ESCHERICHIA COLI O157:H7: PREVALENCE AND SOURCES OF CONTAMINATION OF CATTLE MEAT AT MUNICIPAL ABATTOIR AND BUTCHERIES AS WELL AS ITS PUBLIC HEALTH IMPORTANCE IN DEBRE BREHAN, ETHIOPIA

MScThesis

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As members of the Examining Board of the final MSC open defense, we certify that we have read and evaluated the Thesis prepared by: **Rosa Abdissa** entitled, ***Eschericia coli* O157:H7: prevalence and source of contamination of cattle meat at municipal abattoir and butcheries as well as its public health importance in Debre-Brehan, Ethiopia** and recommended that it be accepted as fulfilling the thesis requirement for the degree of **Masters of veterinary public health.**

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LIST OF ABBRIVATIONS

%	percent
χ^2	Chi square
°C	Degree Centigrade
µL	Micro litter
µm	micrometer
A/E	Attaching and Effecting
AR	Acid Resistance
a_w	Water Activity
BCS	Body Condition Score
CDC	Center for Disease Control and Prevention
Cfu/g	Colony Forming Unit Per Gram
Cm	centimeter
CNS	Central Nervous System
CSLI	Clinical and Laboratory Standards Institute
CT-SMAC	Cefixime Tellurite-Sorbitol Macconkey Agar
CVMA, AAU	Collage of Veterinary Medicine and Agriculture, Adiss Ababa University
DAEC	Diffusellyadhiring *Eschericia coli*
DNA	Deoxyribo Nuclic Acid
E. coli	*Eschericia coli*
EaggEC	Enteroaggravetive *Eschericia coli*
EHEC	Enterohemmorhagic *Eschericia coli*
E-hlya	Enterohaemolysin Gene
EIEC	Enteroinvasive *Eschericia coli*
ETEC	Enterotoxigenic *Eschericia coli*
FDA	Food and Drug Administration Agency
FSAI	Food Safety Authority of Ireland
FSIS	Food Safety and Inspection Service
G+C	Guanine+ Cytocine

ABBREVIATIONS CONTINUED

GAP	Good Agricultural Practice
Gb3	Globotriaosylceramide
GIT	Gastro Intestinal Tract
gm	Gram
GMP	Good Manufacturing Practice
h	Hour
HACCP	Hazard Analysis Critical Control Point
HC	Hemmoragic Colities
HCP	Hemorrhagic Coli Pilus
HUS	Hemolytic Uremic Syndrome
IL	Interleukin
IMS	Immunomagnetic Separation
ISO	International Organization for Standardization
Kda	Kilo dextrose
LEE	Locus of Entrocyte and Effecment
LMIC	Low and Middle Income Countries
LPS	Lipopolysacchride
Mb	Mega Bite
MDR	Multi Drug Resistance
mg /l	Milligram Per Litter
Ml	Milliliter
Mm	Millimeter
MPC-S	Magnetic Particle Concentrator with the Magnetic Strip
MTSB	Modified Trepton Soya Broth
MTSB+N	Modified Trepton Soya Broth + Novobiocin
NPH	National Public Health Service
OIE	Office International des Epizootic/world organization for animal health
OPHS	Office of Public Health and Science

ABBREVIATIONS CONTINUED

PBST	Phosphate Buffered Saline + tween 20
PCR	Polymerase Chain Reaction
PO157	Plasmid O157
RAJ	Recto Anal Junction
rRNA	Ribosmal Ribonuclic Acid
SPATE	Serine Protease Autotransporters Enterobacteriaceae
STEC	Shiga Toxic *Eschericia Coli*
Stx	Shiga Toxin
Tir	Translocated Intimin Receptor
TSB	Trepton Soya Broth
TTP	Thrombotic Thrombocytopenic Purpura
TTSS	Type III Secretion System
USDA	United States Department of Agriculture
VT	vero toxin
VTEC	Vero Toxic *Eschericia Coli*
WBC	White Blood Cell
WHO	World Health Organization

ABSTRACT

The study was carried out from November 2013 to April 2014 with the objectives of determining the prevalence and source of contamination of *E. coli* O157: H7 from municipal abattoir and butcher houses, to assess the public health importance of this organism and to test the drug sensitivity pattern of the isolates in Debre-Berhan. A total of 1,132 samples from abattoir and 40 samples from butcher houses were examined. Samples from abattoir included hide swabs, internal and external carcass swabs, fecal sample, intestinal mucosal swabs and environmental samples (carcass in contacts, i.e. knife, workers hand, and clothing). Likewise, the samples from butcher houses were swab from carcass, butcher man's hand, cutting board and knife. In addition, stool samples from suspect individuals visiting Debre-Berhan referral hospital were collected. The samples were transported in Buffered peptone water to laboratory of the department of Biological science, Debre Berhan University in ice box and scrutinized according to international standards. From 220 animals slaughtered, a total of 1100 samples were taken and only 6 (0.54%) were found to be positive of *E. coli* O157:H7. *E. coli* O157:H7 was isolated from 2 (0.91%) of hide swab samples, 3 (1.36%) of feces samples and 1 (0.45%) of intestinal mucosal swab. There were no isolate found from 32 environmental samples, 20 stool samples and 40 swab samples from butcher shops. Prevalence of *E. coli* O157:H7 was significantly associated with the cleanness of the hide (P=0.036). All *E. coli* O157:H7 isolates were then checked for their susceptibility pattern against ten selected antibiotics. The isolates were completely susceptible to 7 antibiotics tested, namely Tetracycline (TE, 30 µg), Chloramphenicol (C, 30µg), Sulfamethoxazole-Trimitoprim (SXT, 25µg), Ciprofloxacine (CIP, 5µg), Nalidixic acid (NA, 30µg), Kenamycine (K, 30µg) and Sterptomycine (S, 10µg) but they were resistant to Amoxicillin (AML, 25 µg). Though, the prevalence of *E. coli* O157:H7 in present study is low, the impact of the pathogen should not be underestimated.

Key words: *Antibiotic, Buffered peptone, Debre Berhan, E. coli O157:H7, Swab Samples*

1. INTRODUCTION

In the globalized political economy of the late 20th century, increasing social, political and economic interdependence occurred as a result of the rapid movement of people, product and other commodities across national borders. A consequence of increased trade, travel and migration is the growing risk of transmitting biological and other hazards from country to country on a large scale (Harlan and Jacobs, 2008).

Foodborne illnesses are a major health problem in many countries, especially in developing countries. Many foodborne diseases are caused by the ingestion of food contaminated with pathogenic bacteria. The burden of foodborne disease, even in industrialized countries, remains substantial. It is estimated that 76 million cases of foodborne disease occur each year in the USA, with 325,000 hospitalizations and 5000 deaths linked to foodborne and waterborne diseases (CDC, 2006). Food-borne pathogens are able to survive in a diverse range of environmental niches, including those encountered in the mammalian intestine or on the surfaces of different vegetables. The ability to adhere is a vital first step in the successful colonization of these environments. Thus, organisms have acquired an array of fimbrial and afimbrial adhesins that mediate attachment to biotic and/or abiotic surfaces (Sonja *et al.*, 2012).

Most gastrointestinal microbial disease of domestic animals are caused by the bacteria belonging to the family *Enterobacteriacea* which includes most important genera; *Escherichia, Sigella, Salmonella* and *Yersinia* (Quinn *et al.*, 2002). Of which *Escherichia coli* (*E. coli*) is a common inhabitant of the human and animal gut, but can also be found in water, soil and vegetation and is the leading pathogen causing urinary tract infections and is among the most common pathogens causing blood stream infections, wounds, otitis media and other complications in humans causing many deaths in children under the age of five years (Turner *et al.*, 2006).

Most *E. coli* strains are harmless commensals; however, some strains are pathogenic and cause diarrhoeal disease. *E. coli* strains that cause diarrhoeal illness are categorized into

specific groups based on virulence properties, mechanisms of pathogenicity, clinical syndromes and distinct O: H serogroups. These categories include Enteropathogenic *E. coli* strains (ETEC), Enteroinvasive *E. coli* strains (EIEC), Diffuse adhering *E. coli* strains (DAEC), Enteroaggregative *E. coli* strains (EAggEC) and Enterohaemorrhagic *E. coli* strains (EHEC) (Fernandaze, 2008).

EHEC serotype O157: H7 is the most common human pathogen, first identified as a possible human pathogen in 1975 in a California patient with bloody diarrhea and was first associated with a foodborne (ground beef) outbreak of disease in 1982. This serotype (defined by its O and H surface antigens) and some non-O157 serotypes of *E. coli,* produce verocytotoxins, also called Shiga-like toxins because of their similarity to toxins produced by *Shigella dysenteriae*. These *E. coli* are called VTEC (verocytotoxin-producing *E. coli*), STEC (Shiga-toxin producing *E. coli*), and also EHEC (Enterohemorrhagic *E. coli*) because of the symptoms they produce. Serotypes of VTEC bacteria may include different strains that differ in some virulence factors or other characteristics such as motility and sorbitol fermentation (Ellin *et al.*, 2006).

E. coli O157:H7 is gram-negative, rod-shaped, flagellated, motile by peritrichous flagella or nonmotile, oxidase negative, straight cylindrical rods measuring 1.1-1.5 x 2.0-6.0 μm and facultative anaerobe which has both a respiratory and a fermentative type of metabolism. It can grow in temperatures ranging from 7°C to 50°C, with an optimum temperature of 37°C. It is destroyed by thorough cooking of foods until all parts reach a temperature of 70°C or higher (Buxton and Fraser, 1977).

The diseases caused by *E. coli* O157:H7 affect all age groups and the pathogen is exceptional in its severe consequences of infection as a result of its low infectious dose and unusual acid resistance (Fratamico and Smith, 2006). Depending on immune status, general health, dose of bacteria, and virulence factors of the bacteria, persons exposed to VTEC may experience mild diarrhea, severe bloody diarrhea, hemorrhagic colitis, or hemolytic uremic syndrome (HUS) with kidney failure. Some cases, usually among

young children, who are <5.5 years of age are more likely to develop HUS, and older people, are fatal (Boyce *et al.*, 1995).

Cattles (dairy and beef) are the primary reservoir of *E. coli* O157, and ground beef remains a significant source of food-borne transmission with other sources such as fresh vegetables and contaminated water. Cattle that excrete *E. coli* O157:H7 more than 10^4 colony forming unit (CFU)/g of feces have been defined as super-shedders. The super-shedders are responsible for about 90% of the total number of bacteria in the cattle herd and raise the prevalence of cattle infected with this pathogen on farms, making them a high risk factor at the pre-harvest level (Matthews *et al.*, 2006). However, colonization of this pathogen in cattle is usually asymptomatic due to the lack of Shiga toxin receptor, globotriaosylceramide (Gb3), in cattle endothelial cells, that prevents elimination of super-shedding cattle contaminated with this pathogen at farms (Lingwood, 1996).

E. coli O157:H7 in ruminant feces may be directly ingested by persons interacting or working with animals. Fecal material may contaminate meat during slaughter, may enter lakes or drinking water sources by action of rain or wind, and may be deposited on fruits and vegetables inadvertently or by use of manure for fertilization. In addition, some animals may transport these bacteria from a fecal source to drinking water or foods. All of these routes are variations of a pattern "from turd to tongue" (Pennington, 2000).

Person-to-person spread of *E. coli* O157:H7 also has been the primary mode of infection in many outbreaks in day cares, schools and hospitals, particularly where there have been lapses in hygiene. In many other outbreaks, some of the cases who consumed contaminated food or water passed the infection directly to others. Although a majority of children infected with *E. coli* O157:H7 shed these bacteria in their feces for only a few days, in more seriously ill children, cells of *E. coli* O157:H7 may be shed for 20–30 days or longer. VTEC bacteria may be present in stool samples even after children become asymptomatic (Karch *et al.*, 1995).

Depending on the specific strain, *E. coli* O157:H7 can produce both Shiga toxins 1 (Stx1) and 2 (Stx2) that are responsible for hemorrhagic colitis. It has a very low infectious dose

(as low as 50 CFU in one outbreak) and colonizes the intestinal epithelial cells, where it causes attaching and effacing lesions (Nataro and Kaper, 1998).

Antibiotic use in VTEC infections is controversial because some studies have indicated that antibiotics or antimotility agents increase risk for HUS and they have the potential to increase production and secretion of Shiga toxins (Zhang *et al.*, 2000). However antimicrobial resistance in *E. coli* has been reported worldwide and increasing rates of resistance among *E. coli* is a growing concern in both developed and developing countries. Occurrence and susceptibility profiles of *E. coli* show substantial geographic variations as well as significant differences in various populations and environments (Erb *et al.*, 2007).

In Ethiopia, even though the rising incidence and the potentially serious nature of *E. coli* O157:H7 infection is a cause for concern to public health authorities, few studies have been carried out. Study made by Hiko *et al.* (2008), indicated that out of 738 meat samples examined, 31 (4.2%) were found to be contaminated with *E. coli* O157:H7. Beef was found to be most frequently contaminated with *E. coli* O157:H7 (8%) when compared to the other raw meat types examined and again Mersha *et al.* (2009), reported in his finding, which was carried out on sheep and goats, 53 (7.5%) were found to be contaminated with *E. coli* O157:H7. Of the 53 isolates, 16 (30.2%) were from goats, 36 (68.0%) were from sheep and 1 (1.8%) was from another sources. In developing countries of the world, where there is still an alarming rate of insanitary conditions, malnutrition and poor health facilities, there is an urgent need to study this organism and its characteristics with an aim to reduce the human hazard caused by this emerging pathogen.

The objectives of this research are therefore:-

- To estimate the prevalence of *E. coli* O157:H7 in the municipal abattoir in Debre Berhan.
- To identify the source of contamination of meat by *E. coli* O157:H7 in abattoir and butcheries so that major points where safety problem occurs along the meat chain can be determined.
- Survey for the presence of human infection in the area, in question and risk assessment study and its potential implications as health hazard to consumers.
- To identify the antimicrobial susceptibility patterns of *E. coli* O157: H7.

