PREVALENCE OF ENTEROHAEMORRHAGIC *ESCHERICHIA COLI* (EHEC) O157:H7 IN CATTLE MEAT (BEEF) IN TANZANIA.

BY

ABDU AMMAN HAYGHAIMO.

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF VETERINARY MEDICINE OF SOKOINE UNIVERSITY OF AGRICULTURE.

MOROGORO, TANZANIA.

2002.

ABSTRACT

A cross sectional study on the occurrence of EHEC O157:H7 serotype in cattle meat (beef) destined for human consumption was undertaken in two slaughterhouses of Tanzania between December 2000 and June 2001. Surface swabs were taken from 384 beef carcasses immediately after slaughter and were examined for EHEC O157:H7 by first growth enrichment in modified tryptone soya broth (mTSB), followed by immunomagnetic separation (IMS) and finally by culture of bead-bacteria complexes on CHROMAgar ^{TM} 0157 media. Presumptive EHEC O157:H7 were isolated from 25 of 200 (12.5%) beef samples collected from Morogoro slaughterhouse and 21 of 184 (11.4%) beef samples collected from Ukonga-Mombasa slaughterhouse in Dar-es-Salaam. When the presumptive EHEC O157:H7 colonies were subjected to the latex agglutination and biochemical tests for confirmation, 22 (5.7%) isolates showed typical agglutination and biochemical patterns characteristic of EHEC O157:H7. This study suggests that beef in the two slaughterhouses may be contaminated with EHEC O157:H7. Given the hygienic conditions of most slaughterhouses in Tanzania, it can be concluded that beef from slaughterhouses if not properly cooked may present a risk of VTEC O157 infections and associated disease to humans. This is the first report on the isolation of EHEC O157:H7 in cattle meat in Tanzania.

DECLARATION

I, Abdu Amman Hayghaimo, do hereby declare to the Senate of the Sokoine University of Agriculture that, this dissertation is my own original work and has not been submitted for a similar degree award in any other University.

Signature .....

Date 14/11/02

COPYRIGHT

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to Professor Uswege M. Minga of the Department of Veterinary Microbiology and Parasitology, Sokoine University of Agriculture, Morogoro, who supervised the planning and execution of this study. I also cordially acknowledge the cooperation I received from Professor Robert S. Machang'u of the same Department for his valuable advice and supervision during the execution of this project.

I am thankful to Mr D. Tillya and Mr. J. Chilongola of the Morogoro Municipal slaughterhouse and Mr. D. Moshy of Ukonga-Mombasa slaughterhouse Dar- es Salaam, for assistance during sample collection. I would also like to thank Professor J. E. Olsen of The Royal Veterinary and Agricultural University Copenhagen, Denmark for his advice and interest in this study and for providing reference *E.coli* O157:H7 isolates.

The support and cooperation received from the Head of Department of Veterinary Microbiology and Parasitology (Professor P. S. Gwakisa) and the entire staff during the study period is greatly acknowledged. Special thanks are due to Professor S. F. H. Jiwa for his valuable suggestions on verocytotoxin producing *E.coli.* Many thanks are also due to the Principal Technician Mr. M. M. Shoo for his help during sample collection and processing and Mr. M. Shomari for his assistance in media preparation. I also wish to thank my fellow postgraduate students Drs C. Luziga and Kissamo, G. K. M. for their advice during this study.

The encouragement and moral support I received from my wife Hellen and our children Mohamed, Chiku, Amina and Amina-Akhisa is highly acknowledged. I thank them for their patience during the study period.

I would finally wish to express my sincere appreciation and thanks the then Ministry of Agriculture and Cooperatives who sponsored my study through the National Extension Project (NAEP II) and for granting me the study leave.

This study could not have been completed without the great financial assistance provided by the IHEPRUCA (Improving the Health and Productivity of the Rural Chicken in Africa Project) in the procurement of the research equipment and materials.

DEDICATION

To my late parents who laid foundation for my interest in the veterinary medical profession.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND SYMBOLS

Eae gene	*E.coli* attaching and effacing gene
CDC	Center for Diseases Control (US)
CLST	Commercial lattex agglutination test
EHEC	Enterohaemorrhagic *Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
EPEC	Enteropathogenic *Escherichia coli*
ETEC	Enterotoxigenic *Escherichia coli*
SLT	Shiga-like Toxin
STEC	Shiga toxin-producing *Escherichia coli*
Stx	Shiga toxin
SUA	Sokoine University of Agriculture
VT	Verocytotoxin
VTEC	Verocytotoxigenic *Escherichia coli*
USDA	United States Department of Agriculture

CHAPTER ONE

1.0. INTRODUCTION

Food borne pathogenic microorganisms are a public health concern in Tanzania and worldwide. The majority of food-borne illnesses are caused by a few bacterial species, particularly *Escherichia.coli, Salmonella* spp, *Campylobacter jejuni,* and *Listeria monocytogenes*. Other less common pathogens include, *Bacillus cereus* (starchy foods), *Clostridium perfringens* (poultry meat), *Clostridium botulinum* (mostly in home canned foods), *Yersinia enterocolitica* (refrigerated foods), *Staphylococus aureus* (marinated foods), *Shigella dysenteriae* and *Vibrio parahaemoliticus* (sea foods). Other causes of food poisoning are chemicals, heavy metals and fungi. It is has been established that more than 90% of all cases of food poisoning, each year, in the world are caused by bacteria (Wagner, 2000) (Table 1).

The *Salmonellae* are one of the most important pathogens because they have been frequently associated with food poisoning outbreaks in various countries (Hummel, 1974; Saxena *et al.*, 1989; Kayihura, 1982, Ndzinga *et al.*, 1982 and Minga, 1984). Isolation of a specific pathogen like *Salmonella* or *E.coli* from meat is an indicator of contamination by enteric pathogens (Johanson *et al.*1983).

Studies conducted by Maeda (1984) to assess bacterial contamination of cattle carcasses in three slaughterhouses of Tanzania, revealed that 59% of all carcasses processed at the Morogoro slaughterhouse had *E.coli* on their surfaces; while in Vingunguti slaughterhouse (Dar-es Salaam) 100% of the carcasses had *E.coli*. At the

Tanganyika Packers slaughterhouse 7% of the carcasses were contaminated with *E.coli.* Morogoro and Vingunguti slaughterhouses had higher numbers of carcasses contaminated with *E.coli* than the Tanganyika Packers slaughter house mainly because the latter practiced overhead rail dressing.

According to Tauxe (1997), the epidemiology of food borne disease is changing. New pathogens have emerged and some have spread worldwide. Many, including *Salmonella*, *Campylobacter jejuni* and *Yersinia enterocolitica* have reservoirs in healthy food animals, from which they spread to a variety of foods. These pathogens cause millions of cases of sporadic illness and chronic complications as well as large and challenging outbreaks over many states and nations (Tauxe, 1997).

In recent years, a rare but dangerous serotype of *Escherichia coli* known as verocytotoxigenic EHEC O157:H7 has emerged as an important human pathogen. This bacterium was first isolated as a human pathogen in 1982 in Seattle, USA following an outbreak of food borne infection caused by undercooked hamburger (Riley *et al.*, 1983). EHEC O157:H7 is a highly virulent bacterium that has caused serious food borne epidemics in the United States, Japan, and Europe (Doyle *et al.*, 1997). It has been involved in a number of outbreaks of food poisoning following the consumption of undercooked beef. In light of the severity of the illness and the potential for fatalities as a result of food poisoning by this bacterium, it is classed as a serious public health issue within the European Union (Buchanan and Doyle, 1997).

Investigations of disease outbreaks have revealed that cattle frequently shed EHEC O157:H7 in their faeces, which may also represent a source of infection (Wells *et al.*, 1991; Blanco *et al.*, 1996). Cattle are believed to be the main reservoir of this bacterium and it has been frequently found in beef and raw milk (Chapman, 1993). When cattle faeces at slaughterhouses accidentally contaminate beef carcasses, this pathogen may enter the human food supply.

EHEC O157:H7 is highly virulent bacterium and oral exposure to a small number of these invasive bacteria causes severe illness. It is particularly hazardous because of the very low numbers of organisms that are able to cause infection and because of serious complications that can result from the infection, especially in infants and the elderly (Patriquin *et al.*, 2000). The infective dose of EHEC O157:H7 is between ca 100-200 organisms (Nataro and Kaper, 1998). Once introduced into a community, person to person transmission can easily spread the disease.

Many of the slaughterhouses in Tanzania are not built to meet acceptable hygienic standards (personal observation). In most cases, all animals are slaughtered on the same floor and there is no separation between dirty operations (slaughtering, hide removal, and evisceration) and the clean operation (preparation of the carcass), and therefore intestinal contents easily contaminate the meat. Carcasses are only hoisted on the rails during the meat inspection procedures.

An epidemiological survey on the occurrence of EHEC O157:H7 in Tanzania has never been done before, and as such no data is available on the occurrence of VTEC 0157:H7 in beef. At the then Central Veterinary Laboratory (CVL) Dar-es-Salaam between 1965 and 1982, 31.4% of 6,982 bacteria isolated were designated *E.coli* circumstantially based on pure culture isolations from clinical cases of intestinal and extra-intestinal infections (Minga and Kikopa, 1983). However these studies were mainly based on isolation and characterization of *E. coli* in carriers (Jiwa *et al* 1986).

