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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 ADDIS ABABA, ETHIOPIA

MSc Thesis

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LIST OF ABBRIVATIONS

A/E	Attaching And Effecting Lesion
BPW	Buffer Pepton Water
CDC	Center for Disease Control and Prevention
CI	Confidence Interval
CNS	Central Nervous System
CT-SMAC	CefiximeTellurite-Sorbitol Macconkey Agar
DNA	DeoxyriboNuclic Acid
EaggEC	Enteroaggravetive*Eschericia coli*
EHEC	Enterohemmorhagic*Eschericia coli*
EIEC	Enteroinvasive*Eschericia Coli*
ELISA	Enzyme Linked Immunosorbant Assay
EPEC	Enteropathogenic*Eschericia Coli*
ETEC	Enterotoxigenic*Eschericia Coli*
FAO	Food and Agricultural Organization
HACCP	Hazard Analysis Critical Control Point
HC	Hemorrhagic Colitis
HUS	Hemolytic Uremic Syndrome
IMS	.Immunomagnetic Separation
LEE	.Locus of Entrocyte and Effecement
LPS	.Lippopolysaccharide
MTSB	..Modified Trepton Soya Broth
OIE	Office International des Epixotics
PCR	Polymerase Chain Reaction
RNA	RiboNuclic Acid
rRNA	ribosomal ribonucleic acid
STX	SHIGA TOXIN
TTP	Thrombotic Thrombocytopenic Purpura
TTSS	Type III Secretion System
VTEC	VERO TOXIC *E.COLI*
WHO	WORLD HEALTH ORGANIZATION

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ABSTRACT

Escherichia coli O157: H7 is recognized as an important cause of diarrhea, hemorrhagic colitis and hemolytic-uremic syndrome worldwide. The present study was conducted from November 2013 to April 2014 on 150 apparently healthy slaughtered cattle at municipal abattoir, 115 different butcher houses and 50 stool samples from two health centers in Addis Ababa, Ethiopia with the objectives of establishing prevalence of *E. coli* O157: H7 on the carcasses at the municipal abattoir and meat presented for sale in butcher shops, providing information as to the major sources of carcass contamination in abattoir and butcher shops so that to forward strategies in minimizing the contamination and to study the public health importance of the organism in the study area. Different samples was taken for this study; skin swab, fecal samples, intestinal mucosal swabs and the inside and outside part of carcasses as well as carcass in contacts such as workers hands, knife, transporter clothes and vehicles from the abattoir and carcass swab, cutboard, butcher men hand and knife from butcher houses. In addition, stool samples from suspect individuals visiting in both Kazanchis and Kaliti health centers were collected. All the samples were then transported with buffered peptone water to the Microbiology Laboratory of College of Veterinary Medicine, Addis Ababa University for isolation and identification of *E. coli* O157:H7. After enrichment of samples with Triptone soya Broth supplemented with novobiocin and concentration of *E. coli* O157:H7 using Immuno Magnetic Separation it was inoculated on Sorbitol MacConckey agar supplemented with antibiotics, Cefixime and Potassium Telurite. Non-Sorbitol fermenting colonies were tested on latex agglutination test.From all 1380 samples tested only 10(0.72%) were found to be positive of *E. coli* O157:H7.From these 10(0.72%) positive samples 8(1.03) were from abattoir and 2(0.43%) were from butcher houses. No positive isolate were found from both health centers. All *E. coli* O157:H7 isolates were then checked for their susceptibility pattern by 10 selected antibiotics. The isolated strains were found to be susceptible (100%) to Kanamycine, Chloramphenicol, Ciprofloxacin, Sulfamethoxazole-trimithoprim and Nalidixicacid and also a Multidrug resistance to all 10 drugs was detected for all strain. To prevent contamination Hazard Analysis of Critical Control Points (HACCP) should be applied from farm to fork.

Key words: *Addis Ababa, CT-SMAC, E.coli O157:H7, IMS, Latex agglutination*

1. INTRODUCTION

Food borne pathogens are the leading cause of illness and death in developing countries costing billions of dollars in medical care and medical and social costs. Changes in eating habits, mass catering, complex and lengthy food supply procedures with increased international movement and poor hygiene practices are major contributing factors. Contaminated raw meat is one of the main sources of food borne illnesses. The risk of the transmission of zoonotic infections is also associated with contaminated meat. International food management agencies, especially the World Health Organization (WHO), the Food and Agriculture Organization and the International Hazard Analysis Critical Control Point (HACCP) Alliance have already provided guidelines to member countries about safe handling procedures such as HACCP and Good Manufacturing Practices (GMPs) (Nafisa *et al*., 2010).

Despite the extensive scientific progress and technological developments achieved in recent years in developed countries, microbial food borne illness still remains a global concern. Microorganisms of concern to meat processors may originate from the faeces and skin of animals and also include environmental sources like working utensils presented for slaughter and can be transferred to the carcass during skin removal and evisceration (Elder *et al.,* 2000). *Escherichia coli* (*E. coli*) O157:H7 is one of the most important food borne pathogens, causing diarrhea, hemorrhagic colitis and haemolytic uremic syndrome in humans worldwide (Mersha *et al.,* 2010).

Escherichia coli are genetically heterogeneous group of bacteria whose members are typically non pathogens that are a part of the normal microflora of the intestinal tract of humans and animals. However, certain subsets of this bacterial species have acquired genes that enable them to cause intestinal or extra intestinal disease (Bacon *et al*., 2000; Abdella *et al*., 2009). *E coli* that cause enteric disease have been divided into pathotypes, based on their virulence factors and mechanisms by which they cause disease. One of these pathotypes, called Shiga toxin-producing *E. coli* (STEC), refers to those strains of *E. coli* that produce at least 1 member of a class of potent cytotoxins called Shiga toxin. The STEC are also called verotoxin producing *E. coli.* The

name Shiga toxin (Stx), derived from similarity to a cytotoxin produced by *Shigella dysenteriae* serotype 1 and verotoxin (VT), based on cytotoxicity for Vero cells are used interchangeably (Gill *et al*., 1996 ; Gyles, 2007).

Shiga toxin-producing *Escherichia coli* O157:H7 (STEC O157) can cause severe enteric infections. Symptoms may include abdominal pain, bloody diarrhea, hemorrhagic colitis and haemolytic uremic syndrome (HUS) (Nataro and Kaper, 1998; Zhao *et. al*., 1994). Numerous sporadic infections and outbreaks caused by STECO157 have been reported in the United States and elsewhere in worldwide. The majority of STEC O157 infections are food borne; many are associated with bovine sources. STEC O157 was first linked to outbreaks of severe bloody diarrhea in 1982, and is often referred to as a "recently emerged" human pathogen (Wei *et al*., 2006).

Sporadic cases and outbreaks of human diseases caused by STEC have been linked to ground beef, raw milk, meat and dairy products, vegetables, unpasteurized fruit juices and water. Infections can also be acquired by direct contact with animals and by person to person spread. The organism is destroyed in pasteurization process, but insufficient heat treatment of ground meat and raw milk forms a potential infection risk (Rahimi *et al*., 2012).

Detection of *E. coli* O157:H7 in the clinical laboratory is dependent on distinguishing the pathogenic serotypes from normal fecal flora containing commensal strains of *E. Coli (*Chapman *et al.,* 2001; Battisti *et al*., 2006). Fortunately, *E. coli* O157:H7 has two unusual biochemical markers; delayed fermentation of D-sorbitol and lack of β-D-glucuronidase activity, which help to phenotypically separate O157:H7 isolates from nonpathogenic *E. Coli*strains. One of these markers (delayed sorbitol fermentation) enables to develop several selective media (e.g., Sorbitol-MacConkey; SMAC) which aid in the initial recognition of suspicious colonies isolated from bloody stools (Bindu *et al*., 2010). Detection of *E. coli* O157:H7 from food samples requires enrichment and isolation with selective and/or indicator media, but lacks specificity to identify STEC. Thus, more sensitive methods are required to improve the detect ability of STEC O157:H7 from food and environmental samples. Apart from the traditional culture methods relying on biochemical characteristics, various genotypic methods have been proven useful for

species identification, epidemiological typing, and determining genetic relatedness among pathogenic and non-pathogenic bacteria (Ji-Yeon *et al.*, 2005).

The currently accepted methods for the isolation of O157strains consist of assays for the detection of Shiga-like toxins(SLTs), either directly or at the genomic level, coupled with direct plating on sorbitol MacConhkey (SMAC) agar, cefixime-SMAC agar or SMAC agar supplemented with cefixime and tellurite (CT-SMAC) with subsequent stereotyping. Accurate diagnosis of EHEC O157 infections requires the isolation of the pathogen to clarify the etiology of disease and the infectiousness of patients as well as to allow sub-typing of strains for epidemiological purposes. The probability of isolating *E. coli* O157 strains from stool cultures of patients is inversely related to the interval between the onset of diarrhea and the microbiological culture (Helge *et al*.,1995).

Culture proven *E. coli* O157 diarrheal illness has been reported from a number of African countries including South Africa, Swaziland, Central African Republic, Kenya, Uganda Gabon, Nigeria and Ivory Coast (Raji et al., 2006). However, in the presence of all the above situations, little is known about the prevalence, distribution and associated virulent genes of *E. coli* O175: H7 in humans, animals or in foods of animal origin in Ethiopia (Hiko *et a*l., 2008; Mersha *et al*., 2010; Taye *et al*., 2013). Furthermore, it has not been determined well to what extent abattoir and butchery house environments serve as sources of E. coli O157: H7 to red meat contamination. Thus this study was designed with the following objectives:

- To study the prevalence of *E. coli* O157 in the municipal abattoir.
- To determine to what extent the abattoir and butcher house environments serve as sources of *E. coli* O175: H7 particularly to red meat contamination so that to locate the major point where safety problem occurs along the meat chain.
- Survey for the presence of human infection in the area in question and its potential implications as health hazard to consumers.
- To identify the antimicrobial susceptibility patterns of *E.coli O157: H7.*

2. LITERATURE REVIEW

2.1. Historical Back Ground

Escherichia coli were first described in 1885 by Theodor Escherich. 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 (*Kaper, 2005).

Escherichia coli are ubiquitous intestinal bacterial flora of animals and humans. Although comprising a small proportion of the total faecal flora they are the predominant facultative anaerobe in the human colon and presumably exist there symbiotically (Wei *et a*l., 2006). Infants and post parturient animals are normally colonized shortly after parturition, acquiring their mothers' intestinal flora. Pathogenic strains or clones of *E. coli* exist or have developed with the ability to cause a wide variety of diseases in humans (Battisti *et al*., 2006). During the past twenty years, a clonal group of *E.coli* has emerged or been recognized that possess a unique disease causing virulence factor armament, asymptomatically colonize food producing ruminants, and are capable of causing significant morbidity and mortality in humans (Johnsen *et* al.,2001).

*Escherichia coli*O157:H7 is considered a serious threat to public health in developed countries. In the United States alone, it is the single greatest cause of Hemorrhagic Colitis and Hemolytic Uremic Syndrome (HUS).*E. coli* O157:H7 causes the majority and most severe outbreaks of gastrointestinal illnesses related to *E. coli* from infections that range from asymptomatic conditions to mild bloody diarrhea or even severe Hemorrhagic Colitis (Hayashi *et al*., 2001)

2.2. Taxonomy

Escherichia coli is a gram negative, facultative anaerobic, non spore forming rod, which belongs to the Enterbacteriaceae family (Law, 2000).*E. coli* is a Gram negative bacterium in the Phylum *Proteobacteria*, class *Gamma proteobacteria*, order *Enterobacteriales*,f

amily *Enterobacteriaceae* and genus *Escherichia*. In the family *Enterobacteriaceae* most important genera are *E coli, Shigella, Salmonella, Yersinia* which have some common characteristic such as Gram negative rods, non spore forming facultative anaerobic, ferment glucose, simple growth requirements, most are motile with peritrichous flagella and many produce fimbriae (pili), capsules, or both. *E. coli* are one of coliform organisms (Quinn *et al*., 2002).