2. LITRATURE REVIEW

2.1. Etiology

E. coli was first described in 1885 by Theodor Escherich (1857-1911). Escherich, a Bavarian paediatrician, had performed studies on the intestinal flora of infants and had discovered a normal microbial inhabitant in healthy individuals, which he named ''Bacterium coli commune''. In 1919, the bacterium was renamed in his honour to *Escherichia coli* (Karch, 1995).

E. coli are a group of bacteria, with many benefits have been found from *E. coli* in human medicine, food industry, and the water industry. Some studies suggest that *E. coli* can serve as a benefit to the human body by synthesizing vitamin K and by using competitive inhibition to out compete other bacteria that might enter the intestinal tract (Law, 2000). Despite that, they inhabit the intestines of all humans and most animals. Most do not cause disease. The *E. coli* strains that cause diarrheal illness are categorized into pathogenicity groups based on virulence properties, mechanisms of pathogenicity, clinical symptoms and serology (Yngvild, 2001).

Differences between strains of *E. coli* lie in the combination of different antigens they possess. There are three types of antigens: the somatic lipopolysaccharide antigen (O), the flagellar antigens (H), and the capsular antigens (K). There are approximately 174 O antigens, 56 H antigens, and 103 K antigens that have been identified (Fratamico *et al.*, 2002).

E. coli O157: H7 and some non-H7 types are important emerging food-borne pathogens of public health concern in most countries of the world. This was first recognized as a cause of illness in 1982 during an outbreak of severe hemorrhagic colitis and gastroenteritis with a bloody diarrhea traced to consumption of hamburgers at common chains of fast food restaurants in Michigan and Oregon (Armstrong *et al.*, 1996). The causative agent was isolated and recognized to be different in both pathogenicity and

virulence from previously identified *E. coli* strains, expressing new characters as O-antigen 157 and H-antigen 7, and categorized under EHEC which is now believed to account for over 90% of all cases of HUS in industrialized countries (Mead and Griffin, 1998).

From zoonotic point of view, EHEC is the only *E. coli* pathogenicity group of major interest, as the shiga toxin-producing strains are able to cause severe disease in humans when being transmitted through the food chain from their animal reservoirs (Yngvild, 2001). Three unique characteristics of *E. coli* O157:H7 that distinguish it from other *E. coli* strains are its dangerous health implications in which infection can lead to renal (kidney) damage and can be fatal, *E. coli* O157:H7 survives at low temperatures and under acidic conditions and finally it needs very small infective dose (from 10 to 100 cells) which are sufficient to cause disease (Petridis *et al.*, 2002).

EHEC bacteria contain one or more virulence attributes: the ability to produce two types of shiga-like toxin(s), namely Shiga toxin 1 (Stx-1) and/or Shiga toxin 2 (Stx-2), which have been also referred as Verotoxin 1 (VT-1) and Verotoxin 2 (VT-2) respectively responsible for food borne illness, adherence factor(s) and enterohemolysin (Griffin, 1995).

2.1.1. Taxonomy and Classification

E. coli is bacteria in the phylum *Proteobacteria*, class *gamma Proteobacteria*, order *Enterobacteriales*. *E. coli* is the type species of the genus *Escherichia*, which contains mostly motile gram-negative bacilli within the taxonomic family *Enterobacteriaceae* ("the interics") which includes many genera, including known pathogens such as *Salmonella, Shigella,* and *Yersinia* (Ewing, 1986).

E. coli is genetically the most versatile bacteria and is the source of many plasmid and phage mediated genes. Serotyping and serogrouping of *E. coli* is used for subdividing the species into serovars (Yaoxin, 2011). There are several stains of *E. coli* that have been

isolated. The enteric *E. coli* are divided on the basis of virulence properties into enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroinvasive (EIEC), verotoxigenic (VTEC), enterohemorrhagic (EHEC), and enteroaggregative (EaggEC). ETEC can be found in humans, pigs, sheep, goats, cattle, dogs, and horses; EPEC is found in humans, rabbits, dogs, cats, and horses; EIEC and EAggEC are only found in humans; VTEC is found in pigs, cattle, dogs, and cats; while EHEC is found in humans, cattle, and goats and attack porcine strains that colonize the gut in a manner similar to human EPEC strains (Fratamico *et al.,* 2002).

2.1.2. Morphology and Biochemical Characteristics

E. coli O157:H7 is gram-negative, rod-shaped, flagellated, motile by peritrichous flagella or non-motile, oxidase negative, straight cylindrical rods measuring 1.1-1.5 x 2.0-6.0 μm and facultative anaerobe which has both a respiratory and a fermentative type of metabolism (Buxton and Fraser, 1977). The chromosomal size of *E. coli* O157:H7 is 5.5 Mb. This genome includes a 4.1 Mb backbone sequence conserved in all *E. coli* strains. The remaining are specific to *E. coli* O157:H7 (Perna *et al.,* 2001). Additionally, genome comparison of *E. coli* O157:H7 with nonpathogenic *E. coli* shows that 0.53 Mb of DNA is missing for *E. coli* O157:H7, suggesting genomic reduction has also played a role in *E. coli* O157:H7 evolution. The majority of *E. coli* O157:H7 specific DNA sequences (1.4 Mb) are horizontally transferred foreign DNAs such as prophage and prophage-like elements. *E. coli* O157:H7 contains 463 phage associated genes compared with only 29 in non pathogenic *E. coli.* A change in G+C contents is one of the indications that a genomic region has been acquired by horizontal transfer (Wick *et al.*, 2005).

Putonti *et al.* (2006) estimated that at least 53 different species have contributed to these unique sequences in *E. coli* O157:H7. Virulence-associated genes between two sequenced *E. coli* O157:H7 strains are nearly identical (99%). Clearly, both the acquisition and loss of DNA have played an important role in the evolution of pathogenesis of *E. coli* O157:H7.

Since the organism's first recognition as a human pathogen in 1982, diagnostic screening assays that capitalize on this difference have become widely used in clinical laboratories (Wells *et al.*, 1983). Most biochemical reactions of *E. coli* O157:H7 are typical of *E. coli,* with the exception of sorbitol fermentation and β-glucuronidase activity. About 93% of *E. coli* isolates of human origin ferment sorbitol within 24 h; however, *E. coli* O157: H7 was reported as not fermenting sorbitol. Additionally, 93% of *E. coli* possesses the enzyme β-glucuronidase but the vast majority of O157 VTEC does not produce β-glucuronidase (Okrend *et al.*, 1990).

It can grow in temperatures ranging from 7°C to 50°C, with an optimum temperature of 37°C. It is destroyed by thorough cooking of foods until all parts reach a temperature of 70°C or higher (Buxton and Fraser, 1977). Growth studies in tryptone soy broth (TSB) indicated that the organism grows rapidly between 30 and 42°C, with generation times ranging from 0.49 h at 37°C to 0.64 h at 42°C. The organism grows poorly at 44-45°C and does not grow within 48 h at 10 or 45.5°C. Many procedures to detect faecal coliforms and subsequently *E. coli* in food use incubation temperatures in the range of 44-45°C. Hence, *E. coli* O157:H7 would not likely be detected in normal screening for faecal coliforms by standard procedures with incubation at 44.5°C (Doyle and Schoeni, 1984; Raghubeer and Matches, 1990).

2.2. Virulence Factor and Pathogenesis

It is clear that STEC are highly pathogenic and that virulence is not dependent on a single gene or gene product but is a multi-factorial process. The organism can survive in water and a wide range of foods including acidic products and then remain viable after transit through the acidic environment of the stomach, colonization of the bowel, mediated by one or more of a range of potential adhesions then occurs. Following colonization, several toxins and other proteins are produced which may assist with survival and multiplication in the intestinal environment. Elaboration of potent Shiga toxins into the gut lumen causes intestinal damage and eventually systemic complications (Law, 2000). Pathogenicity of *E. coli* strains are due to the presence of one or more virulence factors

including invasiveness factors like invasins, heat labile and heat stable enterotoxins, verotoxins and colonization factors or adhesins (Smith, 1967).

The major virulence factors of *E. coli* O157:H7 have been identified including a pathogenicity island called the Locus of Enterocyte Effacement (LEE), Shiga toxins (Stx) and the plasmid (pO157) encoded enterohaemolysin gene (E-hlyA) that codes for a pore-forming cytolysin. *E. coli* O157:H7 colonization of the intestinal mucosa induces a histopathologic lesion defined as an "attaching and effacing" (A/E) lesion characterized by localized destruction of brush border of microvilli and intimate attachment of the bacteria to host cell plasma membranes (Karpman *et al.*, 2002).

2.2.1. The Locus of Enterocyte Effacement (LEE)

The LEE carries genes for the attaching and effacing phenotype promoting bacterial adhesion and the destruction of human intestinal enterocytes (Vilte *et al.*, 2008). The LEE can be divided into several distinct regions. At one end are genes EspA, EspB and EspD, encoding secreted proteins required for signal transduction and A/E activity. At the other end lie the genes encoding a type III secretion system (TTSS), required for secretion of proteins including the products of EspA, EspB and EspD. A third region between the others contains the *eae* gene encoding intimin, an outer membrane protein required for intimate attachment, and Tir, encoding the protein Tir (translocated intimin receptor) which acts as a receptor for intimin which is a bacterial outer membrane protein that plays a major role in attachment and production of A/E lesions characterized by effacement of microvilli (Kenny *et al.*, 1997; Frankel *et al.*, 1998; Kaper *et al.*, 2004).

DeVinney *et al.* (1999) said that, the pathogenesis process by which these bacteria become attached to the mucosa of the distal ileum and the colon is complex and likely starts by bacterial fimbrial attachment followed by translocation of the bacterial Tir protein to the host cell membrane. LEE is multifilament needle complex, used for insertion of the bacterial effecter proteins EspB, EspD and Tir into the host cell. They genetically govern adhesion and subsequent pathology. Several LEE-encoded genes

mediate the adherence of *E. coli* O157:H7 to intestinal epithelial cells by a characteristic attaching-and-effacing mechanism. Injection of bacterial virulence factors via the TTSS and binding of intimin to Tir leads to a strong interaction between bacteria and host cells (Jerse *et al.*, 1990).

2.2.2. Shiga toxins (stxs)

Once bacteria have adhered to the bowel mucosa they will grow and secrete an array of extracellular products including the potent cytotoxins known as Shiga toxins. There are two major antigenically distinct forms of toxin, Stx1 and Stx2 which are the primary factors responsible for the hemorrhagic aspect of diarrhea and systemic complications (haemolytic uraemic syndrome (HUS)). Although the toxins are probably not necessary for triggering the diarrhea, they most likely cause the intestinal lesions, characterized by hemorrhage and ulcerations, via damaging the micro vasculature of the intestinal wall. Shiga toxins act as N-glycosidases, cleaving ribosomal RNA leading to the inhibition of host cell protein synthesis (Endo *et al.*, 1988; Tesh and O'Brien, 1991).

Stx1 is very similar to the type 1 toxin of *Shigella dysenteriae*; whilst Stx1 is homogeneous there are numerous variants of Stx2; however, Stx1 and Stx2 share approximately 60% DNA and amino acid homology but are immunologically distinct. Possession and expression of the Stx2 gene and the variant Stx2c (which often occurs with Stx2) correlate strongly with the causation of bloody diarrhea and HUS. Both toxins (stx1 and stx2) are compound toxins consisting of an A subunit (32 kDa) and a pentameric B subunit (7.7 kDa monomers). The B subunits form a hollow ring and the C-terminus of the A subunit is inserted into this. The B subunits mediate binding to receptors in eukaryotic cell membranes and the receptors have been identified as globotriaosylceramide (Gb3), although the receptor for one Stx2 variant, Stx2e, is Gb4. Once bound to the target cell membrane, toxin molecules are internalized by a receptor mediated endocytic mechanism. Toxin-containing vesicles are formed; in some cells the vesicles undergo fusion with lysosomes resulting in toxin degradation. In other cells,

following processing in the Golgi apparatus and endoplasmic reticulum, the A subunit is 'nicked' by a protease generating a catalytically active 27-kDaA1fragment and a 4 - kDaA2 subunit. The released A1 subunit has RNA N-glycosidase activity and cleaves a specific bond in 28S rRNA, this cleavage prevents binding of amino acyl-tRNA to 60s ribosomal units, inhibiting the peptide chain elongation step of protein synthesis leading to cell death (Sandvig and Deurs, 1996).

Owing to its pathology, once the gut-blood barrier has been compromised by intestinal da mage, Shiga tox-ins induce an increase in chemokine synthesis from intestinal epithelial cells probably augmenting host mucosal inflammatory responses with release of IL-8, TNF, and IL-1. Activation of human endothelium by TNF or IL-1 leads to an increase in expression of the Shiga toxin cellular receptors which leads to an increased cell death after exposure to the toxins. Since the toxin receptors are widely distributed on various types of cells, thus many host tissues are affected (Meyers and Kaplan, 2000).

In the human kidney, Gb3 is present on glomerular endothelial cells, podocytes, and various tubular epithelial cell types. Shiga toxin binds to these cells in renal sections from patients with haemolytic uraemic syndrome, and damage markers from these cells can be detected in their urine; biopsy samples from these patients show apoptosis of glomerular and tubular cell types and fimbrin-rich glomerular microangiopathy (Pennington, 2010).

2.2.3. Plasmid O157 (PO157)

EHEC O157 possesses a large virulence plasmid of approximately 90 Kb termed pO157. The nucleotide sequence of this plasmid showed that it encodes 35 proteins, some of which are presumably involved in the pathogenesis of EHEC infections (Burland *et al.*, 1998). The *hly* operon encodes four ORF necessary for the synthesis and transport of the enterohaemolysin and confers to EHEC the enterohaemolytic phenotype. The *hly* operon is considered as the best marker of the presence of pO157 and is also present in large plasmids that can be detected in most non-O157 EHEC strains (Brashears *et al.*, 2003). PO157 also carries a type II secretion system related to the pullulanase secretion pathway

of *Klebsiella*, but its function has yet to be elucidated. Other putative virulence factors harbored by this plasmid comprise a katalase-peroxyidase and a serine protease, encoded by *katP* and *espP* genes, respectively (Schmidt *et al.*, 2001).