The only study that suspected the presence of VTEC in Tanzania was that conducted by Jiwa and Musses (1987) on purging dairy goat kids at Magadu Dairy Farm-SUA, in which three out of twenty goat kids were positive for vero toxin producing *E.coli.* However, that study did not indicate which *E.coli* serotype was responsible for the production of the verocytototoxin.

This study was therefore aimed at isolating, characterizing and determining the prevalence of EHEC O157:H7 in beef as well as establishing their zoonotic significance. Findings from this study would bring about awareness of the threat of EHEC O157:H7 to humans and serve as the basis to recommend methods of reducing carcass contamination by this "organism" in Tanzania.

Justification

Since most of the slaughterhouses in Tanzania do not meet acceptable hygienic standards (personal observation), chances are that intestinal EHEC O157:H7 easily

contaminates carcasses during slaughter. The same unhygienic situation was observed in most of the butcheries where meat and offal were sold. Cross contamination is, therefore, a serious concern to the consumer and its evaluation is critical in any discussion of meat hygiene. Some cases of diarrhoea reported in hospitals could be a result of food poisoning by this emerging food pathogen because there are currently no facilities for detecting EHEC O157:H7 (Massi, personal communication).

Given the lack of knowledge on the prevalence of EHEC O157:H7 organisms in beef, it justifies carrying out this pioneer study to determine the presence of EHEC O157:H7 in beef in two slaughterhouses of Tanzania.

Objectives

1.2.1. General objective

To determine the prevalence of Verocytotoxigenic *E.coli* (EHEC) O157:H7 in beef in order to contribute to improved food hygiene in Tanzanias.

1.2.2. Specific objectives

To isolate and characterize verocytotoxigenic *E.coli* (EHEC) O157:H7 from beef in selected slaughterhouses in Tanzania.

To characterise those EHEC O157:H7 isolates.

To determine the prevalence of EHEC O157:H7 in beef.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Foodborne infections

The majority of food borne illnesses are caused by just a few bacterial species particularly *Salmonella* (mostly from cattle meat, poultry meat, eggs, and fish), *Campylobacter jejuni* (associated with poultry products, beef, pork and lamb), Verocytotoxigenic *Escherichia coli* O157:H7 (VTEC O157:H7) (mostly associated with beef) and *Listeria monocytogenes* (dairy products) (Bean and Griffin, 1990). *Salmonellae* are among the top two most frequently isolated bacteria from clinical material in Tanzania, the other being *E.coli* (Minga, 1984). Although Maeda (1984) did not find *salmonella* spp on the surface of the carcass samples taken in Tanzania, earlier studies by Hummel (1974) have indicated a carrier rate of 6.16% in cattle intended for sale in Tanzania. According to Minga (1984), the infection rate of *Salmonellae* in Tanzania ranged from 3% to 9.5% in calves. The overall (calves and adult cattle) infection rates in the *Salmonella* positive herds ranged from 0.9 to 5.6% (Minga, 1984). Common bacterial pathogens causing food borne infections have been summarised in Table 1.

Table 1: Bacterial agents of food poisoning

Bacteria Responsible	Description	Habitat	Types of Foods	Symptoms	Cause	Temperature Sensitivity
Bacillus cereus	Produces a spore and grows in normal oxygen atmosphere.	Soil, dust and spices	Starchy food.	Mild case of diarrhoea and some nausea within 12 to 24 hours.	Improper holding and storage temperatures after cooking.	No growth below 4.4°C
Campylobacter jejuni	Oxygen sensitive, does not grow below 30° C.	Animal reservoirs and foods of animal origin.	Meat, poultry, milk, and mushrooms.	Diarrhoea, abdominal cramps and nausea.	Improper pasteurisation or cooking. cross-contamination.	Sensitive to drying or freezing.
Clostridium botulinum	Produces a spore and is anaerobic. Produces a heat-sensitive toxin.	Soils, plants, marine sediments and fish.	Home-canned foods.	Blurred vision, respiratory distress and possible death.	Improper methods of home-processing of foods.	Type E and Type B can grow at 1.1°C.
Clostridium perfringes	Produces spores and prefers is anaerobic.	Dust, soil and gastrointestinal tract of animals and man.	Meat and poultry dishes, sauces and gravies.	Cramps and diarrhoea within 12-24 hours. No vomiting or fever.	Improper temperature control of hot foods and recontamination.	No growth below 4.4°C.
Enteropathogenic E. coli	Can produce toxins that are heat stable and others that are heat-sensitive.	Faeces of infected humans.	Meat and cheeses.	Diarrhoea, abdominal cramps, no fever.	Inadequate cooking. Recontamination of cooked product.	Organisms can be controlled by heating. Can grow at refrigeration temperatures.
Listeria monocytogenes	Survives adverse conditions for long time periods.	Soil, vegetation and water. Can survive for long periods in	Milk, soft cheeses, vegetables	Mimics meningitis. Immuno- compromised individuals most	Contaminated raw products.	Grows at refrigeration (1.1-4.4°C.)

Bacteria Responsible	Description	Habitat	Types of Foods	Symptoms	Cause	Temperature Sensitivity
		soil and plant materials.	fertilized with manure.	susceptible.		temperatures.
Salmonella spp	Produces an intestinal infection	Intestinal tracts of animals and man	High protein foods - meat; poultry, fish and eggs.	Diarrhoea nausea, chills, vomiting and fever within 12 to 24 hours.	Contamination of ready-to-eat foods, insufficient cooking and recontamination of cooked foods.	No growth below 4.4°C. Bacteria are destroyed by normal cooking.
Staphylococcus aureus	Produces a heat-stable toxin	Nose and throat of 30-50% of healthy population; also skin and superficial wounds	Meat, sea-foods salads., sandwich spreads and high salt foods	Nausea, vomiting and diarrhoea within 4-6 hours. No fever.	Poor personal hygiene and subsequent temperature changes above 4°C.	No growth below 4.4°C. Bacteria are destroyed by normal cooking but toxin is heat stable
Vibrio parahaemolyticus	Requires salt for growth.	Fish and shellfish	Raw and cooked seafood.	Diarrhoea, cramps, vomiting,	Recontamination of cooked foods or eating raw seafood.	No growth below 4.4°C. Bacteria killed by cooking.
Yersinia enterocolitica	Not frequent cause of human infection.	Poultry, beef, swine.	Milk, and pork.	Diarrhoea, abdominal pain,.	Improper cooking. Cross-contamination.	Grows at refrigeration temperatures

2.2.The *Escherichia coli*

2.2.1. General description of *E.coli*

Escherichia coli is among members of the family *Enterobacteriaceae.* It belongs to a genus of gram negative, rod-shaped bacteria measuring approximately 2-3μ in length by 0.6μ in diameter. *Escherichia coli* is a facultative anaerobic bacterium that is found among the normal flora of the intestinal tract of many animals and humans (Johnson and Maloney, 1996). It does not form spores. Many strains of *E.coli* possess peritrichous flagella that make them sluggishly motile. Some strains develop capsules and produce mucoid colonies on solid media. *Escherichia coli* will grow and obtain energy by fermentation, producing characteristic mixed acid and gas as end products.

2.2.2. Culture Media

Escherichia coli can be readily grown on ordinary laboratory media without the addition of blood, serum, ascitic fluid or glucose. A satisfactory medium to use for the isolation of *E. coli* however is MacConkey agar. The presence of lactose sugar and a pH indicator in this medium enables lactose-fermenting organisms such as *E.coli* to be differentiated from lactose non-fermenters such as *Salmonella* and *Proteus*. Growth of *E. coli* will appear as pink coloured colonies due to the fermentation of lactose and the production of acid shown by the pH indicator (neutral-red). Conversely, non-lactose fermenters (NLF) appear as pale-yellowish colonies.

2.2.3. Optimum growth temperature

The optimum temperature for cultivation is 37^0C but growth will occur over a wider temperature range (20-44^0C).

Escherichia coli is relatively susceptible to physical and chemical agents. A temperature of 55^0C for one hour or 60^0C for twenty minutes is lethal to these organisms, and they are killed rapidly by autoclaving at 120^0C. Under natural conditions, *E.coli* may survive for weeks or months in water, faeces and dust in animal houses, however it is highly susceptible to phenol and cresol although the efficacy of these disinfectants is reduced in the presence of mucus and faeces.

2.2.4. Biochemical tests

Biochemical tests are used to differentiate of *E. coli* from other closely related bacteria. 90% of *E.coli* strains ferment lactose. Many diarrhoeagenic strains of *E.coli* do not ferment lactose. 99% of all *E.coli* strains produce indole but fail to produce H_2S and do not grow in citrate media (Nataro and Kaper, 1998). Most strains do not produce urease, give a negative Voges-Proskauer reactions and are positive to methyl red test (Table 2).

Table 2: Some cultural and biochemical criteria for identification of *E.coli*

CRITERIA	REACTION
Motility	+
Glucose	+
Lactose	+ or -ve
Sucrose	v
Mannitol	+
Indole	+
Methyl red	+
Voges-Proskauer	-ve
Citrate	-ve
H_2S	-ve
Urease	-ve

+ = positive reaction or fermentation
-ve = late fermentation
v = variable reaction

Source: Nataro and Kaper, (1998)

2.3. *Escherichia coli* antigens and toxins

Bacterial serotypes are defined by antibodies, which identify specific antigens present in the bacteria. These antigens are employed to serotype these strains: O (heat-stable somatic antigen), K (heat-labile antigen of the capsule) and H (heat-labile flagella antigens). About 164, 100 and 56 of O, K, and H *E.coli* antigens respectively have been described (Johnson and Maloney, 1996). Numbers refer to the known subtypes of antigens that can be differentiated by use of specific antibodies and thus used to identify bacterial serotypes (Johnson and Maloney, 1996).