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) ((Quinn *et al*., 2002).There are approximately 174 O antigens, 56 H antigens, and 103 antigens that have been identified. 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) (Frenzen and Drake, 2005).

Escherchia coli O157: H7 produce toxins which are toxic to Vero (African green monkey kidney) tissue culture cells and are similar to Shiga toxin of Shigella dysenteriae. They have been known as Verotoxin 1 and 2 and as Shiga-like toxin I and II. The strains of *E. coli* that produce these toxins have been known as verotoxin producing *E. coli* (VTEC) or as Shiga-like toxin producing *E. coli* (STEC) (Vidal *et al*., 2004). "Stx producing *E.coli* O157" is synonymous with *E.coli* O157: H7. The term VTEC is still widely used in United Kingdom and many European scientific publications. The term STEC is used especially in American scientific papers. The term *Entero haemorrhagicE.coli* (EHEC) was originally coined to denote strains that cause HC and HUS (Duffy *et al*., 2002).

2.3. Morphology

Escherichia coli are gram negative, Facultative anaerobic and Non sporulating organism. The cells are about 2µ long and 0.5µ in diameter with a cell volumeof 0.6 to 0.7μm^3. Some strains of *E. coli* possess flagella with peritrichus arrangement (Patrick *et al*., 2000).Most *E. coli* are

motile, specially the O157: H7 strain, by means of peritrichous flagellae at opposed to polar, which are strings of protein made in the shape of a corkscrew. They are continuously replenished from inside as they may be broken off. They are attached to a hook like structure embedded in the cell wall, which rotates around 360° causing the screw like flagellae to push the bacteria through the water. In changing the direction, it is reversed along the 360° (Quinn *et al*., 2002).

2.4. Characteristics of *Escherichia Coli* O157: H7

Escherichia coli can be characterized by stereotyping, a method based on differences in antigenic structure on the bacterial surface. The serotype is defined by the bacterium's O-antigen (Ohne), a polysaccharide domain in the bacterium's lipopolysaccharide (LPS) in the outer membrane, and the H-antigen (Hauch) consisting of flagella protein. Serotyping may also include the K antigen (Kapsel) and the F-antigen (Fimbriae) (Ratnam *et al*.,1988)There are many known O, H, K and F antigens and the existing number of different serotypes is known to be very high. Serotyping is an important tool which can be used in combination with other methods to distinguish pathogenic *E. coli* strains as specific pathogenicity attributes are often linked to certain serotypes (Gyles, 2007).

Escherichia coli O157:H7 is an enteric pathogen that can cause diseases ranging from mild diarrhea to hemolyticuremic syndrome, kidney failure, and death. The Shiga toxins, Stx1 and Stx2, are considered to be the primary *E. coli* O157:H7 virulence factors and the cells may harbour genes that express one or both of these toxins. However, the Shiga toxins alone may not be sufficient to cause disease. Additional known virulence factors include intimin and enterohemolysin, products of theeae and hlyA genes, respectively (Nataro and Kaper, 1998).

2.5. Epidemiology

2.5.1. Geographical distribution

Escherichia coli 0157:H7 infections occur worldwide; infections have been reported on every

continent except Antarctica. Other EHEC are probably also widely distributed. The importance of some serotypes may vary with the geographic area (CFSPH, 2009).

The most severe cases of infection typically occur in children <5 years of age, the elderly, and immune-compromised persons. In severe cases of infection, individuals present with hemorrhagic colitis, haemolytic uremic syndrome (HUS is the leading cause of acute renal failure in children),microangiopathic hemolyticanemia (fragmented erythrocytes) and thrombocytopenia (low platelet count). Long term complications of EHEC infection include irritable bowel syndrome (IBS) (Marshall *et al*., 2010). In North America, approximately 75,000 cases of EHEC infections are reported annually. Of these, 10-15% of cases develop HUS, another 5-10% result in long-term complications and 3-5% of HUS cases are fatal EHEC infections account for roughly 250 deaths in North America each year (Serna and Boedeker, 2008).

Most *E coli* O157:H7 infections and HUS occur in the summer and autumn. Non-O157:H7 STEC infections in Australia11 and Montana, USA, had similar seasonality but this pattern did not occur in Seattle, USA (Panos *et al*., 2006). The incidence of HUS probably increased in several regions during the 1970s and1980s, but increasing or decreasing trends have not been proven unequivocally and one population based study found stable incidence during the 1990s (Panos *et al*., 2006). A reported lower likelihood that children of African descent would have HUS was not confirmed in recent series from Natal and North America. The risk of developing HUS relates also to consumptions and behaviours leading to acquisition of infection, so demographic differences in incidence might reflect demographic differences in exposure to the causative agent, rather than differences in genetic propensity to develop HUS once infected (Phillip *et al*., 2005).

2.5.2. Reservoirs of E. coli O157:H7

Understanding the epidemiology of this organism requires knowledge of where these bacteria live and grow in nature (their reservoir) and of how humans come into contacts them. Ruminants have been identified as the major reservoir of *E. coli* O157:H7, with cattle as the most important source of human infections (Calderwood*et al*., 1996) 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 acquired 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 (Kang *et al*., 2004).

2.5.3. 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. Studies on the thermal sensitivity of *E. coli* O157:H7 in ground beef have revealed that the pathogen has no unusual resistance to heat and that heating ground beef sufficiently to kill typical strains of *Salmonella* will also kill *E. coli* O157:H7. Thermal pasteurization of milk has also been determined to be an effective treatment (Dylla*et al*., 1995). The optimal temperature for growth of *E. coli* O157:H7 is approximately 37°C (98.6^{o}F), and the organism will not grow at temperatures below 8°C to 10°C (46°F to 50°F) or above 44°C to 45°C. *E. coli* O157:H7 survives freezing, with some decline in the concentration of *E. coli* O157:H7 (Buchanan and Doyle, 1997).

Escherchia.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 food borne pathogens. Published literature contains conflicting reports about the efficacy of acid spray washing of beef carcasses (Buchanan and Doyle 1997). A study by Brachett *et al.* (1994) found that warm and hot acid sprays did not significantly reduce the concentration of *E. coli* O157:H7 on beef carcasses. Two recent studies have found organic acids to be effective in

reducing the presence of *E. coli* O157:H7 on beef carcasses (Besser *et al*., 2003). These apparently contradictory results may reflect differences in acid resistance among strains of *E. coli* O157:H7 E. coli O157:H7 can survive for extended periods under conditions of reduced water activity while refrigerated; however, the organism does (Bastian *et al*., 1999).

2.5.4. Virulence factor

The Enterohemorrhagic *Escherichia coli* (EHEC) strain O157:H7 is a major food borne pathogen causing severe disease in humans worldwide. Healthy cattle are a reservoir of *E. coli* O157:H7. Bovine food products and fresh products contaminated with bovine waste are the most common sources for Haemorrhagic Colitis (HC) and the Haemolytic Uremic Syndrome (HUS) (Callaway *et al*., 2009).

Three 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 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 microvilli and intimate attachment of the bacteria to host cell plasma membranes. The Locus of Enterocyte Effacement (LEE) genetically governs adhesion and subsequent pathology. It contains the eae gene, encoding the outer membrane protein intimin and its receptor Tir (Trans located intimin receptor). In addition, LEE encodes proteins of the type III secretion system (TTSS), which is made up of an EspA multifilament needle complex, used for insertion of the bacterial effector proteins EspB, EspD and Tir into the host cell. Injection of bacterial virulence factors via the TTSS and binding of intimin to Tir leads to a strong interaction between bacteria and host cells (Cookson and Woodward, 2003).

Virulence arises also from Shiga toxin production, encoded by Shiga toxin genes (stx1 and stx2), which are the primary factors responsible for the hemorrhagic aspect of diarrhoea and systemic complications (HUS). Shiga toxins act as N-glycosidases, cleaving ribosomal RNA leading to the inhibition of host cell protein synthesis (Vilte *et al.*, 2008). Most adults recover from *E. coli*

O157:H7 infections without sequelae. Children and the elderly however, are more likely to experience complications such as HUS and even death. The use of antibiotics in treatment for *E.coli* O157:H7 infections in humans are highly controversial as antibiotics might increase the risk of HUS. Thus, treatment is largely supportive. Nonetheless, innovative therapies such as the use of probiotics, monoclonal antibodies or recombinant bacteria to neutralize or bind toxins, are currently being explored (Bavaro, 2009).

2.5.5. Source of infection and modes of transmission

E. coli O157:H7 has been isolated from the faeces or gastrointestinal tract of cattle, sheep, horses, pigs, turkeys, dogs, and a variety of wild animal species (Heuvelink *et al*. 1999); however, epidemiologic studies have found that cattle manure is the source of most human *E. coli* O157:H7 infections. *E. coli* O157:H7 has also been isolated from bodies of water (e.g., ponds, streams), wells, and water troughs and has been found to survive for months in manure and water trough sediments (Sargeant *et al*., 2000). *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 faeces during the slaughter process (Hancock *et al*., 2001).

Enteroheamorragic *E.coli* is transmitted by the faecal oral route. They can be spread between animals by direct contact or via water troughs, shared feed, contaminated pastures or other environmental sources. Birds and flies are potential vectors. In one experiment, EHEC O157:H7 was transmitted in aerosols when the distance between pigs was at least 10 feet. The organism was thought to have become aerosolized during high pressure washing of pens, but normal feeding and rooting behavior may have also contributed (Dipineto *et al*., 2006).

Enteroheamorragic *Escherchia.coli* O157:H7 is mainly transmitted to humans by the consumption of contaminated food and water, or by contact with animals, faeces and contaminated soil. Person-to-person transmission can contribute to disease spread during

outbreaks; however, humans do not appear to be a maintenance host for this organism. Most human cases have been linked to direct or indirect contact with cattle, but some have been associated with other species including sheep, goats (unpasteurized goat milk), pigs (dry fermented pork salami), deer (venison), horses, rabbits and birds. The infectious dose for humans is estimated to be under 100 organisms, and might be as few as 10 (Ateba and Bezuidenhout, 2008). EHEC O157:H7 can remain viable for long periods in many food products. It can survive for at least nine months in ground beef stored at -20°C (-4°F). It is tolerant of acidity, and remains infectious for weeks to months in acidic foods such as mayonnaise, sausage, apple cider and cheddar at refrigeration temperatures. It also resists drying (Chase-Topping *et al*., 2007).

Some human cases are caused by exposure to contaminated soil or water. EHEC are usually eliminated by municipal water treatment, but these organisms may occur in private water supplies such as wells. Swimming in contaminated water, especially lakes and streams, has been associated with some infections. Soil contamination has caused outbreaks at campgrounds and other sites, often when the site had been grazed earlier by livestock. The reported survival time for EHEC O157:H7 in contaminated soil varies from a month to more than 7 months. This organism can also survive for 2 months or longer in some freshwater sources, especially at cold temperatures, and it may remain viable for two weeks in marine water (DebRoy and Roberts, 2006).

2.5.6. Pathogenesis

Escherchia coli O157:H7 can with stand the acidic environment of the human stomach and begins the arduous and complex process of infection. From the point of ingestion, the incubation period of *E. coli* O157:H7 ranges from 8 hours to 16 days, but the typical incubation period is three to four days. During this time, the bacteria progress through several phases of infection including adherence, colonization and the production and release of Stxs. First, *E. coli* O157:H7 must initially adhere to the microvilli of the host epithelial cells. The association between the bacterial and host cells consequently induces the expression of the TTSS genes located on the LEE. Following their synthesis, the TTSS proteins are systematically assembled (Kaper *et al*., 2004).