An F-like 90-kb plasmid, pO157, is found in most *E. coli* O157:H7 clinical isolates and pO157 shares sequence similarities with plasmids present in other EHEC serotype. Putative virulence factors encoded by pO157 include enterohemolysin (*ehxA*), the general secretory pathway (*etpC* to *etpO*), serine protease (*espP*), catalase-peroxidase (*katP*), a potential adhesion (*toxB*), a Cl esterase inhibitor (*stcE*) and attaching and effacing gene-positive conserved fragments (*ecf*) (Jiyoun *et al.*, 2007).

2.2.4. Other Virulence Factors

While the LEE, pO157 and shiga toxin production is defining virulence factors of *E.coli* O157:H7, other factors contribute to its pathogenecity. Some strains harbors EspP, which belongs to the family of serine protease autotransporters *enterobacteriaceae* (SPATE). This protease cleaves pepsin A and human coagulation factor V, which probably contribute to increased hemorrhage into the intestinal tract. Moreover, EspP cleaves multiple complement system components hence protecting the bacterium from immune system mediated elimination (Orth *et al.*, 2010).

Low *et al.* (2006) showed on the other hand that, in addition to LEE members such as intimin and Tir, bacterial attachment to host intestinal cells is also mediated by a type IV pilus referred to as the hemorrhagic coli pilus (HCP). Multiple fimbriae and afimbrial gene clusters have also been implicated in contributing to adherence of this organism to host cells.

The acid resistance (AR) ability of bacteria is another factor to protect themselves from extremely low pH (<pH 3.0). The low pH in the stomach (pH 1.5 to 3.0) is one of the first host defenses against food borne enteric pathogens. The ability to survive in the acidic environment of the stomach increases the chance of bacteria to colonize the intestines and

cause infection. AR is associated with a lowering of the infectious dose of enteric pathogens. The low infectious dose (10-100 colony forming units (CFUs)) is one of the best known characteristics of *E. coli* O157:H7, making this bacterium highly infectious (Schlech *et al.,* 1993; Mead and Griffin, 1998).

Lipopolysaccharide (LPS) can by itself, independent of Shiga toxins, is another factor that damage endothelial cells, increasing TNF levels, activate platelets and induce the blood coagulation cascade. It can also increase levels of interleukins such as IL-8, which is a potent activator of white blood cells (WBCs). WBCs participate in the pathogenic process by elaborating tissue-damaging enzymes such as elastase (Meyers and Kaplan, 2000).

2.3. Epidemiology

A large number of the bacteria that cause enteric disease in humans and animals, as well as those that comprise the normal gut flora, are members of the family *Enterobacteriaceae.* These are commonly transmitted via contaminated food and water from human sources, or to humans and animals from material contaminated with faces from other animals. The epidemiology of these food borne pathogens depends on locality, food preparation practices, food preferences, hygiene, access to clean water, level of community education and availability of public health services, as well as on the development and enforcement of food and water safety regulations (Bhunia, 2008).

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 the region of South America, especially Argentina, where HUS is endemic (Bolton *et al.*, 1996). The highest incidence occurs in children, elders and immunocompromised people (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 feces of other animal 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 in feces has been also reported in foods of animal origin, in countries like Ireland, Norway, Canada, Spain, Italy, Egypt (DeBoer and Hauvelinek, 2001) and Ethiopia (Mutaku *et al.*, 2005; Hiko *et al.*, 2008; Mersha, 2008) and this generally show that the worldwide distribution of the organism (Baron *et al*, 1994).

The cardinal symptom of food borne diseases is diarrhea. It is estimated that 1400 million episodes of diarrhoea occur annually in children under five years, and up to 70% of these episodes are due to pathogens transmitted through food. One point eight million people in the developing world die each year from complications associated with diarrhea (WHO, 2008). The impact of specific pathogens on human health will depend on the virulence of the pathogen and the susceptibility of the host, as well as on the level of exposure, presence of co-infections and host immune status. The epidemiological picture depends on a wide range of other factors, including the predominant method of disease transmission, the requirement, or not, for disease vectors or intermediate hosts, the role of reservoir hosts, the demographics of the human population that is exposed and whether or not the pathogen is an opportunistic or an obligate pathogen (Susan, 2011). *E. coli* O157:H7 has been isolated from ill people around the world. It tends to be reported more often from more developed countries but this may be an artifact caused by the paucity of sophisticated diagnostic laboratories in developing countries. FoodNet data indicate that *E. coli* O157:H7 causes significantly more cases of sporadic infections than cases linked to an outbreak (CDC, 2006). Factors that cause outbreaks include undercooked hamburgers and exposure to farms and cattle. Some sporadic infections are also associated with use of immunosuppressive medications and dining at table service restaurants (Kassenborg *et al.*, 2004).

More recently a number of outbreaks have occurred among children visiting farms and petting zoos where they come into direct contact with animals carrying *E. coli* O157:H7 and their environment (Grif *et al.*, 2005). The role of environmental sources in the ecology and epidemiology of *E. coli* O157:H7 in cattle operations is still unclear;

however, it's very probable that environmental sources are involved in the maintenance, multiplication, and/or transmission of this organism both within and between cattle farms. Outbreaks may also result from airborne dispersion of bacteria in buildings used to show animals. Finally, direct person to person infection occurs particularly among children and their caregivers, such as in day care facilities and also within families (Bender, 2005).

Pei-ying hong *et al.* (2011) said that, the variable growth of different species under different culture conditions and with different selective media can have a significant impact on reported prevalence's and species distributions around the world. In industrialized countries the infection is often seasonal, and targets mostly young children, but is also found in older people. In developing countries the infection is mostly restricted to children, and does not show clear seasonality. This difference is believed to principally reflect earlier acquisition of immunity in patients in developing countries following multiple exposures to infections and not the existence of geographical variation in strain (patho) types.

2.4. General Framework

2.4.1. Reservoirs and Source of E. coli O157: H7

Several reservoirs and sources of *E. coli* O157:H7 have been identified. Ruminants have been identified as the major reservoir of *E. coli* O157:H7, with cattle as the most important source of human infections. Other ruminants known to harbor these bacteria include sheep, goats, and deer. STEC bacteria are occasionally isolated from other animals but it is believed that the bacteria are present as transients and that the animals acquire these bacteria from meat, foods, or water contaminated by fecal material from ruminants. STEC bacteria usually do not cause illness in animals with a few exceptions such as diarrhea in calves (Sanderson *et al.*, 1999). The association of *E. coli* O157:H7 with undercooked ground beef led to investigations of the role of cattle as a reservoir of the pathogens. Generally, the source-sink model describes the natural history of human infections with *E coli* O157 well in that man is the sink (a dead end in terms of the long-

term survival of the organism) and ruminants, particularly cattle and sheep in which the organism is not pathogenic, the source (Fernandaze, 2008; Pennington, 2010).

Cattle

Cattle are the natural reservoir of *E. coli* O157:H7. Between 1% and 50% of healthy cattle carry and shed *E. coli* O157:H7 in their feces at any given time. Cattle are probably the most important ultimate source of infections for humans. VTEC have been detected in calves, dairy cows and beef cattle worldwide. Prevalence of these bacteria in cattle and their excreta appears to vary seasonally as well as with the age of the animals and other factors. Examination of naturally and experimentally infected calves and cattle demonstrated that most *E. coli* O157:H7 adhere to mucosal epithelium in the lymphoid follicle-dense mucosa at the terminal rectum, called the recto-anal junction (RAJ) mucosa, a short 5 cm long region just proximal from the recto-anal junction. As a result, *E. coli* O157:H7 is present predominantly on the surface of the cow pats (Low *et al.*, 2005).

Three distinct patterns of *E. coli* O157:H7 carriage in cattle has been described. First, animals can be transiently culture positive for short durations of a few days and are considered passive shedders and are likely not colonized at the RAJ mucosa. Second, cattle can be colonized and shed the bacteria for an average of 1 month and typically not longer than 2 months. Third, a few rare animals are colonized for a long duration and shed the bacteria from 3 to 12 months or longer. This unique situation, in which a few animals develop long-duration colonization (>2 months) with *E. coli* O157:H7 is likely due to bacterial association at the RAJ mucosa; however, it may be due to unique colonization by the bacteria at a site(s) in addition to the RAJ mucosa (Cray and Moon, 1995).

On the other hand widespread prevalence in cattle has been attributed to the organism's ability to survive for at least 4 months in water trough sediments, providing an ongoing source of exposure to cattle. *E. coli* O157:H7 is also present in purchased animal feeds;

therefore, such feeds may be an important route by which *E.* coli O157:H7 is disseminated to farms. From the farms, *E. coli* O157:H7 contamination of meat occurs when beef carcasses come into contact with hides and feces during the slaughter process (Hancock *et al.*, 2001).

Cray and Moon, (1995) reported that age, diet and immunity of individual cattle could also potentially affect bacterial colonization. Calves shed *E. coli* O157:H7 longer than adult cattle given the same level of *E. coli* O157:H7 inoculums.

Other ruminants

The prevalence of *E. coli* O157:H7 in small domestic ruminants is less well documented than in cattle, and reports vary considerably that they are another significant source of *E. coli* O157:H7 for human infection (McCarthy *et al.*, 2001).

Mersha *et al.* (2009), investigated that out of 711 different samples examined, 53 (7.5%) were found to be contaminated with *E. coli* O157:H7, of the 53 isolates, 16 (30.2%) were from goats, 36 (68.0%) were from sheep in export abattoirs in Ethiopia whereas, Battisti *et al.,* (2006), reported a prevalence of 0.2% in lambs taken to slaughter in Italy. Sheep products are important sources of *E. coli* O157:H7 and have also been cited as reservoirs for a diverse number of non-O157 serogroups (including O26, O91, O115, O128 and O130) that encode a key colonization factor in common with *E. coli* O157:H7 (Kalchayanand *et al.,* 2007).

Several recent reports have clearly identified goats as sources of *E. coli* O157:H7 infection. In that, goats (as with other ruminants) can be subclinical carriers and excretors of *E. coli* O157:H7. Not only can goats be colonized with *E. coli* O157:H7, but their innately inquisitive behavior means that they are much more likely than sheep to be in regular direct contact with humans, consequently increasing the risk of the direct fecal–oral transmission of zoonotic infection. Outbreaks of human disease have also been linked to cheese made from unpasteurized goats' milk (Fox *et al.,* 2007).

Environment

E. coli O157:H7 can survive and persist in numerous environments such as soil, water and food. *E. coli* O157:H7 has been shown to survive for a year in manure-treated soil and for 21 months in raw manure that had not been composted (Jiang, 2002). *E. coli* O157:H7 can survive for a long time in water, especially at cold temperatures. Water trough sediments contaminated with bovine feces can serve as a long-term (>8 months) reservoir of *E. coli* O157:H7 and the surviving bacteria in contaminated troughs is a source of infection (Lejeune *et al.*, 2001).

E. coli O157:H7 has the ability to adapt to variations or extreme changes in temperature, pH, and osmolarity conditions commonly encountered in nature. These environmental adaptations of *E. coli* O157:H7 play an important role in the persistence and dissemination of this microorganism on farms and the increasing transfer from cattle to cattle. In addition, the ability to survive outside the host reservoir increases the risk that the pathogen may contaminate crops and produce via bovine manure contamination, irrigation with contaminated water, or direct contact with infected animals (Maule, 2000).

2.4.2. Mode of Transmission

There are four principal routes of infection identified for *E. coli* O157:H7 to be transmitted. These are food borne, person-to-person, direct contact with infected animals and transmission through the environment (Boyce *et al.*, 1995; Mead and Griffin, 1998; CDC, 2005). .

E. coli O157:H7 has been a major concern in the meat industry for decades and has increasing concerns with the development of new processing techniques. A cascade effect of *E. coli* O157:H7 can be seen during the slaughter and production process. 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. *E. coli* O157:H7 in the feces of cattle can be transferred to the hide. The feces on the hide are transferred to the carcasses during the de-hiding process and from the carcass the knives and saws become a vector to transfer *E. coli* O157:H7 onto other cuts of meat. The contaminated cuts of meat are then ground and added to other animal's cuts of meat. This is a possible cascade of events that can lead to massive amounts of ground products contaminated with *E. coli* O157:H7 (Laury *et al.*, 2009). Transmission of *Escherichia coli* O157:H7 among reservoir animals is generally thought to occur either by direct contact between a naive animal and an infected animal or by consumption of food or water containing the organism (Johnson *et al.*, 2001).

In man, although a variety of foods have been implicated in *E. coli* O157:H7 associated illness; most outbreaks have been associated with food that has come in contact with contaminated meat or feces (human or bovine). The most commonly identified risk factor in case-control studies of sporadic *E. coli* O157:H7 illness was consumption of undercooked ground beef (Petridis *et al.*, 2002).

