance profiles to the target and is nonpathogenic. While such a 'wish list' is nice in principle, there is no single indicator organism capable of meeting all of these requirements (IFT, 2000; Jay, 2000; Schaffner and Smith, 2004).

These criteria apply to most, if not all, foods that may be vehicles of foodborne pathogens regardless of their source to the foods. In the historical use of safety indicators, however, the pathogens of concern were assumed to be of intestinal origin, resulting from either direct or indirect fecal contamination. Thus, such sanitary indicators were used historically to detect fecal contamination of waters and thereby the possible presence of intestinal pathogens (Jay, 2000; Schaffner and Smith, 2004).

The use of indicator organisms can offer assistance for establishing, monitoring and verification of critical control points in HACCP operations. Indicators can best be used within a HACCP system to control processes that have the greatest influence on the level of microorganisms rather than on determining whether to accept or reject a given lot of product. When used for critical control point evaluation, a large number of samples can be progressively collected throughout a process. HACCP can use index organisms to assess the integrity of give processes by

determining levels of indicator organisms before and after each process is completed. Indicator organisms can be used to establish upper limits for pathogen numbers thus; actions should be implemented that strive to reduce indicator organism numbers to the lowest level possible. It is widely accepted that the regular monitoring of process controls in the meat industry must replace end product testing (Schaffner and Smith, 2004).

2.2.1. Coliforms

Coliforms is not a taxonomic classification but rather a working definition used to describe a group of Gram-negative, facultative anaerobic rod-shaped bacteria under *Enterobacteriaceae* family, that ferments lactose to produce acid and gas within 48 h at 35°C (Feng *et al.*, 2002). The *Escherichia, Citrobacter, Klebsiella* and *Enterobacter* are the four genera of the family *Enterobacteriaceae* that represents by and large coliforms *(*Jay, 2000; Feng *et al.*, 2002; Quinn et *al.,* 2002*)*.

In the earlier time Shardinger (1892) proposed the use of *E. coli* as an indicator of fecal contamination. This was based on the premise that *E. coli* is abundant in human and animal feces and not usually found in other niches. Furthermore, since *E. coli* could be easily detected by its ability to ferment glucose (later changed to lactose), it was easier to isolate than other known gastrointestinal pathogens. Hence, the presence of *E. coli* in food or water became accepted as indicative of recent fecal contamination and the possible presence of frank pathogens (Feng *et al.*, 2002; Schaffner and Smith, 2004).

Later, Smith by the year 1895 suggested a test for this organism as a measure of drinking water. This marked the beginning of the use of coliforms as indicators of pathogens in water and this practice extended to be applied in food safety (Jay, 2000). Although the concept of using *E. coli* as an indirect indicator of health risk was sound, it was complicated in practice, due to the presence of other enteric bacteria like *Citrobacter*, *Klebsiella* and *Enterobacter* that can also ferment lactose and are similar to *E. coli* in phenotypic characteristics so that they are not easily distinguished. As a result, the term "coliform" was established to describe this group of enteric

bacteria. Later the US public health service adapted the enumeration of coliforms as a more convenient standard of sanitary significance (Feng *et al.*, 2002; Schaffner and Smith, 2004).

Coliforms grow well on a large number of media and in food types. They have been reported to grow at temperatures as low as –2°C and as high as 50 °C however, growth is very slow at 5 °C. Its growth pH ranges 4.4 to 9 and grows well on nutrient agar by producing visible colonies within 12-16 hr at 37 °C. Coliforms are capable to grow in the presence of bile salts, the chemical which inhibits the growth of gram-positive bacteria. Therefore this advantage is used and applied for selective isolation of these indicators from various sources. Unlike most other bacteria, they have the capacity to ferment lactose with the production of gas, and this characteristic alone is enough to make presumptive determination (Jay, 2000; Queen *et al.*, 2002).

The general ease with which coliforms can be cultivated and differentiated makes them nearly ideal as indicators, except that their identification may be complicated by the presence of atypical strains. Occasional strain *of Arizona hinshawii* and *Hafnia alvei* ferment lactose but generally not within 48 h and some *Pantoea agglomerans* strains are lactose positive within 48 hr and therefore complicate the role of coliforms as indicators. Although coliforms are easy to detect, their association with fecal contamination is questionable because some coliforms are found naturally in environmental samples. This led to the introduction of the fecal coliforms as an indicator of fecal contamination (Jay, 2000; Yalcin *et al.*, 2001).

2.2.2. Fecal coliforms

Fecal coliform is a subset of total coliforms that grow and ferment lactose at elevated incubation temperature (45.5°C), hence also referred as thermotolerant coliforms, within 48 h. They are grams negative bacilli, non-spore forming, oxidase negative, facultative anaerobic, able to multiply in presence of bile salts and ferment lactose with acid and gas production at specific temperature and time mentioned above (Jay, 2000; Leclercq *et al.*, 2002).

Fecal coliform consists mostly of *E. coli*, but other enteric microorganism such as *Klebsiella* can also ferment lactose at this temperature and therefore, be considered as fecal coliforms (Feng *et*

al., 2002). Enumeration of fecal coliforms in foods can be carried out using a violet red bile agar (VRBA) medium. However, the use of VRBL is difficult because of the need to discriminate colonies on the basis of size and the agar must be cooled to 48°C during plating to avoid bacterial damage (Schaffner and Smith, 2004).

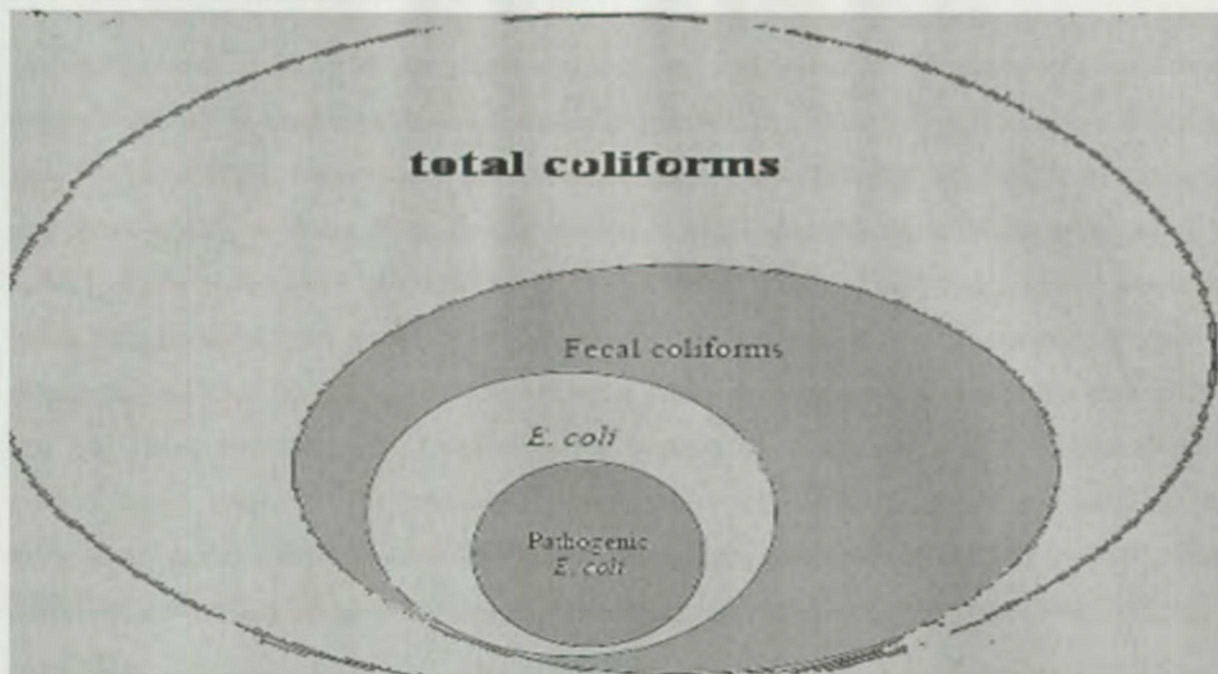


Figure 1: The relationship between coliforms with *E. coli.*

Modified from Schaffner and Smith (2004)

2.2.3. *Escherichia coli*

The first fecal indicator was *E. coli*. When the concept of fecal indicators was applied to food safety, some additional criteria were stressed. However, the inclusion of *Klebsiella* spp in the working definition of fecal coliforms diminished the quality of this group with fecal contamination. As a result, *E. coli* has re-emerged as an indicator, partly facilitated by the introduction of newer methods that can rapidly identify *E. coli.* Since *E. coli* is more indicative of fecal pollution than the 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 *Enterobacteriaceae* and pathogenic *E. coli.* Therefore, increases in the number of *E. coli* during chilling, storage and distribution suggest that meat products has been subjected

to conditions, which would also allow growth of these pathogens (Feng *et al.*, 2002; Schaffner and Smith, 2004).

2.3. Bacterial counting techniques

The examination of foods for the presence, types, and numbers 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 bacterial are capable of multiply and forms visible colonies on or in agar media. In these counting methods the 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 microorganisms in a food product. Therefore, although some methods of analysis are better than others, every method has certain inherent limitations associated to its use (Jay, 2000; Quinn, *et al.*, 2002).

2.3.1. Viable counting methods

Serial ten-fold dilutions of the original fluid containing bacteria must first be made for each method. These must be carried out as accurately as possible to minimize avoidable errors and an aseptic technique should be used (Quinn *et al.*, 2002).

Spread plate method

A range of dilutions is used and an inoculum of known volume of each dilution is placed on the surface of an agar plate. The inoculum is spread rapidly over the entire agar surface using a thin and bent glass rod. At least two and preferably four, plates should be inoculated per dilution. The plates are incubated for 24-48 hours at 25-37°C. The incubation temperature will depend on whether environmental or pathogenic bacteria are being sought. After incubation, plates

inoculated with a sample dilution yielding between 30 and 300 colonies are counted for greatest accuracy. The colony count should be an average of the two or four plates inoculated with the selected dilution (HPB Method 2001; Quinn *et al.*, 2002).

Pour plate method

This method is similar to the spread-plate technique, except that the inoculum in this case is mixed thoroughly with molten agar, which is previously held in water bath at 50 °C. Two or four plates should be inoculated with each dilution. The agar is allowed to set and then incubated at appropriate temperature and time. Plates inoculated with a sample dilution that yields between 30 and 300 colonies per plate should be counted. The colonies will be distributed throughout the agar as well as on the surface (Yalcin *et al.*, 2001; HPB Method 2001; Feng *et al.*, 2002).

Miles-Misra technique

This method is done in such away that lines can be drawn on the bottom of an agar plate with a marker, dividing it into 8 sectors. An inoculum of 0.02 ml, delivered as a drop, is placed on the agar in each sector. At least 4 drops per sample dilution should be used. The inocula are allowed to dry and the plates incubated at appropriate incubation temperature and time. A sample dilution yielding about 30 colonies per drop should be selected. An average colony count from at least 4 drops must be obtained. The calculation is similar to that for the two previous methods, but as the inoculum was 0.02 ml, the conversion factor will be 50 to obtain a figure for the bacteria per ml in the original sample. This method has the advantage of being economical with agar media (Quinn *et al.*, 2002).

Filtration method

This is a useful method for determining the number of bacteria in a water sample or other fluid where the bacterial number is low. A known volume of fluid is passed through a membranes filter of pore size 0.22 μm. The filter will retain the bacterial cells, and is aseptically placed and bacterial-side up on the surface of an agar plate. The medium can be selective or non-selective,

depending on the bacterial species being sought. Colonies will form on the surface of the filter after incubation and can be counted. As the volume of the fluid sample is known, the bacteria per ml of the samples can be calculated (Jay, 2000).

Most probable numbers (MPN)

The MPN method uses a test tube, consisting of media with inverted Durham tubes inside, which captures gas that are released from the growth of coliform bacteria. A series of dilutions and replicates are set up and those producing gas within 24 hrs at 35 °C are counted. These techniques are based on statistical probabilities with the assumption that there is a normal distribution of bacteria in liquid samples. If the liquid sample contains one viable bacterial, its growth and multiplication in a suitable broth can be detected by manifestations such as turbidity or acid and gas production (Jay, 2000; Quinn *et al*., 2002)

In this method, dilutions of samples are prepared as for the standard plate count (SPC). Three serial aliquots are then placed into 9 or 15 tubes of appropriate medium for the three or five tubes method, respectively. Numbers of organisms in the original sample are determined by use of standard MPN tables. The method is statistical in nature (Feng *et al.*, 2002). Although some authors concluded that many MPN values are improbable, this method of analysis has gained popularity. It is relatively simple, reputable than SPC and specific groups of organisms can be determined. However, its drawbacks includes large volume of glassware are required, lack of opportunity to observe the colonial morphology of the organisms and its lack of precision (Jay, 2000; Feng *et al.*, 2002; Quinn *et al*., 2002).

2.3.2. Total (Viable and Non viable) counting methods

These methods do not distinguish between viable and non-viable cells and thus the bacterial count will include both living and dead cells (Quinn *et al*., 2002; Schaffner and Smith, 2004).

Breed direct smear method

This technique is used most commonly for counting bacteria in milk. A microscope slide is placed over a template of 1cm x1cm surface and a 0.01 ml of sample is carefully spread over this area. The smear is allowed to air-dry, fixed by heat and stained with methylene blue for about 1 minute. After air-drying, the stained smear is examined under the oil-immersion objective. The bacterial cells should be counted in at least 50 fields throughout the area of the smear. An average bacterial cell count per field (N) should be obtained. The radius (r) for the particular microscope's oil-immersion field can be found (in mm) using a slide and eyepiece micrometer. The area of the field will be approximately $3.14 \times r^2$ (mm^2) (Quinn *et al.*, 2002).

Counting chamber method

This technique is used for counting bacterial cells and the calculation of bacteria per ml in the liquid sample is similar to that for erythrocyte count. To prevent motility of the bacteria, 2-3 drops of formalin per 10 ml of bacterial suspension can be added. The chamber is filled and viewed under the low-power objective in order to orientate the marked grid. Then the high-dry objective is used to count the bacteria in the five areas in the central region of the grid. Each of these large squares, one at each corner and one central, is divided into 16 smaller squares. Thus, the bacterial cells are counted in 80 (5x16) of the smallest squares in the grid. The average number of bacteria per small square can be calculated (Schaffner and Smith, 2004).

Turbidity standards

It is a convenient and simple method that results in, however, an approximate total bacterial count. This method used a McFarland's (Brown's) opacity tubes, which are commercially available and consist of a series of ten numbered, standard, thin glass tubes containing different dilutions of suspended barium chloride that gives a range of opacities. The test bacterial suspension is placed in a 'blank' tube of similar dimensions to the standards. Rolling the test suspension across a printed page and matching it with a standard of comparable opacity make a visual comparison of opacity. Tables are supplied with the opacity tubes that give the numerical

equivalents (bacteria per ml) of the opacity standards for a certain range of bacteria (Quinn *et al.*, 2002).

2.4. Bacterial attachment and sample taking methods on carcass surfaces

2.4. 1. Bacterial attachment

Bacteria must attach to meat surfaces to remain intact and cause infection. Bacterial 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 (Edwards and Fung, 2006). Attachment to tissue causes bacteria to become trapped in the meat tissue fibrils. The irreversible binding of *E. coli* O157:H7 to the carcass tissue may occur during the skin pulling operation. Bacteria are deposited on the carcass surface through cross contamination from the hide, and over time, the numbers of bacteria that can be removed from the surface dramatically decrease (McEvoy *et al.* 2003).

Bacteria attached to carcasses can be firmly or loosely associated with the nature of the tissues. Fatty tissues may provide a better surface than lean muscle tissues for bacterial attachment since their surface structures are entirely different (Chung *et al.*, 1989). Water washes are intended to remove any bacteria that are loosely associated with the carcass (Dickson, 1992). Prolonged holding times of carcasses may allow bacteria to firmly attach to carcass surfaces and create biofilms that are difficult to remove. The formation of biofilms may facilitate the emergence of resistant bacteria to the decontamination methods employed in slaughter facilities. The efficacy of decontamination decreases as the time between the contamination event and the treatment increases (Sofos *et al.,* 1999).

2.4. 2. 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. Samples from carcass surface can be collected in two different methods: destructive and non- destructive methods. An example of destructive method is

template excision methods where as the non-destructive method includes wet and dry swab, sponge sampling and gauze tampon methods (ISO 17604, 2003).

One of the advantages of destructive methods, for example by excision on carcass surface is it harvests all bacteria while other methods do not. This invariably results in larger bacteria counts. Not all bacteria on the surface may be removed by either method. Repeatability and reproducibility of destructive methods are less variable, whereas the sampling methods used in non-destructive method result in greater operator variability (ISO 17604, 2003).

In other hand destructive method has disadvantages in that only a small proportion of the carcass is sampled by this method, which may result in significant inaccuracies when total contamination is law and heterogeneously distributed, or when the presence of the target pathogen is sparse (ISO 17604, 2003). Secondly it damages the carcass, which may be unacceptable commercially and laborious. Because of this meat industry personnel prefer swabbing-based methods for assessing carcass hygiene (ISO 17604, 2003).

Although, excision is an effective technique for bacterial recovery from meat carcasses (Bolton, 2003; MacEvoy *et al.*, 2003), swabbing with a sufficiently abrasive material may be as effective as excision for bacterial recovery from carcasses. In between there are a variety of sponges, clothes and meshes that range inefficiency depending on their abrasiveness (Byrne *et al.*, 2005).

Of the non-destructive sampling methods, swab sampling is the oldest and the 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 template first. Then the swab will be placed into the diluents that are used to wet the swab and another dry swab will be used in the same site and using the same techniques. Finally both swabs will be placed on the same bottle and stored at refrigerated temperature until plated (Jay, 2000; ISO 17604, 2003).

Exact knowledge of the diagnostic values that is the sensitivity, the specificity, the precision and the predictive value of the classical sampling methods (Table 1) is not available in most literature (ISO 17604, 2003).

Table 1: Evaluation of swabing and excition methods

Method evaluation	Excision	Swabbing
Sensitivity	79%	50%
Specificity	100%	100%
Positive predictive value	100%	100%
Negative predictive value	84%	70%
Precision	90%	77%
Apparent prevalence	37%	23%
True prevalence	47%	47%

Source: (ISO 17604, 2003).

2.5. *Escherichia coli* O157:H7

2.5.1. Historical background

Although *E. coli* was recognized as a cause of urinary tract infection and infantile gastroenteritis in the early times, *E. coli* O157:H7 was first recognized as a human pathogen in 1982 in USA, when it was implicated in two outbreaks of haemorrhagic colitis associated with the consummation of undercooked hamburgers from the same fast food restaurant. Such foods were identified as a vehicle of *E. coli* O157:H7 and it was isolated from patients and a frozen ground beef patty (Buchanan and Doyle, 1997; Mead and Griffin, 1998; Fratamico and Smith, 2006). In 1993, an outbreak of *E. coli* O157:H7 caused 700 illnesses and 4 children died among people who ate undercooked ground beef in other fast food chains in US (Edwards and Fung, 2006).

Shortly after *E. coli* O157:H7 was determined to be a human pathogen; Karmali *et al*. (1983) observed that a stool sample from children with hemolytic HUS has contained a substance that was toxic to Vero cells. This verocytotoxin was produced by *E. coli* isolates with O157:H7 the prominent serotype causing infection. In recognition of its distinct clinical manifestations, *E coli* O157:H7 became the first of several strains referred to as enterohaemorrhagic *E. coli* (EHEC), which are now believed to account for over 90% of all cases of haemolytic ureamic syndrome (HUS) in industrialized countries (Buchanan and Doyle, 1997; Mead and Griffin, 1998).

How long *E. coli* O157:H7 has been causing human illness is still a matter of speculation, though its illness in human was first recognized in 1982. Retrospective surveys of *E. coli* isolates serotyped in different countries showed that serotype O157:H7 had been obtained from an individual in the mid-1970s (Mead and Griffin, 1998). The incidence of HUS has fluctuated in some regions, but studies from developed countries suggested that an overall increase in this condition has been observed. Generally, the available evidence suggests that *E. coli* O157:H7 emerged as an important human pathogen sometime during the past half century (Mead and Griffin, 1998; IFT, 2000).

2.5.2. Classification and nomenclature

Serological identification of *E. coli* involves three surface antigens, which includes "O" (somatic), "K" (capsular) and "H" (flagellar). Although the number provided by different authors in the literature varies, there are around 200 O, 80 K and 56H antigens (Fratamico and Smith, 2006). Some also suggested that the fimbrial (F) surface antigens should be a fourth component of serological testing. The "O" antigen is a lipopolysaccharide molecule on the outer membrane of a cell while "H", "K" and "F" antigens are proteins. Using serotype and serogrouping the species of this organism is subdivided into serovars (Benson, 2003; Fratamico and Smith, 2006).

These antigens have characteristics of immunogenicity and pathogencity, hence, besides serotyping; they help in the diagnosis of the agent (NPH, 2006). Determining the serogroups (O) and the serotype (O, H, and often K antigens) is an important means of defining the various pathogenic strains of *E. coli*. Thus, there are suitable test kits that are used for serotyping based on their antigenic characteristics (NPH, 2006).

Escherichia coli serotyping is important for the proper diagnosis of foodborne out breaks and epidemiological investigations. However, serotyping alone cannot be relied on for categorizing a strain of *E. coli*, and the identification of specific virulence characteristics (genes) must also be performed. Currently about five *E. coli* strains are known to act as intestinal pathogens. These are enterohaemorragic (EHEC), enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroinvasive (EIEC) and enteroaggregative (EAEC) (Besser *et al.*, 2003; Moxley, 2003; Fratamico and Smith,

2006). The EHEC clone apparently expected to be emerged from an EPEC that was lysogenized by bacteriophages encoded shiga-like toxin genes (Perna *et al.*, 1998; IFT, 2000).

The term EHEC refers to *E. coli* serogroups including O26, O111, O103, O104, O118, and O145 (with various H antigen types). Of the subgroups, *E. coli* O157:H7 is the predominant and most virulent serotype in a pathogenic subset of EHEC. *E. coli* O157:H7 is so-named because it expresses the 157th O antigen identified and the 7th H antigen (Morgan *et al.*, 1993; Feng, 1995; Izumiya *et al.*, 1997; Mead and Griffin, 1998; Chapman *et al.*, 2001).