2.3.1. O-antigens

Antigens occurring as part of the bacterial cell wall are referred to as "O" (ohne) and are composed of polysaccharide-phospholipid protein complex. The cell wall also contributes to resistance of the organism to destruction by phagocytosis. The lipopolysaccharide (LPS) endotoxin elicits a pyrogenic response and triggers intravascular clotting. Heating at 100^0C or 120^0C does not destroy those antigens, but the heat enhances their ability to combine with specific antisera.

Variation from smooth to rough colonial form (S-R) is accompanied by a progressive loss of the smooth O antigen to a "rough" (R) bacterial strain whose lipopolysaccharide lacks the O-specific side chains, retaining only the basal core structure. Rough strains do not, therefore, react with O-specific antibody (Buxton and Fraser, 1977).

Some *E.coli* serotypes e.g. *E.coli* 104 and *S.typhimurium* have developed another somatic antigen that is common to different bacterial groups and hence referred to as a "common" antigen. The common antigen is composed of polysaccharide, it is not destroyed at 100^0C and is soluble in 85% ethanol. This antigen plays a significant role in the pathogenesis of diseases because the presence of common antigen derived from one bacterial serotype in the host stimulates an immune response against the common antigen of a second and different serotype which subsequently invades the host's tissue (Buxton and Fraser, 1977).

2.3.2. K-antigens

"Kapsel" (K) antigens occur as envelope or capsule on most strains of *E.coli*. These antigens are composed of polysaccharides, and can prevent agglutination between O-antigens and homologous antisera because they overlie the surface O-antigens. Inhibition of O agglutination by K antigens can be overcome by heating the bacteria to 100^0C for one hour (Ørskov and Ørskov, 1977).

The K-antigens are subdivided into K_1, K_2 and K_3 variants. Not all *E.coli* have K antigens; and when they are present, only one type is found in a given strain. Strains containing K antigens appear, in general, to be more toxigenic, as well as more resistant to phagocytosis and to bactericidal action of antibody and complement (Ørskov and Ørskov, 1977).

Strains carrying K antigens have been incriminated in neonatal meningitis outbreaks (Guinee and Leeuwen, 1976) and certain K antigens influence the adherence to intestinal mucosa in calves and swine. According to Guine'e and Leeuwen, (1976), the synthesis of K99 fimbrial antigen and other K antigens are directed by plasmids.

The polysacharide capsule is produced *in vivo* and is a virulence factor in certain strains of *E. coli* that cause diarrhoea in calves and pigs (Hadad and Gyles, 1982). The capsular material contributes to the formation of micro colonies attached to the intestinal epithelium (Hadad and Gyles, 1982).

2.3.3. H-antigens

The flagella (H) antigens are poorly developed on newly isolated *E.coli* strains and consequently agglutination with homologous H antiserum is weak. Passage of the strains through semi-solid medium often results in increased development of H antigen and motility. More than 56H antigenic types are known (Ørskov and Ørskov 1977).

2.3.4. Flagella

Motile strains of *E.coli* have flagella that project from the entire surface of the bacterium (peritrichous arrangement). There are about twenty genes concerned with the structure and operation of the flagella: the *hag* (H antigen), the *fla* (flagella) gene and the *mot* (motility gene). Motility enhance the virulence of certain bacteria such as in *S. typhimurium*, however, some strains of non-motile *E. coli* are able to cause disease (Ørskov and Ørskov 1977).

2.3.5. Fimbrial antigens

Fimbriae are slender filaments of protein that project from the surface of bacteria and confer adhesive properties on the organism (Gaastra and De Graaf, 1982). Fimbriae are shorter and thinner than flagella and are found over the whole surface of the bacterium. Bacteria possessing fimbriae agglutinate RBC of various animal species and humans due to their ability to adhere to the surface of RBCs. Fimbriae are usually numerous on the bacterial surface whereas the F or sex pilli are limited in number (1-4 per cell) and morphologically differ from common fimbriae. Boiling

destroys the fimbriae, and cells so treated become O-agglutinable (Deneke *et al.* 1984).

2.3.6. Colonization factors I and II

Colonization factors I and II (CFA I and II) are specific fimbrial adhesins that promote colonization by strains of human ETEC (Svendsen *et al.*, 1977),. CFA I and II are plasmid mediated fimbrial structures that are antigenically different, and form the fimbriae that promote attachment to the intestines of various animals. According to Svendsen *et al.* (1977), however, large numbers of ETEC strains colonize the intestines and cause diarrhoea, although they lack specific identifiable adhesins.

2.3.7. Enterotoxins

Enterotoxigenic *E.coli* (ETEC) strains pathogenic for animals and humans produce two classes of plasmid-mediated enterotoxins: heat labile (LT) and heat stable (ST) toxins (Moon *et al.*, 1979). Although many *E.coli* produce both LT and ST, strains producing ST alone are particularly common in isolates from domestic animals (Moon *et al.*, 1979).

2.3.8. Colicin V (Col V) plasmids

Colicins are proteins that have bactericidal activities. They belong to a class of bacterial products known as bactriocins. The first of bacteriocins to be studied were derived from *E.coli* and were termed colicins (Carbonetti and Williams *et al.*, 1984). Their synthesis is plasmid mediated. Colicins bind to receptors on the outer

membrane of susceptible cells following which they contact the cytoplasmic membrane and disrupt its function and cause death of the susceptible bacteria. Colicin production confers selective advantage particularly in the competitive environment of the intestines thus favouring the possessor strain to colonise the gut. This phenomenon is called competitive exclusion of non-favoured bacteria. There are nine known colicins produced by strains of *E.coli* (Carbonetti and Williams *et al.*, 1984).

Plasmids with genes for Colicin V (Col V plasmids) have been associated with strains that cause bacteraemia in calves, lambs and humans (Carbonetti and Williams, 1984). The presence of Col V allows the bacteria to multiply in an environment of limited concentration of free iron such as in the tissues and fluids of the host (Carbonetti and Williams, 1984).

2.3.9. Haemolysins

Some strains of *E.coli* produce haemolysins. The synthesis of haemolysin is controlled by transmissible plasmids (Carbonetti and Williams, 1984). Two kinds of haemolysins are produced by *E.coli* strains (Carbonetti and Williams, 1984): one is designated α haemolysin which is a soluble haemolysin found in cell free culture supernatants and the second one is designated as the β haemolysin which is a cell-bound haemolysin. Alpha (α) haemolysin occurs in two molecular forms that differ in size; sensitivity to trypsin and requirement for calcium ions for activation. The α haemolysin is not toxic to animals and its *invitro* haemolytic activity is neutralized

by homologous antibody (Carbonetti and Williams, 1984). Both α and β haemolysins do not appear to be associated with pathogenesis of infection with haemolytic strains, but have been found to lyse a variety of erythrocytes. Haemolysin production is a common feature of human isolates of *E. coli* involved in extra-intestinal infections and *E. coli* serogroups that produce diarrhoea or oedema disease of pigs. The role of haemolysin in extra intestinal infections is to increase the level of available iron in the host (Carbonetti and Williams, 1984).

2.4. Diarrhoeagenic *Escherichia coli*

Escherichia coli strains that cause diarrhoeal illness are categorized into four specific groups based on virulence properties, mechanisms of pathogenicity, clinical syndromes, and distinct O and H serogroups (Levine, 1987). The specific groups of diarrhoagenic (enteric) *E.coli* are: Enteropathogenic *E.coli* (EPEC), Enterotoxigenic *E.coli* (ETEC), Entreroinvasive *E.coli* (EIEC), and Enterohaemorrhagic *E.coli* (EHEC) (Levine, 1987). *E.coli* is pathogenic both for the intestinal tract (diarrhoagenic *E.coli*) and other tissues (non- diarrhoagenic *E.coli*).

2.4.1. Pathogenesis of diarrhoegenic *E. coli*

E.coli strains associated with diarrhoeal disease in humans and animals exhibit several common properties. The common characteristics of all diarrheogenic *E. coli* are:

The genes for virulence factors are often located on transmissible plasmids.

Specific receptors in the intestines bind each group of *E.coli* and determine host specificity. They interact with the intestinal mucosa.

MultipleX cytotoxin and cytotoxic enterotoxins may be produced.

Within each pathotype the strains fall within certain O:H serotypes (Levine, 1987).

2.4.2. Enterohaemorrhagic *E.coli* (EHEC)

EHECs are defined as Shiga toxin- producing *E.coli* that have been demonstrated to cause diarrhoea in man. EHECs are intestinal pathogenic bacteria producing watery and bloody diarrhoea with colical intestinal pains or the haemorrhagic colitis (HC) syndrome. The infection can develop with life menacing complications in children less than six years old and in elderly people. *E.coli* O157:H7 produces two basic toxins: Shiga-like toxin (SLT 1/Verotoxin 1 (VT 1) or verocytotoxin 1 and SLT 2 or VT 2 (Karmali, 1989). All SLT's are cytotoxic for vero and HeLa cells and are lethal for mice. EHEC strains also produce plasmid-mediated fimbriae and attaching and effacing lesions (*eae*) (Karmali, 1989), which is demonstrable in HEP-2 cells. Like other EHECs, O157:H7 contains a plasmid in the 60 Mega-Dalton (Mda) range that encodes the production of enterohemolysin. It is at this point when the plasmid is regarded as a virulence marker. *Escherichia coli* O157:H7 is one of the several EHEC serotypes that are currently the food borne pathogens of major concern in the world (Fratamico and Strobaugh, 1997).