The membrane-bound proteins first associate and form the foundation of the TTSS followed then by the proteins that form the extracellular channel and by the protein that create the pore in the host cell membrane. Once assembled, a multitude of effector proteins are shuttled through the TTSS channel and into the cytoplasm of the host cell. After the effector proteins invade the cytoplasm, they alter the host cell's normal patterns of signal transduction in order to accommodate bacterial adherence. The alterations in signal transduction are accomplished through the activities of the bacterial effector proteins, and, by selective phosphorylation, the effector proteins force actin topolymerize and the cytoskeleton to reorganize (Jores *et al*., 2004).

Reorganization results in the effacement of host cell microvilli. At this point, the bacteria may superficially adhere to the host cell while simultaneously preparing for intimate adherence. Intimate adherence of the bacterial cell to the host cell requires the orchestrated activities of LEE-encoded intimin and the specialized Tir (Ceponis *et al*., 2005). Intimin is an outer membrane protein whose presence and proper activity arecritical to intimate adherence and the formation of the A/E lesion. A proposed auto transport system is believed to be responsible for shuttling and inserting intimin into the outer membrane of the bacterial cell where intimin then adopts a β–barrel conformation (Garmendia *et al.* 2005).

Although the N-terminus of intimin is highly conserved, the C-terminus is slightly more variable in comparison. As a consequence, approximately 14 subtypes of intimin are now recognized and each variant has a slightly different tissue tropism for the assorted gastrointestinal cells. On the other hand, Tir is more unique, because it is a protein receptor manufactured by the bacterial cell itself, which appears to be a distinctive trait of attaching-effacing bacteria. During infection, Tir is synthesized in the bacterial cell and then translocates through the TTSS to the host epithelial cell. After translocation, Tir inserts spontaneously into the plasma membrane and adopts a hairpin loocon formation as it is presented on the surface of the epithelial cell (Lim *et al*., 2007).

Once inserted, intimin, located on the surface of the bacterial cell, associates with Tir and causes a more exaggerated rearrangement of the host cytoskeleton. This rearrangement forms the characteristic pedestal of the A/E lesion and enables the bacterial cell to intimately adhere to the

host cell. As more *E. coli* O157:H7 intimately adhere to host epithelial cells and form the A/E lesions, the bacteria begin to overwhelm the normal microflora of the intestinal tract and successfully colonize the host (Leblanc, 2003).

However, the exact means by which *E. coli* O157:H7 establishes and sustains colonization in the host remains elusive. Once it has successfully colonized and established itself within the host, *E.coli* O157:H7 produces and releases it Stxs in the intestinal lumen (Mainil and Daube, 2005). The B subunits of the Stxs then bind to their corresponding Gb3 receptors on the surface of epithelial and, unlike the LEE-encoded effector proteins, to endothelial cells as well. The A subunit of the Stxs is then internalized into the host cell through the process of receptor-mediated endocytosis and transported to the Golgi apparatus where furin, a serine protease, cleaves the A subunit. Next, the A subunit is shuttled to the endoplasmic reticulum where a critical disulfide bond is reduced, releasing the active fragment. The active fragment functions as an RNA N-glycosidase that selectively depurinates an adenine residue in the 28S rRNA of the largeribosomal subunit. This prohibits elongation factors 1 and 2 from binding to the ribosome and inhibits host cell proteinsynthesis, ultimately forcing the host cell to undergo apoptosis (Robins-Browne, 2005).

In addition, it is believed that apoptosis results in the local symptoms of disease. Furthermore, the Stxs can translocate from intestinal epithelial cells into the bloodstream. Here, the Stxs bind to the Gb3 receptors on glomerular endothelial cells. The Stxs injure the glomerular cells and cause platelets and fibrin to deposit within the glomeruli. Eventually, the deposits decrease renal filtration and lead to the acute kidney damage characteristic of HUS (Welinder-Olsson and Kaijser, 2005).

2.6. Clinical signs

2.6.1. In Humans

Human can be infected asymptomatically or they may develop watery diarrhea, hemorrhagic

colitis and/ or hemolytic uremic syndrome. Most symptomatic cases begin with diarrhea. Some cases resolve without treatment in approximately a week; others progress to hemorrhagic colitis within a few days. Hemorrhagic colitis is characterized by diarrhea with profuse, visible blood, accompanied by abdominal tenderness, and in many cases, by severe abdominal cramps. Some patients have a low grade fever; in others, fever is absent. Nausea and vomiting may be seen, and dehydration is possible. Many cases of hemorrhagic colitis are self–limiting and resolve in approximately a week. Severe colitis may result in intestinal necrosis, perforation or the development of colonic strictures (Karch *et al.,* 2005).

Hemolytic uremic syndrome occurs in up to 16% of patients with hemorrhagic colitis. This syndrome is most common in children, the elderly and those who are immune compromised. It usually develops a week after the diarrhea begins, when the patient is improving. Occasionally, children develop HUS without prodromal diarrhea. HUS is characterized by kidney failure, haemolytic anaemia and thrombocytopenia (Radostits *et al*., 2000; Quinn *et al*., 2002). The relative importance of these signs varies. Some patients with HUS have haemolytic anaemia and/or thrombocytopenia with little or no renal disease, while others have significant kidney disease but no thrombocytopenia and/or minimal haemolysis. Extra renal signs including CNS involvement with lethargy, irritability and seizures are common (Garcia *et al*., 2006). In more severe cases, there may be paresis, stroke, cerebral edema or coma. Respiratory complications can include pleural effusion, fluid overload and adult respiratory distress syndrome. Elevation of pancreatic enzymes or pancreatitis may also be seen Rhabdomyolysis and myocardial involvement are rare. The form of HUS usually seen in adults, particularly the elderly, is sometimes called thrombotic thrombocytopenic purpura (TTP). In TTP, there is typically less kidney damage than in children, but neurologic signs including stroke, seizures and CNS deterioration are more common. Death occurs most often in cases with serious extra renal disease such as severe CNS signs. Approximately 65–85% of children recover from HUS without permanent damage; however, long-term renal complications including hypertension, renal insufficiency and end-stage renal failure also occur. Residual extra renal problems such as transient or permanent insulin dependent diabetes mellitus, pancreatic insufficiency, gastrointestinal complications or neurological defects such as poor fine-motor coordination are possible (Ejidokun *et al*., 2006).

2.6.2. In animals

*Escherichia col*i cause three main kinds of disease in animals, which are urinary tract infections, neonatal meningitis and diarrheal diseases (Quinn *et al*., 2002). They are mostly associated with coli bacilosis in neonate (Radostits *et al*., 2000; Quinn *et al*., 2002) and associated with different diseases like primary nosocomic pneumonia, wound infections and peritonitis by opportunistic. In addition to these, EHEC O157: H7 causes more rapid and severe neurological disease in suckling neonates (Dean-nystrom *et al*., 2000), edema disease in pig, haemorrhagic entero colitis in calves and post weaning diarrhoea in pigs (Quinn *et al*., 2002). Occasionally *E. coli* O157: H7 toxins that were first identified by their cytopathic effects on Vero cells toxins (VT1, VT2 and VT2e) that damage the vasculature in intestine and other locations. VT and endotoxins of this serotype also affect brain and cause neurological signs in animals (Dean-nystrom *et al*., 2000; Ceponis *et al*., 2005). Dogs that were experimentally inoculated with EHEC O157:H7 developed transient acute diarrhea with decreased appetite and vomiting, but recovered spontaneously without complications in 1-2 days. In the same experiment, dogs inoculated with a non O157 EHEC developed severe disease, with diarrhea and vomiting followed by lethargy and in appetence, dehydration and dramatic weight loss. These dogs also had neurological signs including seizures, cerebral infarction, blindness and coma, and died 5-6 days after the onset of clinical signs (Rasmussen and Casey, 2001).

2.7. Diagnosis

Food and environmental samples may also be tested to determine the source of the infection. EHEC are sometimes difficult to identify. They are a minor population in the fecal flora or food. They also closely resemble commensal *E. coli* except in verocytotoxin production. However, the verocytotoxin alone does not necessarily identify an organism as EHEC; additional virulence factors must also be present (Sass *et al*., 2003).

Many diagnostic laboratories can detect verocytotoxin producing *E. coli* (VTEC) and identify. There is no single technique that can be used to isolate all EHEC serotypes. Carrier animals are usually detected by finding EHEC in fecal samples, which are either freshly voided or taken

directly from the animal. Recto anal swabs may also be used in some cases. Intestinal contents can be collected at slaughter. Repeated sampling, as well as sampling more animals, increases the chance of detection (Keen *et al*., 2006).

2.7.1. Sampling

In most cases, samples taken from animals for VTEC isolation will be faeces collected for surveillance purposes or as part of an epidemiological trace back exercise following an outbreak of disease in humans. Samples may be taken from the rectum or from freshly voided faeces on the farm or from intestinal contents after slaughter (Radostits *et al*., 2000; Quinn *et al*., 2002). A variety of VTEC are present in healthy animals and not all are thought to be pathogenic for humans. *Escherichia coli* O157:H7, which is the most significant VTEC in human disease, is carried sub clinically in animals. Cattle are thought to be the most important reservoir of this serotype. In an infected herd, only a proportion of the animals will be detectably infected, the organism is usually present in carriers in low numbers and is shed intermittently in faeces. Shedding is influenced by the age of the animals, diet, stress, population density, geographical location and season (Meyer-broseta *et al*., 2001). Some animals are thought to contribute disproportionately to transmission of infection and have been termed “super-shedders”. Isolation rates may be improved by taking faeces samples in preference to rectal swabs, by increasing the sample size, by increasing the number of individuals sampled and by repeat sampling. Use of recto anal mucosal swabs is reported to improve detection of colonised as distinct from transiently infected cattle. Precautions should be taken to avoid cross contamination of samples in transit and at the laboratory. Samples should be kept cool and cultured as soon as possible after collection (Rice *et al*., 2003).

2.7.2. Isolation methods

Liquid enrichment media

Clinical samples are routinely plated directly on to solid media for isolation of *E. coli*, but the number of target VTEC organisms in faeces from healthy carriers is usually low and enrichment

in liquid media improves recovery. Commonly used enrichment media are buffered peptone water either un supplemented (which gives good recovery) or supplemented with 8 mg/litre vancomycin, 10 mg/litre cefsulodin and 0.05 mg/litrecefixime (BPW-VCC) to suppress the growth of Gram-positive organisms, *Aeromonas* spp. and *Proteus* spp.; modified trypticase–soy broth (mTSB) supplemented with 20 mg/litre novobiocin or 10 mg/litre acriflavinto reduce the growth of Gram positive organisms; or modified *E. coli* broth with 20 mg/litre novobiocin (mEC+n) (Radostits *et al*., 2000). EHEC *E. coli* grow poorly at 44°C. The optimal incubation for bovine faeces to minimize overgrowth by other organisms is 6 hours at 37°C. For meat samples, enrichment for 6 hours at 41–42°C is used and for water and dairy products, 24 hours at 41–42°C (Quinn *et al*., 2002). Non selective pre enrichment is necessary for the effective recovery of low levels of stressed *E. coli* O157. Enrichment broths should be pre warmed to prevent cold shocking the organisms and slowing their initial growth; 24 hours' incubation may increase recovery if the organisms are stressed (Clifton-hadley, 2000).

Selective culture for *Escherichia coli* O157

There are no biochemical characteristics that distinguish the majority of VTEC from other *E. coli*, however, the inability of most strains of *E. coli* O157:H7 to ferment D-sorbitol rapidly and their lack of betaglucuronidase activity can be exploited in the isolation and identification of these organisms. However, the less common sorbitol fermenting and beta-glucuronidase positive *E. coli* O157: H variants (non motile due to lack of expression of the H7 antigen), will not be identified by isolation in such selective media chosen for these biochemical characteristics (Karch and Bielaszewska, 2001).