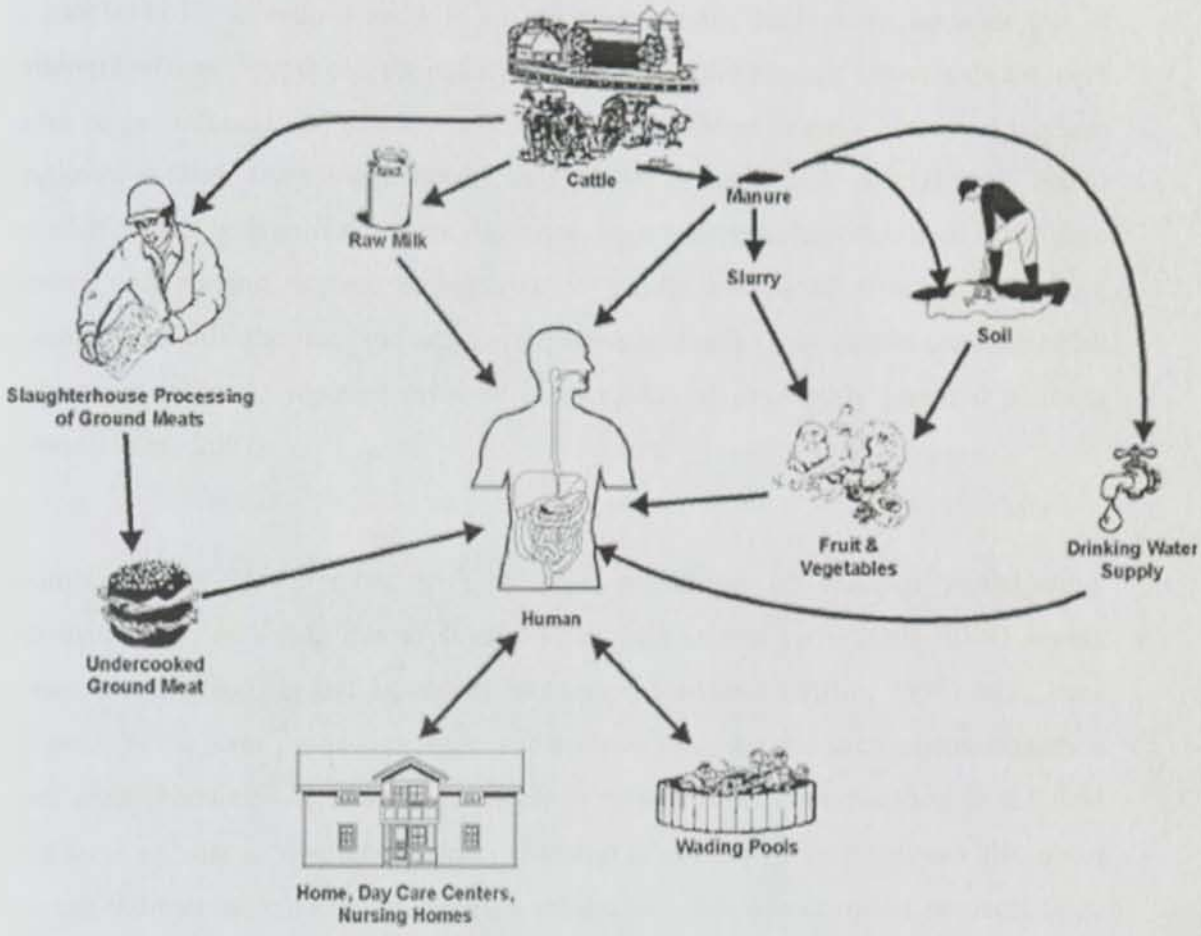

Fig.1. Sources and Transmission route of *Echserichia coli* O157:H7 infection to human. Source: Petridis *et al*., (2002).

E. coli O157:H7 infections also were associated with eating other foods, including vegetables and apple cider due to the use of raw manure or slurry (liquid manure) on or near fruit and vegetable crops, particularly those that are to be eaten raw, is a potential hazard since *E. coli* O157:H7 can be present in animal feces (Collins and Wall, 2004). Serious outbreaks of infection from *E. coli* O157:H7 have also been associated with the consumption of raw or unpasteurized milk (Fernandaze, 2008) and drinking or swimming in contaminated or un-chlorinated water. Because of low infectious dose of the organism, swallowing a small amount of lake water can cause illness (Akashi *et al*., 1994; Feng, 1995).

E. coli O157:H7 infections occur in all age groups; with the highest incidence rate in children less than 5 years old, the elderly and immune-compromised individuals are more likely to get infected and develop complications. Residents of rural areas and workers employed at farms and slaughterhouses have greater exposure to *E. coli* O157:H7 due to possible contact with animal manure. However, large outbreaks have occurred in day care centers and nursing homes, posing risk to people associated with such settings. Consumers of other bovine products (i.e. unpasteurized milk) may also be at a higher risk since most outbreaks reported involved consumption of improperly prepared products (Petridis *et al.*, 2002).

Animal contact is common, with a large proportion of children participating. Occupationally, outbreaks due to direct contact with animal visits (OIE, 2004) among nurses, microbiologists and laboratory workers (Mead and Griffin, 1998) have been reported. While some production 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). Infection can also occur through indirect animal contact, through interaction with contaminated products (e.g., sawdust, shavings, visibly soiled clothes or shoes), or in areas adjacent to contaminating sources, such as playgrounds located near animals. The immediate source of most bacteria on carcasses after slaughter is the contaminated hide (Collins and Wall, 2004).

Another pathway is that of person-to-person transmission of EHEC, which may contribute to outbreaks from a primary source. Asymptomatically infected people may be an unappreciated source of the pathogen due to poor personal hygiene (not washing hands properly after using the toilet and after handling raw ground beef) and unsafe food preparation practices which increase the risk of spreading O157:H7 infection (Armstrong *et al.*, 1996).

The types of food associated with outbreaks and the geographical distribution of cases differ between countries. These differences are an indicator of local food preferences, culinary customs, and patterns of food distribution (Pennington, 2010).

2.4.3. Nature of Illnesses

Gyles, (2007) said that ingestion of *E. coli* O157:H7 results in a wide range of possible outcomes, from asymptomatic infection to death. To cause disease, the *E. coli* O157:H7 must survive acidic conditions within the stomach before moving to distal portions of the gastrointestinal tract. Its principal symptom, diarrhoea, is also a symptom of other gastrointestinal infections.

In Animals

Most *E. coli* O157:H7 infected cattle remain free of disease and are tolerant of *E. coli* O157:H7 for most of their lives. However, *E. coli* O157:H7 does cause fatal ileocolitis in newborn calves. In this regard it is interesting to note that, in contrast to humans, neither clinically affected calves nor older carrier cattle with *E. coli* O157:H7 infections develop extra intestinal vascular lesions. Furthermore, calves clinically infected with other serotypes of Stx-producing *E. coli* do not develop extra intestinal vascular lesions or systemic manifestations of disease. The tolerance of adult cattle for *E. coli* O157:H7 infection and the lack of systemic vascular damage in calves and adult cattle with *E. coli* O157:H7 infection results due to lack of receptors for Stx (Dean-Nystrom *et al.,* 1997).

The preferred receptor for Stx1, Stx2 and for Stx2e (a variant of Stx2 produced by strains of *E. coli* that cause edema disease of swine) is globotetraosylceramide (Gb3). Humans, rabbits, and pigs have vascular receptors for Stx and develop Stx-mediated vascular damage. Rabbits have Stx receptors on intestinal villous epithelial cells, and Stx is enterotoxic (impairs NaCl absorption, causing intestinal fluid accumulation and diarrhea) in rabbits (Kandel *et al.,* 1989; Boyd *et al.,* 1993).

In humans

E. coli O157 infections are associated with a range of illness in humans, although a proportion may be asymptomatic. Where symptoms do occur, the incubation period is 2 to 10 days, with most cases occurring in 3 days. Persons who are infected can develop a range of symptoms, including mild diarrhea or no symptoms at all. Usually the first symptom is non-bloody diarrhea, abdominal cramps and pain, and fever of short duration. Vomiting occurs in about one-half of patients during this first phase of infection. In the following 1-2 days the diarrhea becomes bloody. Normally the disease is self-limiting and the stage of infection lasts for 4-10 days (Gyles, 2007)

However, in 5-10% of cases, primarily in children, elderly and the immuno-compromised, the patients develop a potentially life-threatening complication. The first one is Haemolytic Uraemic Syndrome (HUS), which is defined as the combination of a microangiopathic hemolytic anemia with variable degrees of thrombocytopenia and renal failure. The syndrome usually occurs in previously healthy children and often is preceded by gastrointestinal enteritis. Other systems may be involved, such as the central nervous system (CNS) (WHO, 2013).

James *et al.* (2001) said that HUS has been recognized for more than 45 years and occurs predominantly in children younger than 4 years of age. It is the most frequent cause of acute renal failure in children. The most common form of the syndrome occurs in healthy young children (>6 months to <5 years of age) and is preceded by watery diarrhea that can evolve to hemorrhagic colitis. The diarrhea precedes the hemolysis and thrombocytopenia by 5 to 7 days; oliguria/anuria follows several days later. Although the pathogenesis is unknown, available evidence strongly suggests that endothelial cell damage is necessary. The outcome for most patients who have HUS is favorable: 65% to 85% recover completely, 5% to 10% die (usually during the acute illness), recurrence is uncommon, and only a few patients slowly progress to end-stage renal disease. Clues that a person is developing HUS include decreased frequency of urination, feeling very tired, and losing pink color in cheeks and inside the lower eyelids. Persons with HUS should be

hospitalized because their kidneys may stop working and they may develop other serious problems. Most persons with HUS recover within a few weeks, but some suffer permanent damage or die.

The second is Hemorrhagic colitis (HC) in that the initial symptoms of HC generally occur 1–2 days after eating contaminated food, though longer periods (3–5 days) have been reported. Symptoms start with mild, non bloody diarrhea that may be followed by a period of "crampy" abdominal pain and short-lived fever. The initial diarrhea increases in intensity during the next 24–48 hr to a 4 to 10 day phase of overtly bloody diarrhea accompanied by severe abdominal pain and moderate dehydration (Boyce *et al.*, 1995).

The third is Thrombotic Thrombocytopenic Purpura (TTP), this condition resembles HUS except that it generally causes less renal damage; has significant neurological involvement, e.g., central nervous system deterioration, seizures, and strokes; and is restricted primarily to adults (Fernandez, 2008). About 8% to 10% of patients with TTP, especially young children under 5 years old and the elderly, may progress to HUS. Adults typically shed the bacteria for one week after infection while young children can excrete the bacteria for more than three weeks However, asymptomatic prolonged carriage of *E. coli* O157:H7 is unusual among young children (Reiss *et al.*, 2006).

2.5. Factors Affecting Survival and Growth of *E. coli* O157:H7 in Food

A number of factors have a significant influence on the survival and growth of *E. coli* O157:H7 in food, including temperature, pH, salt, and water activity. *E. coli* O157:H7 has been reported to be more acid resistant than other *E. coli*. Acid resistance enhances the survival of *E. coli* O157:H7 in mildly acidic foods and may explain its ability to survive passage through the stomach and cause infection at low doses. The ability to be acid resistant varies among strains and is influenced by growth phase and other environmental factors. Once induced, acid resistance is maintained for long periods of time during cold storage. Stationary-phase *E. coli* O157:H7 are more resistant than growing cells to acid (Meng and Doyle, 1998).

The presence of other environmental stresses, such as temperature or water activity stress, will raise the minimum pH for growth. *E. coli* O157:H7 survives in such foods as dry salami, apple cider, and mayonnaise, which were previously considered too acidic to support the survival of foodborne pathogens. *E. coli* O157:H7 can survive for extended periods under conditions of reduced water activity while refrigerated; however, the organism does not tolerate high salt conditions (Buchanan and Doyle 1997).

2.5.1. Temperature

EHEC strains respond to temperature in the same manner as non-EHEC strains, with the exception of isolates of serotype O157:H7. *E. coli* are differentiated from other *Enterobacteriaceae* on the basis of their ability to grow and produce gas in *E. coli* broth at 44.5°C. Many O157:H7 isolates, however, do not grow well, if at all, above 44°C. The upper temperature for *E. coli* O157:H7 growth was culture medium– dependent. The minimum growth temperature for *E. coli* O157:H7 under otherwise optimal conditions is approximately 8–10°C (Doyle and Schoeni, 1984).

2.5.2. PH

Growth rates of *E. coli* O157:H7 are similar at pH values between 5.5 and 7.5, but decline rapidly at lower pH values. The minimum pH for *E. coli* growth is 4.0–4.5. This is dependent on the interaction of pH with other growth parameters; for example, additional stresses raise the minimum pH for growth. When the pH falls below the minimum for growth, *E. coli* O157:H7 populations decline over time (Den *et al.*, 1998).

2.5.3. Water activity (aw)

Studies on the effect of water activity 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 to other *E. coli*. The minimum a_w required for growth of *E. coli* is 0.95.

In sub-optimal temperature or pH conditions, a higher a_w value is required for growth of *E. coli* (Desmarchelier and Fegan, 2003).

2.6. Status of *E. coli* O157:H7 in Ethiopia

In many low and middle income countries (LMIC), laboratory diagnosis of enteric disease is rarely attempted and surveillance data are scant. The available data indicate that the pathogen is particularly likely to be associated with illness in young children, and may be frequently found in stools from healthy people. In older children and adults, the frequency among healthy controls was similar to that among people with diarrhea, which may reflect high levels of immunity. In African sites, carriage in healthy people is so frequent that it is difficult to attribute a major fraction of diarrheal illness to *E. coli O157:H7.* If high levels of maternal antibody can mask the clinical burden of *E. coli O157:H7* in the first months of life, during which time high exposure and colonization rates produce some active immunity, then there may be less opportunity for it to cause overt illness, since high exposure rates will continue to boost immunity. In LMIC, the potential value of surveillance is complicated by the scarcity of microbiological laboratories, the lack of supplies and equipment, and the challenge of coordinating the monitoring effort across sectors (WHO, 2012).

In Ethiopia, studies have revealed that diarrhoeal diseases are major causes of infant and child mortality and morbidity. About 39,000,000 episodes of diarrhoea per year were estimated to occur in Ethiopia; out of which 230,000 deaths occur in children below five years of age. Diarrhea was the second leading cause of admission and hospital deaths and *E. coli* O157:H7 is one cause of diarrhoea in the area (Kaba and Ayele, 2000).