2.4.3. Enteropathogenic *E.coli* (EPEC)

Entropathogenic *E.coli* (EPEC) strains associate with intestinal mucosa in a manner known as attachment-effacement, which leads to disruption of the epithelial cell membrane. Although plasmid-mediated factors facilitate bacterial adhesion to the small intestine, attaching-effacing appears to be the most important virulence factor of EPEC strains (Tzipori *et al.*, 1989). EPEC causes diarrhoea different from that of Shigella and different from Enteroinvasive *E.coli* (EIEC). EPEC do not produce enterotoxins. Especially virulent are the EPEC serotypes O55, 0111, O127 (Tzipori *et al.*, 1989).

2. 4. 4. Enterotoxigenic *E.coli* (ETEC)

Enterotoxigenic *E.coli* (ETEC) serogroups have specific adhesion fimbria for intestinal attachment and colonization. Such adhesion fimbria are often host specific and are probably one of the most important factors preventing interspecies transmission of enterotoxigenic *E coli* strains (Wasteson *et al.*, 1990). ETEC strains usually carry five or more plasmids with antibiotic resistance, enterotoxins and adherence antigens on separate plasmids e.g. Col V plasmid (Elwell and Shipley, 1980).

Enterotoxigenic *E.coli* (ETEC) produces two primary enterotoxins: heat labile (LT) containing subunits A and B (LTa and LTb) and heat stable (STa and STb or ST 1/ST 2). LTb is the binding site, while LTa stimulates the adenylate cyclase system. LTa and LTb have immunological properties similar to subunits A and B of the

Vibrio cholerae (Kunkel and Robertson, 1979). LTa and LTb are designated human and porcine strains, respectively. STa elicits secretory response in infant mice while STb elicits secretory responses in swine (Kunkel and Robertson, 1979).

ETEC produce both STa and STb and induce fluid accumulation in the intestines of both weaned and neonatal pigs (Harnett and Gyles, 1983). Class 1 ETEC causes diarrhoea in neonatal and post weaned pigs while class 2 ETEC causes neonatal diarrhoea in pigs (Harnett and Gyles, 1983). According to Larsen (1976), two ETEC strain 0149:K91 were recovered from weaned pigs and differ from those recovered from suckling pigs. The former are usually K88 and colicinogenic, whereas the latter are typically K88 and less frequently colicinogenic.

The edema disease (*E. coli* enterotoxaemia) of weaned pigs is caused by haemolytic *E.coli* strains that belong to O serogroup 138, 139 or 141. The diseases result from the effects of toxins, which are absorbed from the intestines and damage the vascular system (Miller *et al.*, 1994).

The pathogenesis of ETEC is mediated by at least two kinds of virulence factors (Robertson *et al.*, 1985): the fimbriae adhesins that bind to intestinal receptors and determine species specificity, and at least two kinds of cytotoxic enterotoxins; LT and ST. There are four distinct antigenic fimbrial adhesins on ETEC strains isolated from animals namely the K88, K99, 987P and F41 (Robertson *et al.*, 1985).

Three different colonisation factors have been found to be associated with human ETEC strains (CFA/1, CFA/II, and E8775) (Evans and Evans, 1978). Although there are over 10,000 O:K:H *E.coli* serotypes, approximately twelve are responsible for ETEC disease in piglets and eight serotypes are associated with ETEC disease in calves (Ørskov and Ørskov, 1979). Similar strains have been implicated in oedema disease of swine (Marques *et al.*, 1986); however, porcine VT differs from human VT in antigenicity, degree of heat lability and biological properties (Smith *et al.*, 1983).

Enterotoxigenic *E.coli* (ETEC) strains have also often been implicated in the aetiology of human diarrhoea in countries where antibiotic usage is uncontrolled (Armstrong *et al.*, 1981; Elwell and Shipley, 1980). ETECs have serious public health repercussions (Deneke *et al.*, 1984) as well as a serious economic problem in animal industry (Jiwa *et al.*, 1986). ETEC is also a major cause of diarrhoea in infants and in "Traveller's" diarrhoea in regions of poor sanitation (Elwell and Shipley, 1980).

2.4.5. Enteroinvassive *E.coli* (EIEC)

The *E.coli* serogroups possessing invasive properties are designated enteroinvassive *E.coli* (EIEC). These strains often have atypical biochemical characteristics, being lactose–negative or with slow lactose fermentation and are often regarded as non-motile (Olsvik et *al.*, 1991). According to Levine (1987) and O'Brien (1979), the

pathogenesis of EIEC is similar to that of *Shigella spp* since both exhibit the capacity to specifically bind, invade and survive within epithelial cells. Most of those strains also possess genes encoding one or more of the cytotoxins of the Shiga-like toxin family (SLT 1 and SLT 2) (Doyle and Padhye, 1989; Wasteson *et al.*, 1990). Invasiveness of EIEC and *Shigella spp* is due to the presence of a 140-Mda plasmid that codes for several outer membrane proteins. EIEC strains produce multiple cytotoxins referred to as Shiga-like toxin (SLT) or Vero toxin (VT), which are immunologically related but not identical (Konowalchuk *et al.*, 1978). SLTs have been implicated in human diseases such as HC and HUS and in diarhoea in calves and oedema disease in swine (Marques *et al.*, 1986). Invasive *E.coli* strains have so far been found to naturally induce disease in humans similar to Shigella species.

2.4.6. *E.coli* diarrhoea in calves

According to Cray (1995), *E. coli* causes three forms of colibacillosis in calves: septicaemic, enterotoxaemic and enteric colibacillosis. However, studies by Moon (1974) have shown that both enteric forms of the disease are caused primarily by the enterotoxigenic strains of *E.coli* that are non-invasive. The invasive strains of this organism are responsible for the septicaemic form of the disease. Diarrhoea due to *E. coli* occurs most commonly in calves less than one week of age but can be a problem in calves as old as two to three weeks (Acres, 1985). Diarrhoea results from the action of enterotoxin(s) produced by ETEC. A number of pathogens such as ETEC, Rotavirus, Corona virus, BVD-Virus, Cryptosporidium and Salmonellae cause diarrhoea in older calves (Moon *et al.*, 1979).

Septicaemic colibacillosis occurs in all species but is most commonly seen in foals and lambs. The enterotoxic form of colibacillosis occurs most commonly in calves and piglets and are less common in foals and lambs. Calves and piglets that are deficient in immunoglobulins are most susceptible to septicaemia. The clinical findings and lesions in septicaemic colibacillosis are attributed to the effects of endotoxins, which cause shock (Ørskov and Ørskov, 1977).

2.4.7. *E.coli* diarrhoea in other animal species

E.coli is responsible for diarrhoea in diverse animal species including dogs, cats, horses and rabbits. Studies conducted by Moon *et al.* (1979) have shown that the intestines of the young readily permit passage of *E. coli* from the lumen across the epithelium. The *E. coli* strains that cause septicaemia in calves, pigs, foals and puppies possess virulence attributes that are different from those of *E. coli,* that cause enteric disease. Most bovine strains belong to O groups 15, 35, 78 while in pigs, strains 078 and K80 of O the serogroup are commonly encountered. *E.coli* strain O:20 also cause urinary tract infections of dogs while strains 02 and 078:K80 cause poultry colisepticaemia. Pyometra in dogs and cats is caused by *E. coli* strains of O group 2, 4 and 141 and O groups 2 and 22 respectively. Some *E. coli* strains also cause coliform mastitis of cattle and swine (Frost and Hill, 1982).

2.5. Verocytotoxigenic *E. coli* (VTEC) O157:H7

2.5.1. Definitions

VTEC O157:H7 is a specific serotype (expressing the O-antigen 157 and the H-antigen 7) of *E. coli* (Abramov, 1996). VTEC serotype O157:H7 belongs to EHEC and is the commonest serogroup found in human VTEC infections. VTEC O157:H7 is associated with a wide range of clinical illness including asymptomatic shedding of the bacterium (Riley *et al.*, 1983). The subsequent occurrence of large outbreaks and the widespread distribution of cases have led to the designation of *E.coli* O157:H7 as a "newly emerging pathogen". *E.coli* ON: refers to serotypes for which the somatic (O) antigen is non-typeable with standard antisera. Strains that are non-motile are designated as *E. coli* 0157:H- or 0157:NM. They are missing the H antigen, which is the flagella or motility antigen (Griffin, 1995).

This serotype was first isolated as a human pathogen in 1982 in Seattle, USA following an outbreak of food borne infection caused by undercooked hamburgers (Riley *et al.*, 1983). Before then, *E. coli* was considered a harmless inhabitant of the digestive tract of humans and animals. In the mid 1980's it became clear that *E. coli* 0157:H7 was also associated with Human Uraemic Syndrome (HUS) and was capable of producing toxins which were biologically, biochemically and genetically similar to Shiga-toxins (SLT) from *Shigella dysentriae* type 1 strains (Park *et al.*, 1996). Other *E. coli* serotypes have also been associated with HC and HUS and according to Acheson *et al.* (1996) close to one hundred different enterotoxins producing *E. coli* serotypes have been isolated from patients with HC or HUS.