MacConkey agar containing 1% D-sorbitol instead of lactose (SMAC) is a useful and inexpensive medium on which non-sorbitol-fermenting *E. coli* grow as small, round grayish white colonies. Selectivity is improved by the addition of 0.5% rhamnose, and addition of 0.05 mg/litre cefixime (CR-SMAC) inhibits overgrowth by Proteus spp (Radostits *et al*., 2000; Quinn *et al*., 2002). While fewer presumptive colonies require testing on this medium, rhamnose is an expensive supplement. An alternative modification is the addition of 2.5 mg/litre potassium

tellurite in addition to cefixime (CT-SMAC), which has a greater inhibitory effect against *E. coli* non-O157 and other non sorbitol fermenters, such as *Aeromonas*, *Plesiomonas*, *Morganella*and *Providencia*, than against *E. coli* O157. This is currently the most commonly used medium for isolating *E. coli* O157 (Lee and Choi, 2006).Media containing fluorogenic or chromogenic glucuronides are used to distinguish non-beta-glucuronidase producing *E. coli* O157:H7 from beta-glucuronidase-producing *E. Coli* (Tesh *et al.,* 1991).

Sorbitol-fermenting (SF) *E. coli* O157:H7 has been isolated from patients with diarrhoea and HUS but the epidemiology of this infection is poorly understood and only rarely has the organism been isolated from animals, including cattle. (Blackburn and Mccarthy, 2000).

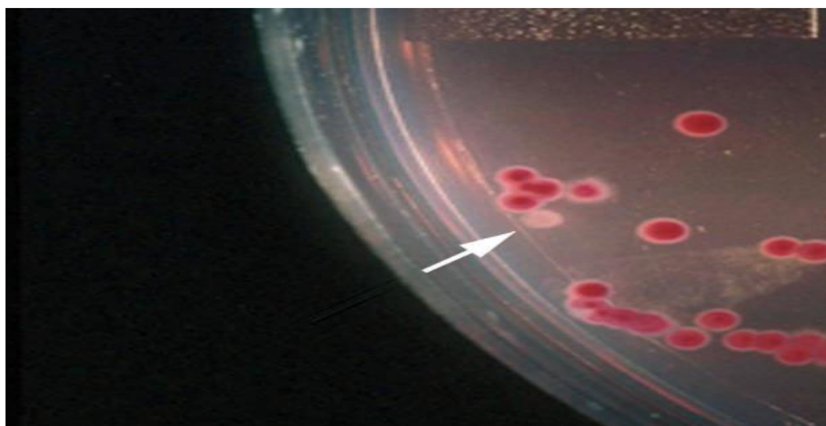


Figure 1: *E. coli* O157:H7 on a sorbitol-MacConkey agar plateArrow indicates distinctive colourless *E. coli* O157:H7 colony (Phillip *et al*., 2005).

2.7.3. Immunologic based methods

Immunological methods are now widely used for the detection of VT. The methods utilize VT specific poly or monoclonal antibodies. There are various assay formats, several of which are commercially available. The assay formats include enzyme linked immune sorbent assays (ELISA) and reversed passive latex agglutination (RPLA) (Beutin, 2003).

Immunoassays to identify O and H antigens and VT may be used to confirm the identity of the organisms once isolated from clinical, food or environmental samples, while others, including

dipstick and membrane technologies, microplate assays, colony immune blotting, immune fluorescence and ELISA, are used as rapid methods for detecting the presence of potential pathogens in samples prior to isolation, thus shortening the time for a presumptive diagnosis. Most assays for somatic and flagellar antigens are designed to detect the O157 LPS and H7 flagellar antigen. Enzyme immunoassays for O157 and VT, visual immunoassays for O157 and agglutination tests for O157, H7 and VT are available commercially as kits (Strockbine *et al., 1998).*

ELISA

Immunoassays such as enzyme-linked immune sorbent assay (ELISA) were very useful for rapid screening of *E. coli* O157:H7 and non O157 in food. ELISA assays are based upon the same reaction, with monoclonal antibody (MAb) reactive with low molecular weight outer membrane antigens of *E. coli* O157:H7, but are performed in microplates and the antibody is coupled with an enzyme that allows colorimetric screening. It has been revealed that the target antigens of the MAb are present in other serotypes of *E. coli* and that their expression and detection are influenced by culture conditions and sample preparation.(Johnson *et al., 1995).*

Immunomagnetic Separation

Immunomagnetic separation (IMS) has been used as a selective concentration technique to improve isolation of *E. coli* O157:H7 where numbers of the organism are low. Commercially available paramagnetic particles or beads coated with anti-lipopolysaccharide antibody are mixed with an aliquot of incubated broth. Beads with bound bacteria are separated from the supernatant by a magnetic field and after washing are plated on to selective agar and incubated for 18 hours at 37°C to isolate suspect colonies. The technique is serogroup specific (Tarr *et al*., 2000). Recovery may be affected by the bead to organism ratio (optimum is 3:1), the enrichment broth used and the problem of nonspecific adsorption of *E. coli* to the magnetic beads, which can be reduced by the use of a low ionic strength solution in the IMS procedure and careful washing. These factors should be taken in to account when trying to maximize the sensitivity of the technique for detecting target E. coli (Church, 2007).

Latex Agglutination Test

The Plasmatic *E.coli* test kit is a latex agglutination test for the rapid identification of *E.coli* sero group O157. The test is best used in conjunction with Sorbitol MacConkey Agar. *E.coli O157* strains cannot ferment sorbitol and will therefore give colourless colonies. The majority of other *E.coli* strains is capable of fermenting sorbitol and therefore, gives characteristic pink colonies. The non-sorbitol fermenting (NSF) colonies were further tested by the latex agglutination method. A positive result is indicated by agglutination with the test reagent, whilst the control reagent should appear milky and smooth (Shelton *et al*., 2004).

PCR

Most detection methods for verotoxigenic *E. coli* are based on cultivation of serotype O157 (Gilgen *et al*., 1998). The low infectious dosage of *E. coli* O157:H7 requires a sensitive detection method (Armstrong *et al.,* 1996). *E. coli* O157:H7 have two verotoxin genes (VT), shiga toxins (Stx_1 and Stx_2). PCR technique allows not only detection of VTECs irrespective of their serotypes, but also detects all different variants of VT (Gilgen *et al*., 1998).

A number of specific PCR procedures are described for detection of genes encoding the virulence factors (Nataro and Karper, 1998; Strockbine *et al*., 1998). Because the genes are well known, the direct detection of the genes encoding the virulence factors by PCR in most laboratories is most convenient (Global Salm-Surv, 2003). PCR can be used on pure or mixed plate or broth cultures, and extracts from food or feces (OIE, 2004). It can also be used to detect genes in non viable organisms. It has the potential to be used to screen samples for EHEC in epidemiological studies. Amplification of target genes in bacterial Deoxyribonucleic acid (DNA) extracts from faeces is less successful than from pure cultures, and careful preparation of the sample is required to improve sensitivity (OIE, 2004). Sensitivity is improved by non selective enrichment prior to testing (Moxley, 2003; OIE, 2004). DNA probes and PCR assays have also been developed to detect other genes in EHEC shown to be associated with virulence in humans (Strockbine *et al*., 1998; OIE, 2004).

Most DNA probes and PCR techniques of EHEC are directed toward the detection of genes encoding Stx. While the presence of Stx-producing strains of any serotype in a clinical specimen is assumed to be significant, the mere presence of such strains of non-O157 serogroups in food or other nonclinical samples is of uncertain significance. Frequent loss of *stx* genes upon subculture can also occur. For these reasons, probes and PCR techniques for additional EHEC virulence factors can often provide crucial information (Inat and Siriken,2010).

2.8. Treatment

Antimicrobial agents have no proven value in the treatment of *E. coli* O157:H7 infections. No randomized clinical trials of the early use of antimicrobial agents in this disease have been performed (Mead and Griffin, 1998). Treatment of hemorrhagic colitis is supportive, and may include fluids and a bland diet. Antibiotics are controversial and are usually avoided: they do not seem to reduce symptoms, prevent complications or decrease shedding, and they may increase the risk of HUS (Boyce *et al*., 1995). The use of anti motility (anti diarrheal) agents in hemorrhagic colitis also seems to increase the risk for developing HUS. (Wilkerson *et al*. (2004) reported that some antimicrobial agents, particularly quinolones, trimethoprim, and furazolidone, were shown to induce toxin gene expression and should be avoided in treating patients with confirmed *E. coli* O157 infections.Patients with complications may require intensive care including dialysis, transfusion and/or platelet infusion. Patients who develop irreversible kidney failure may need a kidney transplant (Scheiring *et al*., 2008).

2.9. Control and Prevention

Close cooperation and communication among clinicians, public health authorities, and clinical microbiologists are needed to help prevent *E. coli* O157:H7 infections. As with many food borne diseases, efforts to decrease contamination of foods throughout the production and distribution chain in both commercial establishment and the domestic environment are necessary to reduce the risk of infection ((Besser, *et al*., 2003).

Effective prevention and control of contamination in abattoirs requires the application of good hygiene practices, the application of Hazard Analysis and Critical Control Point (HACCP) based management practices and risk-based meat inspection practices to minimize faecal contamination of carcasses. In an effort to improve quantity and quality of food, FAO is promoting good management practices in the dairy and beef sector, often in collaboration with the private sector. Examples include the preparation of manuals such as the IDF/FAO Guide to Good Dairy Farming Practice or the development of training material and capacity building interventions in relation to hygienic milk handling and processing but also testing and quality control (Sargeant and Smith, 2003).

Preventions in humans can be achieved through consumption of well cooked beef products, pasteurized milk, milk products, and juices, drinking chlorinated municipal water that has been treated with adequate levels of or other effective disinfectants are also important points (Acha and Szyfres, 2001). In general, a multiple hurdle system for reducing the probability of contamination of carcasses with bacterial pathogens helps directly and/or indirectly in the reduction of risks associated with public and animal health (Smith, 2000). The *E. coli* O157:H7 organism is easily killed by heat. Cooking at 155°F for 0.13minutes will kill the number of organisms usually present in contaminated food products (Jay, 2000). This is easy to accomplish form eat products such as hamburger or sausage. However, for products consumed without cooking such as apple cider or lettuce it presents much more of a problem. Foods such as milk, apple cider, and apple juice should obviously be pasteurized-this is not only important for preventing infection by *E. coli* O157:H7, but also for Salmonella, Campylobacter, and other pathogens. Also, the issue of cleanliness by food handlers and food preparers is central to prevention of any food safety concern (John, 1996). Cook all ground beef and hamburger thoroughly. Ground beef should be cooked until a thermometer inserted into the thickest part of the patty reads at least 160º F on a digital thermometer or until the patty is no longer pink inside. Drink only pasteurized milk, apple juice, or cider. Commercial juice with an extended shelf life that is sold at room temperature (e.g., juice in cardboard boxes, vacuum-sealed juice in glass containers) has been pasteurized, although this is generally not indicated on the label. Juice concentrates are also heated sufficiently to kill pathogens (SCHHSA, 2011).

Vaccines

There are no currently available vaccines to prevent disease due to EHEC (OIE, 2004), but a number of experimental approaches are being investigated in animals. Vaccine development has been severely hampered by the lack of an appropriate animal model, which can develop HUS after exposure to EHEC (Nataro and Kaper, 1998). A crucial antigen in any potential vaccine is the Stx. Parenteral Stx toxoid vaccines have shown protective effects in rabbits (Bielaszewska *et al*., 1994) and pigs (Bosworth *et al*., 1996). Colonization factors, such as intimin (the intestinal adherence factor), as an edible vaccine in transgenic plants have been tried to develop as a vaccine (IFT, 2003; OIE, 2004; Edwards and Fung, 2006). A parenteral vaccine specific for O157 EHEC has been developed based on O157 polysaccharide conjugated to protein carriers (Konadu *et al*., 1994). An ideal broad-spectrum EHEC vaccine should probably engender both systemic immunity against Stx and local intestinal immunity against intimin and other intestinal colonization factors (Nataro and Kaper, 1998; IFT, 2000).