In study made by Hiko *et al,* (2008) out of 738 meat samples examined, 31 (4.2%) were found to be contaminated with *E. coli* O157:H7. Beef was found to be most frequently contaminated with *E. coli* O157:H7 (8%) when compared to the other raw meat types examined (2-2.5%). The rate of *E. coli* O157:H7 isolated from all meat sources was found to be higher from butcher shops (7.8%) than from municipality (6.8%) and export

(2.3%) abattoirs. Furthermore, sheep and goats can be potential sources of *E. coli* O157:H7 for human infection in the country, of which out of the total of 711 different samples examined, 53 (7.5%) were found to be contaminated with *E. coli* O157:H7 and from this 53 isolates, 16 (30.2%) were from goats, 36 (68.0%) were from sheep (Mersha *et al.*, 2009).

On other hand, *E coli* O157:H7 has been isolated from studies made by Tadesse *et al.*, (2005) and Tsegaye and Ashenafi, (2005) from "Borde" and "Shamita", traditionally fermented beverages, and in yoghurt and cheese respectively.

2.7. Economic Impact of *E. coli* O157:H7

Economic and socio-economic threats from livestock diseases come in three broad categories: (i) losses in production, productivity and profitability caused by disease agents and the cost of their treatment; (ii) disruptions to local markets, international trade and rural economies arising from disease outbreaks and the control measures aimed at containing their spread, such as culling, quarantines and travel bans; and (iii) livelihood threats to the poor. The severity and long-term sequelae of infection with *E coli* O157 and other verocytotoxin-producing *E coli* result in high costs in terms of productivity loss, outbreak control costs and medical cost (Pennington, 2010).

There is no published report 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).

Although the American food supply is one of the safest in the world, significant annual economic losses (i.e., approximately 33 million cases of foodborne illnesses; over 9,000 deaths; and an estimated loss between 5.6 and 9.4 billion dollars) are reported due to

consumption of contaminated food (FDA, 1997; Buzby *et al.*, 1996). The annual economic cost of *E. coli* O157:H7 alone is estimated at 216 - 580 million dollars. This cost includes losses at the consumer (e.g., sickness and decreased productivity), producer (e.g., condemnation of contaminated products), and national (i.e., gross national product) levels (Bean and Griffin, 1990; Clarke *et al.*, 1994).

The outbreak surveillance data from CDC indicate that *E. coli* O157:H7 infections are decreasing after the peak in 1999 (Mead *et al.*, 1999). However, large outbreaks and sporadic cases continue to occur. The high cost of illness requires additional efforts to control this pathogen (Frenzen *et al.*, 2005).

Around 5–10% of those who are diagnosed with STEC infection develop a potentially life-threatening complication such as HUS which is the most common cause of acute renal failure in young children in many parts of the world (Tarr *et al.*, 2005). Neurological complications (such as seizure, stroke and coma) occur in around 25% of HUS patients and chronic renal sequelae are seen in around 50% of HUS survivors. The estimated case fatality rate of HUS ranges from 3 % to 5% resulting in high level of economic impact in loss of working hours (WHO, 2013).

2.8. Diagnosis of *E. coli* O157: H7

2.8.1. Culture and isolation

An enrichment/ isolation procedure using Entrohemorrhagic broth (EEB) followed by plating on cefixime tellurite sorbitol macconkey (CT-SMAC) agar has been developed for *E. coli* O157:H7 detection. It has been suggested that enrichment of samples with tryptic soy broth (TSB) containing cefixime (0.05µg/ml) and vancomycin (40 µg/ml) or (TSB) containing the same tellurite (2.5µg/ml, TBS) prior to plating on CT-SMAC, significantly increased the sensitivity of *E. coli* O157:H7 detection (Moxely, 2003).

E. coli O157:H7 share most metabolic and natural characteristics with other *E. coli* strains (Bastian and Sivela, 2000). Appropriate sample, (food, feces or stool), culture on

both blood and MacConkey agar and incubation at 37°C for 24-48 hours, gives pink colonies on MacConkey agar due to lactose fermentation. On biochemical testes such as IMVIC (Indole production, methyl red, Voges-Proskauer and citrate) tests, *E. coli* O157:H7 shows negative for Voges-Proskure and citrate tests and positive for indole production and methyl red tests (Quinn *et al.*, 2002). However, *E. coli* O157:H7 does not ferment rhamnose and ferment sorbitol very slowly and often said to be 'sorbitol negative' unlike other *E. coli* strains (Bastian and Sivela, 2000).

After incubation period on the enrichment broth at 37°C for 24 hours, 0.1ml of appropriately diluted enrichment broth is spread to CT-SMAC agar plate and incubated at 35-37°C for 24 h. Sorbitol fermenting bacteria appear as pink to red colonies on CT-SMAC, typical *E. coli* O157:H7 colonies are colorless or neutral/gray with a smoky center and 1-2mm in diameter (Feng and Weangent, 2002).

2.8.2. Latex agglutination test

The presence of O157 and H7 can be detected by using commercial O157 and H7 antisera respectively. Both prolix *E. coli* O157 latex test reagent kit and RIM *E. coli* O157:H7 latex test give satisfactory results. During the test only single colony should be used, as the use of excessive colony growth (i.e. sweps) may result in positive reaction due to auto-agglutination (Feng and Weangent, 2002).

2.8.3. Polymerase chain reaction (PCR)

PCR has been developed both to confirm the identity of *E. coli* O157:H7 isolates and to directly detect the organism in feces or enrichment broth culture. Because of problems by PCR inhibitors in feces most PCR assay have been used to confirm isolates as *E. coli* O157:H7. A multiplex PCR has been developed to detect *eaeA, Stx* and *ehx*. *E. coli* O157:H7 isolates carry all the three genes, whereas other shiga producing *E. coli* (STEC) isolates were usually negative for at least one. This procedure especially when conducted

both blood and MacConkey agar and incubation at 37°C for 24-48 hours, gives pink colonies on MacConkey agar due to lactose fermentation. On biochemical testes such as IMVIC (Indole production, methyl red, Voges-Proskauer and citrate) tests, *E. coli* O157:H7 shows negative for Voges-Proskure and citrate tests and positive for indole production and methyl red tests (Quinn *et al.*, 2002). However, *E. coli* O157:H7 does not ferment rhamnose and ferment sorbitol very slowly and often said to be 'sorbitol negative' unlike other *E. coli* strains (Bastian and Sivela, 2000).

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on biochemically characterization isolates increase the level of confirmation as *E. coli* O157:H7 (Klerks *et al.*, 2004).

2.9. Antimicrobial Resistant

In the last few years, the emergence and wide dissemination of *E. coli* strains showing resistance to broad-spectrum of antimicrobial agents has been reported. Emergence of resistance to multiple antimicrobial agents in pathogenic bacteria has become a significant public health threat as there are fewer, or even sometimes no, effective antimicrobial agents available for infections caused by these bacteria (Bartoloni *et al.*, 2006). The use of antimicrobials in agriculture, farm management and in veterinary medicine including livestock and companion animals favors dissemination of antimicrobial resistance in animal reservoirs and the environment, leading to increased resistance to antimicrobials in bacteria colonizing humans. The inappropriate use of antimicrobials in human medicine also plays an important role in this complex problem (Belanger *et al.*, 2011). In addition to their therapeutic use in human and veterinary medicine, antimicrobials are routinely used for disease prevention and growth promotion in animal production. It has been shown that repeated sub-lethal exposure to antibacterial agents not only promotes adaptative resistance but also confers decreased sensitivity to antibiotics. This practice leads to a selection of antimicrobial resistance among commensals in the intestinal tracts of food animals, which poses a public health threat (Witte, 1998; Braoudaki and Hilton, 2003).

In the study made by Ibrahim *et al,* (2012) there are high resistance rates of MDR *E. coli* isolates to the first line oral antimicrobial agents such as Amoxicillin, Cefuroxime, Trime thoprim-Sulfamethoxazole, Tetracycline and Nalidixic acid. As such, antimicrobial use should be reduced to a minimum and alternative approaches should be used to limit the spread of antimicrobial resistance in animals and humans.

2.10. Treatment

Treatement of infecton with EHEC strains, including *E.coli* O157:H7 is mainly based on supportive therapy, particularly re-hydration. The uses of anti-motility agent, which inhibit peristalsis and delay clearance of the organism, pose a risk factor for progression to HUS (Bell *et al.*, 1997). Additionally the use of trimethoprim, the quinolones, or furazolidon enhances the production of shiga toxins form *E.coli* O157:H7 in vitro presumably due to lyses of bacterial cells and release of stored toxins (Kimmitt *et al.*, 2000).

This enhanced release of toxins may alternatively be due to induction of Stx producing prophages harbored by the bacterium. This prophage would be activated by the SOS response, a genomic insult which may be extracted by antimicrobial treatment. In light of the difficulties in treating this agent, alternate treatment approaches were investigated by multiple group antibodies, to Stx2 were shown to enhance the survival of infected gnotobiotic piglets (Donohue *et al.*, 1999). These antibodies were also demonstrated to be well tolerated in humans and thus may be useful for preventing HUS in pediatric subjects (Lopez *et al.*, 2010).

On the other hand, Carbosilane dendrimers were shown to specifically bind to Shiga toxins with high affinity and to inhibit cellular entry to the toxin. Intravenous administration of these Carbosilane dendrimers decreased the brain damage prevented the lethal effect of the toxins in infected mice (Nishikawa *et al.*, 2002).

2.11. Prevention and Control

The importance of controlling foodborne pathogenic bacteria postharvest, starting with abattoir processing, is well recognized. However, despite efforts to mitigate pathogens, contaminated meat products still enter the food chain and pathogenic bacteria are frequently detected in food products (Trevor, 2011).

Two different approaches to reduction or elimination of *E. coli* from the food chain may be considered. On one hand are primary prevention efforts aimed at reducing or eliminating colonization of live animals on the farm, while on the other are measures to eliminate or reduce this species from the finished product (decontamination). This is based upon interruption of transmission routes, either from animal reservoirs to humans or from consumption of contaminated foods and water. Major focal points for control include practicing good food hygiene, this includes adequate refrigeration of meat from processing up to preparation, separation of raw and cooked foods, thorough cooking of red meat and obtain a core temperature of 72°C for 1 minute before eating, effective pasteurization of milk and maintaining an uncompromised clean water supply (Butzler and Oosterom, 1991).

Individuals who have diarrheal symptoms should not work as food handlers or day care employees until they have recovered from acute symptoms and should practice good hygiene, Additional precautions and supervision should be exercised in kitchens preparing food for persons at high risk of infection (infants, the elderly, and the immunosuppressed) (Shane, 1994).

Hygiene

Control of *E. coli* throughout the food chain requires implementation of food safety management systems based on well established principles, such as Hazard Analysis Critical Control Point (HACCP) system (FSAI, 2002), Good Agricultural practice (GAP) and Good Manufacturing practice (GMP), at every stage of the meat supply chain, from the farm through to the abattoir, to the retailer, and to those involved with the handling and processing of such raw meat products in the home environment (Hiko *et al.*, 2008).

An important component of hygiene is HACCP application in animal production, which is reducing the carriage of *E. coli* O157:H7 by animals. Two approaches that have potential are competitive exclusion and vaccination. Competitive exclusion involves the use of microbial cultures that out-compete pathogens from colonizing specific niches.

This approach uses defined bacterial cultures that can greatly reduce colonization of *E. coli* O157:H7 (Schoeni and Doyle, 1992).

Vaccination involves exposing an animal to an attenuated pathogen or an antigen of a virulent microorganism to produce immunity. As animals, mainly cattle, are thought to be the reservoirs of infection for the human population, a novel strategy being explored is to vaccinate cattle in order to reduce colonization with pathogenic VTEC and thereby reduce contamination of food and the environment (i.e. to make food safer as opposed to protecting people against their food). One approach is to use a live, toxin-negative colonizing strain as an oral vaccine to induce antibodies against surface components, and another is to deliver colonization factors, such as intimin, as an edible vaccine in transgenic plants (Gayles, 1998).

However, traditional vaccination approaches are not likely to be successful with *E. coli* O157:H7. Recent observations showed that *E. coli* O157:H7 does not form attaching and effacing lesions or colonize mucosal surfaces of the gastrointestinal tract (Brown *et al.*, 1997; Cray and Moon, 1995), and cattle exposed to *E. coli* O157:H7 are not protected from re-infection Hence, innovative approaches will be needed for vaccines to be effective (Johnson *et al.*, 1996).

Further, Gayles, (1998) said that, there are currently no vaccines available to control zoonotic VTEC but 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 (e.g. O157 polysaccharide linked to the B-subunit of VT1 and VT2 as carrier proteins), live-vector vaccines, toxoid vaccine or passive immunization with hyperimmune globulin or monoclonal antibodies against VT (Levine, 1998).

Slaughterhouses

Carcass processing is a promising site for pathogen reduction efforts. The microbial quality of carcasses has been associated with the abattoir where processing occurred.

Treatment of wash water is a potential processing control to reduce contamination (Wedderkopp *et al.*, 2000). Like other *E. coli*, it is assumed that the ultimate source of *E. coli* O157:H7 on carcasses is fecal contamination during animal production and slaughter operations. Fecal contamination 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).

Traditional trimming procedures can reduce *E. coli* O157:H7 levels on areas of the carcass with visible fecal contamination (Hardin *et al.*, 1995). Various alternatives to trimming have been investigated for the removal of enteric pathogens. Recent studies with *E. coli* O157:H7 suggest that rinsing of carcass surfaces with solutions of organic acids may have limited effectiveness. Spray chilling with 1– 2% acetic acid only produced a 1-log cycle (tenfold) reduction of *E. coli* O157:H7 on lean tissue; a slightly greater effect was observed on fat tissue (Dickson, 1991). Investigators found that acid rinses had little effect on eliminating *E. coli* O157:H7 from the surface of beef tissues possibly due to difficulty in removing *E. coli* O157:H7 from beef surfaces previously chilled. Pre-evisceration washing decreased the subsequent attachment of *E. coli* O157:H7 to beef carcasses. Trisodium phosphate has been evaluated as a sanitizing agent for carcass surfaces and equipment. Its overall effectiveness, due to its high pH, was similar to that achieved with organic acids (Fratamico *et al.*, 2002).