2.5.2. Evolution of VTEC O157:H7

Escherichia coli and related gram-negative coliform bacteria predominate among the aerobic commensal flora in the gut of humans and animals and; were until the 1950's recognized as non-pathogenic normal cohabitats (Olsvik *et al.*, 1991). As long as these bacteria do not acquire genetic elements encoding for virulence factors, they remain benign commensals. Strains that acquire bacteriophage or plasmid DNA encoding enterotoxins or invasion factors become virulent and can cause either a plain, watery diarrhoea or an inflammatory dysentery (Evans and Evans, 1990). One serotype has been identified as a newly emerging pathogen is EHEC O157:H7; a member of the family of Vero toxin producing *E. coli* (VTEC) (Chapman *et al.*, 1993).

Bacterial cells are ubiquitous and it is possible for them to acquire genetic information from other sources (bacterial viruses, plasmids or from pieces of chromosomal DNA). In some cases, acquisition of this information may provide an advantage of survival. VTEC O157:H7 appears to have been infected with a bacterial virus (bacteriophage) that had the ability to insert its own DNA into the bacterial chromosome without harming the bacterium (Griffin and Tauxe, 1991). This viral gene contains information for the production of the Shiga-like toxin (SLT). Every time this bacterial cell divides, the viral DNA, being now a part of the bacterial DNA, is passed on to every daughter cell thus creating the enterotoxigenic/verocytotoxigenic VTEC 0157:H7. Consequently, this strain of *E.*

coli and all of its progeny produce this toxin that causes severe damage to intestinal epithelial cells (Griffin and Tauxe, 1991).

Feng *et al*, (1998) assessed the genetic relationships among Shiga-toxin producing O157 strains to elucidate stages in the evolutionary emergence of VTEC 0157:H7. The results support a model which 0157:H7 evolved sequentially from a GUD-positive (B- gaeicirpmodase) and sorbitol positive Enteropathogenic *E.coli* (EPEC) strain of serotype O55:H7; first by acquiring the Shiga toxin 2 gene and then by dividing into two branches. One became GUD-negative and sorbitol-negative resulting in the O157:H7 clone that spread worldwide. The other lost motility leading to the sorbitol fermenting VTEC O157:H- clone that has been identified in Europe.

One hypothesis for the emergence and rapid spread of this organism is that strong mutator alleles enhance genetic variability and accelerate adaptive evolution (Whittam *et al.*, 1993). According to the mutator hypothesis, a pathogen able to enter a transient hypermutable state could overcome the fitness costs of deleterious mutations by acquiring new genetic variation at times critical for survival and colonization of new hosts. In their study, Whittam and co-workers (1993) observed that the main evolutionary benefit of the mutator phenotype is the enhanced ability to acquire useful foreign DNA and not increased rate of point mutation over the long term. Such parallel evolution indicates that natural selection has favoured an ordered

acquisition of genes and the progressive build-up of molecular mechanisms that increase virulence (Reid *et al.*, 2000).

2.6. Virulence factors associated with VTEC 0157:H7

2.6.1. Verocytotoxin (VT)

The term Shiga-like toxin (SLT) is used to describe the toxin produced by *E.coli* strains (Gross, 1991). This is because when VT was first described it was found to be very similar to Shiga toxin produced by strains of *Shigella dysenteriae* type 1 in terms of biological properties, physical characteristics and antigenicity. VT which is neutralized by anti-Shiga toxin is now designated VT1 and a second VT shown in strains of serogroup O157 and not neutralized by anti-shiga toxin is termed VT2. VT1 and VT2 are also known as SLTI and SLTII respectively (Johnson, 1991). SLT/VT have cytopathic effects on vero-cells (African Green Monkey kidney cells). SLTs/VTs affect other cell lines as well (Konowalchuk *et al.*, 1997 and Karmali, 1989). According to Calderwood *et al.* (1996) and Karmali *et al.* (1985), the ability of VTEC strains to cause severe disease in humans is undoubtedly related to their capacity to secrete the VT1/SLT1 or VT2/SLT2. The structural genes for SLTs are encoded on bacteriophages. In contrast, those for Shiga toxin of *S. dysenteriae* type 1 are in the chromosome (Griffin, 1995).

SLT or VT1 and VT2 are made up of A and B subunits. For both toxins, the A subunit possess the biological activities of the toxin while the B subunits are thought to mediate specific binding and receptor-mediated uptake of the toxin. VT1 and VT2

have the same biological activities as Shiga-toxin and that is cytotoxicity for vero and Hella cells, enterotoxicity in ligated rabbit gut loops and mouse paralytic lethality (Johnson, 1991).

VTEC that produce VT2 alone or in combination with VT1 are associated more frequently with HUS than are organisms that produceVT1 alone (Scotland *et al.*, 1987 and Ostroff *et al.*, 1989). VTEC cause disease by direct action of vero toxin on certain cells. Verotoxin bind to a specific glycolipid receptor globotriaosylceramide (Gb3) found on endothelial cells of blood vessels, smooth muscles, renal endothelial cells, as well as red blood cells (Lingwood *et al.*, 1987; Waddell *et al.*, 1988 and Kasai *et al.*, 1985). Once bound, verotoxins inhibit intracellular protein synthesis thereby causing cell death. Because the structures of Gb3 is identical to the PK antigen in the P blood group, P-blood group status appears to influence the risk of developing HUS after VTEC infection (Taylor *et al.*, 1990 and Newburg *et al.*, 1993).

2.6.2. Other VTEC O157:H7 virulence markers

Toxin production alone, however, is not sufficient to make *E. coli* O157:H7 pathogenic, since some SLT/VT producing *E. coli* (STEC) do not appear to be pathogenic to humans (Griffin, 1995). Adherence appears to be another critical virulence factor however, and the most important virulence marker is the chromosomal *eae* gene (for *E. coli* attaching and effacing). *Eae* is a 94 Kda gene responsible for attaching/effacing lesions of epithelial cells that encode the production of intimin, which is an adherence factor (Griffin, 1995 and Tarr, 1995).

That enables the organism to attach to and colonize intestinal mucosal cells. Eae gene is a 94-Kda outer membrane protein involved in the intimate attachment of bacteria to enterocytes (Yu and Kaper, 1992).

VTEC O157:H7 has another virulence marker called enterohaemolysin (hly). Hly is a member of the repeat-in toxin (Rtx) family of cytolysins and has been suspected to have a role in pathogenicity (Schmidt *et al.*, 1995). Brunder *et al.* (1997) described two other putative virulence factors: a serine protease (EspP), which can cleave human coagulation factor V, and a bifunctional catalase peroxidase, (cat p). According to Boerlin *et al.* (1999) there is however, no experimental proof for the role of these factors in the virulence of VTEC O157.

2.6.3. Pathogenesis of VTEC O157:H7 infections

The pathogenicity of VTEC O157:H7 appears to be associated with the VT that it produces (Karmali *et al.*, 1983a, b). The VT receptor is a glycolipid, a compound called globotriaosylceramide (Gb3). Gb3 contains three sugars, namely two galactose residues and one glucose attached to ceramide that contains a fatty acid bound to a sphingosine lipid base. Gb3 is found on the outer surface of host cells where it functions as the receptor for VTs produced by Enterohaemorrhagic *E. coli* including O157:H7. The VT itself is comprised of protein subunits: a single A-subunit with enzymatic activity and a pentamer of non-covalently associated B-subunits that have binding functions (Donahue-Rolfe *et al.*, 1989; Ramotar *et al.*, 1990). In an infection, the B-subunits recognize and bind to the galactose residues of

Gb3 and the toxin is subsequently transported inside the cell (Jacewicz *et al.*, 1986; Lindberg *et al.*, 1987). Inside the cell, the single A-subunit cleaves ribosomes at a specific residue, thus inhibiting protein synthesis (Endo *et al.*, 1988; Saxena *et al.*, 1989).

In young children, the receptor Gb3 is expressed in specialized kidney blood vessels, the glomeruli, while in adults, it is expressed in the kidney tubules rather than in glomeruli (Lingwood, 1994). This differential expression could account for the differences in severity of infection developed by the two age groups.

2.7. Methods for detection and characterization of VTEC O157:H7

Food borne pathogenic microorganism contamination is a public health concern. To minimize possible outbreak of food poisoning by bacteria such as *E.coli* O157:H7, sensitive and rapid detection techniques are needed to alert and apply proper interventions prior to further distribution of contaminated foods (Shui *et al.*, 1999).

Various workers have proposed several rapid methods for the detection of *E.coli* O157:H7. Because cultural isolations of VTEC 0157 from foods and faeces is time consuming, labour intensive and costly, rapid immunological detection kits and immunomagnetic separation (IMS) systems have been developed (Heuvelink *et al.*, 1997). Examples of such test systems include Ampcor ™, *E.coli* O157:H7 kit (Ampcor), Micro-screen ™ for *E.coli* O157:H7 (Neogen), VIP ™Test for EHEC (BioControl Systems), EHEC-TEK ™ (Organon Teknik) and Petrifilm ™ (3M)

(Heuvelink *et al.*, 1997). These commercial test kits reduce analysis time, giving presumptive results after one day compared to cultural procedures that require two days. Detection can be greatly facilitated by use of special media, complemented with serological procedures.