There have been several issues about whether *E. coli* O157:H7 serotype can be compared to its very similar cousin *E. coli* O157:H^-. However, currently they are differentiated based on the biochemical and molecular characteristics of their genome responsible for their toxicity (IFT, 2000). Thus, after EHEC being emerged, it diverts into two branches based on reaction to β-glucuronidase and sorbitol tests. Hence, the negative reacting groups are *E. coli* O157:H7 clone and motile, but the positive reacting groups are non-motile and are *E. coli* O157:H^- clone (Whittam, 1996; Jay, 2000; Leclercq *et al.*, 2001).

2.5.3. General characteristics

Escherichia coli O157:H7 is a Gram's-negative, non-spore forming rod shaped, arranged in pairs or single, is motile by means of peritrichous flagella and may have capsules or microcapsules. It is a facultative anaerobic, chemo-organotrophic microorganism with an optimum growth temperature of 37°C. *E. coli* strains do not grow under refrigeration condition. However, the organism can survive for weeks at 4°C (MOH, 2001).

2.5.4. Colony characteristics

Escherichia coli O157:H7 cannot ferment sorbitol and therefore give colorless colonies on Sorbitol-MacConkey agar (SMAC). However, the majorities of other *E. coli* strains including the serotype O157:H^- are capable of fermenting sorbitol and therefore have a characteristic pink colonies (Chapman, 1994; ISO16654, 2001). Other sorbitol non-fermenting bacteria however, are

found that includes *Morganella morganii* and *Hafnia alivei* appear identical to O157:H7 colonies on SMAC (Chapman, 1994; Feng, 1995). Unlike *E. coli* O157:H7, these groups show Voges-Proskauer positive reactions. Therefore, confirmatory tests must be performed to distinguish O157:H7 isolate from these groups (Okrend *et al.,* 1990; Leclercq *et al.*, 2001).

2.5.5. Biochemical properties

Conventional identification of *E. coli* was done using the indole, methyl red, Voges-Proskauer, and citrate utilization (IMViC) test. Approximately 95 % of *E. coli* strains are indole and methyl red positive, but are Voges-Proskauer and citrate negative.

Table 2: Biochemical profile of *E. coli* O157:H7

Test	**Reaction**	**Test**	**Reaction**
Sorbitol	-	Glucose (gas)	+(98%)*
β-Glucuronidase	-	Indole	+
Salicin	-	Arabinose	+
Esculin	-	Trehalose	+
Arginine dihydrolase	-	Mannitol	+
Adonitol	-	Lactose	+
Inositol	-	Maltose	+
Cellobiose	-	Rhamnose	+
Urease	-	Xylose	+
Citrate	-	Lysine decarboxylase	+
Potassium cyanide	-	Ornithine decarboxylase	+
Sucrose	+ (87%)*	Raffinose	+
Glucose (acid)	+	Dulcitol	+

Source :(Edwards and Fung, 2006)   * Few negative strains have been reported

Other biochemical testes used are urease, glutamate decarboxylase, sorbitol fermentation, and β-glucuronidase tests (Farmer *et al.*, 1985; Okrend *et al.*, 1990; Leclercq *et al.*, 2001; Edwards and Fung, 2006). The unique biochemical properties of the *E. coli* O157:H7 are its failure to ferment sorbitol within 24 h and being β-D- glucuronidase negative (Chapman, 1994; Dontoroua *et al.*, 2004) (Table 2). These are the best and basic principles used in distinguishing these pathogenic strains from other *E. coli* serotypes. Even *E. coli* O157:H7 and *E. coli* O157:H$^-$ strains are separated by these two biochemical characteristics. SMAC media is used for the detection of *E. coli* O157:H7 using the above principles. Further addition of antimicrobial supplements called cefixime and tellurite on SMAC can reduce the number of false-positive isolates (Okrend *et al.*, 1990; Baron *et al.*, 1994; Chapman *et al.*, 1994; Monnet *et al.*, 1994; Leclercq *et al.*, 2001).

2.5.6. Growth and survival

Like all bacteria the survival and growth of *E. coli* O157:H7 in foods are dependent on the interaction of various intrinsic and extrinsic factors such as temperature, pH and water activity (a_w). The Optimum growth temperature for *E. coli* O157:H7 is 37°C, the minimum is 7-8°C and its maximum temperature is 41°C in selective *E. coli* broth and grows only poorly at temperatures greater than this in non-selective media (Chapman, 1994; MOH, 2001). The upper growth temperature for *E. coli* O157:H7 was culture medium–dependent. The organism survives well in chilled and frozen foods (Feng, 1995). For example only little change was noted in numbers of bacteria in hamburgers stored at -20°C for 9 months (Chapman, 1994). The effect of environmental stress and food production processing on the growth, survival and inactivation of *E. coli* O157:H7 are well recognized. The organism is also heat resistant, but influenced by pH, growth condition and growth phase of the cells, and the method of heating (MOH, 2001). Thermal resistance is higher in foods of high fat content or that are packed under low oxygen atmospheres (Chapman, 1994; Boyce *et al.*, 1995; MOH, 2001; Fratamico and Smith, 2006).

Escherichia coli O157:H7 is relatively acid tolerant compared to other foodborne pathogens (Feng, 1995; MOH, 2001). Its pH range is 4.4 to 9.0, the optimum pH being 6-7 and can survive for extended periods in foods at pH levels of 3.5-5.5 (Fratamico and Smith, 2006). Mineral acids such as HCl are less inhibitory than organic acids (e.g. acetic, lactic) at the same pH. The

pathogen's survival in acidic foods is particularly important since several outbreaks have been associated with acidic foods (Feng, 1995). Experimentally, the pathogen has been shown to survive for several weeks to months in a variety of acidic foods, including mayonnaise, sausages, apple cider and cheddar cheese (Feng, 1995). Survival in these foods is extended greatly when stored at refrigeration temperature. Moreover, prior exposure to acidic conditions can increase acid tolerance (Baron *et al.*, 1994). However, acid-sensitive EHEC strains, has also been identified (Boyce *et al.*, 1995).

Optimum growth of *E. coli* O157:H7 is found to be at water activity (a_w) of 0.995 and its minimum a_w is 0.950 (MOH, 2001). Studies on the effect of a_w on the survival and growth of *E. coli* O157:H7 focused primarily on the effect of sodium chloride, though, presumably, *E. coli* O157:H7 behaves similarly with other *E. coli.* It can survive for many weeks when desiccated, particularly at refrigeration temperature (Boyce *et al.*, 1995; Mutaku *et al.*, 2005).

The organism can grow in the presence or absence of oxygen (MOH, 2001). Growth can occur in vacuum-packed meat at 8 and 9°C, but not when the meat is packed under 100% CO_2. Survival on fermented meat was equivalent when packed under air or under vacuum (Boyce *et al.*, 1995; Buchanan and Doyle, 1997). In the absence of organic matter, benzalkonium chloride, chlorhexidine gluconate, ethanol and hot (70°C) water are effective sanitizers against *E. coli* O157:H7 (MOH, 2001). Standard water chlorination is effective against this organism. *E. coli* O157:H7 does not appear to have any increased resistance to antimicrobial food additives (Buchanan and Doyle, 1997; MOH, 2001; Besser *et al.*, 2003).

2.5.7. Epidemiology of *Escherichia coli* O157:H7

Distribution

Human infection with *E. coli* O157:H7 has been reported from in six continents over 30 countries. Since its recognition 1982, it has become an important concern in North America, Europe, South Africa, Japan, South America, and Australia. High rates are present in regions of South America, especially Argentina, where HUS is endemic (Lopez *et al.*, 1989; Bolton *et al.*,

1996). The highest incidence rates occur in children, in elders and immunocompromized peoples, (Mead and Griffin, 1998). Cattle feces are the most important source of *E. coli* O157:H7, however, the presence of *E. coli* O157:H7 in the feces of other animal species has been well recognized. Thus, it is distributed globally in soil, water, vegetation, decaying matter, and the large intestine of most animals and humans (Chapman *et al.*, 1997). *E. coli* O157:H7 has been also reported in ground beef in countries like Ireland, Norway, Canada, Spain, Italy, Egypt (de Boer and Hauvelink, 2001) and Ethiopia (Demisse, 2005; Mutaku *et al.*, 2005; Hiko, 2007) and these generally show the worldwide distribution of the organism (Baron *et al.*, 1994).

Host range

Escherichia coli O157:H7 has been isolated from the feces of a variety of animals, including cattle, sheep, goats (Chapman, 1994; Sargeant and Smith, 2003; Davis *et al.,* 2005; Battisti, 2006; Lenahan *et al.*, 2007), deer, pigs (Dean-nystrom *et al.*, 2000), cats, dogs, chicken, wild birds (Gun *et al.,* 2003; Sargeant and Smith, 2003) and saliva of houseflies (Baron *et al.*, 1994; Ahmad *et al.*, 2007). However, cattle are considered to be the main reservoir of *E. coli* O157:H7 infection for humans (Sargeant and Smith, 2003; OIE, 2004).

Cattle, sheep and goats as reservoir of Escherichia coli O157:H7

Livestock are the most important reservoir of *E. coli* O157:H7 with cattle being the principal sources (Chapman *et al.,* 2001; Sargeant and Smith, 2003). In cattle the fecal prevalence of *E. coli* O157:H7 in early data ranged from 0.1% to 4% for individual animals. With improved techniques in recent studies, the reported range has now increased to 28%. In some groups of animals the rate of excretion may exceed 80% (Gyles, 2004).

Several surveys of *E. coli* O157:H7 from fecal shedding appear to vary by age, with higher prevalence values generally reported in younger animals (Chapman *et al.,* 2001). The reasons for age related difference are not known, but may include age related difference in rumen development, duration or management difference. Prevalence of fecal excretion also varies substantially among positive herds. Fecal shedding of *E. coli* O157:H7 is frequently intermittent

and has short duration. Generally fecal shedding of *E. coli* O157:H7 in cattle has not been associated with disease in field studies and has documented as having a seasonal pattern, with higher prevalence values in warmer months. Generally shedding is influenced by the age of the animals, diet, stress, population density, geographical location and season (Meyer-Broseta, *et al.*, 2001; Sargeant and Smith, 2003; Fratamico and Smith, 2006).

Escherichia coli O157:H7 is confined to the fore stomachs and distal sites the later being the principal sites of localization. Fasting increases the levels of *E. coli* O157:H7 shedding in the feces of some animals. Water has been suggested as a vehicle of transmitting *E. coli* O157:H7 among cattle (Boyce *et al.,* 1995). A diverse population of genetic subtypes is found in dairy and beef herds. More than one strain of *E. coli* O157:H7 can be isolated from feces of the same animal or different animals within the same herd. Even multiple sub type may be present within a farm at a single point in time, and some subtypes appear to be unique to specific herds. The subtype found may change over time (Boyce *et al.,* 1995; Chapman *et al.,* 2001; Sargeant and Smith, 2003).

The epidemiology of these organisms in sheep is apparently similar with cattle and sheep has been identified as a reservoir of *E. coli* O157:H7 (Blanco *et al.,* 2003; Sargeant and Smith, 2003; Battisti, 2006; Bhandare *et al*., 2007; Lenahan *et al*., 2007). A six-month study of healthy ewes revealed that fecal shedding of the pathogen was transient, seasonal and animals showed no signs of disease throughout the study. Orally administered animals at a dose of 10^9 *E. coli* O157:H7 shed the bacteria for up to 92 days. A shedding sheep passed *E. coli* O157:H7 to a non-dosed pen mate. Diet influenced fecal shedding of *E. coli* O157:H7 (Sargeant and Smith, 2003).

Escherichia coli O157:H7 in abattoirs

Skin of animals is an important source for carcass contamination (Elder *et al.,* 2000; Edwards and Fung, 2006). Meat becomes contaminated with *E. coli* O157:H7 when feces or contaminated hides contact the carcass during slaughter. In a study conducted by Elder *et al.* (2000), 11% of *E. coli* O157:H7 was isolated from hide samples of cattle presented for slaughter. In this study *E. coli* O157:H7 was often present on the hide of animals following stunning, and cross

contamination to the carcass was evident in that carcasses sampled immediately after skinning were the most heavily contaminated (Elder *et al.*, 2000). Others also noted that when animals arrive at the abattoir, they carry a wide variety of microorganisms on their hides and hooves, and in their intestinal tracts. Therefore, bulk of microbial contamination occurs during skinning from dust, dirt and fecal material that accumulate on the skin (Buchanan and Doyle, 1997).

Bung tying is a possible source of carcass contamination in the slaughter process, and great care must be taken to prevent bacterial transfer from the anus of the animal onto the edible tissue. Tools or personnel that contact the bung may also contribute to cross contamination. Evisceration is another important source for carcass contamination in the slaughtering process. The evisceration process can transfer potential pathogens form ingesta into carcasses, environment or equipments. Carcass sampling revealed that carcass-to-carcass transfer of *E. coli* O157:H7 does occur in the slaughter line even in the chilling rooms (McEvoy *et al., 2003*).

Environmental source of Escherichia coli O157:H7

Environmental studies have shown that the organism can persist in manure, water troughs, and other places on farms (Sargeant and Smith, 2003). The transient nature of fecal shedding in individual animals, the persistent of specific genetic strains of *E. coli* O157:H7 within farms and the wide distribution of genetic strains suggests that other reservoirs or niches may exist to sustain these bacteria within agricultural environments (Sargeant and Smith, 2003). The organisms may introduce into the environment through the feces including manure used as fertilizer. Rainwater runoff can then spread them to water reservoirs and wells. Alternately, fecal contamination at slaughter may result in meat contamination (Chapman *et al.*, 1997).

Escherichia coli O157:H7 infection in human and sources of transmission

The presence of *E. coli* O157:H7 in animal feces provides the potential for these organisms to enter the food chain by contamination of meat with intestinal contents during the slaughter process, fecal contamination of milk products, or contamination of fruit and vegetables by contact with infected manure. Generally there are four principal routes of infection identified for *E. coli*

O157:H7 to humans. These are person-to-person, foodborne, direct contact with shedding animals and transmission through the environment (Boyce *et al.*, 1995; Mead and Griffin, 1998; CDC, 2005).

In humans, *E. coli* O157:H7 may be shed in the stool for several weeks following resolution of symptoms. In general, young children carry the organism longer than do adults and symptom-free infection occurs frequently (Mead and Griffin, 1998). Bacteria in stools of infected persons can be passed from one person to another if hygiene is inadequate (IFT, 2000; CDC, 2005). It can also be passed from person to person in day care centers and nursing homes, at communal bathroom, through touching of foods etc (Figure.2) (IFT, 2000; CDC, 2005). Thus, fecal-oral person-to-person transmission is often reported in family members of cases who contracted the disease from food or water (MOH, 2001; Gansheroff and O'Brien, 2000).

Most foodborne outbreaks have been traced to contaminated, raw or undercooked ground-beef products (Chapman *et al.*, 1997; Gansheroff and O'Brien, 2000). Foods derived from other species like lambs may also contain the organism. In addition to meat products, raw milk and yoghurt (Morgan *et al.*, 1993), acidic foods, fruits, salad and other vegetables, and water (Feng, 1995; Edwards and Fung, 2006) are all a well-documented route or vehicle for *E. coli* O157:H7 infection in several outbreaks in different parts of the world. This is because of acid tolerance of the organism and its ability to survive fermentation and drying (Feng, 1995).

Occupationally, outbreaks due to direct contact with animals visits (OIE, 2004; Durso *et al.*, 2005) among nurses, microbiologists and laboratory workers (Mead and Griffin, 1998) have been reported. While some produce-associated outbreaks may be due to cross-contamination from meat products, others are more likely to reflect direct contamination in the field with feces of wild or domestic animals (Johnson *et al.*, 2001). *E. coli* O157:H7 can remain viable in soils, water and manure for considerable periods and the organism has been shown to survive for several months in manure and contaminated grassland (Sargeant and Smith, 2003).

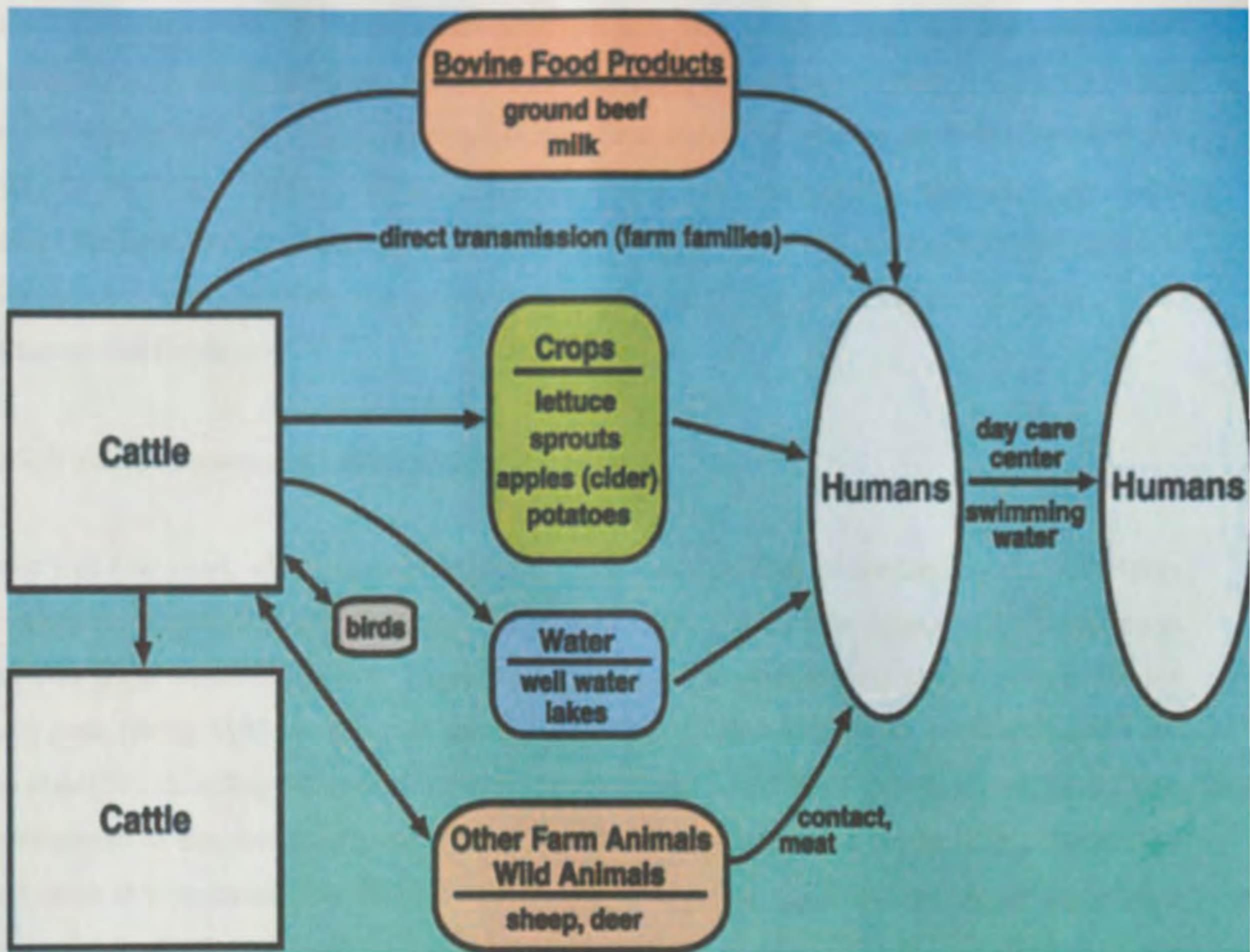


Figure 2: Model for transmission of *E. coli* O157:H7 from cattle to humans
Source: (Gansheroff and O'Brien, 2000).

Several recent incidents showed that both drinking and recreational unchlorinated water could serve as vehicles for transmitting *E. coli* O157: H7 infections. Because of low infectious dose of the organism, swallowing a small amount of lake water can cause illness (Akashi *et al.*, 1994; Feng, 1995).

2.5.8. Infectious dose

The infectious dose for *E. coli* O157:H7 has been reported to be low and might be as low as 10 organisms (Bolton *et al.,* 1996; Doyle *et al.,* 1997). Analysis of foods implicated in disease outbreaks have reveled that foods with as low as 0.3-0.4 cells per gram of food have been involved in outbreaks in humans (Sargeant and Smith, 2003; Fratamico and Smith, 2006). Other

studies (Willshaw *et al.*, 1994) have indicated that less than 2 bacteria per 25 gram of foodstuff were sufficient to cause infection. A direct transmission from person to person and through water can also suggest that *E coli* O157 have a low infectious dose (Willshaw *et al.*, 1994; Bolton *et al.*, 1996; Doyle *et al.*, 1997). The low infections dose demonstrates that infection may occur without bacterial growth occurring in contaminated food and its epidemiological significance of environmental contamination with relatively low levels of *E. coli* O157:H7 (Boyce *et al.*, 1995; Buchanan and Doyle, 1997).

2.5.9. Virulence factors and pathogenesis

In the past few years, scientists have made significant advances in understanding the underlying virulence mechanisms of EHEC strains. For *E. coli* O157:H7 to cause disease it must possess at least two major virulence factors. These are the shiga toxin gene and the attaching and effacing (A/E) gene (Feng, 1995; Gansheroff and O'Brien, 2000). However, other virulence factors are also identified including the enterohemolysis factor. Genes responsible for shiga toxin production is designated as *stx,* for attachment is *eaeA* and for hemolysis is *hlyA* (Feder *et al.*, 2003). The *eaeA* gene is also called the locus for enterocyte effacement (LEE) pathogenecity Island that enable the bacteria to adhere to epithelial cells (Beutin *et al.*, 1995; IFT, 2000; Smith and Sargeant, 2003; Dziva *et al*, 2007). Genes located within the LEE of EHEC have been found to play key roles in colonization of the intestines (Beutin *et al.*, 1995).

Shiga toxin synthesis and secretion

Toxins produced by EHEC are called shiga toxin (stx), also referred as Vero toxins (because of their effect on cultured vero cells). It is one of the major characteristics that discriminate EHEC from other A/E- forming bacteria, and this difference is generally accepted as the explanation for why EHEC are capable of causing extra intestinal complications such as HUS and TTP (Izumiya *et al.,* 1997; Benson, 2003).