The use of steam to briefly heat carcass surfaces to temperatures sufficient to inactivate *E. coli* O157:H7 while maintaining the raw character of the animal tissue is a new method for reducing the presence of enteric pathogens on meats. Steam vacuum systems are used for spot removal, and steam pasteurization cabinets are used for whole carcass treatments. The steam vacuum system is reportedly capable of achieving a 5-log cycle (100,000-fold) reduction of *E. coli* O157:H7 on inoculated beef surfaces (Dorsa *et al.*, 1996).

3. MATERIALS AND METHOD

3.1. Study Area

Debre Berhan is located at 130 km from Addis Ababa on the main road Addis Ababa-Dessie -Mekele road, 9 ° 41 ° N latitude and 39°31 ° E longitude (Figure 2). An average elevation is between 2800 and 2845 meters above sea level for the district. The mean annual temperature ranges between 5 ° C and 23 ° C. The relative humidity is 73.3% (MOWR, 1995). The district is one of 24 districts of north shewa zone of Amhara region and it has 9 peasant associations. According to the Debre Berhan town administrative agricultural office (DTAO), in 2005 the human population in the urban area were 35265 males and 37267 females and in the rural area 5983 males and 6429 females. The numbers of animals in the area are 21,641 cattle, 26,920 sheep, 1,100 goats, 56,576 poultry and 4,970 equines. In urban area people practice milk production from dairy cattle and keep sheep. Livestock provide meat, milk, labor, income and clothing (DTAO, 2005).

Figure 2. The map of the study area, Debre brehan, North Showa, Amhara, Ethiopia. Source: CSA, (2007).

3.2. Study Population

The study was conducted on apparently healthy adult cattle slaughtered in Debre Berhan municipal abattoir and meat (beef meat) sold in butcheries from November 2013 to Aprile 2014. The cattle are slaughtered in the abattoir originating from Shoa Robit, Debresina and in and around Debre Berhan. On average 15-30 cattle are slaughtered every day except Tuesday and Thursday and the meat is presented for local markets in and around the town. Slaughtering operations are performed on the floor of the abattoir (Annex 6).

3.3. Study Design

A cross sectional study was conducted to estimate the prevalence of *E. coli* O157:H7 in the municipal abattoir and to assess the source of contamination of the meat presented for sell in the butcher houses. Simple random sampling was used to select animals for sampling. The sampling was done twice a week during which 10 animals were selected. From each selected animals, fecal sample and swab samples of the hide, carcass (internal and external), intestinal mucosa and samples of abattoir environment, which have contact with the carcass (i.e. knives, workers' hands and clothing) were collected.

On the other hand, legally registered butcher shops obtaining carcasses from the municipal abattoir were selected using simple random sampling technique. Swab samples from the knives, cutting board, butcher mans' hands and carcasses while sold to the consumers, was collected to determine to what extent butchery house environments serve as sources of *E. coli* O157: H7 for meat.

Furthermore, a purposive study was conducted to see the public health importance of *E. coli* O157: H7 in the study area. Stool samples from suspected individuals with clinical manifestation at Debre Berhan referral hospital were collected. But first the patient were informed verbally and in written form about this study that plans to investigate diarrheic patients' opinion towards *E. coli* O157:H7. So, questionnaire survey was performed

along with sample collection to see if the cases fit with the result. The questionnaire contained questions about demographic information, health details, food and water related history, contact with farm animals and with symptomatic individuals, travel history etc. (Annex 5).

All samples for the study were then transported in ice box to College of Biological Science, Debre Berhan University, Microbiology laboratory and stored at 4°C until processed. Culture of the sample and isolation and identification of *E. coli* O157:H7 were then performed. The positive isolates were transported by cold chain to Addis Ababa University, College of Veterinary Medicine and Agriculture (CVMA, AAU) Microbiology laboratory to conduct antibiotic susceptibility tests.

3.4. Sample Size Determination

The approximate sample size required for study animals was determined based on the expected prevalence of *E. coli* O157:H7 and the desired absolute precision using the formula stated on Thrustfield (2005);

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

Where, n = required sample size.

P_{exp} = expected prevalence.

d=desired absolute precision.

Previous study made in Debre Zeit abattoir showed the prevalence of *E. coli* O157: H7 to be 8% in cattle (Hiko *et al.*, 2008). Therefore, by using this 8% expected prevalence, at a confidence level of 95% and required absolute precision of 5%, a total of 114 animals were sampled to demonstrate the prevalence of *E. coli* O157:H7. By considering the variation in weather conditions between the two towns (Debre Zeit and Debre Berhan)

and also, to reduce the error occurs as a result of sampling, the sample size was increased to 220 animals. Swabs of hide, carcass inside, carcass outside and intestinal mucosa and fecal samples were collected from each 220 slaughtered cattle. Whereas, carcass in contact surfaces sample were taken once for each sampling day.

3.5. Sample Collection

Hide swab samples

Hide swab samples were taken according to McEvoy *et al.* (2003), by using 2x3 cm sterile cotton tipped swabs soaked in approximately 10ml of buffered peptone water (Oxoid Ltd., Hampshire, England). Hide were swabbed from the neck of animals over the line of bleeding before slaughtering near the bleeding area at an area of approximately 10x10 cm. The vertical part of the hide of the animal was also swabbed similarly at the mid line to determine its contact to the carcass during flaying (Annex 6). The shaft of the swab was then broken by pressing it against the inner wall of the test tube and disposed.

Intestinal mucosal swab and Fecal sample

For each fecal sample collection, the distal colon were legated and transected approximately 750 cm proximal to the rectum, before complete evisceration (Annex 6) and the colorectal tissues were placed in individual clean plastic bags. Within 2 h of sampling, approximately 25gm of fecal samples were taken aseptically by opening the colon. The lumen was also swabbed by using sterile swab. The swab was then introduced in to approximately 10 ml buffered peptone water in a sterile test tube and kept in an icebox for transportation to the laboratory.

Carcass swab samples

Selected carcasses were swabbed using the method described in ISO17604 (2003) and Commission Decision 2001/471. The external part of the carcass (neck, brisket, flank,

and rump) was swabbed using sterile cotton tipped swab (2X3cm) fitted with shaft, which was first soaked in an approximately 10 ml of buffered peptone water (Annex 6). The swab was moistened for at least 5 seconds in the diluents and rubbed initially horizontally, then vertically, then diagonally not for less than 20 seconds across the entire meat surface and as much pressure as possible were used to sample the carcass. 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. For the internal part of the carcass, where contacts with other carcass is not possible, the thoracic and the pelvic parts were swabbed using the same procedure above. Both the inside and outside carcasses was swab sampled separately (Annex 6).

Environmental samples

Similarly Carcass in contacts like; knife (junction of blade and handle), workers hands (whose hands have direct access to the carcass) and clothing (aprons), were also swabbed as a sample using sterile cotton swabs soaked in approx. 10 ml of buffered peptone water. A pooled sample was taken from knives used for evisceration and carcass trimming, workers hands and clothing on each sampling day, and finally all swab samples were placed in an icebox to be transported (Annex 6).

Butcheries

Swab samples were taken from the carcass, knife, cutting board and the butcher man's hands and transferred to separate a screw-capped test tube containing 10 ml buffered peptone water. All swab samples were placed in an icebox to transport to the laboratory for further analysis.

A sterile latex glove was used to avoid cross contamination and every procedure was done as aseptic as possible. During the sample collection process hide swab, carcass swabs and fecal samples from animals was labeled with the same matching number in order to avoid possible confusion of samples. Each sample were labeled legibly and accompanied by necessary identifying information, which includes date of sampling, type of sample, sex, BCS, breed and hide cleanliness of animal from which the sample was

obtained. Swabs with in sterile test tubes and fecal samples in separate sterile plastic bags are placed, to prevent spilling and cross contamination and transported immediately to the laboratory with in an ice box for further processing.

Samples from Debre Berhan Referral Hospital

Stool samples that were sent to laboratory with a physician's order to screen for enteric pathogens were included. Stool samples were collected from patients who present with diarrhea, especially bloody in nature. The samples were added to sterile test tubes and transported immediately to the laboratory within an ice box for further processing. A questionnaire was also administered to the sampled patient.

3.6. Isolation and Identification of *E. coli* O157:H7

3.6.1. Sample preparation and enrichment

Twenty five grams of fecal content were taken 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 containing 20 mg/l novobiocin (mTSB+n) as a selective enrichment was added. The resulting mixture was then agitated using stomacher at low speed for 30 seconds. Stool samples collected from hospital was also processed by the procedure mentioned above, but the ratio was dependent on the amount/type of stool collected. In to all other swab samples 90 ml of mTSB+n was added and homogenized. Then all sample types were incubated at 41.5°C for 24 hrs (OIE, 2004). Microbiological samples for the isolation and identification of this bacteria was processed as described in ISO 16654(2001), OIE (2004), and OPHS (2002), FSIS (2002), USDA (2002).

3.6.2. Immunomagnetic Separation (IMS)

The use IMS has been suggested as a method of reducing total analysis time improving sensitivity of detection. It recovers target cells from the enrichment broth using

paramagnetic beads. These beads are coated with polyclonal antibodies specific for a particular VTEC sero-group. Para-magnetic particles coated with antibodies specific to a target organism are added to a food system. The target organism is captured onto the magnetic particles and the whole complex removed from the system by application of a magnetic field. Target organisms are thus removed from food debris and background micro-organisms which potentially will interfere with the detection system and by re-suspending isolated cells in a reduced volume (ISO 16654, 2001).

After 24 h of incubation all enriched broth culture were subjected to IMS using Dynabeads anti-*E.coli* O157 (Dynal Biotech AS, Oslo, Norway) as follows. Both enriched broth culture and the paramagnetic beads were vortexed to be homogenized (Annex 6) and 1ml of the enriched culture was put in to a sterile screw cupped eppendorf tube, using a micro pipette with a sterile disposable tip. A 20µl of re-suspended paramagnetic beads was then transferred in to the same eppindorf tube. Then each tube was briefly vortexed for about 30 minutes at room temperature, for the bacteria to attach to antibody surface on the beads. The tubes were then put in to the manual magnetic particle concentrator (MPC-S) with the magnetic strip in place, inverted 3 to 4 times and left to settle for about 5 minutes. It was then gently rotated for the magnetic beads to concentrate at the back of the tube. The cap of the tube then carefully opened and the supernatant was discarded by carefully aspirating it with sterile fine tipped pipette, without touching the back wall of the tube. 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 another disposable fine tipped pipette. It was then inverted 3 times after the tubes were closed, the magnetic strip replaced and the above step repeated at least twice. To prevent cross contamination the PBST was reduced to other small container and for each sample (TSB enriched sample and those samples to be discarded from the eppindorf) and for the addition of PBS in each eppindorf tubes new pipette tips were used. Finally the supernatant was aspirated (again by separate/different pipette tips); the magnetic strip was removed and about 100µl of PBST was added in each tube and mixed gently.

3.6.3. Culture plating

Around 50 µl of the suspension (IMS bead and bacteria complex) were streaked onto Sorbitol MacConkey agar (Oxoid, Basingstoke, Hampshire, England) containing 0.05 mg/l cefixime and 2.5 mg /l potassium tellurite (Dynal Biotech ASA, Oslo,Norway) (CT-SMAC). Culturing was done carefully to obtain pure colonies (Annex 6) and plates were incubated at 37°C for 20–24 h. The CT-SMAC agar plates were examined for the presence of non sorbitol fermenting colonies (ISO 16654, 2001; OIE, 2004; OPHS, 2002).

The non sorbitol fermenting colonies on CT-SMAC appear as slightly transparent, almost colorless with a weak pale brownish appearance with a diameter of one mm (ISO16654, 2001; OIE, 2004; FOOD-CT, 2006). Such colonies are sub cultured on CT-SMAC for further a confirmatory test.

3.6.4. Confirmatory test

Latex agglutination test was conducted using Latex Test Kit for the confirmation of *E. coli* O157:H7. *E. coli* O157 latex kit consists of three components which are: the test latex particle which is sensitized with rabbit antibody reactive with the O157 somatic antigen, control latex which consists of latex particle sensitized with pre-immune rabbit globulin, positive control suspension of activated *E. coli* O157 and negative control suspension of *E. coli* O116; and reaction card. The test kit was used according to the manufacturer instruction (Oxoid Ltd, Hampshire, England).

The Latex kit was checked for its performance by using the control suspensions in the kit, the test was continued after the positive control reacts with the test latex showing positive result. A drop of test latex and 0.85% sterile saline water were dispensed in to the reaction card separately. A few Presumptive colonies of *E. coli* O157 was taken and emulsified in to the saline water on the latex card, then its slowly mixed with the test latex using the sterile loop and check for agglutination within two minutes (Annex 6).

Isolates showing visible agglutination by reacting with the test latex solution are considered *E. coli* O157:H7.

Test portion, 25g + Modified TSB+n (225ml) swab samples+ Modified TSB+n in 1:9 ratios

⇩

Homogenization and incubate at 41.5°c for 24hrs.

⇩

Capture of *E. coli* O157 by using immunomagnetic beads.

⇩

Inoculation of 50 µl immunomagnetic beads complex on selective media (CT-SMAC) and incubate at 37°C for 24hrs.