2.7.1. Pre-enrichment of sample

The isolation of *E. coli* O157:H7 from complex samples (environmental, animal faeces, food and clinical specimens) is a major challenge. Samples often contain low numbers of VTEC with very high levels of background flora and natural inhibitors, which can interfere with isolation and subsequent detection of the pathogen (Duffy, 1999). VTEC are often present in an injured or stressed condition and will not be recovered, hence resulting in false negative results. A recovery step must therefore be built into the protocol (Duffy, 1999).

The low numbers and the injured form of VTEC O157:H7 in samples therefore, require the use of enrichment media when screening for the presence of these organisms in foods and faeces (Heuvelink *et al.*, 1997). Selective enrichment media that can undergo IMS with Dynabeads anti-*E.coli* O157 (Dynal, Oslo) include modified tryptone soya broth (mTSB) with acriflavin, vancomycin, cefsulodin or cefixime, buffered peptone water (BPW) with cefsulodin and cefixime and Brilliant-Green Bile Broth (BRILA) (Fratamico *et al.*, 1997).

2.7.2. Growth in Sorbitol MacConkey Agar (SMAC)

Enterohaemorrhagic *E. coli* (EHEC) strains belonging to serogroup O157 lack the ability to ferment sorbitol. The development of sorbitol Mac Conkey Agar (SMAC) has enabled many laboratories to isolate these organisms (March and Ratnam, 1986).

E.coli O157:H7 rapidly ferment lactose and is indistinguishable from most other *E. coli* on traditional lactose-containing media. However, unlike approximately 80% of other *E.coli,* nearly all isolates of VTEC O157:H7 ferment d-Sorbitol slowly or not at all. Sorbitol-MacConkey agar was developed to take advantage of this characteristic by substituting the carbohydrate sorbitol for lactose in SMAC (March and Ratnam, 1986). Sorbitol-negative colonies will appear colourless on SMAC. Isolates agglutinating in O157 antiserum or O157 latex reagent should be identified biochemically as *E. coli* since strains of several species e.g. *Proteus spp* cross-react with O157 antiserum (Lior and Borczyk, 1987 and Bettelheim, 1998a).

2.7.3. CHROMagar ™ O157

Traditional methods for detecting *E. coli* are tedious and usually require heavy overload with experimental studies of many colonies. CHROMAgar ™ O157 (Prod. No 704.02) is a non-selective medium for the differential isolation of VTEC O157 from most other non-sorbitol fermenting bacteria. IMS-plating onto CHROMAgar ™ O157 will result in maximum sensitivity and less confirmation work (Padhye and Doyle, 1991). It offers increased sensitivity (down to 1 viable organism per 25 gm of

sample or 100 organisms/ml of pre-enrichment broth) and a significant reduction in background flora for more reliable results (Padhye and Doyle, 1991).

CHROMAgar O157 is a chromogenic medium with very high specificity and a very high sensitivity. CHROMagar ™ O157 has therefore been developed for the isolation of enterohaemorrhagic *E. coli* O157 and their direct differentiation (Bettelheim, 1998a). This medium contains a special chromogenic mix, and all SLT-producing strains of *E. coli* O157 (H7 and H-) give characteristic pink to purple (mauve) colonies on CHROMagar ™ O157 (Appendix 3). Results can be read under normal lighting. Studies conducted by Bettelheim (1998a) have shown that, of the strains of *E.coli* O157, which included O157:H7 and O157:NM, there was a strong correlation between SLT production and the formation of pink-coloured colonies. The three non-SLT-producing O157 strains investigated were all environmental, and they gave the same characteristic biochemical reactions as the typical SLT-producing O157 strains. Bettelheim (1998b) speculated that those strains were capable of acquiring the extra chromosomal genetic elements determining SLT production and other virulence factors required for human infection.

According to Bettleheim (1998ab), virtually all SLT-producing strains of VTEC O157 (both H7 and H-) give characteristic pink to purple (mauve) colonies on CHROMagar ™ O157. A few other EHEC serotypes particularly O111, also give pink to purple colonies on CHROMagar™ O157 and would not be missed also. If SLT producing *E. coli* serotypes are suspected, they would grow on this medium and

the colonies could then be tested by other means for SLT production (Table 3 below).

Table 3: Interpretation of EHEC O157:H7 colonies on CHROMagar TM O157

Colony colour	Presumptive microorganisms.
Mauve	E.coli O157
Steel blue	Coliforms
Colourless to grey	Proteus

Source: Dynal Biotech, Oslo.

2.7.4. Dynabeads® anti-*E.coli* O157

Dynabeads® anti-*E.coli* O157 are designed for rapid selective concentration of VTEC O157 directly from a pre-enriched sample aliquot using IMS (Appendix 1). Dynabeads are incubated with an aliquot of the pre-enriched sample and the antibodies coated onto the beads will specifically bind the target bacteria. The bead bacteria complexes are subsequently separated by using a Magnetic Particle Concentrator (Dynal MPC®-S).

2.7.5. Immunomagnetic Separation (IMS) technique

The Immunomagnetic Separation (IMS) technique has been developed by Dynal Biotech, Oslo, and is a promising tool for detection of pathogenic bacteria (Gilhuus-

Moe *et al.*, 1989; Olsvik and Skjerve, 1989). It is a rapid isolation technique for specific bacterial microorganisms. The IMS technique is used to physically separate specific biological entities from a sample using magnetism. It has been widely and successfully used in many biological fields including molecular biology, immunology and microbiology. The use IMS technique has been reported to reduce the total test time and improve the sensitivity of microbiological tests on foods (Vernozy-Rozand *et al.*, 1997).

Dynabeads® immunoseparations are based upon the interactive binding between unique cell surface antigens and antibody coated magnetic beads. Dynabeads® bacterial products are pre-coated with high affinity antibodies against surface markers of live bacteria (Dynal Biotech, Oslo). The products are designed for IMS of bacteria, which cause gastrointestinal illnesses directly from pre-enriched food or environmental samples (Appendix 2).

2.7.6 Principles of IMS

Immunomagnetic Separation (IMS) technique involves the use of small uniform polystyrene super paramagnetic beads coated with specific antibodies against surface antigens of the target bacterium (Appendix 2) (Gilhuus Moe *et al.*, 1989; Olsvik and Skjerve, 1989). The bacteria bound to the beads grow well and the technique gives several advantages (Dynal Biotech, Oslo). First, the target bacteria are separated from the contaminated environment and are concentrated from a large volume to an immunomagnetically-purified volume suitable for cultivation on plates or broth. In a

study conducted by Vernozy-Rozand *et al.* (1997) to demonstrate the ability of Dynabeads® immunomagnetic separation, it was possible to detect VTEC O157:H7, which were inoculated in 25g of raw minced beef after enrichment in modified tryptone soya broth (mTSB) and incubated at 42^0C for only four hours. The IMS technique is rapid, technically simple and is specific for the isolation of VTEC O157. It is, therefore, useful in epidemiological studies (Wright *et al.*, 1994).

2.7.7 The commercial latex agglutination test

Since *E.coli* species consists of both pathogenic and non-pathogenic strains, and due to the fact that the latter constitute a part of the normal intestinal flora, differentiation between pathogenic and non-pathogenic strains is of particular importance. Traditional cultivation from food samples using selective enrichment broths has been shown to favour strains of environmental origin over strains of human origin (Hill and Carlise, 1981).

Since VTEC O157:H7 does not ferment sorbitol, the non-sorbitol fermenting colonies can then be tested with the latex reagents to determine whether the isolate belongs to the O157 serogroup and therefore a potential VT-producing strain. The Oxoid *E.coli* O157 latex test (Oxoid DR 620) detects by slide agglutination *E.coli* strains possessing the 0157-serogroup antigen (Oxoid Limited, UK) (Appendix 5).

Chapman and co-workers (1989) evaluated the performance of the Commercial Latex Slide Test (CLST) (Oxoid DR620M) for identifying VTEC O157 and concluded that the latex slide test offers a rapid and economical alternative to tube agglutination for the identification of VTEC O157. Previous studies conducted by Sowers *et al.* (1996) to evaluate the performance of the commercial latex reagents for identification of O157 and H antigens of *E.coli* had also shown that CLSTs were good alternatives to standard serological methods.

2.7.8 Biochemical tests for the confirmation of VTEC O157 isolates

Due to cross-reactivity between the O157 antigen with other *E. coli* serotypes and other members of the *Enterobacteriaceae*, biochemical confirmation of the isolates is mandatory (Rice *et al.*, 1992b; Park *et al.*, 1998 and Bettleheim, 1995). When Bettelheim (1998b) conducted a study to assess biochemical characteristics of 585 *E. coli* strains obtained from a variety of sources from around the world including Africa, Asia, Australia, Europe and North America, all the strains were characterized as being *E.coli* on the basis of their characteristic reaction on Triple Sugar Iron agar (TSI), their ability to produce indole and to split ortho-phenyl galactoside (ONPG) and their inability to split urea (Appendix 4). They were then serotyped for their "O" and "H" antigens (Bettelheim and Thompson, 1987) and for their ability to produce SLT (Konowalchuk *et al.*, 1997) and specifically SLT 1 and SLT II (Perera *et al.*, 1988). Generally, full agreement between the tests for the presence of SLT by the three methods was achieved. Isolates confirmed as *E. coli* 0157:H7 should then be tested for toxin production as not all strains elaborate SLT/VT.

2.8. Other methods for the detection of VTEC O157

2.8.1. DNA hybridization and the Polymerase Chain Reaction (PCR)

The occurrence of phenotypic variants of *E. coli* O157 and other VTEC serotypes necessitate the use molecular techniques to detect virulence characteristics such as the presence of Shiga toxin (Stx) genes and the *eae* gene (Heuvelink *et al.*, 1997).