The toxin name is drive from the prototype shiga toxin from *Shigella dysenteriae* (Feng, 1995). Two types of toxins (stx1 and stx2) are produced by shiga toxin producing *E. coli* (STEC). Stx1

is indistinguishable from shiga toxin produced by *Shigella dysenteriae*, whereas stx2 shares only 56 % amino acid identity with shiga toxin (Benson, 2003). Most *E. coli* O157 strains produce stx2 and it is more divergent, more toxic than stx1 and has a higher propensity to cause HUS than does stx1 (IFT, 2000; Benson, 2003). Both toxins are composed of five B subunits and a single A subunit. The B subunit confers tissue specificity, enabling the toxin to adhere to a specific glycolipid receptor on cell surfaces. The active (A) portion of the toxin is then delivered into the host cell where it inhibits protein synthesis, ultimately killing the host cell. The toxins target certain cells, including the endothelial cells of blood vessels and the kidney where those specific receptors are found in large number (IFT, 2000; Edwards and Fung, 2006). As EHEC are not highly invasive, their toxin production occurs exclusively in the colon of infected people, not in foods (Izumiya *et al.,* 1997; Nataro and Kaper, 1998; Benson, 2003; Fratamico and Smith, 2006).

Intestinal colonization and production of attaching-effacing (A/E) lesions

Mead and Griffin (1998) and Buchanan and Doyle (1997), described that in addition to shiga toxins production there are other factors thought to contribute to the virulence of *E. coli* O157 include a virulence plasmid (pO157) and LEE. The *eae* chromosomal gene, for example, is ubiquitous among EHEC strains, encoding for an outer membrane protein associated with attachment. A plasmid-encoded enterohemolysin is characteristic of EHEC. The haemolysin may allow *E coli* O157:H7 to use blood released into the intestine as a source of iron. The LEE contains genes is used for an adhesion molecule (intimin) and for other factors important to the production of attaching-effacing (A/E) lesions (Gyles, 2004). The organism is believed to adhere closely to mucosal cells of the large bowel and disrupting the brush border (Buchanan and Doyle, 1997; Benson, 2003).

Following ingestion, the shiga toxins have both local and systemic effects on the intestine and are probably critical to the development of bloody diarrhea. The histopathological sequence of events begins by formation of microcolonies at sites in the large intestine, followed by subsequent formation of attaching and effacing (A/E) lesions, a hallmark of pathogenesis (Benson, 2003; Gyles, 2004; Fratamico and Smith, 2006). Within the A/E lesions, bacteria became tightly bound to host cells via specialized host derived pedestal structures. Microvilli are displaced from

regions adjacent to the bound bacteria and local cytoskeletonal rearrangements lead to formation of pedestals at the site of bacterial attachment. Though EPEC and EHEC share the A/E characteristics, HUS and TTP have been associated only with EHEC and specifically with the ability of EHEC populations to synthesize shiga toxins (Benson, 2003; Law, 2000).

Toxins, which are secreted by the bacteria colonizing the colon, pass through the intestinal wall via transcytosis and subsequently spread haematogenously to target tissues. Ultimately, the toxins preferentially attack renal tissue because of their high affinity for receptors that are found predominantly in this tissue. After internalization by receptor-mediated endocytosis, the active subunit of the toxin catalytically inactivates ribosome. Stx-induced apoptosis (cell death) may be present in epithelial cells of the renal tubules and the glomeruli and necrosis may be observed in enterocytes as well as in the renal cortex (Gyles, 2004). Apoptosis with in the glomeruli ultimately results in tissue damage that can be further complicated by the subsequent inflammatory response (Benson, 2003; Moxley, 2003). These vascular damage and secondary complications is known as hemolytic uraemic syndromes (HUS), the leading cause of acute renal failure in childhood, and thrombotic thrombocytopenic purpura (TTP) in adults. The damage caused in the large intestine is characterized by erosion and haemorrhagic of the mucosal lining and the effect produced is named as haemorrhagic colitis (Gyles, 2004).

2.5.10. Clinical features

In animals

Escherichia coli O15:H7 in cattle is not associated with clinical signs in field studies. It rather persists in cattle without causing disease (Chapman, 1994) because cattle lack a receptor for the illness producing shiga toxins (IFT, 2000). Therefore, the organisms did not colonize mucosal surfaces and did not form A/E lesions (IFT, 2000). Under experimental conditions, oral inoculation of calves with *E. coli* O157:H7 induced and sustained in serum antibodies against O157 antigens and to a lesser extent to stx1 (Fratamico and Smith, 2006; Ahmad *et al*., 2007). The serological responses, however, do not correlate with elimination of carriage by cattle or protection of calves against reinfection by the same strains. The ability of *E. coli* O157:H7 to persist and reinfect cattle is likely contributed to the introduction and persistence of infection in

herds. As many as half of all cattle may carry O157:H7 at some time in their lives and some observers suggest that nearly all cattle have been exposed to EHEC (IFT, 2000).

However, under experimental conditions colostrum deprived neonatal calves found to develop diarrhea 18 hrs following inoculation with 10^{10} CFU of *E.coli* O157:H7 and may develop A/E lesions in large intestine (Fratamico and Smith, 2006). Similarly, intragastric inoculation of *stx2* has induced diarrhea and inflammation. In other experiment, piglets infected orally with *stx2* producing *E. coli* O157:H7 developed gastrointestinal illness and lesions in the kidney, and intramuscular injection of *stx1* producing *E. coli* O157:H7 developed muscular damage and necrosis in the intestine and brain (Benson, 2003; Fratamico and Smith, 2006).

In humans

Escherichia coli O157:H7 infection in humans shows clinical manifestations that range from carrier state to more complicated forms of the disease that includes hemorrhagic colitis (HC), hemolytic uraemic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP) and finally death (Izumiya *et al.,* 1997; Mead and Griffin, 1998). The disease affects any age groups however, most frequently in infants (less than 4 years), elders (greater than 65 years) and immunocompromized individuals (Boyce *et al.*, 1995; Mead and Griffin, 1998; CDC, 2001; CDC, 2005; Moses *et al.,* 2006).

The average incubation period of the disease has been reported to be three days followed by HC, HUS, and TTP (Boyce *et al.*, 1995; Mead and Griffin, 1998; CDC, 2001; CDC, 2005). Most patients with HC recover spontaneously within seven days. Illness typically begins with abdominal cramps and nonbloody diarrhoea (Mead and Griffin, 1998). Bowel movements may become bloody over the next 1–2 days, with the amount of blood varying from a few small streaks to stools that are almost entirely blood. Vomiting occurs in 30–60% of cases. Fever may be absent or mild (Izumiya *et al.,* 1997; Mead and Griffin, 1998; Fratamico and Smith, 2006). Initial laboratory diagnosis is generally unrevealing. The percentage of cases that progress to HUS ranges from 3 to 20% or more. HUS is typically diagnosed six days after the onset of diarrhea (Boyce *et al.*, 1995; Mead and Griffin, 1998).

Healthy adults typically recover completely from *E. coli* O157:H7 illness within a week (Mead and Griffin, 1998). However, in some people, especially young children and the elderly, the illness can progress to HUS, a condition that can lead to serious kidney damage and even death. Some patients have incomplete form of HUS. Acute neurological complications such as stroke, seizure, and coma develop in 25% of patients (Mead and Griffin, 1998). Occasionally, patients with *E. coli* O157:H7 infection is diagnosed as having TTP, a condition similar to HUS but with more prominent neurological findings and less renal involvement. This type of syndrome is more common in elders. TTP is due to hemolytic anemia as a result of fragmentation of red blood cells and shortage of platelets (thrombocytopenia) with easily bleeding, neurological abnormalities, impaired kidney function and fever, however, there is no prior sign of diarrhea (Boyce *et al.*, 1995; CDC, 2001). Among patients with HUS, about 3–5% dies acutely and a similar percentage develops end-stage renal disease. Other long-term sequelae include cholelithiasis, colonic stricture, chronic pancreatitis and glucose intolerance (Boyce *et al.*, 1995).

A number of infectious and non-infectious causes of colitis in humans can be listed as a differential diagnosis of *E. coli* O157:H7. From the infectious causes it is possible to suspect diseases caused by *Shigella, Salmonella, Campylobacter, Yersinia enterocolitica and Entamoeba histolytica.* From the non-infectious cause ulcerativecolitis, intussusceptions, ischemic colitis and appendicitis should be considered (Boyce *et al.*, 1995).

2.5.11. Detection of *Escherichia coli* O157:H7 by culturing

The usual samples collected and used to isolate *E.coli* O157 include fecal, stool, carcass swabs, suspected food items etc (Chapman, 1994; Chapman *et al.*, 2001). In addition to isolating the organisms, diagnosis must include the demonstration of known virulence factors in the isolates (Law, 2000; OIE, 2004).

The organism is usually present in carrier groups in low numbers and is shaded intermittently in feces. Therefore, isolation rates may be improved by increasing the sample volume, the number of individuals to be sampled and by repeat sampling (OIE, 2004). As shown in figure 3, the

isolation and detection of *E. coli* O157 involves selective enrichment, concentration of the enrichment culture by immunomagnetic separation (IMS) beads followed by plating onto a selective agar and biochemical and serological confirmations (Chapman, 1994; Chapman *et al.*, 2001; ISO16654, 2001; McEvoy *et al.*, 2003).

Selective enrichment

Clinical samples are routinely plated directly onto solid media for isolation of *E. coli* O157, but the number of target organisms in feces from healthy carriers is usually low and enrichment in liquid media improves recovery (Chapman, 1994; Chapman and Cudjoe, 2001; Chapman *et al.*, 2001). Commonly used enrichment media are modified trypticase-soy broth (mTSB) supplemented with 20 mg/l novobiocin or 10 mg/l acriflavin to reduce the growth of Gram-positive organisms; buffered peptone water supplemented with 8 mg/l vancomycin, 10 mg/l cefsulodin and 0.05 mg/l cefixime (BPW-VCC) to suppress the growth of Gram-positive organisms such as *Aeromonas* spp. and *Proteus* spp. or modified *E. coli* broth with 20 mg/l novobiocin (mEC+n) (Chapman, 1994; Chapman and Cudjoe, 2001; Chapman *et al.*, 2001). EHEC grows poorly at 44°C (Chapman, 1994).

The optimal incubation temperature for feces, meat and skin sample is 6 hours at 41.5° to minimize overgrowth by other organisms. For dairy products, 24 hours at 41-42°C is recommended for the same purpose. Enrichment broth should be pre-warmed to prevent cold shocking of the organisms and slowing their initial growth. Overnight incubation may increase recovery if the organisms are stressed (Chapman, 1994; McEvoy *et al.*, 2003; Tutenel *et al.*, 2003; OIE, 2004).

Immunomagnetic separation (IMS)

Because of the presence of the organisms in low number in bovine feces and other samples, attempts were made to increase the sensitivity of detection for the presence of the organisms. For this purpose IMS was developed (Chapman, 1994; Chapman and Cudjoe, 2001; ISO16654, 2001; NPH, 2006). The use of IMS followed by plating onto CT-SMAC increases the sensitivity more

than one hundred fold times (Chapman, 1994). IMS is based on magnetic particle beads coated with antibodies specific for *E. coli* O157:H7. The use of this technique on fecal samples, food and other complex matrices results in concentration of the target bacteria and sequestrating from non-target organisms (Chapman, 1994; Chapman *et al.*, 1994; Sargeant and Smith, 2003; Fratamico and Smith, 2006). It is a rapid, technically uncomplicated method and it is specific for *E. coli* O157:H7 and yields an isolate of the organisms that may be useful in epidemiological studies (Chapman, 1994; Bolton *et al.*, 1996).

Commercially available paramagnetic particles or beads coated with anti-lipopolysaccharide antibodies are mixed with the test sample. Magnetic beads with bound bacteria are separated from the supernatant by a magnetic field and after washing plated on to selective agar and incubated for 18 hours at 37°C to isolate suspect colonies. The technique is serogroup specific (Chapman and Cudjoe, 2001). Recovery may be affected by the bead-to-organism ratio, the enrichment broth used and nonspecific adsorption of *E. coli* to the magnetic beads. The problem of this non-specific absorption can be reduced by the use of a low ionic strength solution in the IMS procedure and by careful washing of the complex formed (Chapman, 1994; Chapman *et al.*, 1994; ISO16654, 2001; OIE, 2004).

Plating out and identification

The failure of most strains of *E. coli* O157: H7 to ferment D-sorbitol rapidly and their lack of beta-glucuronidase activity are best exploited in the isolation and identification of these organisms (Chapman, 1994; Karch and Bielaszewska, 2001). This is currently the most commonly used medium for isolating *E. coli* O157:H7. MacConkey agar containing 1% D-sorbitol instead of lactose is a useful and inexpensive medium for the isolation of these pathogens. On this media non-sorbitol fermenting *E. coli* grow as small, round, shinny, grayish-white to colourless colonies (Chapman, 1994; Feng *et al.*, 2002). Selectivity is improved by the addition of 0.05mg/litre cefixime and 2.5 mg/liter potassium tellurite on SMAC, which has a greater inhibitory effect against non-O157 *E. coli* and other non-sorbitol fermenters, such as *Aeromonas, Plesiomonas, Morganella* and *Providencia* (Zadik *et al.*, 1993; Chapman, 1994; Sargeant and Smith, 2003; OIE, 2004).

Media containing fluorogenic or chromogenic glucuronides are used to distinguish non-beta-glucuronidase-producing *E. coli* O157:H7 from beta-glucuronidase-producing *E. coli.* Hydrolysis of 4-methylumbelliferyl-beta-D-glucuronide (MUG) by beta-glucuronidase activity produces a fluorescent compound visible under UV light. However, the additives used may adversely affect isolation rates of *E. coli* O157:H7, particularly for the stressed bacteria (Blackburn and McCarthy, 2000).

Confirmation

Escherichia coli O157:H7 is non-sorbitol fermenters, therefore, they produce colorless colonies on cefixime and tellurite containing SMAC (CT- SMAC). Colonies growing on solid media that are suspected to be *E. coli* O157:H7 must be confirmed biochemically as *E. coli* (Chapman, 1994; ISO 16654, 2001; OIE, 2004).

A biochemical test is applied through inoculation of suspected isolates on biochemical medias such as IMVIC test media. Since *E. hermannii* serologically and biochemically cross-react with *E. coli* O157:H7 (Chapman, 1994), differentiation is required. This can be done in such away that *E. hermannii* can presumptively be differentiated on the basis of the appearance of its colonies, which are gold colored (Chapman, 1994; Feng, 1995; Moxley, 2003). In addition, *E. hermannii* changes a nutrient media into green unlike *E. coli* (OIE, 2004).

Serological tests are also required to confirm cultures after plating on differential media. Sorbitol negative (colorless) colonies on CT- SMAC can be picked and characterized for the presence of O157 and H7 antigens using commercially available latex agglutination kits or antisera, including the *E. coli* O157:H7 latex test (Moxley, 2003). A latex test kit is an agglutination test for the rapid identification of *E. coli* serogroup O157 (Chapman, 1994; Chapman and Cudjoe, 2001; Chapman *et al.,* 2001). The principle behind this test is that latex particles are coated with an antiserum against *E. coli* O157 antigen. When the latex particles are mixed with fresh colonies of *E. coli* serotypeO157 the bacteria will bind the antiserum, causing the latex particles to visibly agglutinate (positive). Bacteria, which are not O157 serotype, will not bind to the antiserum and will not result in agglutination (negative). One has to remember that a positive identification of *E.*

coli strain by latex methods does not directly indicate that the isolate is a toxin producing strain (Moxley, 2003).

Latex tests should be carried out according to the manufacturer's instructions and should incorporate positive and negative control organisms and control latex. Since O157 antiserum has been observed to cross-react with other organisms including *E. hermanii*, *Salmonella* O group N, *Yersinia enterocolitica* serotype O9 and *Citrobacter freundii* (Chapman, 1994; Chapman and Cudjoe, 2001; Chapman *et al.*, 2001; OIE, 2004), it requires the need to confirm presumptive EHEC colonies as *E. coli*.

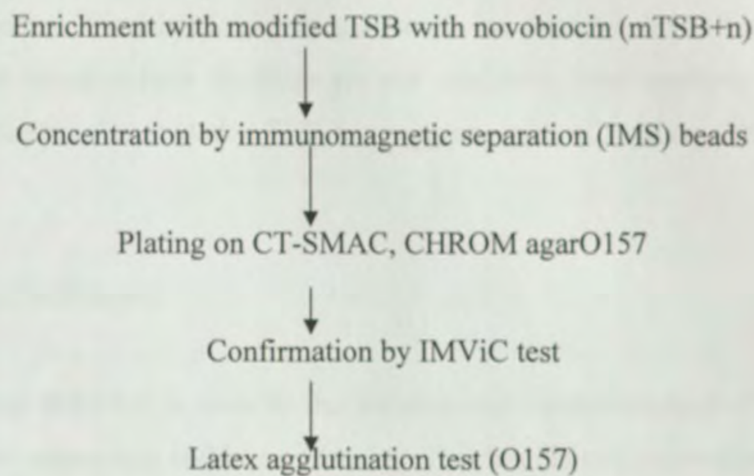


Figure 3: Flowchart showing *Escherichia coli* O157:H7 isolation procedure on CT-SMAC

Source: ISO16654, 2001

Detection of shiga toxins by PCR and Vero cell assay

A number of specific PCR procedures are described for detection of genes encoding the virulence factors (Nataro *et al.*, 1998; Strockbine *et al.*, 1998). Because the genes are well known, the direct detection of the genes encoding the virulence factors by PCR in most laboratories is most convenient (Global Salm-Surv, 2003). PCR can be used on pure or mixed plate or broth cultures, and extracts from food or feces (OIE, 204). It can also be used to detect genes in non-viable organisms. It has the potential to be used to screen samples for EHEC in epidemiological studies. Amplification of target genes in bacterial DNA extracts from feces is less successful than from

pure cultures, and careful preparation of the sample is required to improve sensitivity (OIE, 2004). Sensitivity is improved by nonselective enrichment prior to testing (Moxley, 2003; OIE, 2004). DNA probes and PCR assays have also been developed to detect other genes in EHEC shown to be associated with virulence in humans (Nataro *et al.*, 1998; Strockbine *et al.*, 1998; OIE, 2004).

The Vero cell assay remains as alternative method for the confirmation of stx production. The assay can be used on fecal suspensions, cultures filtrates or live cultures. In mixed fecal cultures, treating the suspension with polymyxin B to release cell-associated toxin properly increases sensitivity of the assay (OIE, 2004). While the test is sensitive, it is not available in all routine diagnostic laboratories. It is labor intensive and results can take 3-4 days after the cell culture is inoculated. Where tissue culture facilities are not available, other methods may be used for detecting stx production that includes ELISA or agglutination (Chapman, 1994; Moxley, 2003; OIE, 2004).

2.5.12. Non-culture techniques

Definitive diagnosis of EHEC is done by the isolation and characterization of pure cultures. But this method is time-consuming and labor intensive. This has led to the development of a range of immunological and nucleic acid hybridization tests for rapid identification of O and H antigens, and *stx* producing genes in the sample. However, as the tests have a detection level above the numbers at which the target organism is normally present in the food or faces, nonselective enrichment step is required to increase the numbers prior to testing (OIE, 2004).

Of the non-culture techniques, immunological test can be used in the diagnosis of this organism. Although all are not validated for fecal samples, immunoassays (to identify O and H antigens and *stx*) may be used to confirm the identity of the organisms once isolated from samples (OIE, 2004). Most assays for somatic and flagellar antigens are designed to detect the O157 LPS and H7 flagellar antigen. Toxin assays have the advantage of detecting all EHEC (Nataro *et al.*, 1998; Strockbine *et al.*, 1998).

2.5.13. Treatment

Treatment of *E. coli* O157 infections in humans with antimicrobial agents is controversial (Mead and Griffin, 1998) and extremely limited. It is thought to worsen the illness, presumably by breaking up the bacteria, which releases more toxins and increases toxin expression (IFT, 2000). Wilkerson *et al.* (2004) reported that some antimicrobial agents, particularly quinolones, trimethoprim, and furazolidone, were shown to induce toxin gene expression and should be avoided in treating patients with confirmed *E. coli* O157 infections. Similarly, in-vitro studies of *E. coli* O157:H7 and clinical experience with *S. dysenteriae* have raised concern that antimicrobial therapy may increase the release of shiga toxin and thus increase the risk of HUS. Therefore, management of patients with *E. coli* O157:H7 infection is supportive. Patients with documented infection should be monitored for dehydration and other signs of symptoms that might suggest HUS (Siegler, 1988; Boyce *et al.*, 1995; Mead and Griffin, 1998)

2.5.14. Antimicrobial resistance

Over the past few years an increased antibiotic resistance in *E. coli* O157:H7 isolates has been observed. Resistance to tetracycline was the most common observations found in bovine and human isolates followed by resistance to streptomycin and ampicillin (Fratamico and Smith, 2006). Some suggests that selection pressure imposed by the use of antimicrobials, including tetracycline derivatives, sulfa drugs and penicillin in human and veterinary medicine results in the selection of antimicrobial resistant strain of EHEC (Fratamico and Smith, 2006).

2.5.15. Prevention and control

Vaccines

Natural infection with *E. coli* O157:H7 does not confer immunity, and no human effective vaccine is currently available (OIE, 2004). However, various approaches to the immunological control of EHEC infections in humans are being explored. These are aimed at preventing colonization, intestinal disease or the serious sequelae of HUS and TTP. They include the use of conjugate vaccines, live-vector vaccines, toxoid vaccine or passive immunization with hyper

immunoglobulin antibodies against stxs (OIE, 2004). In March of 2006, a vaccine eliciting an immune response against the *E. coli* O157:H7 O-specific polysaccharide was reported to be safe and immunogenic in children and in adults (OIE, 2004). A phase III clinical trial to verify the large-scale efficacy of the treatment is planned (Nataro and Kaper, 1998; IFT, 2000).

Despite such attempts even in the presence of effective vaccine, there is debate about the social, political and economic consequences of widespread vaccination of people against pathogens in their food (IFT, 2000; OIE, 2004). As cattle are the main reservoirs, a novel strategy being explored is to vaccinate cattle in order to reduce colonization with *E. coli* O157:H7 and thereby it reduces food and environment contamination. One approach is to use a live, toxin-negative colonizing strain as an oral vaccine to induce antibodies against surface components. Another approach is the use of competitive exclusion that involves the use of microbial cultures that out-compete pathogens from colonizing specific niches so that exclude the pathogen from attaching to the gut lining. It works by beneficial bacteria colonizing the gut, competing for nutrients, producing bacteriocins and stimulating the immune system. The principle for the later approach is to deliver colonization factors, such as intimin, as an edible vaccine in transgenic plants (IFT, 2000; OIE, 2004; Edwards and Fung, 2006). In January 2007 the Canadian bio-pharmaceutical company announced the development of *E. coli* O157:H7 vaccine for cattle which reduces the number of bacteria sheded in feces by a factor of 1000 (Pearson, 2007).