As cattle are the main reservoirs, a novel strategy being explored is to vaccinate cattle in order to reduce colonization with E. coli O157:H7 and thereby it reduces food and environment contamination. A Canadian bio-pharmaceutical company thereby announced in January, 2007, the development of E. coli O157:H7 Type III secreted protein vaccine for cattle which reduces the number of bacteria shaded in feces by a factor of 1000 (Pearson, 2007).

2.9. Economic Significance*Escherichia coli* O157:H7

Escherchia coli O157: H7 is the most common serotype of shiga toxin producing *E. coli*. The incidence was estimated to be 110,000 cases annually in the United States. Uncommonly reported in patients in less industrialized countries and are estimated 3,200 hospitalizations annually in the United States. Patients who develop HUS often require prolonged hospitalization, dialysis, and long term follow up, which are expensive in all directions (CDC, 2005). Diarrhoea also remains an important problem in developed countries, but the course of disease is generally

mild, and the mortality has decreased drastically over time. However, economic losses due to the cost of medical care and absence of patient from work or school may be considerable (Keskimaki, 2001).

The cost of *E. coli* O157:H7 to the food industry as a result of recalls, destroyed food, control measures and lost demand associated to loss of consumer confidence is estimated to be in the billions of dollars in the U.S. alone (Frenzen and Drake, 2005).

2.10. Status of *Escherichia coli O157:H7* in Ethiopia

Certain studies have been conducted at some areas on different types of samples in Ethiopia concerning the EHEC strain *E. coli* O175: H7. Thus, prevalence of *E. coli* O175: H7 from cattle, sheep and goat meat at export and municipal abattoirs in East Showa (Hiko *et al.,* 2008), occurrence of *E. coli* O157:H7 in feces, skin and carcasses of small ruminants at an export abattoir in Modjo (Mersha *et al.,* 2010) and Study on Carcass Contaminating *Escherichia coli* in Apparently Healthy Slaughtered Cattle in Haramaya University Slaughter House with Special Emphasis on Escherichia coli O157:H7 (Taye *et al*., 2013) have been studied. Also, the survival of *E. coli* O157: H7 during the making of yogurt and cheese, in Ethiopian cottage and during storage of these products at both ambient and refrigeration temperatures have been studied (Tsegaye *et al*., 2005). Similar study was also conducted by Mutaku *et al*. (2005) concerning the growth potential of *E. coli* O157: H7 in fresh tropical fruit juices.

3.1. Study Area

3. MATERIALS AND METHODS

3.1. Study Area

The study was carried out in Addis Ababa. Addis Ababa is the capital city of Federal Democratic Republic of Ethiopia and it has an area of 51,000 hectare in the central highlands with an average altitude of 2000-2560 meters above sea level .The area is characterized by bimodal rainfall with an average of 1100 mm, the highest percentage of rain falls is during the long rainy season from June to September. The short rainy season is from February to April. Its annual average minimum and maximum temperature are 10.7^{0}c and 23.4^{0}c, respectively (NMSA, 2003).

Figure 2: Map of Addis Ababa (NMSA, 2003).

Addis Ababa abattoir was established in 1956 as a private share company and was taken over by the government during the derg regime. Now it is Addis Ababa municipality. The types of species of animals slaughtered in Addis Ababa abattoirs are bovine, ovine, caprine and swine. The source of these animals is from different part of the country. The main purpose of the abattoir are processing of one or several classes of livestock in to fresh meat for human consumption, hygienic processing and storage of meat and edible by products, exercise close control over environmental condition at all stages of processing and break down the transmission

of zoonotic meat borne disease through meat inspection. At Addis Ababa abattoir daily 700 cattle, 250 sheep and 75 goats are slaughtered. On average 153,000 cattle, 39,000 sheep, 3200 goats and 750 pigs are slaughtered annually

3.2. Study population

The study was carried out on a healthy slaughtered cattle, apparently healthy abattoir personnel, abattoir environment, retailer shops and health centers. The cattle slaughtered at Addis Ababa abattoir originated from different parts of the country.

3.3. Study Methodology

3.3.1. Study Design and sample

A cross sectional study was conducted on apparently healthy slaughtered cattle, abattoir environment, butcher shops and health centers at Addis Ababa from November 2013 to April 2014. On each sampling day, usually once a week, 7-10 animals were randomly selected. The output variable was the status of *E.coli* in abattoir, butcher house and health centers. The sample types were skin swab, cattle faeces, intestinal mucosa swab, carcass swab from slaughtered animal and transport material, eviscerating knife, eviscerater hands, transporter clothes, swab from abattoir environment and knife, hands of the butcher man, cut board, carcass swab from butcher shop sand stool from health centers. All samples for the study were then transported in ice box to Microbiology Laboratory of College of Veterinary Medicine and Agriculture, Addis Ababa University (CVMA, AAU) and stored at 4°C until processed. Culturing, isolation and identification were then performed to determine the presence of *E. coli* O157:H7 in each sample.

3.3.2. Sampling method and Sampling size

Systematic random sampling was used to select the sampled animals. From each selected animals faecal samples and skin, carcass and intestinal mucosal swab samples were collected. Besides, swab from abattoir environment, which are in contact with the carcass, were sampled once on each sampling day. Knives, Eviisceretor hands, Transporters cloth and Vehicles were considered to be carcass in contacts.

Butcher shops obtaining carcasses from the municipal abattoir were selected using simple random sampling technique. Knives, cutting board, butcher men's hand and carcasses were collected to determine to what extent butchery house environments serve as sources of *E. coli* O175: H7.

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 Adis Ababa (Kaliti and Kazanchis) health centers were collected to estimate *E. coli* O157 prevalence. The samples were tested if the cases fit with a pre tested questionnaire prepared. The questionnaire include questions about clinical manifestation, exposures of the past days, such as contact with symptomatic individuals, travel, food consumption such as beef and lamb, eating in a restaurant, contact with farm animals or manure, water related activities etc. (Annex 3).

3.3.3 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);

$$n=\frac{1.962\ Pexp(1-Pexp)}{d2}$$

Where: n=required sample size

Pexp =Expected prevalence

d = desired absolute precision

Previous study made in Bishoftu 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%, the minimum sample size was 114 and we sampled 150 animals. Therefore, skin, carcass inside, carcass outside and intestinal mucosal swab and fecal samples were collected from 150 slaughter cattle. Whereas carcass in contact surface swabs were taken once for each sampling day.

3.3.4. Sample Collection Procedure

Faecal Sample collection

Faecal samples were collected according the method described by Elder *et al*. (2000). Briefly, the distal colon was legated and transacted approx. 750 cm proximal to the rectum after complete evisceration; and the colorectal tissues were placed in individual sterile plastic bags and kept in an icebox until processing.

Intestinal mucosal

For intestinal mucosal swab sample, the distal colon was legated and opened using a sterile surgical blade approx. 750 cm proximal to the rectum and the lumen was swabbed by using sterile swab. The swab was introduced in to approximately 10 ml buffered peptone water in a sterile test tube.

Skin swab samples

Hide samples were collected from the brisket area of individual animals for detection of *E. coli* O157:H7 following the procedure indicated by McEvoy *et al*. (2003). The samples were collected immediately before bleeding. This area was chosen because it is an area that has been identified previously as one of the most heavily contaminated and it is one of the initial opening cut sites for removal of the hide. The area along the breastbone between the front legs (along the incision line for hide removal) was sampled using a moist swab. Briefly, a sterile pre-moistened

with 20 ml sterile buffered peptone water (BPW) were used to sweep the area in a Z pattern in one streak, followed by turning the sponge and retracing the Z pattern in the opposite direction. The Z pattern were cover approximately 1,000 cm^2, and a different angle for the Z pattern were applied for each sampling to avoid re sampling the same area.

Carcass swab sample

Selected carcasses were swabbed using the method described in ISO17604 (2003) and Mersha *et al*., (2010) by placing sterile template (10 x 10 cm) on specific sites (neck/brisket and rump) of a carcass. A sterile cotton tipped swab (2X3cm) fitted with shaft, was first soaked in an approximately 10 ml of buffered peptone water rubbed first horizontally and then vertically several times on the carcasses. On completion of the rubbing process, the shaft was broken by pressing it against the inner wall of the test tube and disposed leaving the cotton swab in the test tube. A second dry sterile cotton swab of the same type will be used as before over the entire sampled area. 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 mentioned above.

Carcass in contact surfaces' both from the abattoir and butchery houses

Carcass in contact surfaces including knife swab, personnel's hand swab, transporter clothes and vehicle swabs were obtained by swabbing using a cotton swab pre moistened in buffer peptone water. Similarly, butcher men hand swab, knife swab, cutting board swab and meat swab from legally registered butcher house were collected by swabbing using a cotton swab pre moistened in buffer peptone water.

During the sample collection process skin swab, carcass swabs, intestinal mucosal swabs and faecal samples from animals were labelled with the same matching number in order to avoid possible confusion of samples. Each sample was labelled legibly and accompanied by necessary identifying information, which includes date of sampling, type of sample, origin of the animal and age of animal from which the sample was obtained. All samples were placed in separate

sterile plastic bags to prevent spilling and cross contamination. Finally the collected samples were transported to the microbiology laboratory of the College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu, for microbiological analysis, using icebox in cold chain. Upon arrival, the samples were stored in a refrigerator at 4°C. The samples were processed within 24 hours.

3.3.5. Laboratory methods

Sample processing and selective enrichment

Twenty five grams of fecal sample was 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 (Oxoid Ltd., Hampshire, England) containing 20 mg/l novobiocin (mTSB+n) was added. The resulting mixture was agitated using stomacher (Stomacher 400, Sewared Medical, England) at low speed for 30 seconds.When the sample was less than 25 gm, mTSB+n was added to the samples in 1:9 ratios.

Ninety ml of mTSB+n was added to each test tube containing swab samples and homogenized. Then all sample types were incubated at 41.5°C for 24 hrs.

Immunomagnetic Separation

After enrichment, a 1 ml aliquot of each sample type was subjected to immunomagnetic separation (IMS) using immunomagnetic beads coated with an anti *E.coli* O157 antibody (Dynabeads anti *E. coli* O157; Applied Biosystems®) according to the manufacturer's instructions. Briefly, the paramagnetic beads coated with anti *E. coli* O157 were re suspended by gentle vortex mixing to ensure that the pellet at the bottom of the vial was completely suspended. Twenty micro litter of re suspended para magnetic beads were transferred to a screw top Eppendorf tubes.One ml of the enriched culture was added in to the Eppendorf tubes. Then, each tube was briefly vortexed for 10-30 minutes at room temperature.The tubes were then transferred to the manual magnetic particle concentrator (MPC-S) with the magnetic strip in place, inverted 3-4 times and left to separate for 3 minutes.

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

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. The tubes were closed again and the MPC-s inverted three times with the tubes still in place in order to re suspend the beads. The magnetic strip was replaced and the above steps were again performed three times. To prevent cross contamination a universal tube of PBST and separate sterile micropipette tips were used for each sample. Finally the supernatant was aspirated, the magnetic strip was removed and approximately 100 µl of PBST was added to each tube and mixed carefully.

Plating out on selective media

Fifty microliters of the bead suspension was spread plated onto CT-SMAC (sorbitol MacConkey plates supplemented with cefixime (0.05 mg/liter) and potassium tellurite (2.5 mg/liter), Oxoid Ltd., Hampshire, England). This was spread into one half of the media using sterile cotton tipped swab then further spreads in to the remaining second half using wire loop to obtain discrete colonies.The inoculated plates were then placed in an incubator at 37°C for 18 h to 20 h.