⇩

Latex agglutination test

Figure 3: Flow diagram showing the procedure for isolation of *E coli* O157:H7
Source: ISO16654, 2001 (modified form)

3.6.5. Antimicrobial Susceptibility test

Antibiotic susceptibility tests were performed on all *E. coli* O157:H7 isolates to determine their antibiotic resistant profiles using standard agar disc diffusion method, CSLI (2008). Briefly, once isolates were grown on sorbitol MacConkey agar at 37°C for 18 to 24 h. Pure colonies, incubated for 6 h in Tryptone Soya Broth (Oxid, England) were made to have a turbidity of 0.5 McFarland standards (approximately $3x10^8$ CFU per ml) in a sterile saline solution. And it is inoculated on already prepared Muller-Hinton agar (Bacton Dickinson Company, Cockeysville, USA), which were prepared according to the manufacturer's instruction. The inoculation was performed using sterile cotton swab, making sure that all the surface of the media is immersed with the solution. Antibiotic

discs were then dispensed onto the inoculated agar plate and pressed down to ensure complete contact with the agar surface and incubated for 24 hours at 37^0c. After incubation, the antibiotic inhibition zone diameters (IZD) were measured. Results obtained were used to classify isolates as being resistant, intermediate or susceptible to a particular antibiotic using standard reference values given by CSLI (2008) (Table 1). Diameters of the zone of inhibition were measured and the results were classified as resistance, intermediate and susceptible after the zone of inhibition of bacterial growth is appreciated, hence a standardized table is given by the manufacturer for this classification (CSLI, 2008).

Table 1. Antimicrobials used, their symbols and inhibition zone size interpretation

Antimicrobial agents	**Symbol**	**Diameter of zone of inhibition in mm**		
		Resistant(≤)	Intermediate	Susceptible (≥)
Amoxicillin (25 µg)	AML	13	14-16	17
Cefotaxim(5 µg)	FOX	14	15-17	18
Chloramphenicol (30 µg)	C	12	13-17	18
Ciprofloxacine(5µg)	CIP	20	21-30	31
Kanamycine (30µg)	K	13	14-18	18
Nalidixic acid (30 µg)	NA	13	14-18	19
Nitrofurantion (300 µg)	F	14	15-16	17
Streptomycine (10µg)	S	11	12-14	15
Sulfamethoxazole-trimithoprim (25µg)	SXT	10	11-15	16
Tetracycline (30µg)	TE	11	12-14	15

3.7. Data Management and Statistical Analysis

The obtained data was entered to Excel spread sheet Microsoft Office 2007 program and analyzed by STATA version 11.0 (2013). Over all and sample-specific prevalence were determined by dividing the number of positive samples to the total number of samples examined times 100. The association of risk factors with *E. coli* O157:H7 status was determined using Chi square (χ^2) test. Significant level was set at $\alpha < 0.05$.

4. RESULTS

4.1. Overall Prevalence of *E. coli* O157:H7

From the total of 1,192 samples examined for bacteriological status of *E. coli* O157:H7 only 6 (0.53%, 95%CI: 0.11%, 0.95%) were positive: three fecal (1.36%), two hide swabs (0.9%) and one intestinal mucosal swab (0.45%) (Table 2). The animal level prevalence of *E. coli* O157:H7 was 1.82% (95%CI: 0.04%, 3.60%) at abattoir. None of the samples taken from butcher houses and hospital were positive.

Table 2. Prevalence of *E. coli* O157: H7 by Sample Types Examined

		No. of Samples		
Sample Source	**Sample Type**	**Examined (No.)**	**Positive (%)**	**p-value***
Abattoir	Faecal sample	220	3 (1.36%)	0.303
	Hide swab	220	2 (0.91%)	
	Intestinal mucosal swab	220	1 (0.45%)	
	Carcass internal swab	220	0 (0%)	
	Carcass external swab	220	0 (0%)	
	Environmental swabs	32	0 (0%)	
	Subtotal	1132	6 (0.53%)	
Butcher house	Carcass	10	0 (0%)	
	Hands	10	0 (0%)	
	Cutting board	10	0 (0%)	
	Knife	10	0 (0%)	
	Subtotal	40	0 (0%)	
Hospital	Stool	20	0 (0%)	
	Total	1192	6 (0.53%)	

*Fishers exact

4.2. Risk Factors

In this study different suspected risk factors (Sex, Breed, BCS and Hide cleanness) were studied to determine whether there is a significant variation in *E. coli* O157:H7 infections among the different group of animals.

4.2.1. Prevalence of E. coli O157:H7 versus animal level risk factors

The entire positive samples to *E. coli* O157:H7 originated from male animals. However, the difference between the two sexes were not significant ($P>0.05$) (Table 3). Likewise, though there was no significant difference, highest prevalence was observed in animals with good body condition. Besides, the difference in the *E. coli* isolation rate was not significant for the two breeds.

Statistical analysis of the hide cleanness on overall *E. coli* O157:H7 status showed significant difference ($P < 0.05$). Cattle were visually inspected in the lairage of the abattoir and assigned to a category ranging from 1 (very clean) to 5 (very dirty) depending on the observed cleanliness of the hide, based on Irish Department of Agriculture and Food Livestock Cleanliness Categories (Anon, 1997). Animals from categories 2, 3, and 4 were slaughtered. The procedure requires that category 5 animals are rejected from slaughter, while category 4 can be slaughtered under special conditions, which involve increased workstation hygiene and slower line speeds. Cattle assigned to other categories (1-3) are processed as normal (Table 3).

Table 3. Prevalence of *E. coli* O157:H7 based on different risk factors (Sex, BCS, Breeds and Hide Cleanness) in Debra Berhan abattoir.

Risk factor	Categories	No. of samples	No. of Positive (%)	P-value	$\chi 2$
Sex	Male	163	2.40%	0.256	0.575
	Female	57	0%		
BCS	Poor	2	0%	0.103	0.101
	Medium	157	0.64%		
	Good	61	4.92%		
Breed	Local	187	2.14%	0.396	1.000
	Cross	33	0%		
Hide cleanness	1	1	0%	0.036	0.078
	2	55	1.82%		
	3	113	0%		
	4	51	5.88%		
	5	0	0%		

4.3. Antibiotic susceptibility test

The isolates were resistant to one antibiotic tested which is approved to treat *E. coli* infections in feedlot animals. Again the isolates had intermediate resistance to two of the antibiotics, which are not approved to be used in animals. But they were susceptible to Tetracycline (TE, 30µg), Chloramphenicol (C, 30µg), Sulfamethoxazole-Trimithoprim (S XT, 25µg), Cifrofloxacine (CIP, 5µg), Nalidixic acid (NA, 30µg), Kanamycine (K, 30µg) and Streptomycin (S, 10µg) (Table 4).

Table 4. Antimicrobial susceptibility of all isolates

	No of isolates, All (n=6)					
	Drug family name	Antibiotics tested	No. of isolate tested	S. (No.)	I. (No.)	R. (No.)
Approved for feed lot animals (*E. coli* targeting)	Tetracycline	TE	6	6	0	0
	Penicillins	AML	6	0	0	6
	Aminoglycosides	S	6	6	0	0
		K	6	6	0	0
Not approved for feed lot animals (*E. coli* targeting)	Quinolones	NA*	6	6	0	0
		CIP	6	6	0	0
	Nitrofuranotoins	F	6	0	6	0
	Amphenicol	C*	6	6	0	0
	Cephalosporins	FOX	6	0	6	0
	Sulfonamides	SXT	6	6	0	0

S. = Susceptible I. = Intermediate, R. = Resistant, *not authorized, but used in some animals.

5. DISCUSSION

In the present study out of 1,172 samples collected from abattoir and butcher houses in Debre Berhan, *E. coli* O157:H7 was isolated from six samples (0.53%). The animal level prevalence in the abattoir was 1.82%. Our finding is in line with Miyao *et al.* (1998), from Japan and Eriksson (2010) from Sweden, where they recorded prevalence of 1.8% and 1.2% from intestinal and fecal samples correspondingly. But it is lower than the findings of Schouten *et al.* (2004) in Netherlands, Vidovic and Korber (2006) in Canada, Hiko *et al* (2008) in Ethiopia and Tahamtan, *et al.* (2011) in Iran, in that they reported 7.2%, 8%, 15.6% and 53.84% respectively in samples taken from feces, meat and recto anal mucosal swab. This difference might be due to variations in precipitation and humidity in the study areas as well as differences in soil composition which may affect survival and/or proliferation of the organism in the environment (Magwira *et al.*, 2005). *E. coli* O157 occurrence was apparently affected by temperature since there was a trend toward higher prevalence in the warmer spring and summer months. Sampling methods, sample type, sample size, food preferences and management practices related to hygiene in different geographic location might also be the grounds for these differences, suggesting that carriage of the organism by cattle is widespread.

The prevalence of *E. coli* O157:H7 in fecal sample in the present study was 1.36%, which is almost similar with Lahti *et al.* (2001) in Finland (1.31%) and Albihn *et al.* (2003) in Sweden (1.2%). Kaddu-Mulindw *et al.* (2001) from Uganda, Osek *et al.* (2001) from Poland and Dontorou *et al.* (2004) from Greece studied on the status of fecal carriage of *E. coli* O157:H7 in cattle and found 0%. On the other hand, higher prevalence rates were reported: 10.8% in Germany (Montenegro *et al.*, 1990), 9% in Spain (Blanco *et al.*, 1993), 16.6% in Italy (Bonardi *et al.*, 1999) and 12.9% in Britain (Chapman *et al.*, 2001). The differences in isolation rates of *E. coli* O157:H7 from cattle feces among these studies might be due to environmental conditions, seasonal variation, isolation methods employed in detection of the organism, geographical location and more importantly patterns of fecal shedding of STEC which are affected by diet. Dietary shift causes changes in the microbial populations of the GIT; ruminal and intestinal volatile fatty acid

(VFA) concentrations limit *E. coli* populations because they are toxic. The trend of fasting animals 48 hours before slaughtering operation increases their susceptibility to colonization by *E. coli* O157:H7. Withdrawal and/or starvation results in decreased VFA concentrations in the gut, subsequently, increasing shedding of EHEC (Van Immerseel *et al.*, 2006). An individual animal shading large numbers of *E. coli* O157:H7 will arguably pose a greater risk than the combined output of many animals excreting at low levels, with respect to both potential food contaminations and transmission to other animals.

The hide of cattle is known to be a source for the microbial contamination of beef, with the potential for the microorganisms to be transferred onto the carcass during slaughtering and dressing processes. In the present study, the percentages of hide samples that contained *E. coli* O157:H7 was 0.91% which is in agreement with Brichta-harhay *et al.* (2008) in USA which ranged from 0.4% to 6% in four meat processing plants that mainly slaughter cull cows and bulls. However, our finding is lower than the observations of Reid *et al.* (2002) and Osaili *et al.* (2013) who reported 22.2% in South-West of England and 10% in Amman, Jordan respectively. The marked differences in the results may be due to a variety of factors which can potentially affect hide contamination, including faecal shedding, abattoir management system, farming systems, lairage-related conditions, duration of farm/market-to-abattoir transport (and related levels of hide contamination), and hygienic conditions along unloading-to-stunning areas.

As mentioned above, hide contamination occurs from direct and indirect faecal contamination in cattle production and lairage environments. The effect of super shedders on hide contamination was simulated by Stephens *et al.* (2008), who detected high level of inoculums in hide samples from the high level inoculums group one day after deposition of fecal pats. With grouping of cattle in close quarters during transport and holding, significant cross-contamination of hides with feces occurs, thus increasing the apparent prevalence on hides. One explanation for this apparent discrepancy in the reports is due to choice of sampling site. Hide samples were taken from the ventrum of the animal over the sternum (brisket) on the assumption that as cattle rested in sterna

recumbency, this site would be in contact with faecal matter on the ground, in effect swabbing the pen floor and maximizing hide contamination.

Further, Gregory *et al.* (2000) said that, feed could also be another factor for the status of *E. coli* O157:H7 on the hide, in that when cattle arrived at the slaughter plant the hides of cattle fed hay for 48 h prior to transport were as clean as the hides of fasted cattle, and were significantly cleaner than pasture-fed cattle. In different environments, individual animals shedding *E. coli* O157:H7 at high levels can have a disproportionate effect on cattle hide. Woerner *et al.* (2006) demonstrated that when a pen had a >20% fecal incidence rate, then the percentage of hides positive for *E. coli* O157:H7 rose to 26%; however when the fecal incidence rate in the pens was <20% then the hide contamination level was only 5%.

In the current study the isolation rates of *E. coli* O157 from intestinal mucosal swabs were 0.45%. But in previous studies by Chapman *et al* (1993) and Fox (2007), 4% in South Yorkshire, England and 8.7% in Kansas, USA was reported. The variation might be due to difference in the sample types analyzed, in that the part where the intestine is swabbed has significant impact on the result achieved.

The most important source of infection for *E. coli* O157:H7 is food of animal origin especially meat and meat products (Bindu *et al.*, 2010). But in the present study, *E. coli* O157:H7 was not isolated from swabs of carcasses (inside and outside) and carcass in contacts (knives, aprons and workers hand). One percent, 2.65% and 9.74% of the carcasses of cattle slaughtered in Turkey (Inat and Siriken, 2010), Ethiopia (Taye *et al.*, 2013) and Iran (Tahmatan *et al.*, 2006) had *E. coli* O157 respectively. Carcass can be contaminated during slaughter by exposure to feces, hides or abattoir environment containing *E. coli* O157:H7. This variation in the results might be due to cross contamination of carcass surfaces from the hide during slaughter or evisceration, diversity in geographical origins of cattle, number of cattle slaughtered per day, study design, season and abattoir conditions. Fecal samples harboring highest incidence of the EHEC in the abattoir may directly contaminate the carcass. But this is not the fact in this

study because of two possible reasons. First, most fecal sample examined for the status of *E. coli* O157:H7 have lower prevalence and the other, even if the slaughtering operation is performed on the floor, the GIT from the internal part of the carcass is removed with precaution not to puncture the GIT while the organ is inside the carcass and further processing of the GIT is carried out in opposite direction from carcass operation area in the abattoir.