Hygiene

Factors associated with animal production practices and with the distribution, marketing, and consumption of the final products have to be considered in a farm to-table Hazard Analysis and Critical Control Point (HACCP) plan in the control of *E. coli* O157:H7. Its low infectious dose in combination with the disease severity requires successful prevention strategies that focus on reducing or eliminating *E. coli* O157:H7 rather than on preventing pathogen growth (Sargeant and Smith, 2003).

The HACCP system continues to be the most effective means for systematically developing food safety protocols that can reduce the risk of *E. coli* O157:H7 infections. These pathogens however,

pose some unique problems when developing and implementing HACCP plans. For example, the low incidence of *E. coli* O157:H7 in food makes direct microbiological testing for the pathogen as a means of verifying the effectiveness of a HACCP program of limited benefit. In such instances, verification based on microbiological analysis would have to depend on the use of an appropriate indicator organism that could provide a measure of how well a process controls factors associated with risk of *E. coli* O157:H7 contamination. An important component of HACCP application in animal production at farms is reducing the carriage of *E. coli* O157:H7 in animals. Two approaches that have potential are competitive exclusion and vaccination (Sargeant and Smith, 2003).

At slaughterhouse, the ultimate source of *E. coli* O157:H7 on carcasses is fecal contamination. It is associated primarily with contamination of the carcass during hide removal and spreading of contamination to other carcasses by equipment and workers' hands (Dickson and Anderson, 1992; Elder *et al*., 2000; Reid *et al*., 2002). Traditional trimming procedures of the visible fecal contamination, pre-evisceration washing, rinsing of carcass surfaces with solutions of organic acids on carcass surfaces and equipment can reduce *E. coli* O157:H7, with different degree of effectiveness (Besser, *et al.*, 2003). Most desirable are a process that is lethal to the pathogen and a critical control points associated with preventing pathogen growth. Such plan shall include skinning, post-skinning rinsing/bactericidal spray, evisceration, final bactericidal spray, chilling and maintenance of refrigeration as likely critical control points (Sargeant and Smith, 2003).

At food processing center, *E. coli* O157:H7 can be controlled readily through traditional thermal processing techniques; however, the organism's low infectious dose requires effective processing that eliminates the pathogens completely (MOH, 2001). Food handling and preparation practices can contribute to *E. coli* O157:H7 infections and conversely play an important part in their prevention. Raw and/or undercooking has been an important contributing factor in *E. coli* O157:H7 outbreaks associated with beef products. Infected food handlers could potentially serve as foci for *E. coli* O157:H7 infections.

Proper food handling practices at food service and home preparation are the most and last line of defense in the prevention of *E. coli* O157:H7 infection. Generally, adequate cooking temperatures

and times, prevention of cross contamination between raw and cooked, and proper storage are key factors for reducing the risks associated with *E. coli* O157:H7 (Buchanan and Doyle, 1997).

2.5.16. Economic impact

There is no study about the economic impact of *E. coli* O157:H7 undertaken under Ethiopian conditions, however, in other countries for example in the United States, the severity of serotype O157:H7 infections in the young and the elderly have had a tremendous impact on human health, food industry, and federal regulations regarding food safety (CDC, 2005; Frenzen and Drake, 2005). Since January 2000, more than 20 million pounds of beef have been recalled in North America. Of still greater concern is the cost of treating infected individuals. A recent U.S. study estimated the annual cost of *E. coli* O157:H7 illnesses to be $405 million, in 2003 (Frenzen and Drake, 2005). In addition to the direct human costs due to *E. coli* O157:H7 infection, cattle and dairy producers, meat packers and dairy processors, meat and milk distributors etc incur direct and indirect costs associated with this foodborne disease threat. The cost of *E. coli* O157:H7 to the food industry as a result of recalls, destroyed food, control measures and lost demand associated to loss of consumer confidence is estimated to be in the billions of dollars in the U.S. alone (Frenzen and Drake, 2005).

2.5.17. Status of *Escherichia coli* O175:H7 in Ethiopia

Very few attempts have been made to identify *E. coli* O175:H7 under Ethiopia conditions. Isolation of the agents from stool samples at Bishoftu Hospital and from minced beef collected at supper markets by Demissie (2005) and from cattle, sheep and goat meat at export and municipal abattoirs in East Showa by Hiko (2007) have been reported. These reports indicated the presence of *E. coli* O175 in the country. On other hand, *E coli* O157:H7 has been isolated and studied from "Borde" and "Shamita", a traditional fermented beverages (Tadesse *et al.*, 2005), its survival in the processes of yoghurt and cheese makings (Tsegaye and Ashenafi, 2005) and concerning its growth potential in fresh tropical fruit juices (Mutaku *et al.*, 2005) in the country.

3. MATERIALS AND METHODS

3.1. Study area

This study was conducted at an export abattoir in Modjo, Ethiopia. Modjo is the center of Lume District, Eastern Showa administrative zone of Oromia Regional State. It is located 73 Kms South East of Addis Ababa at an altitude of 1777 meters above sea level. The average minimum and maximum temperatures are 18 °C and 28 °C respectively. It experiences a bimodal rainfall patterns with the main rainy season occurring between June and September and short rainy season from March to May. The average annual rainfall is of 800mm (ILRI, 2005).

At this abattoir, 500-1500 goats are slaughtered every day coupled with 200-600 sheep twice per week depending on the importers demand. Unscheduled slaughters, which sometimes may include small groups of camels, cattle and calves, are performed for the local supermarkets, hotels and institutions.

Sheep and goats are slaughtered separately and the slaughter hall floor was washed with pressurized tap water with hand held hoses. The same personnel are involved in slaughtering both groups of animals. Slaughtering operations are performed on overhead rails. Animals are bled using "Halal" methods. The skins are washed with tap water before flaying. Pressurized cold water is used for washing carcasses after evisceration. Trimming and cleaning with wiping towels are used before weighing. Thereafter carcasses are kept in the chilling room. Relatively clean areas for bleeding, dressing, evisceration and meat inspection are available.

3.2. Study design and methods

A survey was undertaken to determine coliform loads from carcasses before and after wash and prevalence of *E. coli* O157:H7 from fecal, skin and carcasses between October 2007 and April 2008. Systematic random sampling of slaughter animals was used to collect all the types of samples (fecal, hide and carcasses) once a week in each selected animals. Fecal samples and skin and carcass swabs were aseptically collected exactly from the same animals following their

identification tag across the line of operation. Further, water samples were collected in each sampling day in the abattoir.

3.3. Study animals

This study was carried out on apparently healthy slaughtered sheep and goats at an export abattoir. Animals slaughtered at the abattoir are originated from different parts of the country mainly from Geanear (Bale), Awash-Metehara, Borana, Arbaminch, Measo, Babele and Bati (Wollo). Only male and adult animals are slaughtered in the abattoir.

Figure 4: Catchments areas for sheep and goats slaughtered at the abattoir

3.4. Sample size determination

The numbers of study animals was determined based on the expected prevalence of *E. coli* O157:H7 and the desired absolute precision following the formula published in Thrusfield (2005) as follows:

$$n = \frac{1.96^2 P_{exp} (1-P_{exp})}{d^2}$$

Where:

n = required sample size

P_{exp} = expected prevalence

d = desired absolute precision

A previous study on *E. coli* O157:H7 in Modjo and Debre-zeit export and municipality abattoirs reported a prevalence of 2% and 4.1% in goats and sheep respectively (Hiko, 2007). Therefore, using these two expected prevalence, 95% confidence interval and 5% absolute precision the number of sampled goats and sheep needed to demonstrate the presence of *E. coli* O157:H7 was estimated to be 30 and 56 respectively. However, these numbers were increased to 60 goats and 112 sheep for the purpose of increasing precision.

3.5. Sample collection

Fecal samples were collected in the slaughterhouse according to Elder *et al*. (2000). For each fecal sample, the distal colon was ligated and transected approximately 750 cm proximal to the rectum after complete evisceration and the colorectal tissues were placed in individual sterile plastic bags and kept in icebox after proper labeling.

Skin swab samples were obtained following the method described in McEvoy *et al.* (2003) from animals in the bleeding area using 2X3 cm sterile cotton tipped swabs soaked in approximately 10 ml of buffered peptone water (Oxoid Ltd., Hampshire, England). An area of approximately 100 cm^2 of the neck over the line of bleeding was swabbed. On completion of swabbing, the shaft was broken by pressing it against the inner wall of the test tube and disposed, leaving the cotton

swab in the test tube. A second dry sterile cotton swab of the same type was used as before over the entire sampled area. Each test tube were capped with sterile gauze, placed in icebox and transported to the laboratory at the Addis Ababa University, Faculty of Veterinary Medicine

Selected carcasses were swabbed using the method described in ISO17604 (2003) by placing sterile template (10 x 10 cm) on specific sites of a carcass. A sterile cotton tipped swab (2X3cm) fitted with shaft, was first soaked in an approximately 10 ml of buffered peptone water (Oxoid Ltd., Hampshire, England) rubbed first horizontally and then vertically several times on the carcasses. On completion of the rubbing process, the shaft was broken by pressing it against the inner wall of the test tube and disposed leaving the cotton swab in the test tube. A second dry sterile cotton swab of the same type was used as before over the entire sampled area.

Based on these methods the same carcass was sampled before carcass wash and after carcass wash. Carcass swabs before wash samples were collected after evisceration but before carcass wash. In this case approximately 10X10 cm areas of the rump, midline and brisket from one half of each carcass were sampled. The after carcass wash samples were taken after pressurized water wash, trimming, drying with wiping towel and weighing but before entering the chilling room. Sampling was performed on the same sites and areas as described for carcass before wash but on the opposite half of the same carcasses. Thus, the two types of samples were matched by carcass based on its identification number. Sterile gloves were used and changed between sampling.

Water samples were collected aseptically using duplicate sterile test tubes directly from the mouth of water tap. Before taking samples the tap water was allowed to flow freely and at the middle of the flow samples were collected.

3.6. Bacteriological sample processing

All the samples were transported to microbiology laboratory, Faculty of Veterinary Medicine, Addis Ababa University in icebox with in one hour after collection and processed immediately. However, samples that were not to be processed within one hour of collection were stored at 4 ^{O}C

for less than 24 h. only carcass swabs and water samples were used for the enumeration of coliforms, whereas for isolation and detection of *E. coli* O157:H7 all the sample types were used.

Table 3: Samples types collected from sheep and goats at an export abattoir

No	**Sample Type**	**Processed for**		
		***E. coli* O157:H7**	**Total Coliform**	**Fecal Coliform**
1	Fecal	+*	- **	-
2	Skin swab	+	-	-
3	Pre carcass wash Swab	+	+	+
4	Post carcass wash Swab	+	+	+
5	Water	+	+	+

*Examined **Not examined

For coliform counts, carcass swabs and water samples were homogenized with vortex mixer for 7 seconds and one ml was transferred into sterile test tube with 9 ml maximum recovery diluents that were used for further dilutions. The remaining portion of the test samples was used for *E. coli* O157:H7 isolation. A twenty five grams of fecal content was taken for *E. coli* O157:H7 isolation by direct puncturing of the colorectal tissue with sterile blade and transferred into a sterile stomacher bag and about 225 ml of modified tryptone soya broth (Oxoid Ltd., Hampshire, England) containing 20 mg/l novobiocin (Sigma, Steinheim, Germany) (mTSB+n) was added. The resulting mixture was agitated using stomacher (Seward Stomacher 400, London, UK) at low speed for 30 seconds. Onto each skin and carcass swab samples 90 ml of mTSB+n was added and homogenized with vortex mixer. For each 25 ml of water samples 225 ml mTSB+n was added before incubation. Whenever samples were less than 25 gm/ml, mTSB+n was added to the samples in 1:9 ratios.

3.7. Bacteriological isolation

3.7.1. Coliform count

Preparation of decimal dilutions

Preparation of decimal dilutions, identification and enumeration of coliforms were done following the methods described in Feng *et al.* (2002) and HPB Method (2001). Series of sterile test tubes were filled with 9 ml of maximum recovery diluents (MRD) and labeled as 10^{-1}, 10^{-2}, 10^{-3} etc. depending on the expected bacterial load. Using sterile pipette, 1 ml was transferred from properly homogenized initial test samples into test tubes labeled as 10^{-1}. Then again with new sterile pipette, 1 ml was transferred from 10^{-1} to 10^{-2} test tubes and so on. Further dilutions were prepared using the same techniques. Each test tube was homogenized carefully using a vortex mixer for seven seconds after the addition but before the transfer of an aliquot.

Enumeration of total coliforms on VRBA using pours plate technique

The bacteriological media used in different stages were prepared according to the manufacturer's instructions (Appendix 3). Each sample was processed and analyzed separately. Enumeration of coliforms was done according to the methods described by Feng *et al.* (2002) and HPB Method (2001), using violet red bile agar (VRBA) (Oxoid Ltd., Hampshire, England). Briefly a prepared, homogenized, and decimally diluted sample as described above was used, so that isolated colonies were obtained after plated. Two 1 ml aliquots of the required dilutions were transferred into labeled and sterile petri dishes in duplicate. Approximately ten ml of VRBA tempered to 48°C in water bath was poured into each plate, mixed by moving clockwise horizontal and anticlockwise direction and allowed to solidify on a leveled surface. To prevent surface growth and spreading of colonies, an overlay of additional 5 ml VRBA was poured again. One ml of the original water sample was transferred to sterile petri dishes in duplicates and VRBA was poured in to for coliform count like the procedure mentioned above. Solidified plates were inverted and incubated at 35°C for 18-24 h. Plates that had 25-250 number of purple-red colonies of, 0.5 mm

or larger in diameter and surrounded by zone of precipitated bile acids were considered to be coliforms and therefore counted using colony counter.

Confirmation of total coliform colonies

To confirm that the colonies were coliforms, at least 10 representative colonies were picked and transferred each into a tube of Brilliant Green Lactose 2% Bile (BGLB) broth (Oxoid Ltd., Hampshire, England). The tubes were incubated at 35°C for 24 h and examined for gas production. Gas-negative tubes were further incubated for an additional 24 ± 2 h, re-examined, and recorded as additional gas-positive tubes to the result obtained above. Gram stains were performed, if gas-positive BGLB tube showed a pellicle to ensure that gas production was not due to Gram-positive, lactose-fermenting bacilli (Feng *et al*., 2002; HPB Method, 2001).

Enumeration of fecal coliforms on VRBA using pours plate technique

Enumeration of fecal coliforms was done according to the method in Leclercq *et al*. (2002) and Bhandare *et al*. (2007). Generally, the type of media used, identification, confirmation and enumeration of fecal coliform colonies were used uniformly with that of the total coliforms except that the incubation temperature used in this case was at 45°C.

Determining the number of coliforms

Number of coliforms per 100 cm^2 area of a carcass was calculated by multiplying the average VRBA count of each dilution times the percent of BGLB tubes confirmed as positive multiplied by the dilution factor (DF)(Feng *et al*., 2002; HPB Method, 2001). Then this value was multiplied by ten and divided by hundreds to obtain the value of coliforms per centimeter square area of carcasses. Similarly number of coliforms per ml of water was calculated by multiplying the average VRBA count of each dilution times the percent of BGLB tubes confirmed as positive multiplied by the dilution factor (Feng *et al*., 2002; HPB Method, 2001)

3.7.2. Isolation and identification of *E. coli* O157:H7

Microbiological samples for the isolation and identification of *E. coli* O157:H7 were processed as described in Chapman (2001), ISO 16654(2001), USDA, OPHS and FSIS (2002), OIE (2004) and NPH (2006).

Selective Enrichment

As mentioned above, 25 g fecal samples were homogenized using stomacher (Stomacher 400, Seward Medical, England) and enriched in 225 ml of mTSB+n in stomacher bag. For each 25 ml of water samples 225ml mTSB+n was added.

Ninety ml of mTSB+n were added to each test tube containing hide and carcass swabs, homogenized by vortex mixer and all sample types were incubated at 41.5°C for 6.

Isolation by immunomagnetic separation (IMS) beads

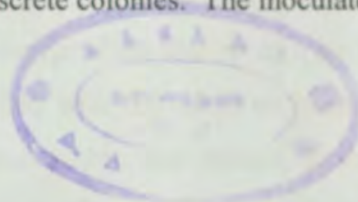
The enriched broth culture was subjected to IMS (Dynal Biotech, Oslo, Norway) after 6 h. of incubation as follows (ISO16654, 2001; NPH, 2006; UK NRM, 2006). The paramagnetic beads coated with anti *E. coli* O157 were re-suspended by gentle vortex mixing to ensure that the pellet at the bottom of the vial was completely suspended. A 20 µl of resuspended para magnetic beads were transferred to a screw top eppendorf tubes using pipette and disposable tips. One ml of the enriched culture was added using another pipette and disposable tips in to the eppendorf tubes. Then, each tube was briefly vortexed and loaded onto the sample mixer for 10-30 minutes at room temperature. The tubes were then transferred to the manual magnetic particle concentrator (MPC-S) with the magnetic strip in place, inverted 3-4 times and left to separate for 3 minutes.

With the magnetic strip still in place it was gently rotated and the MPC-s inverted three times to concentrate the beads into a small pellet at the back of the tube. The cap of the tube was carefully opened, aspirated and the supernatant was discarded using new disposable fine tipped pipette.

Then magnetic strip was removed and 1ml of phosphate buffered saline containing 0.05% tween 20 (PBST, Sigma chemicals Co. Saint Louis, USA) was added to each tube using a disposable fine tipped pipette. The tubes were closed again and the MPC-s inverted three times with the tubes still in place in order to re-suspend the beads. The magnetic strip was replaced and the above steps were again performed three times. To prevent cross contamination a universal tube of PBST and separate sterile micropipette tips were used for each sample. Finally the supernatant was aspirated, the magnetic strip was removed and approximately 100 μl of PBST was added to each tube using a disposable fine tipped pipette and mixed carefully.

Plating out on selective media

Around 50 μl of beads - bacteria complex was transferred with pipette onto Sorbitol MacConkey agar (Difco, Becton Dickinson, Claix, France) containing 0.05 mg/l. cefxime (Dynal biotech ASA, Oslo, Norway) and 2.5 mg/l potassium tellurite (Dynal biotech ASA, Oslo, Norway). This was spread into one half of the media using sterile cotton tipped swab then further spreads in to the remaining second half using wire loop to obtain discrete colonies. The inoculated plates were placed in an incubator at 37°C for 20-24h.



Biochemical identification

On CT-SMAC, the majority of *E.coli* O157 strains appear as slightly transparent, almost colorless with a weak pale brownish appearance with a diameter of one mm (Chapman, 1994; ISO16654, 2001; USDA, OPHS and FSIS, 2002; OIE, 2004). Such presumptive colonies were subcultured onto nutrient agar (Oxoid Ltd., Hampshire, England) and incubated at 37°C± for 16-24 h. From nutrient agar biochemical tests that included indole, methyl red, Voges-Proskauer reaction and citrate utilization (IMViC) tests and H_2S production test were preformed. Indole and H_2S productions were done using SIM media (BBL®, Becton Dickinson, USA) while methyl red and Voges-Proskauer reactions tests were done using MR-VP broth (Titan Biotech Limited, Bhiwadi, India). Using Simon's citrate agar (Difco, Detroit, USA) citrate utilization test was done. The test reagents used were Kovac's reagent for indole test, methyl red for methyl red test, and VP-1 and VP-2 chemicals for Voges-Proskauer reaction tests (Appendix 4). The H_2S

production and citrate utilization tests results were observed and interpreted according to Quinn *et al.* (2002).

Confirmatory test

Latex agglutination test was employed using latex kit for the confirmation of *E. coli* O157:H7. This latex kit consists of four components namely the latex test reagent, it is a latex particles sensitized with specific rabbit antibody against O157 antigen, the latex control reagent consisting of latex particles sensitized with pre-immune rabbit globulin, positive and negative controls which are suspension of inactivated *E. coli* O157:H7 cells and a suspension of inactivated non-specific *E. coli* cells and reaction slides.

The test was performed according to the manufacturer instructions (Oxoid Ltd, Hampshire, England) and its sensitivity and specificity was reported as 100% and 99.0%, respectively. Briefly, a drop of test latex and 0.085% sterile saline water were dispensed into the reaction card separately. Up to five presumptive *E. coli* O157:H7 colonies were picked from each nutrient media. Colonies were picked by lightly touching the center of the colony with sterile inoculating needle. The picked colonies were thoroughly emulsified with the saline on latex card and then finally with the test latex. The results were examined within one minute. Before testing the isolates proper working of the test latex were checked by the positive control. Positive isolates were bound to the test latex and causing the latex particles to visibly agglutinate and those, which were not, did not agglutinate (negative). Test positive isolates were subcultured onto nutrient slant to be kept for further virulent gene identification.