Confirmatory Test by *E. coli* O157 Latex agglutination test

Latex agglutination test was employed using latex kit for the confirmation of *E. coli* O157:H7. Up to three sorbitol-negative (clear) colonies exhibiting colony morphology typical for EHEC O157 per plate was picked and spread plated on CT-SMAC. Then after, a single colony was picked and subjected to latex agglutination using an *E. coli* O157 latex kit (Oxoid Diagnostic Reagents, From Thermo Scientific).

The latex kit consists of four components namely the latex test reagent, it is a latex particles

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

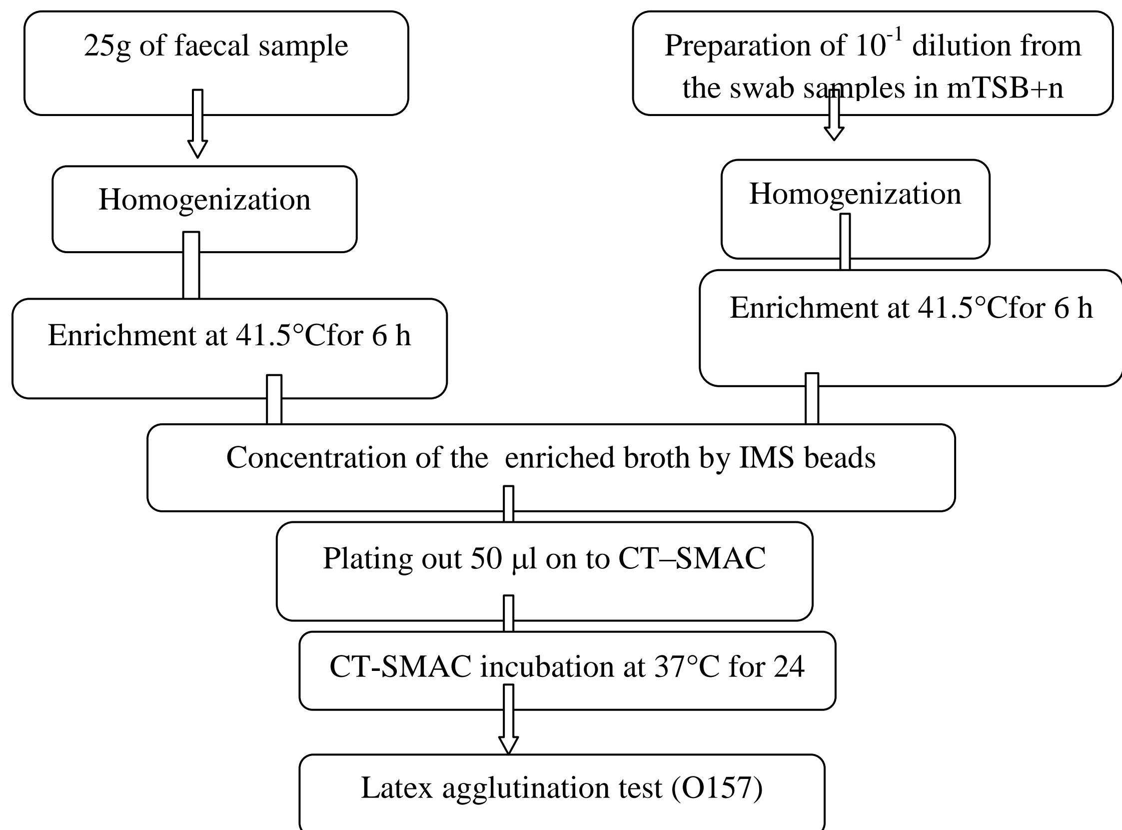


Figure 3: Flow diagram for the detection and isolation of *E. coli* O157:H7 used in the laboratory. **Source:**(ISO16654, 2001).

3.4. Antimicrobial Susceptibility

The antimicrobial susceptibility test was performed following the standard agar disk diffusion method according to (NCCLS, 2008) using commercial antimicrobial disks (Table.1).

Table 1: Antibiotic disks used to test *E. coli* O157:H7 and their respective concentrations

NO.	Antibiotic disks	Disc code	Concentration	Diameter of Zone of inhibition in mm		
				Resistant ≤	Intermediate	Susceptible ≥
1	Tetracycline	TE	30µg	14	15-16	17
2	Chloramphenicol	C	30µg	13	14-17	18
3	Sulfamethoxazole-trimithoprim	SXT	25µg	14	15-17	18
4	Amoxicilline	AML	25µg	14	15-17	18
5	Nitrofurantion	F	50µg	12	13-17	18
6	Cifrofloxacine	CIP	5µg	15	16-20	21

7	Nalidixic acid	NA	30µg	13	14-18	19
8	Kanamycine	K	30µg	11	12-14	15
9	Streptomycine	S	10µg	11	12-14	15
10	Cefotaxime	FOX	30µg	10	11-15	16

Each isolated bacterial colony from pure fresh culture was transferred in to a test tube of 5 ml Tryptone Soya Broth (TSB) (Oxid, England) and incubated at 37°C for 6 hours. The turbidity of the culture broth was adjusted using sterile saline solution or added more isolated colonies to obtain turbidity usually comparable with that of 0.5 McFarland standards (approximately $3x10^{8}$ CFU per ml). Mueller-Hinton agar (Bacton Dickinson and Company, Cockeysville USA) plates were prepared according the manufacturer's instruction (Anex 3). A sterile cotton swab was immersed into the suspension and rotated against the side of the tube to remove the excess fluid and then swabbed in three directions uniformly on the surface of Mueller-Hinton agar plates. After the plates dried, antibiotic discs were placed on the inoculated plates using sterile forceps. The antibiotic discs was gently pressed onto the agar to ensure firm contact with the agar surface, and incubated at 37°C for 24 hours. Following this the diameter of inhibition zone formed around each disc was measured using a black surface, reflected light and transparent ruler by lying it over the plates. The result was classified as sensitive, intermediate, and resistant according to the standardized table supplied by the manufacturer (NCCLS, 2003).

3.5. Data storage and analysis

All the research findings were stored in Microsoft Excel spread sheet. The analysis was carried by Stata version 11.0(2013). The prevalence of *E.coli* O157:H7 was computed the number of positive samples divided by the total number of examined samples times 100. Association isolation frequency and considered variables (sample types, sample origin, sex...) determined by Chi-square and Fitsher's exact tests. The significance level was set at $\alpha < 0.05$.

4. RESULTS

The present study was conducted on 150 apparently healthy slaughtered cattle at Addis Ababa municipal abattoir and 115 number butcher houses from November 2013 to April 2014. Of the total of 1380 samples examined for bacteriological status of *E. coli* O157:H7, 10(0.72%, 95%CI: 0.27, 1.16%) positive isolates were found from abattoir and butcher houses. Eight samples (1.03%, 95% CI: 0.32, 1.73) from abattoir and two samples (0.43%, 95% CI 0.69, 4.16) from butcher houses had *E. coli*O157:H7. There is no positive result from stool samples taken from health centres. (Table3).

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

Of the different sample types taken from 150 randomly selected animals the prevalence of *E. coli* O157:H7 at animal level was 4% (95% CI: 0.83%, 7.17%). Highest isolation rate was observed in faecal samples (2.66%) compared to the other two (Table 2)

Table2: Isolation rate of *E. coli* O157: H7 for different types of samples that indicate bacterial status of animals slaughtered in Addis Ababa abattoir.

	Sample types	Number of samples	Number of positive (%)	95% CI
Abattoir	Carcass internal	150	2(1.33%)	0.161,4.73
	Fecal	150	4(2.66%)	0.73,6.68
	Intestinal mucosal swab	150	2(1.33%)	0.161,4.73

An animal was considered *E. coli* O157: H7 positive when it was bacteriologically positive either for faecal sample and/or intestinal mucosal swab. Skin and carcass inside and carcass outside *E. coli* O157: H7 statuses were considered indicators of contamination and were not used for the calculation of prevalence. All the swab samples from carcass outside, skin and including environmental samples (knife, personnel's hands, transporter's clothes and vehicles) were found to be negative for *E. coli* O157: H7.The level of carcass inside contamination was considered as an outcome variable taking, fecal sample as risk factor for carcass contamination but associations of carcass contamination with the risk factor were assessed no stasticall significant association (P>0.05).

From a total of 460 samples examined from butcher houses for bacteriological status of *E. coli* O157:H7 only 2 (0.43%) isolates were found. Of the two isolates 1(0.2%) from meat swab and 1(0.2%) from cut board swab. When we compare the status of *E.choli* O157:H7 between butcher houses and abattoir there is no association between the two (p>0.05).

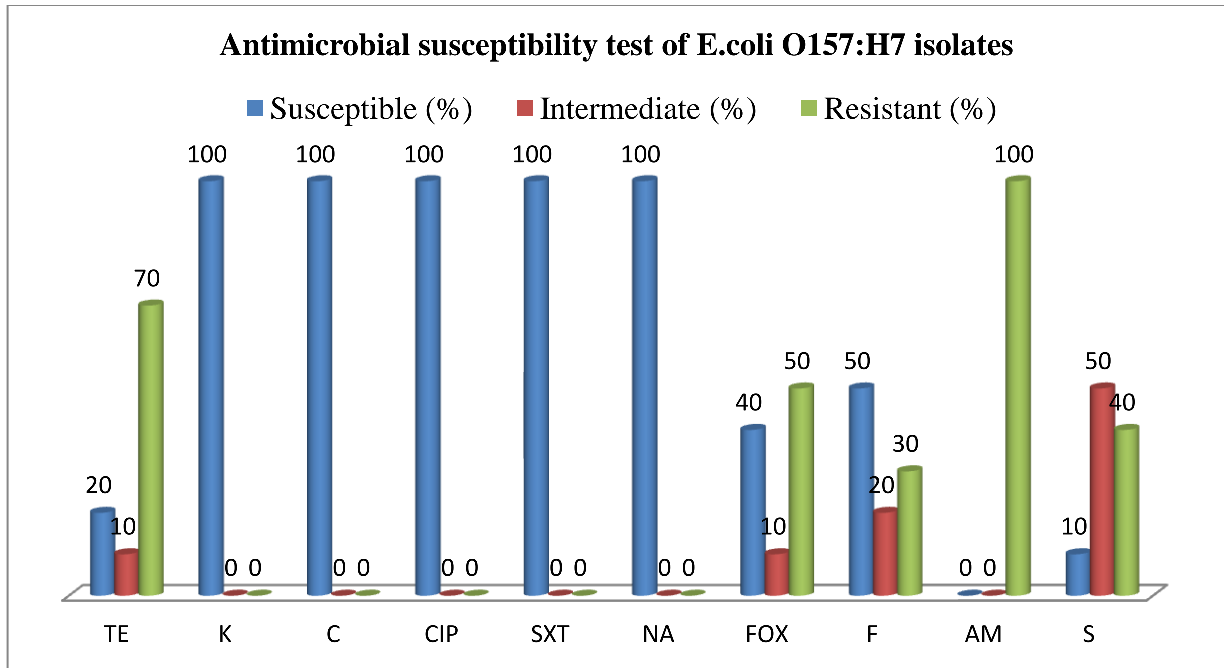
Table 3: Isolation frequency of *E. coli* O157:H7 and its association with sample types in abattoir and butcher houses from Addis Ababa

Sample source	Sample Type	Number of samples	Number of positive isolates (%)	Chi-square	p-value
Municipal	Skin swab	150	0	7.68	0.175
Abattoir	Carcass inside swab	150	2(1.33)		
	Carcass outside	150	0		

	swab				
	Intestinal Mucosal swabs	150	2(1.33)		
	Fecal sample	150	4(2.66)		
	Knife swab	30	0		
	Personnel hand swab	30	0		
	Transporters' cloth swab	30	0		
	Vehicle swab	30	0		
Butcher shop	Knife swab	115	0	2.00	1.00
	Butcher men hand swab	115	0		
	Cutting board swab	115	1(0.87)		
	Carcass swab	115	1(0.87)		
Hospital	Stool	50	0		
Total		**1380**	**10(0.72)**		

4.2. Antimicrobial Susceptibility of the Isolates

The 10 *E. coli* O157: H7 isolates were subjected to antimicrobial susceptibility test, using 10 selected antimicrobials.The isolated strains were susceptible (100%) toNalidixic acid (NA30µg),Kanamycine (K30µg), Sulfamethoxazole trimithoprim (SXT25µg), Ciprofloxacin (CIP5µg) and Chloramphenicol (C30µg) and were (50%) susceptible to both Cefoxatime (FOX30µg) and Nitrofurantion (F50µg) followed by Tetracycline (TE30µg) (20%) and Streptomycin (S10µg)(10%).From all antimicrobials used Amoxicillin (Aml25µg) (100%) resistance to all isolates followed by Tetracycline (TE30µg) (70%), Streptomycin (S10µg) (40%) and Nitrofurantion (F50µg) (30%).