In the present work, different risk factors were studied to determine whether there is significant variation in *E. coli* O157:H7 isolation rate. In addition to the bacterial factors, animal genetic and physiological factors contribute to the prevalence of this pathogen in cattle (Magwira *et al.,* 2005). With regard to sex, the study shows no significant association; but male animals outnumbered females. Our observation is in line with the work done by Yilmaz *et al.* (2002) in Istanbul, but different from Luga *et al.* (2007) in Nigeria in that seroprevalence of *E. coli* O157:H7 in cattle is significantly associated with sex ($P < 0.05$). The variation between these studies may be due to the fact that more samples are analyzed from male animals than females, and it may be due to climatic variation which plays important role (Magwira *et al.,* 2005).

Status of *E .coli* O157:H7 varies among individuals and different breeds of cattle. Exotic or cross breed of cattle may be more susceptible to any infections including by *E. coli* O157 than the local breeds (Luga *et al.,* 2007), however, the present study shows higher level of isolation rate in local breeds of cattle. Jeon *et al.* (2013) reported that local breed (Brahman) among the Angus-Brahman multi-breed excreted the lowest level of *E. coli* O157, suggesting this breed is less prone to colonization by this pathogen. Although no definitive conclusions can be drawn from this study regarding local breeds of Ethiopia, the variation might be due to the fact that the owners provide special attention to the cross breed of cattle due to their higher milk yield and food conversion capacity than local ones.

The isolation rate of *E. coli* O157:H7 was significantly related with hide cleanness ($p<0.05$). Heavy amounts of adherent dirt/faeces on fore and hind legs, underside of the

abdomen and the lower surface of the ribcage was more prevalent (5.9%) in the current study, which is in line with study made by McEvoy *et al.* (2000) in category that the briskets from excessively dungy cattle were significantly more contaminated than briskets from cattle which did not have large amounts of dung on the hide ('normal') which accounted 0.53- 0.62 cfu/ cm^2. The hide swab samples taken might harbor *E. coli* O157:H7 even if the hide seems clean, in terms of given category, so it might not be always true that clean hide are free from *E. coli* O157:H7. Contamination of the hide might occurs when animals lie down on contaminated ground/floor either on the farm, during transportation, in lairage, and/or in slaughterhouses due to the landing area (area of the floor where carcasses fall immediately after stunning) if it is not cleaned after each carcass.

In the present study, *E. coli* O157:H7 was not isolated from samples taken from butcher houses in Debre Berhan. However, previous studies done by Hiko *et al.* (2008) in Bishoftu and Balcha *et al.* (2014) in Mekelle, from Ethiopia, by Rahim *et al.* (2012) in Iran, by Cagney *et al.* (2004) in Republic of Ireland and by Adwan and Adwan (2004) in Palestine showed isolation rates of 34.4%, 18 % 2.8%, 3.14% and 27.3% from raw beef meat correspondingly. The reasons of low detection rate in this study could be due to limited sampling sources, not much diversified sample types, cold weather condition and small sample size. Also, most meat products were obtained from small-scaled meat shops unlike the other studies.

The public health importance of shedding *E. coli* O157 in transmission among humans is well established especially in families and daycares (Doyle *et al.*, 2006). Even though, in Ethiopia, due to low awareness of society and customary condition, raw/under cooked meat is consumed in the form called "*tire siga*" or " *kitfo*", and again because of unaffordable price of cooling materials, by most Ethiopia people, and fluctuating electric power in the country the probability of consuming cold food is higher, which are the main two reasons of human infection. In the current study, however, *E. coli* O157:H7 was not isolated from stool samples from hospitals. This is in agreement with report of Huruy *et al.* (2011) in Gondar teaching hospital, northwest Ethiopia in that *E. coli* O157:H7 was

not found in any of the stool samples tested. But this finding deviates from the reports of Simor *et al.* (1990) who recorded 0.3% from the total of 16,125 stool specimens within 16 month survey in Toronto and surrounding communities, 0.07% - 3.6% was reported by Sehgal *et al.* (2008) within in ten years survey in India, 60% was also reported by Karch *et al.* (1996) in Germany from stool samples taken from pediatric patients. This variation is best explained by differences in local preference of food, life style, study area (urban or rural), the immune status and age group of the patients from whom the stool was taken. Repeated exposure to infection occur in our country, due to low hygienic practices and close contact with farm animals, which confer immunity to infections, subsequently reducing in the number of the *E. coli* O157:H7 recovered from the stool samples.

Antimicrobial resistance pattern of *E. coli* O157:H7 isolates from animal and human sources have been reported in Ethiopia by Hiko *et al.* (2008). The bacteria seems to be resistant to only one antibiotic used in this study, namely Amoxicillin, but all the rest of the antibiotics used have ultimate effect on it. On study done by Mora *et al.* (2005) in Spain 58 of 141 (41%) STEC O157:H7 isolates from human, cattle, ovine and food were shown to be resistant to at least one of the 26 antibiotics tested. Difference in resistance among different antimicrobials tested might be explained by the fact that since bovines constitute an important reservoir for STEC pathogenic to humans, these antimicrobial agents are used in cattle for treatment or as growth promoters, as a result the resistance phenotypes originated in cattle. However, we cannot determine conclusively whether the resistance observed in this study among STEC isolates can be attributed to the use of these drugs in cattle production of our country. These data raise important questions about the potential impact of antibiotic use in animals and the possible entry of resistant pathogens into the food chain.

6. CONCLUTION AND RECOMMENDATIONS

In the current study, *E. coli* O157: H7 was identified from the feces, intestinal mucosal swab and hide swab samples taken from cattle slaughtered in Debre -Brehan municipal abattoir. Isolation of this organism from the fecal sample and intestinal mucosal swab indicates carriage of *E. coli* O157: H7 by the animal. The investigated contamination points are environmental samples taken from the abattoir namely, the workers hands, clothing and knifes which are used in the slaughtering operations. Though, the prevalence of *E. coli* O157:H7 in present study is low, the impact of the pathogen should not be underestimated, owing to the low infective dose of *E. coli* O157: H7, prevailing habit of consuming raw meat and low level awareness of the society about food-borne infection. The use of prohibited drugs in food animals subsequently enters in the food chain, resulting adverse human health effects.

Therefore based on the above conclusion, the following recommendations are forwarded:

- Strict prevention and control measures to reduce the public health risks arising from *E. coli* O157:H7 should be done by reducing fecal prevalence and avoiding cross contamination following good manufacturing practice and hygiene at all lines of operations in the abattoirs.

- Informing national governments, public health officials, food producers, retailers and the general public worldwide, about the importance of *E. coli* O157:H7 in enteric disease so that, epidemiologic and trace back investigations could be conducted at regional and federal level.

- National governments should ensure that *E. coli* O157:H7 is ranked first as a leading enteric bacterial pathogens, to reflect its public health importance and it should be nationally notifiable in that early reporting to the local public health authorities must be encouraged so that outbreaks can be contained.

- Regular inspection of meat and hygienic practices of the abattoir must be conducted by Ministry of Health and the respective Regional Bureaus, for corrective action, and detailed study of microbiological safety of meat in Ethiopia particularly in Debre-Berhan must be carried out on large scale to counter the lack of surveillance and response capacity in our country.

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PPENDICES

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

n collection							Laboratory work													
Sample ID	Breed	Sex	BCS	HD. Cleeanness	Sample	Sample source	Enrichmen	IMS	Colony character (CT-SMAC)	Latex Aggl.	Antibiotic			Susceptibili ty						

= body condition score, HD= hide, IMS=Immunomagnetic separation

ex 2: Media used for isolation and identification of *E. coli* O157: H7

uffered peptone water (Oxoid Ltd., Hampshire, England)

osition (g/l): Peptone 10.0; Sodium chloride 5. Final PH: 7.2 $\pm$ 0.2.

on: Add 20g to1 litter of distilled water. Mixed it well and sterilized by autoclaving at 121°C for 15 minutes.

iptone Soya Broth (TSB) (Oxide, England)

ion: Pancreatic digest of casein (17.0 g), peptic digest of soyabean meal (3.0 g), sodium chloride (5.0 g), Di-Base potassium phosphate (2.5 g), Glucose (2.5 g).

on: Suspend 30 g of power in 1 litter of Distelled water. Mixed thoroughly, Heated with frequent agitation and boiled for 1 minute, autoclave at 121°C for 15 minutes.

- Sorbitol MacConkey agar (Oxide, England)

ition (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.

ion: 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 minute. Then Potassium tellurite (2.5 mg/l) and Cefixime (0.05mg/l) were added on the prepared base media tempered at 50-55°C in water bath. Finally gently shacked and poured into Petri dishes.

. Body Condition Score.

ndition Score (BCS)	Body Fat, %	Shrunk body weight, % of BCS
1	3.77	77
2	7.54	81
3	11.30	87
4	18.07	93
5	18.89	100
6	22.16	108
7	26.38	118
8	30.15	130
9	33.91	144

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4: Irish Department of Agriculture and Food Livestock Cleanliness Categories (source: Anone, 1997).

y 1:- No evidence of adherent faecal material and limited amounts of loose straw/bedding. Animals in this category are accepted for slaughter.

y 2:- A light covering of dried faecal material and limited amounts of loosely adherent straw/bedding. Animals in this category are accepted for slaughter.

y 3:- A significant amount of loose straw/bedding/dirt over a large body area. Animals in this category are accepted for slaughter under normal conditions when presented in a dry state.

y 4:- Heavy amounts of adherent dirt/faeces on fore and hind legs, underside of the abdomen and the lower surface of the ribcage. During dressing, line speed is reduced, additional space is allowed between carcasses and the use of workstation hygiene is increased. Hide clipping may also be practiced before animals are permitted for slaughter

y 5:-Very heavy amounts of adherent dirt/faeces. Balling of adherent dirt/faeces may be evident on the underside of the abdomen. Animals are rejected for slaughter and returned to premises of origin or subjected to hide clipping

ıex 5: *E. coli* O157: H7 Questionnaire

ʻIOGRAPHIC INFORMATION

Community Area: _____

Name: _____

Address: _____

DOB: dd/mm/yyyy _____

Sex: M_____ F_____

Occupation: (retired, unemployed, housewife, student, other_____)

:SONAL DETAILS

Date of onset of first symptom: **(dd/mm/yyyy)**:_____

Does anyone else in the house hold or other close contacts have similar symptoms in the 2 weeks before or the week after you was ill.

YES **NO**

ıLTH DETAILS

	YES	NO
.1. Diarrhea (3 or more loose Stools in a 24 h period)	_____	_____
.2. Abdominal pain/cramp	_____	_____
.3. Blood in stools	_____	_____
.4. Fever	_____	_____
.5. Nausea	_____	_____
.6. Vomiting	_____	_____
.7. Headache	_____	_____

D HISTORY

3.1. Did you eat any beef or food containing beef in 7 days before becoming ill?

YES **NO**

3.2. Was any of this meat rare or undercooked?

YES **NO**

3.3. Do you have clean drinking water for a drink, cook and bath?

YES **NO**

3.4. Did you have Raw (unpasteurized) milk or other dairy products made from(unpasteurized milk)

YES **NO**

MALS

e10 days before illness began, did you:

	YES	**NO**	**UNKNOWN**
Visit or live on a farm?			
Have contact with any cows or cattle?			
Touch any cow manure?			
Contact with pet animal?			

5.4.1. If yes specify.

5.4.2. Where any of these animals ill with diarrhea in 7 days before illnesses?

.VEL DETAIL

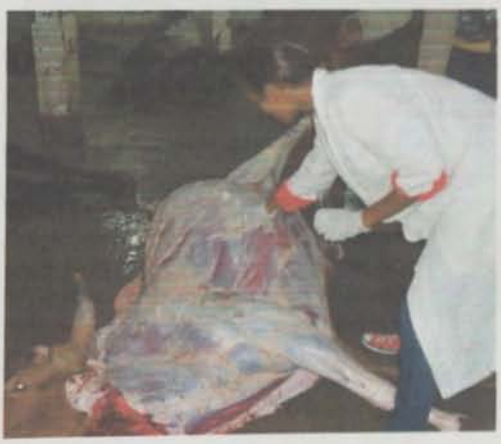
.1. Did you spend any time abroad or elsewhere in the country?

YES **NO**

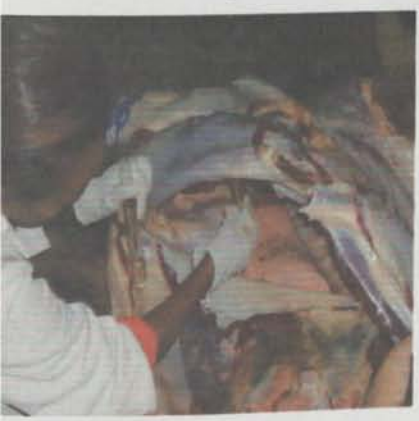
ex 6. Photo pictures showing sample collection and laboratory activities

. Environmental Swab Samples (Personnel clothing, hand and Knife from left to right.)

.2. Hide swab sample



Pic. 3. Carcass external swab sample



4. Carcass internal swab sample

Pic.5. Slaughtering operation performed on the hide of cattle, on the floor.

'ic. 6. Vortex mixing of sample

pic 7. Concentration of *E.coli* O157: H7 by IMS

3. Culturing of IMS complex on CT-SMAC

Pic. 9. Lattex Agglutinatin Test

RRICULUM VITAE

ERSONAL INFORMATION

ıe	Rosa Abdissa
phone/e-mail	0913413599/radflg@gmail.com
ɔnality	Ethiopian
tal status	single
;uages	Amharic and English
th status	Excellent
of birth	30.12.1982
: of birth	Addis ababa

DUCATION

-2004	Doctor of Veterinary Medicine
-1999	Ethiopian Higher Education Entrance Examination Certificate
1997	Ethiopian General Secondary School Leaving Certificate

kill

ɔuter skill

search Experiences and papers

- Study on the prevalence of Ixodid ticks on cattle in Welmera district, west Shoa zone of Ethiopia.
- Sperm sexing: current advancement in artificial insemination.

References

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