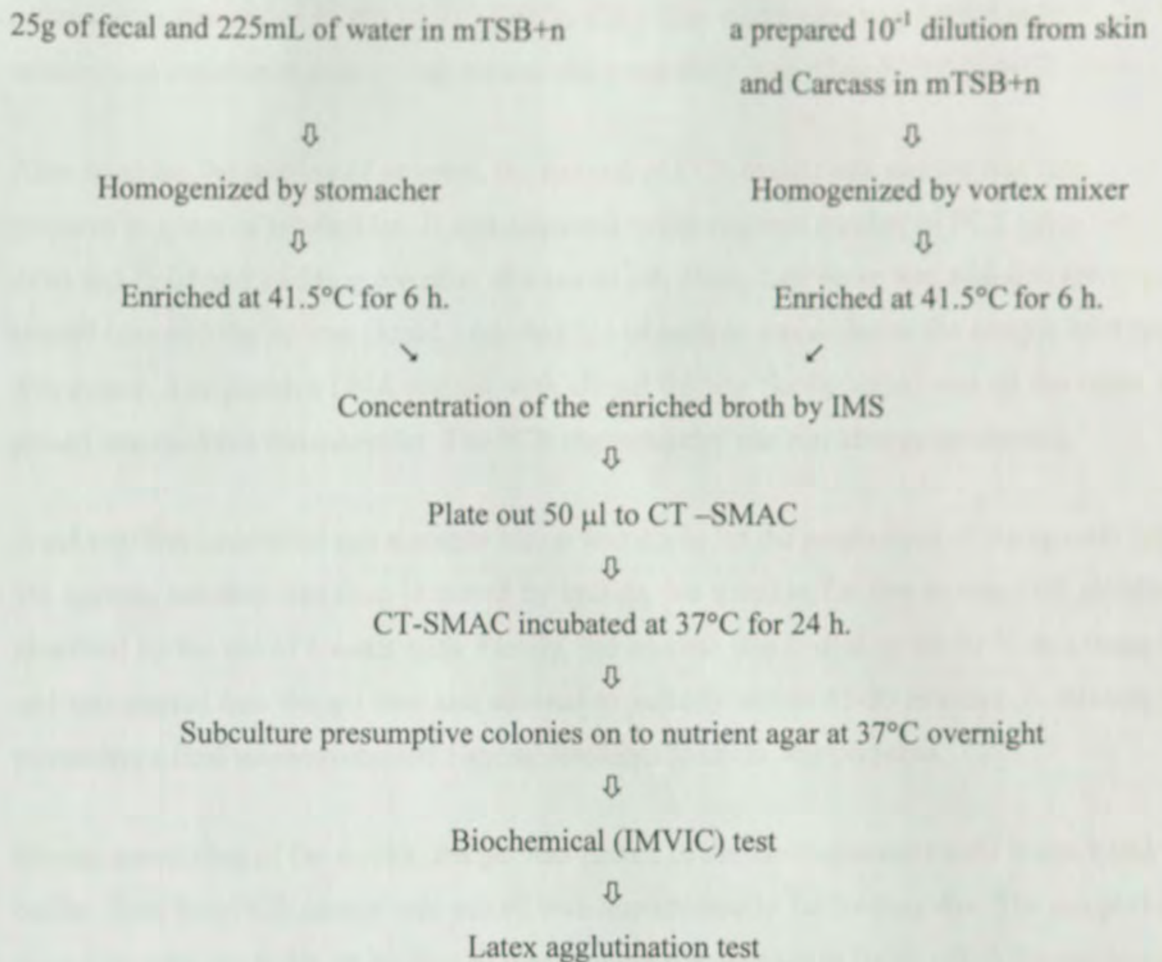


Figure 5: Flow diagram for the detection and isolation of *E. coli* O157:H7 used in the laboratory
Source: ISO16654, 2001; NPH, 2006

Isolated colonies were sent to National Food Institute, Copenhagen, Denmark for further identification of virulence gene.

Procedures for the detection of E. coli O157virulence gene by PCR

E. coli O157:H7 virulence gene identification was performed by PCR analysis according to the procedures recommended by Global Salm-Surv (2003). For the preparation of the samples, one ml of PBS and a loopful of bacteria that was picked from a plate were transferred to an eppendorf tube (1.5 ml) for centrifugation at 14000 rpm for 5 min. The supernatant was discarded and the

pellet was re-suspended in 100 µl Tris-EDTA (TE). The suspension was heated at 95°C for 5-10 minutes and transferred directly into ice and the lysed DNA was diluted 10 fold in TE buffer.

After checking the number of samples, the amount of PCR master mix needed was calculated and prepared in a tray of crushed ice. It was aliquoted to the required number of PCR tubes (48 µl per tube) and finished by adding one drop of mineral oil. Then, 2-µl water was added to the negative control tube and the lid was closed. Another 2µl of sample was added to the sample tube and lid was closed. The positive DNA control with closed lid was finally added and all the tubes were placed into the PCR thermocycler. The PCR thermocycler was run after programming.

A gel tray was assembled and a proper set-up was made for the preparation of the agarose gel. A 1% agarose solution was then prepared by boiling the solution for few minutes till completely dissolved by the use of a water bath. Finally, the agarose was cooled to 40-50 °C in a water bath and was poured into the gel tray and allowed to solidify within 15-30 minutes. A staining bath containing a final concentration of 5-ug/ml ethidium bromide was prepared.

During assembling of the results, the gel was placed in the electrophoresis until it was filled with buffer. Each 8-ul PCR sample was mixed with approximately 2ul loading dye. The samples were loaded into the gel wells by loading at least 1 molecular marker to finish off. After replacing the lid of the unit, the gel was run by starting the electrophoresis process. Thereafter, the lid of the unit was removed after 30-45 minutes complete run, placed the gel in a straining-bath for about 30 minutes and rinsed shortly in water before visualizing the bands. The gel tray was placed on the top of the UV trans-illuminator and the results were visualized by UV-lamp. Finally the presence of specific bands was observed.

3.8. Data management and analysis

The establishment of computer database and the necessary manipulations such as variable coding was performed using MS Excel. The database was transferred to SPSS version 11.5 (2002) for analysis.

Descriptive statistics such as means, proportions, standard deviations and 95 % confidence intervals were performed. Over all and sample-specific prevalence were determined by dividing the number of positive samples to the total number of samples examined. Differences among and between proportions of the groups with certain determinant factor are determined by logistic regression. ORs were calculated using univariable logistic regression to determine the degree of associations of carcass contamination with fecal and skin status. Differences in means of sample-specific coliform data were performed using paired t- test and ANOVA after normalizing the data by taking the $\log_{10}$ of them

4. RESULTS

4.1. Means of total and fecal coliforms

A total of 300 carcass swabs before and after pressurized water wash were collected from sheep and goat carcasses at an export abattoir, Modjo, Ethiopia. Coliforms were counted as CFUs per cm^2. These values were transformed into $\log_{10}CFUcm^{-2}$ to normalize the data.

The log means of total coliform counts before and after carcass wash were 3.15 (SD= 0.77) and 2.97 (SD = 0.82) respectively. The minimum counts of total coliform before and after carcass wash were 1.4 and 1.3 $\log_{10}$ CFU cm^{-2} respectively while the respective maximum values were 5.3 and 5.0 $\log_{10}$CFU cm^{-2}. Similarly the log means of fecal coliforms before and after carcass wash were found to be 2.96 (SD= 0.88) and 2.77 (SD=0.91) respectively (Table 4).

Table 4: Summary of descriptive statistics for the overall total and fecal coliform counts before and after carcass wash

Type of Sample	Number of Sample	Mean ($\log_{10}$ CFUcm^{-2})	95% CI For the Mean	SD	Min.	Max.
TCBW	150	3.15	3.03-3.27	.776	1.4	5.3
TCAW	150	2. 97	2.84-3.10	.822	1.3	5.0
FCBW	150	2. 96	2.82-3.11	.881	1.3	4.9
FCAW	150	2. 77	2.62-2.91	.906	1.3	4.6

TCBW = total coliforms before wash, TCAW = total coliforms after wash, FCBW= fecal coliforms before wash, FCAW = fecal coliforms after wash, CI = confidence interval, SD = standard deviations, Min. = minimum value, Max. = maximum value

The log means of total coliforms before and after carcass wash from goats were 2.85 (SD=0.72) and 2.87 (SD=0.87) respectively whereas from sheep the respective values were 3.35 (SD=0.75) and 3.04 (SD=0.79). Similarly fecal coliforms before and after carcass wash from goats were 2.59 (SD=0.84) and 2.57 (SD=1.0) respectively while respective 3.21 (SD=0.82) and 2.89 (SD=0.79) were found from sheep carcasses (Table 5).

Table 5: Summary of descriptive statistics of species-specific total and fecal coliform counts before and after carcass wash after log transformed

Sample type	**Species**	**Number of Sample**	**Mean (log_{10} CFUcm^{-2})**	**95% CI For the Mean**	**SD**
TCBW	Goats	60	2.85	2.67-3.04	0.72
	Sheep	90	3.35	3.19-3.50	0.75
TCAW	Goats	60	2.87	2.64-3.09	0.86
	Sheep	90	3.04	2.87-3.20	0.78
FCBW	Goats	60	2.59	2.37-2.80	0.84
	Sheep	90	3.21	3.04-3.38	0.82
FCAW	Goats	60	2.57	2.30-2.83	1.02
	Sheep	90	2.89	2.73-3.07	0.79

TCBW = total coliforms before wash, TCAW = total coliforms after wash, FCBW= fecal coliforms before wash, FCAW = fecal coliforms after wash, CI = confidence interval, SD = standard deviations

4.2. Comparison of the means of total and fecal coliforms before and after carcass wash

Mean differences for coliform counts before and after carcass washes were compared using paired t- test. As a result statistically significant difference was observed between the means of total coliform counts (P=0.002) and between the means of the fecal coliform counts (P=0.001) before and after carcass wash considering the two species together (Table 6).

A similar statistical method was used to compare the effect of washing on coliform loads in sheep and goats carcasses separately. In sheep, statistically significant difference was observed between

the means of total coliform counts (P=000) and fecal coliform counts (P=000) before and after carcass wash. However, in goats no significant differences (P>0. 05) were observed for both total and fecal coliform counts before and after carcass wash (Table 6).

Table 6: Paired comparison of mean differences in total and fecal coliform counts before and after carcass wash swabs after log transformed

Sample types	Sample Size	*Mean of the differences	SD	95% CI	T-test	df	P-value
TC	150	0.18	.69	0.07-0.29	3.22	149	.002
FC	150	0.19	.69	0.08-0.31	3.50	149	.001
TC (Goats)	60	-.01	.71	-0.2-0.17	-0.15	59	.879
FC (Goats)	60	.02	.70	-0.16-0.20	0.25	59	.803
TC (Sheep)	90	.31	.65	0.17-0.45	4.53	89	.000
FC (Sheep)	90	.32	.66	0.18-0.45	4.52	89	.000

* $Log_{10}CFUcm^{-2}$ values TC= total coliforms, FC= fecal coliforms, SD= standard deviation of the mean difference, CI= confidence level of the mean difference, df = degree of freedom

4.3. Comparison of the means of coliforms between species

ANOVA was used for the comparison of the means of coliform loads between sheep and goat carcasses before and after wash. Therefore, statistically significant differences (P<0.05) were observed for total coliform counts before wash, fecal coliform before wash and fecal coliform after wash between sheep and goat carcasses (Table 7). Both types of coliform counts were higher from sheep carcasses than that of the goats.

Table 7: Comparison of the mean coliform loads between sheep and goat carcass using ANOVA.

Sample type		Sum of Squares	df	Mean Square	F	P-value
TCBW	Between*	8.89	1	8.89	16.3	.000
	Within	80.83	148	0.55		
TCAW	Between	1.04	1	1.04	1.5	.216
	Within	99.76	148	0.67		
FCBW	Between	14.01	1	14.01	20.4	.000
	Within	101.67	148	0.69		
FCAW	Between	3.98	1	3.98	5.0	.027
	Within	118.31	148	0.80		

TCBW= total coliforms before wash, TCAW=total coliforms before wash, FCBW= Fecal coliforms before wash, FCAW= fecal coliforms after wash, * = Species, df = degree of freedom

Of the total 21 water samples tested, total coliforms were observed in 9 (42.9%) of them. The highest number counted was 8 CFU per ml (Fig. 6). However, no fecal coliforms were found from all water samples analyzed.

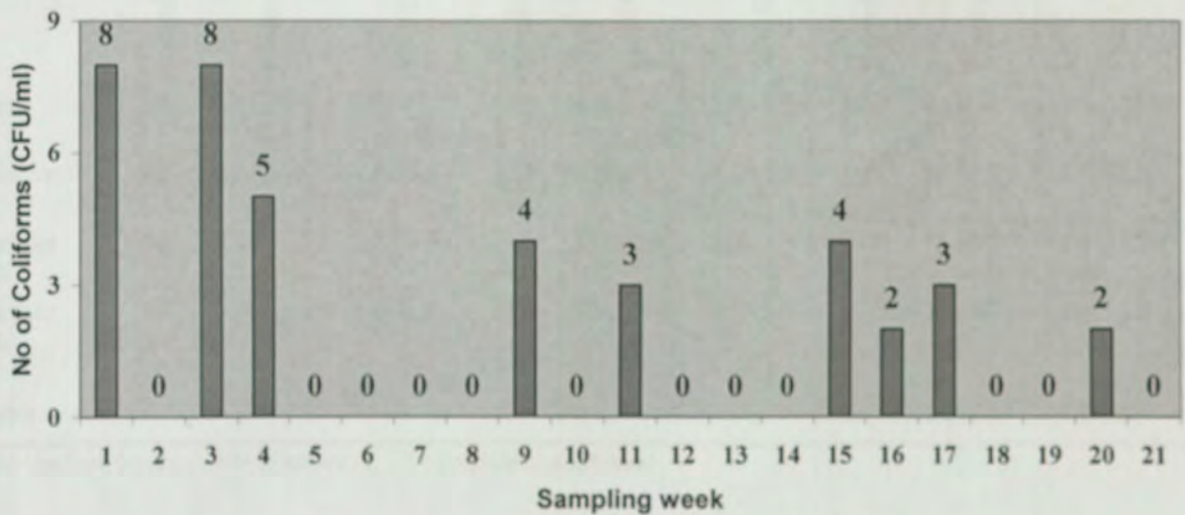


Figure 6: Number of coliforms counted from abattoir water samples

4.4. Prevalence of *E. coli* O157:H7 in feces, skin and carcass samples

Of the total 711 samples examined for bacteriological status of *E. coli* O157:H7 an overall prevalence of 7.5% (53/711) was recorded of which 6.6% (16/240) were from goats, 8.0% (36/448) were from sheep and 4.2% (1/23) was from water samples (Table 8). By sample type, 8 (4.7%) fecal, 15 (8.7%) skin swabs, 14 (8.1%) carcass swabs before wash and 15 (8.7) carcass swabs after wash, and 1 (4.2%) water samples were positive for *E. coli* O157:H7 (Figure 7).

In goats, *E. coli* O157:H7 was isolated at prevalence of 3.3% (2), 10% (6), 5% (3) and 8.3% (5) from fecal samples, skin swabs, carcass swabs before and after wash respectively whereas in sheep, the respective prevalence were 5.4% (6), 8.0% (9), 9.8% (11) and 8.9% (10) (Table 8).

Table 8: Prevalence of *E. coli* O157 from sheep and goat samples at an export abattoir

Sample Type	Number of samples								
	Goats			Sheep			Total		
	Examined	Positives (%)	95% CI	Examined	Positives (%)	95% CI	Examined	Positives (%)	95% CI
Fecal	60	2 (3.3)	0.9- 11	112	6 (5.4)	2.4-1.2	172	8 (4.7)	2.4- 9.0
Skin Swab	60	6 (10.0)	4.6-20.1	112	9 (8.0)	4.2-4.5	172	15 (8.7)	5.4-13.9
Carcass BW	60	3 (5.0)	1.7- 3.7	112	11 (9.8)	5.5-6.7	172	14 (8.1)	4.9-13.2
Carcass AW	60	5 (8.3)	3.6-8.0	112	10 (8.9)	4.9-5.6	172	15 (8.7)	5.4-13.9
Water							23	1 (4.2)	1.0-18.4

BW= before wash, AW= after wash, CI= confidence interval

Six animals were found to be positive both for skin and carcass swabs. However, only three animals were positive both for fecal samples and carcass swabs and only one animal was positive

for both fecal and skin swabs. Fifteen carcass positive samples were negative for fecal and skin samples.

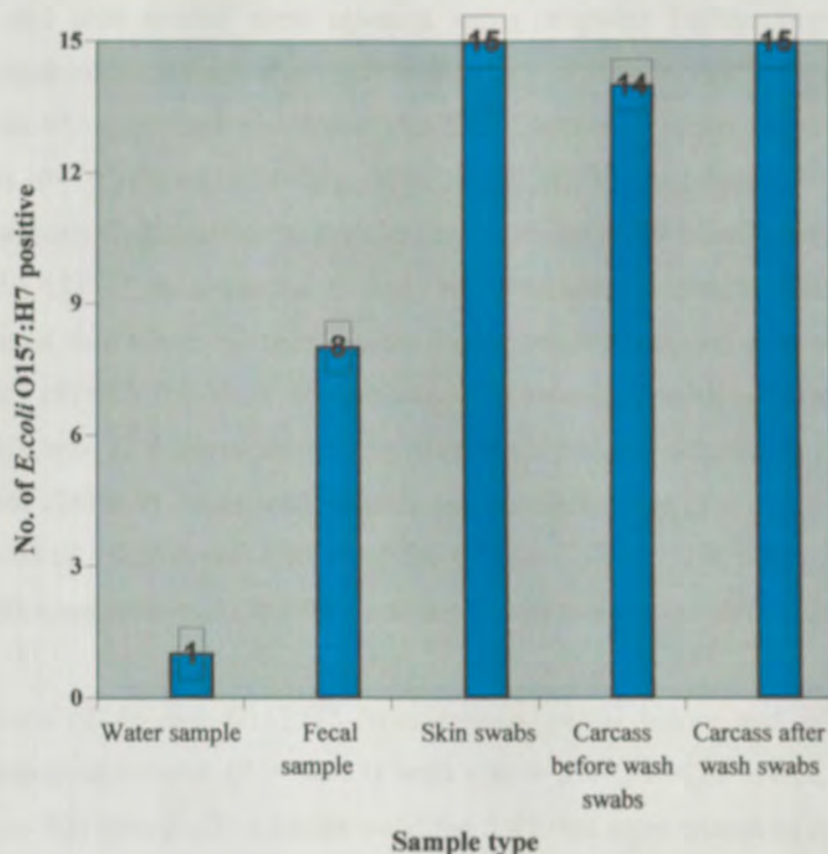


Figure 7: Distribution of *E. coli* O157: H7 positive isolates from711 samples by sample type

No statistically significant difference was found (P >0.05) between sheep and goats in *E. coli* O157:H7 prevalence (Table 9).

Table 9: Summary of descriptive statistics to compare in the prevalence of *E. coli* O157:H7 between sheep and goats in different samples

Sample type	OR	95% CI	P-value
Fecal	1.64	0.32-8.4	0.427
Skin swab	0.79	0.27-2.33	0.663
Carcass before wash	2.07	0.55-7.73	0.212

4.5. Association of *E. coli* O157:H7 status in feces and skin with carcass contaminations

The associations of carcass contamination (dependent variable) with the potential risk factors (fecal and skin swabs) were assessed using stepwise logistic regression test. Carcasses contaminations before and after wash were compared with the risk factors separately. As a result, a statistically significant association was found between carcass swabs before wash and fecal samples (P = 0.019) with OR= 8.4 (95%CI =1.8-39). The calculated OR indicated that carcasses obtained from fecal positive animals had a probability of 8.4 times more likely to be positive for *E. coli* O157:H7 as compared to fecal negative animals. Similarly a statistically significant association was found between carcass swabs before wash and skin swabs (P = 0.000) with OR=12.4 (95%CI=3.5-43.5). The probability of carcasses before wash to be positive for *E. coli* O157:H7 was 12.4 times more from skin swab positive animals as compared to skin swab negatives (Table 9). Statistically significant association was also found between carcass swabs after wash (P = 0.029) with OR= 4.8 (95% CI=1.3-17.7) and skin swabs. However, no significant association was observed (P>0.05) between carcass swabs after wash and fecal samples.

Prevalence of *E. coli* O157:H7 from carcass swabs before and after wash found to be significantly associated (P = 0.023) with OR = 5.4 (95%CI =1.4-19.8). This indicated that carcasses that were positive before wash has 5.4 times more chance of being positive after wash as compared to carcasses which were initially negative.

Table 10: Summary of descriptive statistics for the associations of carcass contamination with fecal and skin *E. coli* O157:H7 status using step wise logistic regression

Compared variables	Number of Samples	OR	(95% CI)	P-value
Carcass Before Wash * Fecal Sample	172	10.8	1.9-60.7	0.007
Carcass Before Wash* Skin Swab	172	14.3	3.7-53.9	0.00
Carcass after Wash* Skin Swab	172	4.8	1.3-17.7	0.03
Carcass swabs Before * after Wash	172	5.4	1.4-19.8	0.023
Carcass After Wash* Fecal Sample	172	0.0	0.0-	0.99♦

* With ♦, No significant association

No significant association ($P>0.05$) was observed between fecal and skin swab samples, however relatively higher prevalence of *E. coli* O157:H7 from skin swabs (8.7%) than from fecal samples (4.7%) was found.

Statistically significant association ($P=0.029$) was found between *E. coli* O157:H7 carcass contaminations with total abattoir slaughter volume however, no statistically significant association ($P>0.05$) between total and fecal coliform loads with abattoir slaughter volume.

From the total of 53 *E. coli* O157:H7 isolates that were sent to the National Food Institute, Copenhagen, Denmark, only one (1.8%) has both *stx*1 and stx2 (figures 7 and 8) and was negative for the *eae* and *hylA* virulent genes using PCR. This strain was isolated from goat carcass. The remaining isolates were free of any virulent factors. As the numbers of *E. coli* O157:H7 isolates of having the virulent gene was only one, antimicrobial susceptibility test was not performed.

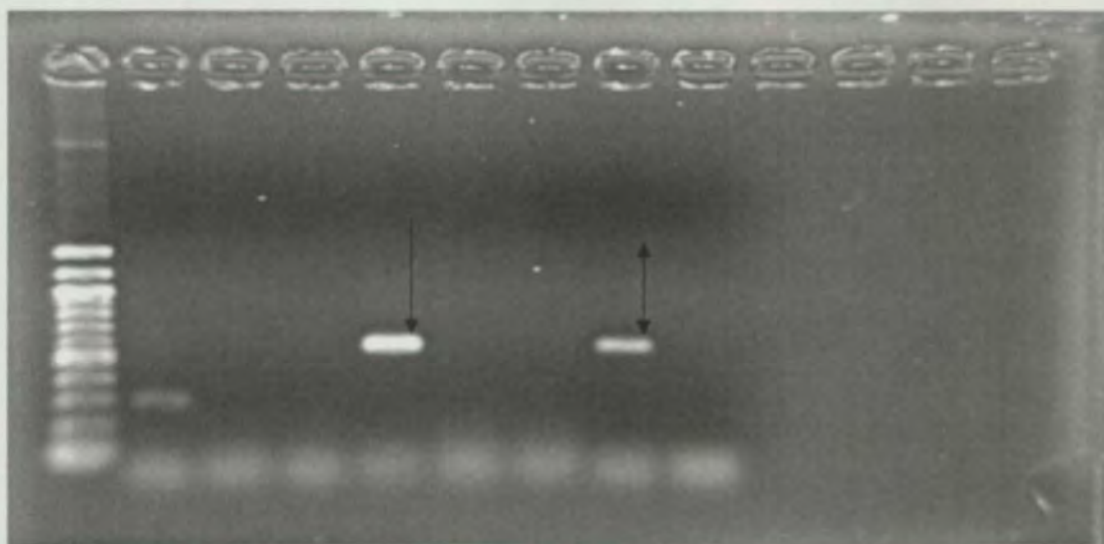


Figure 8: *E. coli* O157:H7 *stx2* virulent gene isolated from goat carcass by PCR with 584 bp (single arrow) and positive control (double arrow)

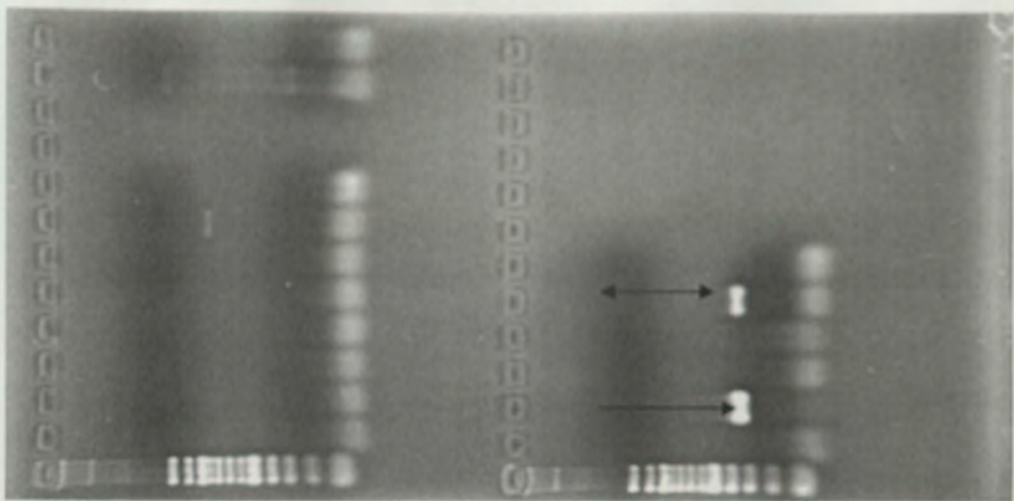


Figure 9: *E. coli* O157:H7 *stx1* virulent gene isolated from goat carcass by PCR with 348 bp (single arrow) and positive control (double arrow)

5. DISCUSSION

5.1. Coliforms

Carcass contamination with coliforms may occur during slaughtering operations as a result of direct contact with contaminated materials such as skin, fecal debris, knives, workers' hands and so on. High total coliform count indicates poor hygienic quality of slaughtering processes whereas fecal coliform indicates the presence of recent carcass contaminations by fecal materials during slaughtering operations.