TE-Tetracycline,K-Kanamycine,C-Chloraphenicol,CIP-Ciprofloxacin, SXT-Selfamethoxazole trimetophrim, NA-Nailidixic acid,FOX-Cefoxatime, F-Nitrofuration,AM-Amoxicillin,S-Streptomycine.

Figure 4: Antimicrobial susceptibility pattern of *E. coli* O157: H7 isolated from abattoir and butcher houses in Addis Ababa .

All of the isolates showed multiple drug resistance.Multi-drug test done shows that almost 100% of the *E.coli O157:H7* isolates were found resistant to two and above drug combinations. The most frequently resistant combinations were Amoxicillin, Tetracycline and Cefoxatime which have 40% of the*E.coli* O157:H7 isolates. Outof ten drug combination the four drugs were form resistance to a single isolate of*E.coli*O157:H7.

Table 4: Biogram of multi drug resistance of *E. coli* O157:H7 from abattoir and butcher hoses in Addis Ababa

Antibiotics	**Resistant strains**	
	Frequency	%
AML,S	1	10%
AML,N	1	10%
AML,TE,	1	10%
AML,F,S	1	10%
AML,TE,FOX	4	40%
AML,TE,S,FOX	1	10%
AMl,TE,FOX,F	1	10%
Total	**10**	**100%**

5. DISCUSION

The present study was conducted to establish prevalence of *E. coli* O157: H7 on samples collected from abattoir and butcher houses in Addis Ababa. The presented study *E. coli* O157:H7 was isolated from 4 (2.66%) faecal samples, 2(1.33%) from intestinal mucosal swabs and 2 (1.33%) from carcass inside swabs. At abattoir level the prevalence of *E. coli* O157:H7 was 1.03% (8/780). This result is in line with 1.2% prevalence in USA (Barckocy-Gallagher *et al*., 2003), 1.4% in England (Chapman *et al.,* 2001), 1.5% from Turkey (Gökhan and Belgin, 2010) and 1.6% from Canada (Power *et al*., 2000). But our finding is lower than some reports from previous works: 3% from Ireland (Carney *et al*, 2006), 6.4% from Isfahan (Rahmi *et al*., 2008), 8% from Ethiopia (Hiko *et al*., 2008), 8.3% from Iran (Hashemi *et al*., 2010) and 9.6% from Iran (Tahamtan *et al*., 2006). In contrast the finding of the present study is a bit higher than prevalence of 0.4% from France (Guyon *et al.,* 2001).

Observed variation in prevalence among studies could be attributed to difference in sampling and isolation procedures, variability in sampled populations, diverse geographical origins of cattle, numbers of cattle, study design, season, abattoir conditions and treatment with antimicrobial substances during the process. A number of studies have confirmed that the prevalence of *E. coli* O157:H7 varies among studies due to the above mentioned reasons (CDC, 1996; Chapman *et al*., 2001 and Varela-Hernández *et al.,* 2007).

Even though, the highest prevalence was observed in faecal sample (2.66%) among the different sample types from abattoir in this study the difference was not statically significant (p>0.05). Similar findings were reported by Mcevoy *et al*. (2003) from United Kingdom (2.4%) and Conedera *et al*. (1997) in Irish abattoir (3%). It is also comparable with the results from Great Britian (4.7%) reported by Piaba *et al*.,(2003) and (4.7%) in Manhattan reported by Greenquist *et al*.,(2005).Conversely, our finding is lower than the reports by Synge *et al*. (2000) in United Kingdom (8.6%), Omisakin *etal*.,(2003) in United Kingdom (7.5%),Callaway *et al*.(2006) in United State (11.3%), Paiba *et al.* (2002) in United Kingdom(40.4%), Elder *et al*. (2000) in United State 28%, Strachan *et al*. (2002) in United Kingdom 23.7%. The differences in isolation rate of *E. coli* O157:H7 from fecal samples from abattoir different studies are possibly due to differences in husbandry practices, agroclimatic variation, seasonal differences, sampling times, breeds and age of animals, sampling technique and so on. A number of studies have also showed that prevalence of *E. coli* O157:H7 shed from animal faeces can vary significantly in relation to time, age of animals, nature of feeds etc. (Chapman, 1994; Chapman *et al*., 2001; Reid *et al*., 2002).

Escherchia coli O157:H7 prevalence of 1.33% from carcass inside swab in the current finding was comparable to a previous study done in Rome (1.9) (Maria *et al.,* 2011). Higher prevalence rates were reported from Ethiopia by (Hiko *et al*., 2008) and (Taye *et al*., 3013), 8% and 2.65%, respectively and from other countries, 18% in United State (Elder *et al*., 2000), 8.3% in Iran (Hashemi *et al.,* 2010) and3.2% in United Kingdom (McEvoy *et al*., 2003). On the other hand a Lower prevalence than the present finding was reported in United State (1%) (Mildred *et al*., 2004), in south Yorkshire of Britain (1.1%) (Chapman *et al*., 2000), in United Kingdom 0% (Chapman and Ashton, 2003).

The differences in the reported prevalence could be due to the used culture, the age of the animals and season in which sample is collected (Zhao *et al*., 1995, Mead and Griffin, 1998). The greater prevalence encountered in the present study as compared with the previous once may be due to the presence of IMS technique of isolation methods. Several enrichment culturing methods and isolation methods have been developed but IMS technique is the most sensitive (Weagant *et al*., 1995; Tutenel *et al*., 2003). On the other hand the present study prevalence lower than the previous mentioned at the above paragraph it might be due to seasonal variation. The present sample was collected during the winter season. Seasonal distribution of *Escherchia coli* O157:H7 has been reported previously by (Cagney *et al*., 2004) with highest prevalence in summer and lowest in winter so it is possible that the contamination rate lower than previous studies.

Association of several risk factors with carcass contaminations has been reported by several researchers. Associations with feces (Elder *et al*., 2000; Gansher off and O'Brien, 2000) and with skin (Reid *et al*., 2002) and *E. coli* O157:H7 has been reported to spread easily on to carcass surfaces from the hide or during evisceration (Arthur *et al*., 2007, Elder *et al*., 2000 Gun *et al*., 2003). The result of the present study does not support that contention because positive carcasses were found from animals that were *E. coli* O157:H7 negative from their faecal, mucosal swab or skin samples. This finding strongly suggests the presence of *E. coli* O157:H7 on carcass might be due to cross contamination with abattoir environmental samples but the environmental samples (knife, eviscerater hand, trasporter cloth and vehicle) were encountered 0 prevalence. There are some confounders like water which does not included in our sample type studies. So, the carcass contamination might come from potable water that the abattoir used for washing the carcass part. A potable water wash can cause an increase or decrease in the prevalence of pathogens at particular carcass sites (Prasai *et al*., 1995; Bell 1997). A Study which agrees with redistribution of contamination is reported to occur in a posterior to anterior direction as the wash water runs down the carcass surface (Bell, 1997). But for conclusion that the carcass contamination might be came from potable water, future studies must be conducted in water samples.

From a total of 460 different samples taken from butcher houses, the *E. coli* O157:H7 isolates were found in 1(0.43%) meat swabs and in 1(0.28%) cut board swab. In previous reports the prevalence of *E. coli* O157:H7 on cattle carcasses were from 0.0% to 27.8% (up to 68% in heifers) (Chapman *et al*., 1997; Jo *et al.*, 2004 and Madden *et al*., 2001). The presence of *E. coli* O157:H7 in carcass surface swabs is comparable with the findings of Chapman *et al*. (2000) in United Kingdom (1.1%), Stephen *et al., (*2009) in Nigeria (2.78%), (Chinen *et et al*., (2001) in Argentina (3.8%). But it is higher than the reports from Ireland, 0.17% (Walsh *et al*., 1997) and (0.12%) in France (Vernozy-Rozand *et al*., 2002).

Direct comparison of results is difficult due to the occurrences of E. coli O157:H7 might be attributed to different sampling techniques, such as, differences in sample size, type of sample and when it is collected laboratory methodologies, agro ecology of the area, hygienic condition, bacterial load in the sample and handling of the meat at the retail shops (Hussein, 2007). The high prevalence observed in the present study compared to the studies of Walsh *et al*., (1997) and (Vernozy-Rozand *et al*., (2002) is due to the difference in the isolation methods, for instance, the use of IMS technique. In the present study we used IMS technique to isolate E. coli O157:H7. The detection of higher proportions of *E. coli* O157:H7 in more recent studies is more probably associated with the wider use of more sensitive detection methods such as IMS (Chapman *et al*., 2001).

In the present study, the prevalence of *E. coli*O157:H7 in abattoir and butcher houses has no significant difference ($p>0.05$). The carcass samples from abattoir had higher *E.coli* O157: H7 prevalence (1.03%) than samples from butcher houses (0.22%). This result is consistent with the work of Agbeyegbe and Uraih (1982), whose study showed high prevalence rate of *E. Coli* O157:H7 in meat samples than samples from abattoir. But the difference between the results were not statically significant ($p>0.05$) which revealed that these results may be an indication of the poor sanitary environment under which the animals are slaughtered in abattoir. These animals were slaughtered on the abattoir floor that is not properly disinfected after every kill, with butchers and retailers walking between carcasses as they transact their business, while those in the market are displayed on tables in the open for sale. So our findings also supported by (Nkanga and Uraih ,1981) reported that meat is frequently found to be contaminated due to poor

sanitary environment during slaughter, transportation and usage and through handling.According to the report by (Bassam *et al.,* 2012), the infective dose of the pathogen is < 10 cells for humans. Considering this very low infective dose of this pathogen, its detection in the butcher houses and abattoir of this study poses public health risks.

From 50 stool samples taken from humans in this study , which might be due to From a total of 50 stool samples taken from humans in this study *E. coli* O157:H7 was not isolated. The same result was reported by Abdolvahab *et al*., (2008) in Iran. There are different studies their prevalence rate differed from 0% were in Japan, rates of symptomatic and asymptomatic infection of *E. coli* O157: H7 ranged from 0.5 to 4 cases per 100,000 people in 2000(Anonymous, 2001). In a large study in the United States, *E. coli* O157:H7 was isolated from 118 patients (0.39%) (Slutsker *et al*., 1997) and was the most common pathogen isolated from visibly bloody stool specimens (39%) (Slutsker *et al*., 1997).

A variety of factors may be responsible for undetected *E. coli* O157:H7 in the present study was number of the sample size there was small number of samples processed as compared to previous studies. Another reason for under diagnosis is that the organism is shed primarily in the early period of illness and is cleared rapidly from the gastrointestinal tract (Tarr, 1995).Therefore *E. Coli* O157:H7 may not be detected if a stool culture is not done during the early period of illness.