Using DNA based polymerase chain reaction (PCR) methods, identification of VTEC O157: H7 can be done rapidly and with high specificity and sensitivity. One multiplex PCR method amplifies simultaneously three different DNA sequences of VTEC O157:H7 a specific fragment of the *eae* gene, conserved sequences of Vero toxin 1 and 2, and a fragment of the 60-MDa plasmid (Deng and Fratamico, 1996). Since this test detects other virulence markers besides verocytoxin, it is more specific than tests that only identify verocytoxin genes. Studies conducted by Olsvik *et al.* (1990ab) have shown that using PCR, one might be able to identify genes encoding virulence factors directly in food or stool samples without necessarily cultivating microorganisms on media.

2.8.2 DNA fingerprinting

Inters train differentiation of *E. coli* O157:H7 can be done using molecular methods. These methods are used in distinguishing between outbreaks due to related and unrelated isolates. The most commonly used DNA fingerprinting tests are based on restriction fragment length polymorphism (RFLP) method where restriction enzyme are used to cut genomic DNA into fragments that are separated by agarose gel electrophoresis. A pattern or "fingerprint" is resolved for particular bacterial strains

(Johnson *et al.* 1995). Two RFLP methods are commonly used: one uses pulsed field gel electrophoresis (PFGE) and the other one uses conventional gel electrophoresis (Samadpour, 1995)

2.8.3 The BIA core-biosensor

The BIA core biosensor uses a detection system in which bacteria can be detected directly without the use of enzyme or radioactive labels. VTEC O157 detection systems using this biosensor have allowed simple rapid screening of foods and environmental samples for the presence of this pathogen (Fratamico *et al.*, 1996).

2.8.4 Enzyme linked immunosorbent assay (ELISA)

Enzyme linked immunosorbent assay (ELISA) methods are also used to detect and identify suspect colonies (Johnson *et al.*, 1995). Immunoassay methods are used to detect the presence of verocytotoxins (Su and Brandt, 1995) so as to identify VT producing serotypes other than O157:H7.

2.8.5 Methylumbelliferyl-B-D- glucuronide (MUG) test

Some laboratories also test VTEC O157 strains for the enzyme B-glucuronidase using broth or agar medium containing the substrate 4-methylumbelliferyl-B-D-glucuronide (MUG) (Thompson *et al.*, 1990). When this enzyme cleaves MUG, a fluorescent product is produced that is detectable with ultraviolet light. Unlike the majority of *E. coli* that are MUG positive, VTEC O157:H7 and VTEC O157 NM

strains that produce SLT are MUG negative. For this reason, the MUG assay used in conjunction with testing for sorbitol fermentation and agglutination with *E. coli* O157 antiserum is a useful screening test for toxigenic strain of O157 (Thompson *et al.*, 1990).

2.9 Identification of SLT1/VT1 and SLT 2/ VT 2

Shiga-like toxins (SLT) are important virulence factors associated with VTEC O157 infections. Although VTEC O157 is the most common serotype associated with disease outbreaks, other serotypes of *E. coli* are known to produce VTs/SLTs (Heuvelink *et al.*, 1996). However, according to Heuvelink *et al.* (1996) some VTEC O157 isolates from raw meats do not produce SLT. It is therefore important to be able to identify pathogenic markers like toxins and adhesion of pathogenic strains, and differentiate them from non-pathogenic strains originating from animals or the environment (Sussman, (1985). Some of the recommended methods for this purpose are described below.

2.9.1 Plasmid profiles and probes

Plasmid profiles and probes have been used to identify EPEC strains that caused an outbreak in a nursery ward for pre- term neonates in Nairobi (Senerwa *et al.*, 1989a).

According to Senerwa *et al.* (1989a), genetic probing using polynucleotide fragments that have been sequenced and oligonucleotides produced in automatic synthesizers might soon be a routine diagnostic tool in microbiological laboratories.

2.9.2 The Premier™ EHEC assay (Meridian)

Another test kit for the rapid identification of VTEC O157 is the Premier™ EHEC assay kit (Meridian). According to Kehl *et al.* (1997), the Premier™ EHEC assay kit is a sensitive and specific method for the detection of all VTEC isolates.

2.9.3 Verotox F™ (Denka Seiken)

Another test for identifying SLT type of O157 VTEC isolates is the use of Verotox F™ (Denka Seiken) technique. Verotox F™ technique is said to be a rapid and a simple test for the identification of SLT type from O157 VTEC isolates and gives results within 48 hours. It is therefore, consistent with PCR results, which can be obtained after 26-48 hours (Heuvelink *et al.*, 1997). This is advantageous over the general vero cell assay that may require up to days to produce results.

Despite their sensitivity and specificity, both PCR and vero cell assays are too expensive and technically demanding to be widely used in most routine laboratories. PCR is based on detection of *Stx* genes, but does not provide information on the expression of the genes. Although the Verotox F™ test seems a good alternative for vero cell assays and PCR it has a limited scope since several different antigenic variants or types of toxins exist (Heuvelink *et al.*, 1997).

2.9.4 Other SLT/VT toxin-detection methods

Other methods for the detection of SLT/VT have been developed and tested. According to Mackenzie *et al.*, (1998), each of these methods has limitations including: cost, labour turnaround, time and requirement for special equipment and technical expertise. Mackenzie *et al.* (1998) recommended a commercially available assay (Premier EHEC™, Meridian Diagnostics) that uses monoclonal antibodies against *Stx*1 and *Stx*2 and a commercially available toxin detection kit VTEC-RPLA™ (Oxoid, UK) that employs a reverse, passive latex agglutination-principle. However, when Heuvelink *et al.* (1997) evaluated the performance of a variety of commercial test systems in the detection of *E. coli* O157 strains, it was concluded that the Petrifilm ™ Test Kit-EHEC and the Dynabeads ™ anti-*E.* VTEC O157 proved to be significantly more sensitive in the detection of O157 VTEC strains than Verotox F ™ and VTEC-RPLA kits.

2.9.5 H7 serology and toxin testing

Confirmation of VTEC O157:H7 also requires identification of the H7 flagella antigen. Testing for the H7 antigen as well as for the production of SLTs that are associated with pathogenic strains is carried out in reference laboratories because VTEC O157 isolates often require multiple passages before the flagella antigen is detected (CDC, 1994). Isolates that are non-motile or that are negative for the H7 antigen should be tested for the production of the SLT to identify pathogenic strains. According to CDC (1994), toxin testing of VTEC O157 strains that have the H7 antigen is sometimes not necessary because virtually all these strains produce the

SLTs. Some strains of VTEC O157 have other H types and do not produce SLT, but that is rare and such strains are not recognized as pathogens (CDC, 1994).

2.10 Other VTEC (non-O157) serotypes

SLT-producing VTEC strains of different serotypes have been increasingly isolated from humans with disease and from healthy domestic animals (Chapman *et al.*, 1992). There are however, other strains and serogroups of *E.coli* that have been identified as VTEC. These include 0N:H-, O26:H11, O11:H8, and sorbitol positive O157:H- (Whittam, 1993). Other *E.coli* serotypes have been shown to cause both sporadic outbreaks, notably serotype O111 in Italy (Caprioli *et al.*, 1994), Japan (Kudoh *et al.*, 1994) and Australia (Cameron *et al.*, 1995). A review of the literature by Bettelheim (1995) indicates that there may be at least 100 VTEC serotypes present around the world, many of which have been associated with human disease. For example, out of 4810 bovine carcasses examined in one of the slaughterhouses in Tobu, Japan in 1997, nine (0.2%) were positive for verocytotoxin producing *Escherichia coli* (VTEC). The isolates were serotypes O26:H11 (1 strain), O111: H8 (4 strains), O128: H2 (1 strain) and O157:H7 (3 strains) (Kanda *et al.*, 1997). Elsewhere, *E.coli* serotype O157:H7 has been incriminated in outbreaks and sporadic cases of food borne infections (Chapman *et al.*, 1993).

According to Feng (1995), most research focus has been on the serotype O157:H7 because of its frequent association with human infection, there are however, over 60 non-O157 vero-toxin producing *E.coli* serotypes. Tarr (1995) and Feng (1995) have

cited various reports that indicate the importance of non-O157 serotypes as causes of HUS. In 1995, the largest community outbreak of HUS in Australia, which affected 23 people was attributed primarily to the serotype O111:NM. The disease was associated with consumption of uncooked semi-dry fermented sausage product produced locally (Cameron *et al.*, 1995). According to Moore and co-workers (1995), the first outbreak of non-O157 VTEC causing haemorrhagic colitis (HC) in humans occurred in Montana, US in 1994 where *E. coli* O104:H21 was confirmed in eleven cases. The illness was associated with consumption of a particular brand of milk.

According to studies by Riley *et al.* (1983) as cited by Fukushima *et al.* (2000), most other VTEC O157 strains isolated in the US belonged to serotypes O26:H11, O103:H2, O111:H, O113: H21, H145:H and O157:H7, which in humans can cause severe disease such as HC and HUS. Other serotypes of VTEC are either associated with disease at low frequency or have not been implicated in disease (Boerling *et al.*, 1999). Furthermore, the SLT genes have been detected in members of approximately 90 to 100 *E. coli* "O" serogroups (Blanco *et al.*, 1996; Pradel *et al.*, 2000). Most VTEC strains of bovine origin have been described to belong to 20 "O" serogroups: O4, O8, O22, O25, O32, O45, O82, O84, O103, O111, O113, O116, O121, O136, O146, O153, O157, O171, O172, and Ox3 (O174). (Blanco *et al.*, 1993; and Pohl, 1991).