A relatively lower numbers of total and fecal coliform counts were obtained after carcass wash (log mean of 2.97 and 2.77 $CFUcm^{-2}$ respectively) than before carcass wash (respective log mean of 3.15 and 2.94 $CFUcm^{-2}$) from sheep and goat carcasses. However, the decreases in the coliform load after washing of carcasses in our finding might not necessarily signify the effectiveness of washing. It could also be due to longer holding time of carcasses that could decrease the effectiveness of swabbing. Coliforms that could be picked by swabbing might be attached with carcasses irreversibly. As it was explained by Dickson (1992), water washes are intended to remove any bacteria that are loosely attached with carcasses. Moreover, Sofos *et al.* (1999) also reported that prolonged holding times of carcasses may allow bacteria to firmly attach to carcass surfaces and create biofilms that are difficult to remove by swab samples.

A decrease in the number of coliform load after carcass wash in the current report was in agreement with other works (Gill *et al.* 1996; Bacon *et al.*, 2000; Yalcin et *al.*, 2001). Yalcin *et al.* (2001) reported that washing could bring reduction in the number of fecal coliform counts. Gill *et al.* (1996) also showed that the number of bacteria could be approximately halved after washing of carcasses. However, others on the contrary have reported that washing might not brought much reduction in bacterial counts (Narasimha and Ramesh, cited by Yalcin *et al.*, 2001). An increase in the number of coliform counts after washing than before washing at the brisket region has been reported by Yalcin *et al.* (2001) and Mengistu (2007). This might be due to the inadequate washing and the greater difficulty of cleaning this part as compared to other parts. The use of wiping towel, which we observed for drying of carcasses in the study abattoir, might have contributed for further contaminations. This further decreases the effectiveness of

carcass washing. As explained by Midgley and Small (2006), a very important source of carcass contamination on the outside surface of a carcass is from the wiping cloths used by abattoir workers when cleaning up the carcasses.

The means of total and fecal coliform counts on sheep carcasses (3.35 and 3.21 $log_{10}CFUcm^{-2}$ respectively) were much higher than that of goat carcasses (respective 2.85 and 2.59 $log_{10}CFUcm^{-2}$) before wash. Similarly after carcasses wash the means of $log_{10}CFUcm^{-2}$ total and fecal coliform values in sheep (3.04 and 2.89 respectively) were higher than in goats (respective 2.87 and 2.57). This difference might be associated with the nature of skin and volume of wool present in sheep, their fatty content on their carcass and types of feed difference, which in turn affect intestinal coliforms content. In this study observation of flaying operations showed that, goats' skins were easily removed and therefore the speed of skinning operation was very fast. However, it was relatively difficult to remove sheepskins. This longer duration of flaying might have allowed more carcasses to aggregate in the line of operations and hence more chance of cross contamination of sheep carcasses. In addition, fat tissue (the case of sheep) may have provided a better surface than lean muscle tissue for bacterial attachment as it was explained by Chung *et al.* (1989) that their surface structures are entirely different. Midgley and Small (2006) also reported a higher bacterial load on carcasses derived from woolly lambs than from non-wooly lambs.

The number of total coliform counts obtained in goats was comparable with previous work in Ethiopia (Mengistu, 2007) that reported mean values ranged from 2.4 to 2.9 $log_{10}CFUcm^{-2}$ at different abattoirs and sites of carcasses. However, coliform values of the current report were lower than those reported in India (Yadav *et al.*, 2006; Bhandare *et al.*, 2007). Yadav *et al.* (2006) reported a mean of 4.1 $log_{10}\ g^{-1}$ total coliforms from ready-to-sale sheep carcasses whereas Bhandare *et al.* (2007) reported an average fecal coliform mean count of 3.3 $log_{10}CFUcm^{-2}$. On the contrary, the current report was significantly much higher than the reports in US and European countries (Roberts, 1980; Sofos *et al.*, 1999; Duffy *et al.*, 2001; Yalcin *et al.*, 2001; Byrnea *et al.*, 2007), where they reported means that ranges from 0.02 - 1.63 $log_{10}CFUcm^{-2}$. This difference might be associated with hygienic and microbial standards they set and followed in

their abattoirs, and difference in geoclimatic conditions as compared to the hygienic conditions and control schemes lacking in many developing countries like Ethiopia.

Generally due to poorly organized farm to table production chain, poor standard sanitary operations procedures practiced by the abattoir personnel that includes poor personnel hygiene, improper use of hot water for knife sterilization, over crowding of animals at the bleeding stage, failure to separate dirty and clean working areas, improper disposal of meat trims, failure in the use of overhead rail up to the chilling room, frequent visitors, use of wiping cloth, etc are some of the risk factors which could contribute to the high bacterial load obtained in the current finding. This suggests the need to improve the microbiological quality of the meat products in order to maintain the export standards and to remain competent in the international market.

By considering the effect of washing on goat carcasses separately, differences in the number of total coliform counts before (mean of 2.85 $log_{10}CFUcm^{-2}$) and after (2.87 $log_{10}CFUcm^{-2}$) carcass washing were not observed. This showed that washing had little effect on goat carcasses. This might be due to the difference in the speed of operations during goat carcass processing. It is worthy note that goats and sheep were slaughtered in the abattoir separately one after the other. As mentioned above skinning operations of goats was relatively easier and not time consuming. As indicated by Midgley and Small (2006), slowing the speed of operation could reduce carcass microbial contamination however, the faster slaughtering operation observed in the current study could reduce the effectiveness of pressure washing in goat carcasses. Further more Gill *et al.* (1996) also indicated the effectiveness of washing of carcasses varies with the time spent on washing, the volume, pressure and temperature of the water. Duffy *et al*. (2001) also showed that the effectiveness of using both trimming and washing together (not separately) for the elimination of pathogens such as *E. coli* O157:H7, and the efficacy depends very much on the skill of the operator, the extent of visible contamination compared with non-visible contamination, and the temperature, angle and pressure of the wash waters used.

Water samples were tested for coliforms in order to find out its role in carcass contaminations. However, fecal coliforms were not detected in any of the water samples tested. This confirmed that abattoir water did not significantly play a role in carcass contaminations with fecal coliforms

after wash. Although few total coliforms were found from water samples, it could not affect the coliforms loads on carcass after wash, as the counts were significantly low (maximum count was 8 CFU /ml) in most of the water samples analyzed.

5.2. *Eschericha coli* O157:H7

IMS is used for the first time for the concentration and isolation of *E. coli* O157:H7 from skin swabs, fecal samples, carcass swabs and water samples in Ethiopia from samples at an export abattoir in Modjo. While cattle are generally regarded as the main reservoir of *E. coli* O157:H7 for human infection, the results of the present study indicates that sheep and goats may also be significant contributing sources. In addition *E. coli* O157:H7 is present in samples such as feces, skins, carcass swabs and water used in the abattoir.

In the current study no statistical significant difference was found in the prevalence of *E. coli* O157:H7 between sheep (5.4%, 8.0%, 9.8% and 8.9%) and goats (3.3%, 10%, 5% and 8.3%) from fecal, skins, carcass before and after wash respectively and this finding showed that both species are equally important as sources of human *E. coli* O157:H7 infections.

A 5.4% fecal prevalence of *E. coli* O157:H7 from sheep in this study was comparable with reports of surveys conducted in other countries. *E. coli* O157:H7 was isolated from 4% of ewes and 4% of lambs in The Netherlands (Heuvelink *et al*., 1998), 3% of lambs in Spain (Blanco *et al*, 2003), 1.4% of sheep with monthly variations of zero to 4.8% in UK (Chapman *et al.* , 2001; Paiba *et al*., 2002) and 0.2% in Italy (Battisti *et al*., 2006). However, a prevalence of 31% in USA (Kudva *et al.*, 1996) and 68% from sheep flocks in Australia (Sidjabat-Tambunan and Bensink, 1997) from fecal samples were reported. These reports are much higher than the present finding. On the other hand, zero *E. coli* O157:H7 prevalence from sheep fecal samples was reported in Norway (Johnsen *et al*., 2001), Scotland (Synge and Hopkins, 1994), Ireland (Lenahan *et al*., 2007), Greece (Dontorou *et al*., 2004) and United States (Keen *et al*., 2007).

A 3.3 % faecal *E. coli* O157:H7 prevalence in goats in this study was lower than the reports in other countries. A prevalence ranging from 55 to 95% were reported in French (Bastian *et al*.,

1999) and 40% in Australia (Fagan *et al.*, 1999) in goats by flocks. In contrast, lower prevalence than the present finding was reported in Greece (1 out of 81) associated with human outbreaks from goats by Dontorou *et al.* (2004). Similarly, Keen *et al.* (2007) reported no *E. coli* O157:H7 from 526 goats' in US zoological parks.

Marked differences in the prevalence of *E. coli* O157:H7 from fecal samples were observed. The differences in the prevalence of *E. coli* O157:H7 observed between the current study and those previous reports are possibly due to differences in husbandry practices, agroclimatical variation, seasonal differences, sampling times, breeds and age of animals, sampling technique and so on. A number of studies have also shown that shedding of *E. coli* O157:H7 from animal feces can vary significantly in relation to time, age of animals, and nature of feeds and so on (Chapman, 1994; Chapman *et al.*, 2001; Reid *et al.*, 2002). Similarly, Battisti *et al.* (2006) indicated sheep husbandry as a possible reasons for *E. coli* O157 fecal prevalence differences in US as compared to European countries. No report is available in Ethiopia to compare the occurrence of *E. coli* O157 in sheep and goat fecal samples.

Escherichia coli O157:H7 was isolated with an overall prevalence of 8.7% from sheep and goats skin swabs. Isolation of *E. coli* O157:H7 from skin of these species is very rarely described and only a report from Ireland was found so far. In Ireland as reported by Lenahan *et al.* (2007), a 5.8% prevalence of *E. coli* O157:H7 from fleece, sampled by shaving was reported. However, in hides zero to 22% *E. coli* O157:H7 prevalence were reported from different sites of hides in US (Elder *et al.*, 2000; Reid *et al.*, 2002).

A prevalence of 8.7% from carcasses before wash and 8.1% after wash of *E. coli* O157:H7 in the current finding was much lower than the report in Australia (Sidjabat-Tambunan and Bensink, 1998), who reported 29.2% (31/106) of *E. coli* O157:H7 from sheep carcasses. On the other hand, much lower prevalence than the present find was reported in Ireland (Lenahan *et al.*, 2007), in England (Chapman *et al.*, 2001) and in Ethiopia (Hiko, 2007) that ranges between 0.7 and 4 % from goats and sheep carcasses. Similarly Chapman *et al.* (2001) in UK reported 0.21%, 1, 22% and 1.17% prevalence of *E. coli* O157:H7 from minced, burger and sausages of lambs respectively.

Differences in the reported prevalence could be due to difference in the number of samples, sampling sites, the skill of operators and so on. For example, MacEvoy (2003) recovered only one among the nine positive samples by swabbing but by excision the author recovered six among the nine positive samples. This shows a difference in the prevalence of a given study due to difference in sampling methods. The surface swabbing method, which was used in this study however, was also used by others (Gun *et al.*, 2003) and depending on the degree of abrasiveness the method is recommended as an alternative method since it is cost effective and nondestructive.

Associations of carcass contamination with the potential risk factors (feces and skin) were analyzed using logistic regression. As result all the risk factors were found to be associated with carcass contaminations except fecal samples with carcass after wash contaminations.

In the current study *E. coli* O157:H7 from fecal samples was found to be significantly (P=0.007) associated with carcass swabs before wash. This finding was in agreement with previous studies (Elder *et al.*, 2000; Gansheroff, and O'Brien, 2000). Elder *et al.* (2000) reported that from the 21 fecal *E. coli* O157 positive batches of cattle presented for slaughter in US, 19 (90%) were also positive for pre eviscerated carcass swabs. The association between fecal prevalence and carcass contamination indicates the need for the control of *E. coli* O157:H7 on the farm toward reducing the risk of human infection. However, an effective method of controlling *E. coli* O157:H7 on farm is not yet established specially in developing countries.

In this study, skin prevalence was significantly associated with carcasses before (P=0.000) and after (P=0.03) wash contaminations. Such association of skin with carcass swabs before wash was also reported by Reid *et al.* (2002). Moreover it has been indicated that one site on the skin may have higher levels of contamination than the others and therefore, greater risks for carcass contamination. Those studies reported a prevalence of 22.2% *E. coli* O157:H7 at the brisket area of hides and incriminated skin as a major source of microbiologic contamination of carcasses after demonstrating their associations.

In our finding a higher prevalence of *E. coli* O157:H7 from skin swabs (8.7%) than from fecal samples (4.7%) was observed as a result, level of carcass contamination was more associated

with skin sources than fecal. These observations were in contrast with Elder *et al.* (2000), where they reported higher prevalence in feces (28%) than on hides (11%). It is true that the skin of animals could have a number of sources to carry *E. coli* O157:H7 such as the soil, feed, water, feces etc. but animals could shed *E. coli* O157:H7 seasonally in their feces and as a result might be negative for the organisms on sampling time. The skin of a given animals could be contaminated by fecal sources from themselves and other animals. Therefore, during transportation and in the lairage cross contamination of skins could occur due to a more close contact of animals and as result an increase in the apparent prevalence of *E. coli* O157:H7 on skin relative to feces could be observed.

On the contrary, absence of association between hide prevalence and carcass contamination was reported by Elder *et al.* (2000), and in those reports hide prevalence of *E. coli* O157:H7 was much lower than fecal prevalence. Possible explanations for this apparent discrepancy might be a difference in the selection of skin sampling site. A difference in the sampling site could affect isolation rate of a given pathogen. For example, Reid *et al.* (2002) isolated 3.3% from rump, 4.4% from flank and 22.2% from brisket of *E. coli* O157:H7 by swabs on the same animals. This might be due to difference in the survival rates of *E. coli* O157: H7 in different site of the skin of animals. Moreover it has been indicated that one site on the skin may have higher levels of contamination than the others and therefore, greater risks for carcass contamination.

Similar with carcass before wash, level of carcass after wash contamination with *E. coli* O157:H7 was significantly associated (P=0.03) with skin prevalence but not with fecal (P=0.9). This indicates that skins are more important than fecal sources for carcass after wash contaminations. This might be explained by a difference in visible contamination verses non-visible contaminations. Contamination from skin source may not be grossly visible whereas fecal matters are. The possibility of removal of grossly visible contaminations by abattoir personnel through washing as in the case of fecal matter could be stronger and effective while from skin sources might not be. However, further research is required to arrive at a conclusion. As mentioned above Gill *et al.* (1996) and Yalcin *et al* (2001) reported that washing could bring reduction in the number of coliform counts and thereby pathogens. However, the effectiveness of washing varies with the time spent on washing, and the volume, pressure and temperature of the

used water and design of the spray equipment. Bell (1997) also reported that the areas of high contaminations on carcasses were the sites where cuts are made through the skin.

Almost similar prevalence of *E. coli* O157:H7 on carcasses before wash (8.1%) and carcasses after wash (8.7 %) were observed that indicates the absence of effective interventions that reduce the pathogen across the line of slaughtering operations. This was in contrast to the study by Elder et al. (2000), which was carried out in US abattoirs where samples were taken at three stages of operations and anti microbial interventions were applied to reduce carcass pathogen numbers. Those authors demonstrated a reduction in the prevalence of *E. coli* O157:H7 on carcasses from 43% (at pre evisceration) to 18% (at post evisceration) and finally 2% after antimicrobial intervention (Elder *et al.*, 2000). This difference indicates the less effectiveness of using pressure cold-water wash alone in reducing *E. coli* O157:H7 prevalence in the abattoir where the present study was undertaken.

In the abattoir where these samples were collected, cold pressurized water wash was used to avoid visible contaminants such as blood clot, fecal debris, GIT continents so on. This was done at after evisceration and inspection. However, the whole carcasses surfaces were not washed with this pressure wash. Non-visible contaminants might pass this point and washing may spread contaminants further in to the other carcass surfaces. This allowed to the spread of contamination within or to the other carcasses. In addition to this, after washing carcass trimming was performed using one knife. During the study period we observed limited use of hot water for knife sterilization. After trimming wiping towels were used to dry carcasses. Carcasses are transferred manually by carrying from the overhead rails to the chilling room after weighing. Swabs after carcass wash were collected (second sampling point) at this stage of operations. As carcasses successively pass through all these processing lines, bacterial recontamination might occur and spread from one carcass to others. These all might be the possible reasons for the uniform prevalence of *E. coli* O157:H7 on carcass after wash than carcass before wash.

In addition, 15 positive carcasses were found from animals that were *E. coli* O157:H7 negative from their fecal and skin samples. This finding suggests the presence of carcass cross-contamination in the abattoirs. The prevalence level detected in the fecal samples was generally

lower than those detected on the carcasses from the same animals. This finding may also reflect some level of cross-contamination between carcasses, and it indicated that before wash carcass contamination might be a critical control point for further intervention strategies. Reducing the amount of *E. coli* O157:H7 in live sheep and goats' likely lower contamination not only of meat but also of other foods and water supplies that come into contact with animals' fecal matter.

Statistically significant correlation ($P<0.05$) was found between *E. coli* O157:H7 prevalence on carcasses with total slaughtered volume in the abattoir. These could indicate as the slaughter volume per day increases, the rate of carcass contaminations increases. These might be associated with the speed of operators. As mentioned above, Midgley and Small (2006) forwarded that increasing the speed of operations could increase carcass microbial contamination. However, no correlation was found ($P>0.05$) between slaughter volumes with carcass coliform loads.

Of the total 23 water samples collected in the abattoir, only one isolate was positive. This isolate was free of the virulent gene. No *E. coli* O157:H7 was isolated from the abattoir water collected in Istanbul (Gun *et al*., 2003). However, a study under taken in South Africa (Müller *et al*., 2003) reported only one positive *E. coli* O157 isolate from sewages that contained virulent gene and none from river water. In the current study, water source for the abattoir is under ground water in dug in the compound and from this stored in reservoir tankers. However, occasionally water transported from external sources was used when failure occurs. Transportation from other sources might contaminate abattoir water with pathogens such as *E. coli* O157:H7. Waterborne transmission of *E. coli* O157:H7 has been reported from both recreational and contaminated drinking water (Feng, 1995), however, its importance as source for carcass contaminations in abattoirs was ignored. Water to be used in the abattoirs in general should be potable and free of pathogenic bacteria and control measures to reduce the public health risks arising from *E. coli* O157:H7 must address at slaughterhouse by monitoring the water quality.

Of the total 53 *E. coli* O157:H7 isolates in this study, only one was carried the virulent gene. It has been indicated that the virulence of *E. coli* O157:H7 has been mainly associated with the production of shiga toxins encoded by *stx1* or *stx2* genes, the presence of the pathogenicity island LEE, responsible for attaching and effacing lesions and the *ehxA* gene, encoding

enterohaemolysin (IFT, 2000; Chahe *et al.*, 2006). In our finding only one isolate from goat carcass had both stx1 and *stx2* but not the *eae* gene. Similarly a study undertaken in Greece (Dontorou *et al.*, 2004) reported only *stx2* from a single *E. coli* O157:H7 isolate obtained from goat feces. Of the total 33 isolates in France (Lenahan *et al.*, 2007) from sheep, only 5 of the isolates carried the *stx1* and *stx2*. The identification of this virulent gene in this study from goat carcass indicates the potential of goat carcasses as source of *E. coli* O157:H7 for human infections in the country and its effect to the growing meat export market. It also suggests the need for further detailed epidemiological studies on *E. coli* O157:H7 in Ethiopia involving different export abattoirs and species of animals.

6. CONCLUSIONS AND RECOMMENDATIONS

In general total and fecal coliform counts found in both sheep and goats after carcass wash was relatively lower than before carcass wash. But total and fecal coliform counts on sheep carcasses were much higher than in goats'. Fecal coliforms were not detected in water samples and only few total coliforms were counted in these water samples that could not overall affect carcass coliform loads.

E. coli O157: H7 was present in the feces, skin and carcasses of sheep and goats and water at export abattoir. This pathogen was isolated from carcasses before and after wash that indicated the presence of carcass contamination during slaughter operations. Thus, interventions to reduce the occurrence of *E. coli* O157:H7 were less effective or require additional stringent approaches.

A strong association was observed between the prevalence of *E. coli* O157:H7 in the fecal and skin of animals with carcass contaminations. This indicates the need for reduction of these pathogens in live animals that are presented for slaughter.