Antimicrobial resistance pattern of *E. coli* O157:H7 isolates from animal and human sources have been reported in Ethiopia by Hiko *et al*. (2008),In the present study, all of the 10 isolates were highly susceptible to Tetracycline (TE30µg), Nalidixic acid (NA30µg), Kanamycine (K30µg), Sulfamethoxazole-trimethoprim (SXT25µg), Ciprofloxacin (CIP5µg) and Chloramphenicol (C30µg). This finding agrees with the work of (Hiko *et al*., 2008; Osaili*et al.,* 2013). However, the study conducted in Saudi Arabia (Naser and Wabel, 2007), revealed that there was resistant strain to the drugs such as Tetracycline (TE30µg), Nalidixic acid (NA30µg), Kanamycine (K30µg), Sulfamethoxazole-trimethoprim (SXT25µg), Ciprofloxacin (CIP5µg) and Chloramphenicol (C30µg).This variation probably attributed to the expression of resistant gene code by the pathogen which associated with emerging and re emerging aspects of the isolates

with the regards of different agro ecology (Reuben andOwuna, 2013). On the other side, the current study revealed that all isolates were highly resistant to Amoxicillin (AML25µg). Similar findings were reported by many researchers (Mora et al., 2005;Srinivasan *et al.*, 2007;Taye *etal*., 2013). This might be due to the use of inappropriate antibiotics for treatment of diseases (Sharada *et al*., 2010) and also excessive use of antimicrobials for therapeutic and prophylactic treatment (Majalija *et al*., 2010).

In the present study, almost all isolates have multiple drug resistance. This finding comparable related with previous findings (Zhao *et al*., 2005; Salehi and Bonab, 2006; Guerra *et al*., 2007; Akond *et al*., 2009). This multi drug resistance occurred might be due to administration of multiple antibiotics for prophylaxis or infection, discriminant use of antibiotics in the farms and another possibility is that cattle are being treated with antibiotics for other conditions, thereby selecting for resistant populations of *E. Coli* O157:H7. Such multi drug resistance may apparently be occurred which may ultimately replace the drug sensitive microorganisms from antibiotic saturated environment (Van De Bogaard and Stobberingh, 2000).

The information from this study can contribute to the development of a surveillance program in Ethiopia for antimicrobial resistant bacteria. Additionally, this information can be used in food safety risk assessment modelling and in identifying areas for further detailed investigation, such as on farm risk factors that lead to the development of resistance.

6. CONCLUSION AND RECOMMENDATIONS

Most human diseases are caused by pathogens from animal and/or animal products like meat and meat products. However, the contaminated one acts as source of emerging potentially pathogenic microorganisms such as *E.coli* O157: H7 which needs preventive actions at any point in the food production chain. *E. coli* O157: H7 was present in the faeces, intestinal mucosal swab carcasses swab and cut board at municipal abattoir and butcher houses. This pathogen was isolated from carcasses, from the inside parts that indicated the presence of carcass contamination during slaughter operations and at the butcher house the presence*E. coli* O157: H7 due to environmental contamination. The isolated bacteria were susceptible to most of the drugs used and multi drug resistance was occurred in all isolates for in vitro testin this study.

Based on these conclusions the following recommendations are forwarded:

- The most important practice that should be considered in animal slaughtering are cleaning dirty animals before slaughtering, skinning while being on the rail, separating carcasses from each other and avoiding contact between the external surface of the hide and carcasses. Hygiene measures must be sufficient to prevent from contamination via hands, knives, saws, equipments, clothing and Regular medical check up of personnel working in both abattoir and butcher houses.
- Good Agricultural practice, good Manufacturing practice and Hazard Analysis of Critical Control Points (HACCP) at every stage of the beef supply chain, from the farm, through the abattoir, to the butcher houses, and those involved with the handling and processing.
- Education and awareness for the public, for consumption of sanitary and well cooked food; abattoir workers, about appropriate slaughter procedures, sanitary and hygienic method of production, and the risk of food borne diseases should be given.
- Further investigation on the future research such as molecular or epidemiologic works as well as to develop strategies for minimizing cross contamination during the process should be undertaken in meat and other food items of animal origin.

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8. APPENDICES

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

No	Data collection															
	Date	Spp.	Sample type	Sam. ID	Enrichment	IMS	Colonychar. CT-SMAC)	Latex Agglu.	Antibiotic Susceptibility							

IMS=Immunomagnetic separation

Annex 2: **Questionnaire**

1. DEMOGRAPHIC INFORMATION

1.1. Community Area: ____

1.2. Name: ______________________________________

1.3. Address: _______________________________________

1.4. DOB: dd/mm/yyyy ______________

1.5. Sex: M________ F____________

1.6. Occupation: (retired, unemployed, housewife, student, other_______________)

2. PERSONAL DETAILS

2.1. Date of onset of first symptom: **(dd/mm/yyyy)**:____________________

2.2. 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**

3. HEALTH DETAILS

	YES	**NO**
3.1. Diarrhea (3 or more loose Stools in a 24 h period)	________	______
3.2. Abdominal pain/cramp	________	______
3.3. Blood in stools	________	______
3.4. Fever	________	______
3.5. Nausea	________	_______
3.6. Vomiting	________	_______
3.7. Headache	________	_______

4. FOOD HISTORY

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

YES **NO**

4.2.Was any of this meat rare or undercooked?

YES **NO**

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

YES **NO**

4.4.Did you haveRaw (unpasteurized) milk or other dairy products made from(unpasteurized milk)

YES **NO**

5.ANIMALS

In the10 days before illness began, did you:

	YES	**NO**	**UNKNOWN**
5.1. Visit or live on a farm?			
5.2. Have contact with any cows or cattle?			

5.3. Touch any cow manure?

5.4. 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?

6.TRAVEL DETAIL

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

YES **NO**

Annex 3: Media used for isolation and identification of *E. coli O*157: H7

1. Buffered peptone water (Oxoid Ltd., Hampshire, England)

Composition (g/l): Peotone 10.0; Sodium chloride 5. Final PH: 7.2 + 0.2
Preparation:- Add 20g to1 litter of distilled water. Mixed it well and sterilized by autoclaving at 121°C for 15 minutes.

2. Triptone Soya Broth (TSB) (Oxide, England)

Composition: 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).
Preparation: Suspend 30 g of power in 1 litter of purified water. Mixed thoroughly. Heated with frequent agitation and boiled for 1 minute. Autoclaved at 121°C for 15 minutes.

3. CT- Sorbitol MacConkey agar (Oxide, England)

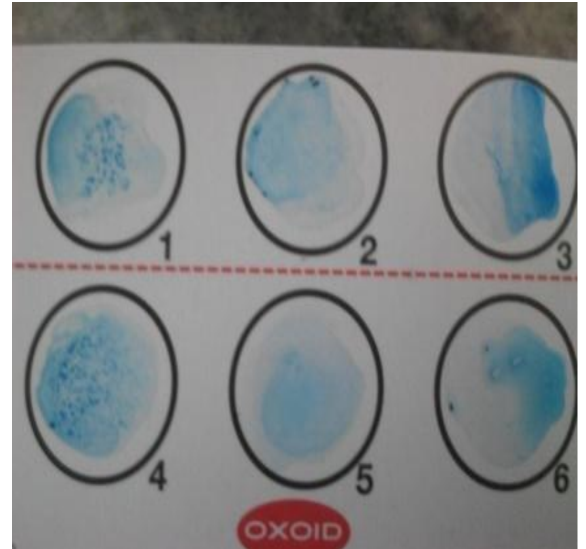
Composition (g/liter): Peptone 15.5g; Proteose Peptone 3 g, D-sorbitol 1.0 g; Bile salts 1.5 g; Sodiumchlorid 5.0 g; Neutral red 0.03.

Preparation: 50 g of the powder was suspended in 1 liter of distilled water. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Autoclave at
121°C for 15 minutes. Then Potassium tellurite (2.5 mg/l) and Cefixime (0.05mg/l) were added on the prepared base media tempered at 50-55OC. gently shacked and poured into Petri dishes.

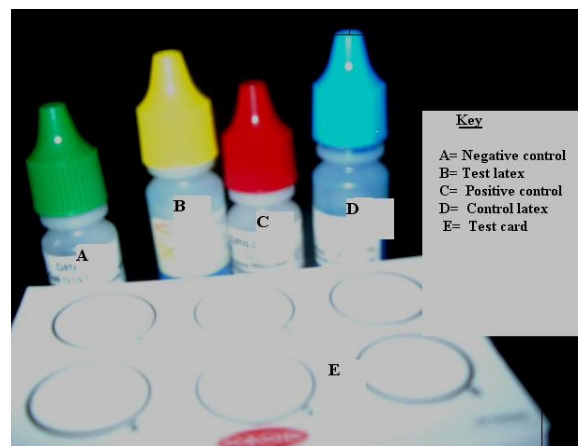
4. Muller-Hinton agar preparation (Oxoid, England)

Preparation: Suspend 38 g of the medium in one liter of purified water. Heat with frequent agitation and boil for one minute to completely dissolve the medium. Autoclave at 121°C for 15 minutes.
Formula (g/l): Beef Extract 2, Acid Hydrolysate of Casein 17.5, Starch 1.5, and Agar 17. Final.PH 7.3 ± 0.1 at 25°C

Annex 4: Photo pictures

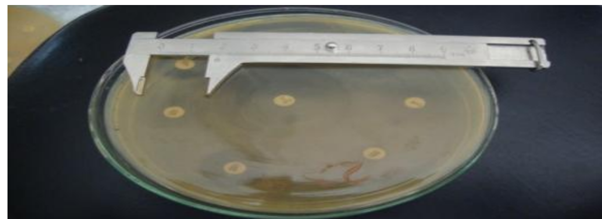


Picture 1:Latex agutination test picture 2: When agultination occur



Picture 3: Concentration of *E. coli* O157:H7 technique

Picture 4: Latex agglutination kit using IMS



5: Measuring the diameter of inhibition zone by using calliper

9. CURRICULUM VITAE

I. Personal Information

- Name Woynshet Haile
- Sex Female
- Date of birth 12/Mar/1982 E.C
- Nationality Ethiopian
- Religion Orthodox
- Marital status single
- Telephone mobile:0913156635 Home: 0473310304
- Contact address woyni2gabon@yahoo.com

II. EDUCATIONAL BACK GROUND

- Scholar on DVM degree in veterinary medicine in Addis Ababa University
- 19196-1999 E.C: Bonga Bishaw W/Yohannes Secondary School (High school and preparatory school certificates)
- 1988-1995E.C: Bonga Grazmach Phawlos primary school (primary school Certificates)

III. Researches

- DVM seminar, Duration: 2003 E.C

  Parasitic diseases:a global concern on parasitic resistance

- DVM thesis,duration:2004 E.C

  An invitro anti bacterial effect of xanthium strumarium and grewia bicolor juss on staphylococcus aureus isolated from bovine clinical mastitis.

Iv.language proficiency	**Speaking**	**listening**	**writing**	**reading**
• English	Excellent	Excellent	Excellent	Excellent
• Amharic	Excellent	Excellent	Excellent	Excellent
• Kafinono	V.good	V. good	Excellent	Excellent
• Oromifa	V.good	V. good	Excellent	V. good

V. Personal skill (Social skill):

- High level of interaction in multicultural environment, positions where communication is important and situations where teamwork is essential.

Technical skills and expertise

- Basic computer application Microsoft office (Ms-word, excels, access and power point.)
- Internet service

VI. Campus clubs membership

- Member of anti-AIDS club in AAU, School of Vet. Medicine
- Member of Veterinary students Association in AAU, School of vet. Medicine
- Member of animal welfare club in AAU, School of Vet. Medicine
- Member of girls club
- I take march project training

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10. SIGNED DECLARATION SHEET

Statement of author

First, I declare that this thesis is my bonafide work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an advanced (MSc) degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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Name:_______________________ Signature:______________

College of Veterinary Medicine and Agriculture, Bishoftu

Date of Submission:_________________________