E. coli O157:H7 *stx1* and *stx2* genes were identified from goat carcass. The identification of these virulent factors indicates the potential of sheep and goats as sources of *E. coli* O157:H7 other than cattle for human infection in the country. Based on these conclusions the following recommendations are forwarded: -

- Meat product quality and microbial standards should be established and improved in the country in order to effectively competent for world market for meat and meat products.

- Sheep and goats should be considered as reservoirs of *E. coli* O157:H7 in addition to cattle.

- Control measures to reduce the public health risks arising from *E. coli* O157:H7 in reservoir animals must be addressed at abattoirs by reducing skin and fecal prevalence and avoiding carcass cross contaminations at different stages of slaughter operations.

- Abattoir workers need to be educated about appropriate slaughter procedures, sanitary and hygienic method of production, and the risk of foodborne diseases. There should also be regular monitoring and inspection of workers by the responsible bodies to reduce public health hazards associated with foodborne pathogens using systems such as HACCP.

- Responsible bodies, both in the veterinary and public health sectors need to be aware of the presence of *E. coli* O157:H7 in food animals and animal products in Ethiopia and measures should be taken to strengthen the surveillance and diagnostic facilitates in the country to further promote the local and export trade of meat and meat products with in and out side the country.

- Further detailed research involving different abattoirs, different types of food animals and their products should be undertaken to determine the epidemiology of *E. coli* O157:H7 so that appropriate and effective control and preventive measures could be designed.

7. REFERENCES

Ahmad, A., Nagaraja, T. and Zurek, L. (2007): Transmission of *Escherichia coli* O157:H7 to Cattle by Houseflies. *Prev. Vet. Med.*, **80**: 74–81.

Akashi, S., Joh, K. and Tsuji, A. (1994): A Severe Outbreak of Hemorrhagic Colitis and Haemolytic Uraemic Syndome Associated with *Escherichia coli* O157:H7 in Japan. *Eur. J. Pediatr.*, **153:** 650–55.

Bacon, R., Belk, K., Sofos, J., Clayton, R., Reagan, J. and Smith, G. (2000): Microbial Populations on Animal Hides and Beef Carcasses at different Stages of Slaughter in plants employing Multiple sequential interventions for Decontamination. J. Food Prot., **63:** 1080–1086.

Baron, E., Peterson, L., and Finegd, S. (1994): *Entrobacteriacae*. In: Railey and Scott's Diagnostic Microbiology 9th (ed) Mosby, London, Pp 590-633.

Bastian, S., Carle, I., Grimont, F., and Grimont, P. (1999): Diversity of Shiga Toxin-Producing *E. coli* in Herds of Dairy Cows and Goats. *Acta Clin. Bel.*, **54**: 49-50.

Battisti, A., Lovari, S., Franco, A., Diegidio, A., Tozzoli, R., Caprioli, A. and Morabito, S. (2006): Prevalence Of *Escherichia coli* O157 in Lambs at Slaughter in Rome, Central Italy. *Epidemiol. Infect.*, **134**: 415–419.

Bell, R. (1997): Distribution and Sources of Microbial Contamination on Beef Carcasses. *J. Appl. Microbiol.*, **82**: 292-300.

Benson, A. (2003): Molecular and Population Genetics of Virulence Traits in *E. coli* O157:H7. In: Torrence, M. and Isaacson, R. (eds.). Microbial Food Safety in Animal Agriculture Current Topics, Iowa State Presses. A Blackwell Publishing Company, USA, Pp 155-166.

Besser, T., Lejeune, J., Rice, D. and Hancock, D. (2003): Prevention and Control of *E. coli* O157:H7. In: Torrence, M. and Isaacson, R. (eds). Microbial Food Safety in Animal Agriculture Current Topics, Iowa State Presses. A Blackwell Publishing Company, USA, Pp 155-166.

Beutin, L., Geier, D., Zimmermann, S. and Karch, H. (1995): Virulence Markers of Shiga-Like Toxin-Producing *Escherichia coli* Strains Originating from Healthy Domestic Animals of Different Species. *J. Clin. Microbiol.*, **33:** 631– 635.

Bhandare, S., Sherikar, A., Paturkar, A., Waskar, V. and Zende, R. (2007): A Comparison of Microbial Contamination on Sheep/Goat Carcasses in a Modern Indian Abattoir and Traditional Meat Shops. *Food Control,* **18:** 854–858

Blackburn, C. and McCarthy, J. (2000): Modifications to Methods for the Enumeration and Detection of Injured *E. coli* O157:H7 in Foods. *Int. J. Food Microbiol.*, **55:** 285-290.

Blanco, M., Blanco, J., Mora, A., González, E., Bernárdez, M., Alonso, M. ,Coira, A., Rodríguez, A., Rey, J., Alonso, J. and Usera, M. (2003): Vero toxin-producing *Escherichia coli* (VTEC) in Spain: Prevalence, Serotypes, and Virulence Genes of O157:H7 and non-O157 VTEC in Ruminants, Raw Beef Products, and Humans. *Exp. Bio. and Med.*, **228**:345-351.

Bolton, D. (2003): The EC decision of the 8th June 2001 (2001/471/EC): Excision Versus Swabbing. *Food control,* **14:**207-209.

Bolton, F., Crozier, L. and Williamson, J. (1996): Isolation of *Escherichia coli* O157 from Raw Meat Products. *Lett. Appl. Microbiol.*, **23**:317-321.

Boyce, T., Swerdlow, D. and Griffin, P. (1995): *Escherichia coli* O157:H7 and the Hemolytic–Uremic Syndrome. *N. Engl. J. Med.*, **333:** 364 –368.

Buchanan, R. and Doyle, M. (1997): Foodbore Disease Significance of *Escherichia coli* O157:H7

Byrne, B., Dunne, G., Lyng, J. and Bolton, D. (2005): Microbiological Carcass Sampling Methods to Achieve Compliance with 2001/471/E.C. and New Hygiene Regulations. *Res. Microbiol.,* **156**: 104-106.

Byrne, B., Dunnea, G., Lyngb, J., Boltona, D. (2007): The Development of A 'Clean Sheep Policy' in Compliance with the New Hygiene Regulation (EC) 853/2004 (Hygiene 2). *Food Microbiol.*, **24**:301–304.

CDC (Centers for Disease Control and Prevention) (2001): Preliminary Food Net Data on the Incidence of foodborne Illnesses-Selected Sites, United States. MMWR. **50**:241-246.

CDC (Centers for Disease Control and Prevention) (2005): Outbreaks of *Escherichia coli* O157:H7 associated with Petting Zoos -North Carolina, Florida, and Arizona, 2004 and 2005 Department of Health and Human Services. *MMWR,* **54**:1277-1280.

Chahe, A., China, B., Mainil, J. and Daube, G. (2006): Prevalence of Enterohaemorrhagic *Escherichia coli* from Serotype O157 and Other Attaching and Effacing *Escherichia coli* on Bovine Carcasses in Algeria. *J. Appl. Microbiol.*, **101:** 361–368.

Chapman, P. (1994): Isolation, Identification and typing of Vero cytotoxin-Producing *Eschericha coli* O157. *PHLS Microbiol. Digest.*, **11**:13-17.

Chapman, P. and Cudjoe, K. (2001): Evaluation of Bead retriever TM, an Automated System for Concentration of *Escherichia coli* O157 from Enrichment Cultures by Immunomagnetic Separation. *J. Rapid Meth. Autom. Microbiol.***9**: 203-214.

Chapman, P., Cerda´n Malo, A., Ellin, M., Ashton, R. and Harkin, M. (2001): *Escherichia coli* O157 in Cattle and Sheep at Slaughter, On Beef and Lamb Carcasses and In Raw Beef and Lamb Products in South Yorkshire. *Int. J. Food Microbiol.*, **64:** 139–150.

Chapman, P., Siddons, C., Cerdan Malo, A. and Harkin, M. (1997): A 1-Year Study of *Escherichia coli* O157 in Cattle, Sheep, Pigs and Poultry. *Epidemiol. Infect,* **119**:245–250.

Chapman, P., Wright, D. and Siddons, C. (1994): A Comparison of Immunomagnetic Separation and Direct Culture for the Isolation of Verocytotoxin-Producing *Escherichia coli* O157 from bovine feces. *J. Med. Microbiol.*, **40**: 424-427.

Chung, R., Dickson, J. and Crouse, J. (1989): Attachment and Proliferation of Bacterial On Meat. *J. Food prot.*, **52**: 173-177.

Davis, M.A., Cloud-Hansen, K., Carpenter, J., Hovde, C.J. (2005): *Escherichia coli* O157: H7 in Environments of Culture-Positive Cattle. *App. Environ. Microbiolo*. **71**: 6816-6822.

Dean-Nystrom, E., Pohlenz, J., Moon, H., O'brien, A. (2000): *Escherichia coli* O157: H7 Causes More-Severe Systemic Disease in Suckling Piglets than in Colostrum-Deprived Neonatal Piglets. *Infect. Immun.*, **68**: 2356-2358.

DeBoer, E., and Heuvelink, A. (2001): Food as vehicles of VTEC Infection. In: Duffy, G., Garvey, P., and McDowell, D. (Eds). Verotoxigenic *E. coli.* Food Science and Nutrition Press. USA, Pp 120.

Demisse, D. (2005): Prevalence and Distribution of Enterohemorragic *E. coli* O157:H7 and *Salmonella* Serotypes Isolated from Selected Samples in Debre Zeit and Addis Ababa, Ethiopia. DVM Thesis, Faculty of Veterinary Medicine, AAU, Debre-Zeit, Ethiopia.

Dickson, J. and Anderson, M. (1992): Microbiological Decontamination of Beef Carcasses by Fecal Coliforms. *J. Food prot.*, **55**: 133-140.

Dickson, J. and Anderson, M. (1992): Microbiological Decontamination of Food Animal Carcasses by Washing and Sanitizing Systems. *J. Food Prot.*, **55**:133–140.

Dontoroua, A., Papadopouloua, C., Filioussisb, G., Apostoloua, I., Economoua, V. and Kansouzidouc, A. (2004): Isolation of a Rare *Escherichia coli* O157:H7 Strain from Farm Animals in Greece. *Comp. Immunol. Microbiol. Infect. Dis.*, **27:** 201–207.

Duffy, E., LeValley, S., Kain, M., Belk, K., Tatum, J., Sofos, J., Smith, G. and Kimberling, C. (2001): Microbial Contamination on Lamb Carcasses Processed in the United States. *Food Prot.*, **64:** 503-508.

Durso, L., Reynolds, K. and Keen. J. (2005): Shiga-Toxigenic *Escherichia coli* O157:H7 Infections among Livestock Exhibitors and Visitors at a Texas County Fair. *Vector-Borne Zoonotic Dis.*, **5:** 193-201.

Dziva, F., Vlisidou, I., Crepin, V., Wallis, T., Frankel, G. and Stevens, M. (2007): Vaccination of Calves with *EspA*, a Key Colonization Factor of *Escherichia coli* O157:H7 Induces Antigen-Specific Humeral Responses but does not Confer Protection against Intestinal Colonization. *Vet. Microbiol.*, **123:** 254–261.

Edwards, J. and Fung, D. (2006): Prevention and Decontamination of *Escherichia coli* O157:H7 on Raw Beef Carcasses in Commercial Beef Abattoirs. *J. Rapid Meth. Autom. Microbiol.*, **14:** 1–25.

Elder, R., Keen, J., Siragusa, G., Barkocy, G., Koohmaraie, M. and Laegreid W. (2000): Correlation of Enterohemorrhagic *Escherichia coli* O157 Prevalence in Feces, Hides and Carcasses of Beef Cattle during Processing. *Proc. Natl. Acad. Sci.* **97:**2999-3003.

Fagan, P, Hornitzky, M., Bettelheim, K. and Djordjevic, S. (1999): Detection Of Shiga-Like Toxin (*Stx1* and *Stx2*), Intimin (*eae*), and Enterohemorrhagic *Escherichia coli* (EHEC) Hemolysin (*hly*) Genes in Animal Faeces By Multiplex PCR. *Appl. Environ. Microbiol.* **65**, 868-872.

Farmer, J., Iii, J., Davis, B., Hickman-Brenner, F., McWhorteh, A., Huntley-Carter, G., Asbury, M., Riddle, C., Wathen-Grady, H., Elias, C., Fanning, G., Steigerwalt, A., O'Hara, C., Morris, G., Smith, P. and Brenner, D. (1985): Biochemical Identification of New Species and Bio-Groups of *Enterobacteriaceae* Isolated from Clinical Specimens. *J. Clin. Microbiol.*, **21**:46-76.

Feder, I., Wallace, F., Gray, J., Fratamico, P., Fedorka, P., Pearce, R., Call, J., Perrine, R. and Luchansky, J. (2003): Isolation of *Escherichia coli* O157:H7 from Intact Colon Fecal Samples of Swine. *Emerg. Infect. Dis.*, **9**:381-384.

Feng, P. (1995): *Escherichia coli* Serotype O157:H7: Novel Vehicles of Infection and Emergence of Phenotypic Variants. *Emerg. Infect. Dis.,* **1**:13-15.

Feng, P., Weagant, S. and Grant, M. (2002): Bacteriological Analytical Manual 8th edition: US Food and Drug Administration Center for Food safety and Applied Nutrition, Washington D.C, USA, Pp 24-26.

Fratamico, P. and Smith, J., (2006): *Escherichia coli* infections. In: Riemann, H. and Cliver, D. (eds). FoodBorne Infections and Intoxications, 3rd Edition. New York: Elsevier science

Frenzen, P. and Drake, A. (2005): Economic Cost of Illness due to *Escherichia coli* O157 infections in the United States. *J. Food Prot.*, **68**: 2623-2630.

Gansheroff, L. and O'Brien, A. (2000): *Escherichia coli* O157:H7 in Beef Cattle Presented for Slaughter in the U.S. Higher Prevalence Rates than Previously Estimated. *Proc. Natl. Acad. Sci.*, **97:** 2959–2961.

Gill, C., Badoni, M. and Jones, T. (1996): Hygienic Effects of Trimming and Washing Operations in a Beef -Carcass-Dressing Process. *J. Food Prot.*, **59**: 666-669.

Global Salm-Surv (2003): PCR for identification of *Escherichia coli* toxins VT1, VT2 and EAE. A global Salmonella surveillance and laboratory support project of the World Health Organization Laboratory Protocols Level 4 Training Course 2nd ed. Copenhagen, Denmark, Pp 1-8.

Gun, H., Yilmaz, A., Turker, S., Tanlasi, A. and Yilmaz, H. (2003): Contamination of Bovine Carcasses and Abattoir Environment by *Escherichiacoli*O157:H7 in Istanbul. *Int. J. of Food Microbiol.*, **84**:339-344.

Gyles, C. (2004): Pathogenesis, Epidemiology, and Control of VTEC Infection in the Cattle Industry. In: Proceedings of the 23rd World Buiatrics Congress. July 11-16, 2004. Québec, Canada. Pp 12-15.

Hansson, I., Hamilton, C., Ekman, T. and Forslund, K. (2000): Carcass Quality in Certified Organic Production, Compared with Conventional Livestock Production. *J. Vet. Med.*, **47**: 111-120.

Heuvelink, A., Flam, V. and de Boer, E. (1998): Isolation and Characterization of Verocytotoxinproducing *Escherichia coli* O157 strains from Dutch cattle and sheep. *J. Clin. Microbiol.*, **36**: 878–882.

Hiko, A. (2007): A Cross Sectional Study of *E. coli* O157:H7 in Cattle, Goats and Sheep Carcass in East Showa, Ethiopia. MSc. Thesis, Faculty of Veterinary Medicine, AAU, Debre-Zeit, Ethiopia.

Hopkins, K. and Hilton, A. (2000): Methods Available for the Sub-typing of *Escherichia coli* O157. *World J. Microbiol. Biotechnol.*, **16**: 741-748.

HPB (Health Products and Food Branch) Method (2001): Determination of Coliforms in Foods using Violet Red Bile Agar Canadian Food Inspection Agency, Ottawa, Canada, Pp 1-7.

IFT (Institute of Food Technologists) 2000: Expert Report on Emerging Microbiological Food Safety Issues. Implications for Control in the 21st Century, S. Lowry/Univ. Ulster/Stone Pp1-32

ILIRI (International Livestock Research Institute) (2005): Ada'a-Liben Woreda Pilot Learning Site Diagnosis and program Design. Addis Ababa, Ethiopia, Pp1-8.

ISO (International Organization for Standardization) 16654 (2001): Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Detection of *Escherichia coli* O157. Pp 1-13.

ISO (International Organization for Standardization) 17604 (2003): Microbiology of Food Animal, Feeding Stuffs-Carcass Sampling For Microbiological Analysis, Pp 2-12

Izumiya, H., Terajima, J., Wada, A., Inagaki, Y., Itoh, K., Tamura, K. and Watanabe, H. (1997): Molecular Typing of Enterohemorrhagic *Escherichia coli* O157:H7 Isolates in Japan using Pulsed-Field Gel Electrophoresis. *J. Clin. Microbiol.*, **35:** 1675–1680.

Jay, J. M. (2000): Modern Food Microbiology. 6th ed. Aspen Pub. Maryland, USA. Pp 2- 50.

Johnsen, G., Wasteson, Y., Heir, E., Berget, I. and Herikstad, H. (2001): *Escherichia coli* O157:H7 in Feces from Cattle, Sheep and Pigs in the Southwest Part of Norway during 1998 and 1999. *Int. J. Food Microbiol.*, **65**: 193–200.

Karch, H. and Bielaszewska, M. (2001); Sorbitol-Fermenting Shiga toxin-producing *Escherichia coli*O157:H-strains:Epidemiology, Phenotypic and Molecular Characteristics and Microbiological Diagnosis. *J. Clin. Microbiol.*, **39**: 2043-2049.

Karmali, M., Petric, M., Lim, C., Fleming, P., Arbus, G. and Lior, H. (1985): The association between Idiopathic Haemolytic Uraemic Syndrome and Infection by Verotoxin-producing *Escherichia coli*. *J. Infect. Dis.*, **151:** 775-782.

Keen, J., Durso, L. and Meehan, T. (2007): Isolation of *Salmonella enterica* and Shiga-Toxigenic *Escherichiacoli* O157 from Feces of Animals in Public Contact Areas of United States Zoological Parks. *Appl. and Envir. Microbiol.*, **73**: 362–365

Kiraa, H., Arthaud, J. and Fournaud, J. (1985): Contamination and Bacterial Retention Capacity of Beef Carcasses at the Abattoir. *J. Appl. Bacterio,* **59**: 23-28.

Kudva, I., Hatfield, P. and Hovde, C. (1996): *Escherichia coli* O157:H7 in Microbial Flora of Sheep. *J. Clin. Microbiol.*, **34**: 431–433.

Law, D. (2000): Virulence Factors of *Escherichia coli* O157 and Other Shiga Toxin-Producing *E. coli*. *J. Appl. Microbiol.*, **88**: 729-745.

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. *Appl. and Enviro. Microbiol.*, **68:**1631–1638.

Lenahan, M., O'Brien, S., Kinsella, K., Sweeney, T. and Sheridan, J. (2007): Prevalence and Molecular Characterization of *Escherichia coli* O157:H7 on Irish Lamb Carcasses, Fleece and in Faeces Sample. *J. Appl. Microbiol.*, **103**: 2401-2409.

Lopez, E., Diaz, M. and Grinstein, S. (1989): Hemolytic Uremic Syndrome and Diarrhea in Argentine Children: The Role of Shiga-Like Toxins. *J. Infect. Dis.*, **160**: 469–475.

Mc Evoy, J., Doherty, A., Sheridan, J., Thomson-Carter, F., Garvey, P., McGuire, L., Blair, I. and McDowell, D. (2003): The Prevalence and Spread of *Escherichia coli* O157:H7 at a Commercial Beef Abattoir. *J. App. Microbiol.*, **95**: 256–266.

Mead, P. and Griffin, P. (1998): *Escherichia coli* O157:H7.*The Lancet,* **352:**1207–1212.

Mengistu, T. (2007): Microbial Load on Goat Carcasses Slaughtered in Selected Abattoirs of East Showa Administrative Zone, Ethiopia. MSc. Thesis, Faculty of Veterinary Medicine, AAU, Debre-Zeit, Ethiopia.

Meyer-Broseta, S., Bastian, S., Arne, P., Cerf, O. and Sanaa, M. (2001): Review of Epidemiological Surveys on the Prevalence of Contamination of Healthy Cattle with *Escherichia coli* serogroup O157:H7. *Int. J. Hyg. Environ. Healt.*, **203**: 347-361.

Midgley, J. and Small, A. (2006): Review of New and Emerging Technologies for Red Meat Safety. Meat and Livestock Australia Limited, Sydney, Australia. Pp 5-25 and 41-53.

MoARD (Ministry of Agriculture and Rural development)(2007): Annual report of the Fiscal year 2006/7.Addis Ababa, Ethiopia, Pp 7-9.

MOH (Ministry of Health) (2001): *Escherichia coli* O157:H7. Prepared for the Ministry of Health by MOH, ESR). Data Sheets Contain a Summary of Information available In the Literature. ESR Ltd. Pp 1-5.

Morgan, D., Newman, C., Hutchinson, D., Walker, A., Rowe, B. and Majid, F. (1993): Verotoxin Producing *Escherichia coli* O157 infections associated with the Consumption of Yoghurt. *Epidemiol. Infect.*, **111**: 181-187.

Moses, A., Garbati, M., Egwu, G. and Ameh, J. (2006): Detection of *E. coli* O157 and O26 Serogroups in *HIV*-infected Patients with Clinical Manifestation of Diarrhoea in Maiduguri, Nigeria. *Res. J. Med. and Med. Sci.*, **1**: 140-145.

Moxley, R. (2003): Detection and Diagnosis of *Escherichia coli* O157:H7 in food-producing Animals. In: Torrence, M. E. and Isaacson, R.E. (eds). Microbial Food Safety in Animal Agriculture Current Topics, Iowa State Presses. A Blackwell Publishing Company. USA, Pp 143-154.

Müller, E., Grabow, W. and Ehlers, M. (2003): Immunomagnetic Separation of *Escherichia coli* O157:H7 from Environmental and Wastewater in South Africa. *Water S.A.*, **29**: 427-432.

Mutaku, I., Erku, W. and Ashenafi, M. (2005): Growth and Survival of *Escherichia coli* O157: H7 in Fresh Tropical Fruit Juices at Ambient and Cold Temperatures. *Int. J. Food Microbiol.*, **56**: 133-139.

Nataro, J. and Kaper, J. (1998): Diarrheagenic *Escherichia coli. Clin. Microbiol. Rev.*, **11**: 142-201.

NPH (National Public Health Service for Wales)(2006): Detection of *Escherichia coli* O157 by Automated Immunomagnetic Separation. Standard Method. Issued by Standards Unit, Evaluations and Standards Laboratory With the Regional Food, Water and Environmental Coordinators Forum. SOPs from the Health Protection Agency, Wales, UK, Pp 1-15.

OIE (Office for international des epizootics) (2004): Verocytotoxigenic *Escherichia coli.* Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Geneva, Switzerland. Updated:

23. 07. 2004. Part 2. Section 2.10.3. htt. //www.oie.int/eng/norms/manual (A-0007) htm retrived on Augest, 12, 2007.

Okrend, A., Rose, G. and Lattuada, C. (1990): Use of 5-bromo-4-chloro-3-indoxyl-D-glucuronide in Mac Conkey Sorbitol to Aid in the Isolation of *Escherichia coli* O157: H7 from Ground Beef. *J. Food Prot.*, **53**: 941-948.

Paiba, G., Gibbens, J. and Pascoe, S. (2002): Fecal carriage of Verocytotoxin-Producing *E. coli* O157 in Cattle and Sheep at Slaughter in Great Britain. *Vet. Rec.*, **150**: 593–598.

Pearson, H. (2007): "The Dark Side of *E. coli*". *Nature*, **445**: 8-9.

Perna, N., Myheew, G. and Pofai, G. (1998): Molecular Evolution of a Pathogencity Island from Enteroheamorrahgic *Escherichia coli* O157:H7. *Infect. Immunol.*, **66**: 3810-3817.

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

Reid, C., Small, A., Avery, S. and Buncic, S. (2002): Presence of Foodborne Pathogens on Cattle Hides. *Food Control.*, **13**: 411–415.

Roberts, T. (1980): Contamination of Meat. The Effect of Slaughter Practices on the Bacteriology of the Red Meat Carcasses. *J. Royal Soc. Health*, **100**: 3-9.

Sargeant, J. and Smith, D. (2003): The Epidemiology of *Escherichia coli* O157:H7. In: Torrence, M. and Isaacson, R. (eds). Microbial Food Safety in Animal. Agriculture Current Topics, Iowa State Presses. A Blackwell Publishing Company, USA, Pp 131-174.

Schaffner, D. and Smith, S. (2004): Indicator Organisms /Microbiological Analysis. Enc. Meat Sciences, Elsevier Ltd. New Jersey, USA, Pp1-6.

Sidjabat-Tambunan, H. and Bensink, J. (1997): Vero toxin-producing *Escherichia coli* from the Faeces of Sheep Calves and Pigs. *Aust. Vet. J.*, **75:** 292-293.

Siegler, R. (1988): Management of Hemolytic-Uremic Syndrome. *J. Pediatr.*, **112**: 1014-1020.

Sofos, J., Kochevar, S., Reagan, J. and Smith, G. (1999): Extent of Beef Carcass Contamination with *Escherichia coli* and Probabilities of Passing United States Regulatory criteria. *J. Food Prot.*, **62**: 234-238.

SPSS (statistical package for the social sciences), Inc (2002): SPSS for windows (version 11.5.0), Chicago, Illinois, USA.

Strockbine, N., Wells, J., Bopp, C. and Barrett, T. (1998): Overview of Detection and Subtyping Methods. In: Kaper J. and O'Brien A. (eds). *Escherichia coli* O157:H7 and Other Shiga Toxin-Producing *E. coli* Strains. ASM Press, Washington, D. C., USA, Pp 331-356.

Synge, B. and Hopkins, G. (1994): Studies of Verotoxigenic *Escherichia coli* O157 in Cattle in Scotland and Association with Human Cases. In: Karmali, M. and Goglio, A. (Eds.), Recent Advances in Verocytotoxin Producing *Escherichia coli* Infections. Elsevier Science, New York, USA, Pp 65-68.

Tadesse, G., Ephraim, E. and Ashenafi, M. (2005): Assessment of the Antimicrobial Activity of Lactic Acid Bacteria Isolated from Borde and Shamita, Traditional Ethiopian Fermented Beverages, On Some Foodborne Pathogens and Effect of Growth Medium on the Inhibitory Activity. *Int. J. Food Saf.*, **5**: 13-20.

Thrusfield, M. (2005): Veterinary Epidemiology. 3rd ed. Blackwell Science Ltd., London, England, Pp 228-246.

Tsegaye, M. and Ashenafi, M. (2005): Fate of *Escherichia coli* O157:H7 During the Processing and Storage of Ergo and Ayib, Traditional Ethiopian Dairy Products. *Int. J. Food Microbiol.*, **103**: 11-21.

Tutenel, A., Pierard, D., Vandekerchove, D., Van Hoof, J. and de Zutter, L. (2003): Sensitivity of Methods for the Isolation of *Escherichia coli* O157 from Naturally Infected Bovine Faces. *Vet. Microbiol.* , **94:** 341–346.

UK NRM (UK National Reference Method) (2006): *E. coli* O157 Isolation by Immuno-Magnetic Separation and Presumptive Identification. The Veterinary and Public Health Test Standardization Group. Issue no: 3, Reference no: NRM 001, Pp.1-11.

United States Department of Agriculture (USDA), Office of Public Health and Science (OPHS), Food Safety and Inspection Service (FSIS) (2002): Detection, Isolation, and Identification of *E. coli* O157:H7 and O157: NM from Meat Products Microbiology Laboratory Guidebook Notice of Change. Limits of Detection to Meet the Requirements of ISO 17025. Pp1-13.

Whittam, T. (1996): Genatic Variation and Evolutionary Processes in Natural Population of *Escherichia coli.* In: Neidhardt, F. (ed), *Escherichia coli* and *Salmonella*. Celluler and Molcular Biology. 2nd ed. ASM Press. Washington, DC. Pp 2708-2720.

Wilkerson, C., Kirk, N. and Roberts, M. (2004): Antibiotic Resistance and Distribution of

Tetracycline Resistance Genes in *Escherichia coli* O157:H7 Isolates from Humans and Bovines. Antimicrob. Agents Chemother., **48**:1066–1067.

Willshaw, G., Thirlwell, J., Jones, A., Parry, S., Salmon, R. and Hickey, M. (1994): Verocytotoxin–Producing *Escherichia coli* O157 in Beef Burgers linked to an Outbreak of Diarrhoea, Haemorrhagic Colitis and Haeamolytic Uremic Syndromes in Britain. *Lett. Appl. Microbiol*., **19:** 304-307.

Yadav, M., Tale, S., Sharda, R., Sharma, V., Tiwari, S. and Garg, U. (2006): Bacteriological Quality of Sheep Meat in Mhow Town of India. *Int. J. Food Sci. and Technol.*, **41**, 1234–1238.

Yalci, S., Nizamlioclu, M. and Gu"rbuz, U. (2001): Fecal Coliform Contamination of Beef Carcasses during the Slaughtering Process. *J. food Saf.*, **21:** 225-231.

Zadik, P., Chapman, P. and Siddons, C. (1993): Use of Tellurite for the Selection of Verocytotoxigenic *Escherichia coli* O157. *J. Med. Microbiol.*, **39**: 155-158.

8. APPENDICES

Appendix1: Sample collection and laboratory activities work sheet for isolation of *E.coli*O157:H7

No	Data collection			Laboratory activities										
	Date	Spp.	Sample type	Sam. ID	Enrichment	IMS	Plate out	Colony char.	I	MR	VP	Ci	H_2S	Latex Agglu.

Spp=Species of animals, IMS=Immunomagnetic separation, I= Iodole test; MR=Methyl Red test, VP= Voges-Proskauer test, Ci=Citrate utility test, H_2S= Hydrogen Sulfide production.

Appendix 1: Sample collection and laboratory activities work sheet for enumeration of total and fecal coliforms

No	Data collection				Laboratory activities			
	Date	Spp.	Sample type	Sample ID	Preparation Of Dilution	Colony Counting	Growth On BGLB	Recording

Appendix 2: Media used and its preparations for the enumeration of total and fecal coliform and isolation of *E. coli* O157:H7

1. Peptone Water (Oxoid Ltd., Hampshire, England)

Composition (g/l): Peptone 10.0; Sodium chloride 5. Final pH: 7.2±0.2. *Preparation*: Add 15 g to 1 liter of distilled water. Mix it well and sterilize by autoclaving at 121°C for 15 minutes. Finally dispensed in to test tubes each with 9 ml, near a flame.

2. Violet red Bile2%agar (VRBA) (Oxoid Ltd., Hampshire, England)

Composition (g/l): Yeast extract3.0 g, Peptone or gelysate7.0 g, NaCl 5.0 g, Bile salts1.5g, Lactose10.0 g, Neutral red 0.03 g, Crystal violet0.002 g and Agar15.0 g. *Preparation*: 52g of the ingredients were suspended in1000 ml of distilled water and mix thoroughly and adjust to pH 7.4 ± 0.2. Heated with agitation and boiled for 2 min. Not sterilized. It was cooled at 48°C in water bath and used as plating medium. After solidification, approximately 3.0 to 4.0 ml of the medium was added as a cover layer to prevent surface growth and spreading of colonies.

3. Brilliant green lactose bile (BGLB) broth (Oxoid Ltd., Hampshire, England)

Composition (g/l): Peptone10 g, Lactose10 g, bile salt 20 g and Brilliant green0.0133 g. *Preparation*: 40 g of the ingredients were suspended in 1000 ml of distilled water, mixed carefully and autoclaved at 121°C for 15 min, dispensed into fermentation tubes, making certain that fluid level covers inverted vials.

4. Buffered peptone water (BPW) (AES laboratory, Cedex, France)

Composition (g/liter): Peptone from casein 10.0; sodium chloride 5.0; di-sodium hydrogen phosphate 3.5; potassium dihydrogen phosphate1.5. *Preparation:* Twenty grams of this medium was dissolved in one liter of distilled water and sterilized by autoclaving at 121 °C for 15 minutes.

5. Modified Tryptic Soy Broth with Novobiocine(mTSB+n)

Tryptic soy broth (Oxoid Ltd., Hampshire, England) *Composition (g/liter)*: Pancreatic digest of casein 15.0; enzymatic digest of soybean meal 5.0; sodium chloride 5.0; Di potassium hydrogen phosphate 2.5, Glucose 2.5g,. Bile salt (Oxoid Ltd., Hampshire, England) 1.5 g, Di potassium hydrogenphosphate (Techno pharmachemicals, Bahadurgarch, India) 1.5 g and Novobiocine (Sigma, Steinheim, Germany) *Preparation:* 30 g of the tryptic soy both, 1.5 g of bile salt and 1.5 g of K_2HPO_4 were measured, mixed and dissolved in one liter of distilled water and sterilized by autoclaving at 121° C for 15 minutes. 400 mg of novobiocine in 100 ml of distilled water was mixed and membrane filtered and added to the prepared media tempered at 50 ^{O}C.

6. Sorbitol-MacConkey agar (Difco, Becton Dickinson, Claix, France) containing cefixime and tellurite (Dynal biotech ASA, Oslo, Norway).

Composition (g/liter: Peptone 15.5g; Proteose Peptone 3 g, D-sorbitol 1.0 g; Bile salts 1.5 g; Sodiumchlorid 5.0 g; Neutral red 0.03. *Direction*: 50 g of the powder was suspended in 1 liter of distilled water. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Autoclave at 121°C for 15 minutes. Filter-sterilized Potassium tellurite (2.5 mg/l) and Cefixime (0.05mg/l) were added on the prepared base media tempered at 50-55°C. gently shacked and poured into Petri dishes.

7. Nutrient agar ((Oxoid Ltd., Hampshire, England)

Composition (g/liter): Meat extracts 10.0 g, Peptone 10.0 g, Sodium chloride 5.0 g, Agar 15.0 g, Final pH 7.5 ± 0.2 at 25°C. *Preparation:* Suspend 28grams of the powder in 1 liter of purified water, mix thoroughly. Heat with frequent agitation and boil for 1minute to completely dissolve the powder. Autoclaved at 121 ^{o}C for 15 minutes and gently shacked and poured into Petri dishes.

8. SIM (Sulfide Production, Indole and Motility Test Medium (BBL®, Becton Dickinson, USA).

Composition (g/liter): Pancreatic digest of casein 20.0g; peptic digest of animal tissue 6.1g, Ferrous Ammonium Sulfate 0.2g; Sodium thiosulfate 0.2g; Agar 3.5g. Final pH = 7.3 ± 0.2°C for 15 minutes.*Direction*: Suspend 30g of powder in 1L of purified water. Mix thoroughly. Heat with

frequent agitation and boil for 1 min. Autoclave at 121°C for 15 minute. Gently mixed and poured into sterile test tubes.

9. MR-VP medium (Titan biotech Ltd, Bhiwadi, India)

Composition (g/liter): Peptone 7.00; Dextrose 5.00; Dipotassium phosphate 5.00; Final pH = 6.9 $\pm$ 0.2 at 25°C. *Preparation*: Dissolve 17g in 1000 ml distilled water. Gently heat to dissolve the medium completely. Sterilize by autoclaving at 121°C for 15 minute. .Gently shacked and poured into sterile test tubes near a flame.

10. Simmon's Citrate Agar (Difco, Detroit, USA).

Composition (g/liter): Magnesium sulfate 0.2g; Ammonium dihydrogen phosphate 1g; Dipotassium phosphate 1g; Sodium citrate 2g; Sodium chloride 5g; Bacto agar 15g; Bacto Bromothymol Blue 0.08g. Final pH = 6.8 $\pm$0.2 at 25°C. *Direction:* Suspend 24.4gram in 1 liter of distilled or deionized water and boil to dissolve completely. Sterilize at 121-124°C for 15 minutes. Gently shacked and poured into sterile test tubes near a flame.

11. Tryptic Soy Agar (Difco, Sparks, USA)

Composition (g/liter): Pancreatic digest of casein 15.0; enzymatic digest of soybean meal 5.0; sodium chloride 5.0; agar 15.0 *Preparation*: forty grams of the powder was suspended in 1 liter of distilled water and mixed thoroughly. The medium was heated with frequent agitation and boiled for 1 minute to completely dissolve the powder. Then autoclaved at 121°C for 15minutes, dispensed into criovial tubes and allowed the medium to solidify.

12. Phosphate Buffer Saline with Tween 20(Sigma chemicals Co. Saint Louis, USA)

Composition (0.01MPBS / L): Sodium chloride, 0.138M; Potassium chloride 0.0027M; Tween 20 0.05%. *Preparation:* one sacate of this composition was suspended in 1 liter of distilled water

and mixed thoroughly. Sterilize by autoclaving at 121°C for 15 minute. Gently shacked and poured into sterile universal tubes near a flame.

Appendix 3: Chemicals and reagents used for *E. coli* O157:H7 biochemical test

1. Kovac's reagent

Preparation: Dissolve 10g *P*-dimethylamino benzaldehyde (Sigma, Steinheim, Germany) in 150 ml ethanol alcohol, and slowly add 50ml concentrated hydrochloric acid while constantly stirring the mixture. Finally, pale color was formed and stored in brown bottle at refrigerator.

2. Methyl Red reagents

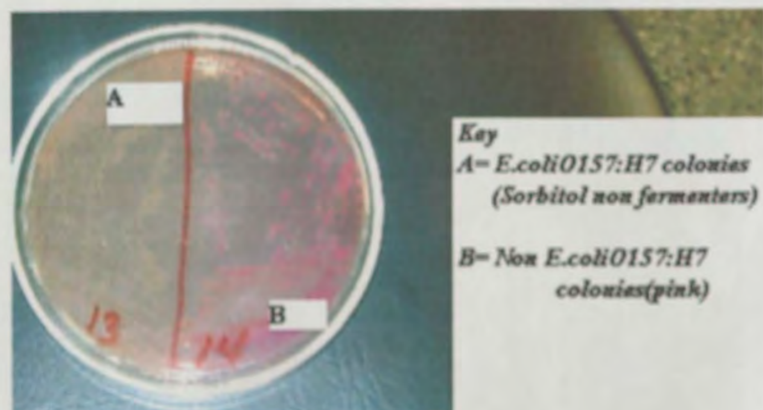
Preparation: Dissolve (0.1g) methyl red in 300ml alcohol and finally add 200ml-distilled water. Finally, red color was formed and stored in brown bottle at refrigerator.

3. Voges-Proskauer (VP) reagent

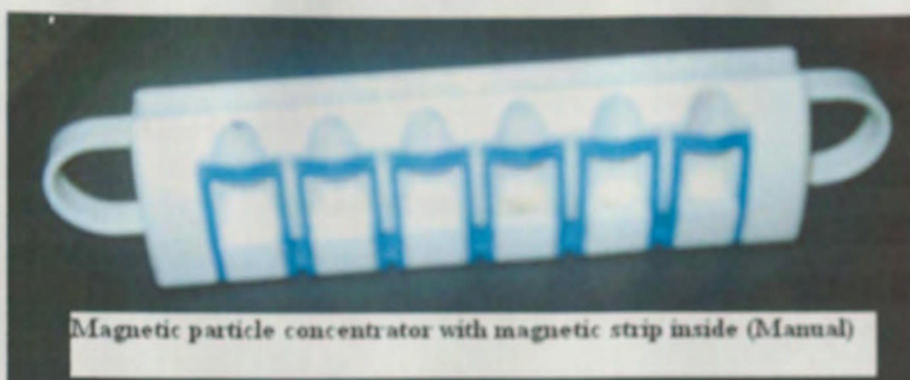
Preparation: *a) VP-reagent-1*: Dissolve 5g α-Naphthol (Sigma, Steinheim, Germany) in small amount of ethyl alcohol and bring to 100ml in flask. Alcohol should be color less. Then store in Brown bottle and in refrigerator. *b) VP-reagent-2*: Add less than 100ml distilled water to 40g pellets of KOH in cold water bath to prevent over heating and bring to 100ml. Finally 40% solution that store in polyethylene bottle at refrigerator.

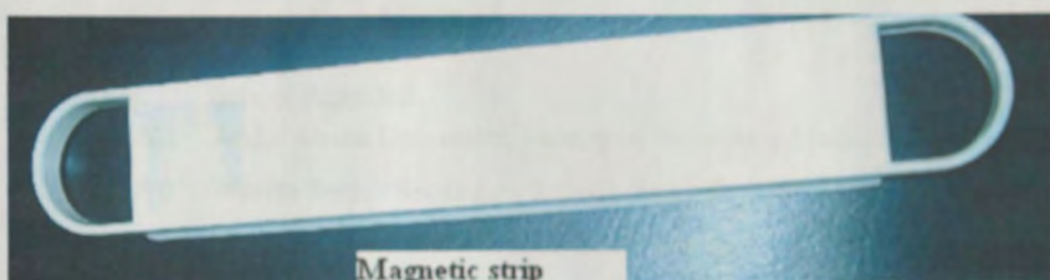
Appendix 5: Photo pictures showing colony morphology and materials used for the isolations of *E. coli* O157:H7

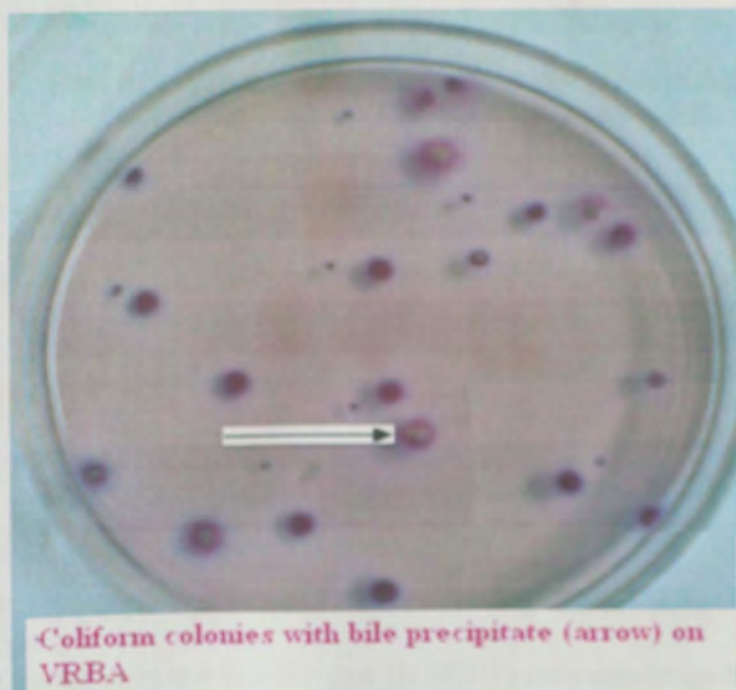
Latex agglutination test kits (full set)



*E.coli*O157 colonies on SMAC


Magnetic particle concentrator with magnetic strip inside (Manual)

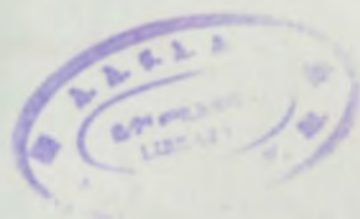

Magnetic strip


Coliform colonies with bile precipitate (arrow) on VRBA

9. CURRICULUM VITAE

A. Biographical Data:

Name	Gashaw Mersha Tessema
Date of birth	May11, 1977
Place of birth	Woreta, South Gonder, Ethiopia
Marital status	Single
Nationality	Ethiopian
Profession	Veterinarian
Occupation	Instructor



B. Educational background

Year	School Attended
1995- 2001	Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia
1989-1995	Woreta Senior Secondary School, South Gonder
1983- 1988	Seyifatra Elementary School, South Gonder

C. Work Experience

November, 2001 to June, 2002	Government employed field veterinarian in Lay Gayint District, North Gonder, Ethiopia
Since July 2002 to date	Senior instructor at Alage Agricultural Technical and Vocational Training Collage, MOARD

D. Research output/Technical paper

Gashaw Mersha and Moges Woldemeskel (2008): Study on Small Ruminants Dermatophillosis in and around Kombolcha, North East Ethiopia. *Eth. Vet. J.*, **12**:37-50.

E. Membership to professional societies

Member of Ethiopian Veterinary Association.
Member of Ethiopian Animal welfare association
Member of World Food Protection Association.

F. Language: Amharic and English (Writing and Speaking)

Contact address = E- mail: gmt_042001@yahoo.com or gashaw.m.tessema@freemail.et

10. 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. Moses N. Kyule (BVM, MSc., MPVM, PhD, Assoc. Prof.) ______________________

Dr. Daniel Asrat (MD, MSc., PhD. Assist. Prof.) ______________________