Infections with non-O157 serotypes may be under-reported because the isolations require techniques not generally available in clinical laboratories. Furthermore, most non-0157 serotypes do ferment sorbitol and therefore are not detected by sorbitol MacConkey medium screening (Tarr, 1995). VTEC O157:H7 is easily differentiated from other *E. coli* by its inability to rapidly ferment sorbitol but non-O157 VTEC are phenotypically similar to commensal non-pathogenic *E. coli* and are not detected with either sorbitol MacConkey agar or CHROMagar ^{TM} O157 media. To detect non-O157 VTEC, non-culture methods such as enzyme immunoassay (EIA) or Polymerase Chain Reaction (PCR) have to be used. These tests are typically performed only in reference laboratories (Fey *et al.*, 2000).

2.11 Factors affecting survival and growth of VTEC O157:H7

2.11.1 Temperature

Most strains of VTEC O157:H7 posses microbiological characteristics uncommon to most other *E.coli*. VTEC O157:H7 does not grow well at 44-45.5 ^{0}C which is the usual temperature for recovery of *E.coli* from food samples (Doyle and Schoeni, 1984). Raghubeer and Matches (1990) found that VTEC O57:H7 is excluded using standard faecal coliform enumeration procedures for foods and water. The organism can grow from around 7-10^{0}C to 50 ^{0}C with optimum temperature of 37^{0}C. VTEC can grow in acidic foods down to a pH of 4.4 and in foods with a minimum water activity (AW) of 0.95. Thorough cooking of foods until all parts reaches a temperature of 70^{0}C or higher destroys it (Doyle and Schoeni, 1984).

Studies on thermal sensitivity of VTEC O57:H7 in ground beef have revealed that the pathogen has no particular resistance to heat and heating ground beef sufficiently to kill typical strains of salmonella will also kill VTEC O57:H7. Thermal pasteurisation of milk has also been shown to be an effective treatment (Doyle *et al.*, 1997). It was found that VTEC O57:H7 in ground beef was more sensitive to heat than *salmonellae,* but can survive for nine months at 20°C (Doyle and Schoeni, 1984 and Buchanan and Doyle, 1997).

2.11.2 Low pH

The importance of gastric juice in controlling the outcome of food borne infections is well recognized. Benjamin and Data (1995) found that to cause human illness at the intestinal level, an invading organism must survive the acidic environment of the stomach. The acidity of gastric juice therefore provides the first line of defence against food borne pathogens.

Unlike most food borne pathogens, VTEC O57:H7 is tolerant to acidic environments and outbreaks have been associated with consumption of acidic foods such as dry salami and apple cider. Inoculation studies have also shown that VTEC O57:H7 can survive fermentation, drying and storage of fermented sausage (pH 4.5) for up to 2 months at 4°C with only 100-fold reduction in cell population (Doyle *et al.*, 1997) Hot acid sprays have been demonstrated to be ineffective in decontaminating VTEC O57:H7 on beef. This acid tolerance appears to be associated with proteins that can be induced by re-exposing the bacteria to acid conditions (Doyle *et al.*, 1997).

2.11.3 Environmental

Studies conducted by FDA (1992) have shown that these bacteria can survive in inoculated soil samples for at least 18 weeks and can persist in drying manure for at least 70 days depending on temperature and perhaps available moisture. Kudva *et al.* (1998) found that VTEC 0157:H7 may survive in unaerated sheep manure pile incubated outside, under fluctuating environmental conditions for 21 months. VTEC O157:H7 is able to survive in water trough sediments for at least four months and appears to multiply in this environment (Le Jeune *et al.*, 1997).

2.11.4 Dietary effects

A simple change in cattle diet few days before slaughter may reduce the risk of *E.coli* infections in humans. By feeding hay to cattle for 5 days before slaughter, the number of acid resistant *E. coli* can be dramatically reduced (Russell *et al.*, 1998). Cattle are fed starch-containing grains to increase growth rate and produce tender meat. Some undigested grain reaches the colon where it is fermented to produce acetic, propionic and butyric acids. These accumulate in the animal's colon favouring the growth of acid resistant types of *E. coli.* High nutrient and low fibre feeds therefore increase the concentrations of volatile fatty acids (VFA) and decrease the pH in the ruminant gut (Russell *et al.*, 1998 Diez-Gonzalez *et al.*, 1998). This finding was supported by Van Donkersgoed *et al.* (1999) who concluded that faeces and rumen contents are sources of *E. coli* and that there were significantly fewer *E. coli* isolated from steers changed to an alfalfa hay diet for three weeks than for steers

that stayed on silage/grain. Humans find a natural barrier that kills food borne bacteria in the acidic and gastric juices of the stomach. However, *E. coli* can withstand "acid shock" by adapting to the presence of fermentation acids (Russell *et al.*, 1998).

However, there has been conflicting information on the effect of diet on *E. coli* shedding from cattle. Published data on VTEC O157:H7 tends to contradict or does not support the effects of dietary change proposed by Diez-Gonzalez *et al.* (1998). Buchko and co-workers (2000) experimentally inoculated *E. coli* O157:H7 isolates that were acid resistant to steers that were grain or and found that the hay-fed animals shed VTEC O157:H7 longer than the grain fed and irrespective of the diet; the bacteria were equally acid resistant. Hancock *et al.* (1999) contends that VTEC O157:H7 uses several mechanisms to survive acid environments, some of which are innate and are not influenced by the environment. Therefore, the induced acid resistance of beef contaminating *E. coli* O157:H7 is likely to related to the pH of its ancestral colonic environment. Hancock *et al.* (1999) goes further to say that the *E. coli* that contaminate beef, typically originates from the hide, the hooves, or the equipment used in slaughter and processing, rather than directly from the colon, and likely replicate in environments and not in the colon. There is therefore, a need for further study to confirm that cattle feeding management practices can be manipulated to decrease the risk of food-borne illness from *E coli* (Weber, 1990).

2.11.5 Horizontal transmission of VTEC O157 and Management factors

In a study by Heuvelink *et al.* (1998) it was found a predominance of a single strain type in most of the farms thus supporting the idea that there is a horizontal transmission among animals sharing the same farm buildings (Faith *et al.*, 1996). VTEC strains have also been isolated from faecal samples from horses, ponies, and sheep and from milk filters and stable flies. When Heuvelink and co-workers (1998) characterized the VTEC O157 isolates for VT production and type, it was found that no overlapping strain types were identified among isolates from different farms except one. The predominance of a single type at each sampling suggests that horizontal transmission is an important factor in dissemination of VTEC O157 within a farm. The presence of more than one strain type, both simultaneously and over time, suggests that there could be more than one source of VTEC O157 on the farms.

Management factors such as grouping of animals in close contact and communal housing may favour the horizontal transmission of VTEC 0157 among animals within a farm (Kudva *et al.*, 1997). Faith *et al.*, (1996) and Kudva *et al.*, (1997) were able to isolate VTEC O157 strains that were identical to the strains isolated from animals from common water troughs. They concluded that contaminated animal drinking water could be an important mode of dissemination of O157 VTEC among animals on farms.

2.12 Risk factors associated with VTEC O157

2.12.1 Risk Factors for VTEC O157 shedding in cattle

Dietary stress and feed type may alter *E. coli* shedding in cattle through their effects on the ruminal environment. These factors alter the concentration of volatile fatty acids and pH in the rumen, which in turn influence bacterial growth (Kudva *et al.*, 1995; Armstrong *et al.*, 1996). Cray *et al.* (1995) found that four calves fasted for 48 hours prior to oral inoculation with VTEC O157:H7 shed the organism for a significantly longer period compared to four non-fasted controls.

Although ground beef is the most frequently identified source of outbreaks, surveillance data indicate that the majority of VTEC O157:H7 infections are sporadic, with no identified link to any other case (Mac-Donald *et al.*, 1998 and Ostrof *et al.*, 1989). Furthermore, sporadic disease may reflect entirely different food vehicles, mechanisms, or sources of infection than those responsible for outbreaks. According to Russell *et al.* (1998), dietary stress and feed type may alter *E. coli* shedding in cattle through their effects on the ruminal environment. Stressed cattle were found to shed VTEC O157:H7 for a significantly longer period compared to four non-fasted controls (Buchko *et al.*, 2000).

2.12.2 Host susceptibility factors

Host susceptibility factors, inoculum size, virulence of the strain or other unknown factors may account for the selective development of HUS among those infected with *E. coli* O157:H7. Patients at extremes of age (young and old) may be at risk of

VTEC O157:H7 associated diarrhoea as well as of HUS, thrombotic thrombocytopaenic purpura (TTP) and death (Su and Brandt, 1995).

E.coli 0157:H7 causes two conditions: HC and HUS. TTP is one of the conditions that constitute HUS, it is not a separate disease caused by *E.coli* 0157:H7. The other disease conditions that define HUS are acute renal failure and microangiopathic haemolytic anaemia (Nataro and Kaper, 1998).

Strains that produce relatively more Shiga-toxin 2 are also suspected to be more virulent than those that produce relatively more Shiga toxin 1. Antibiotic treatment during the exposure period and before symptom onset was reported to be a risk factor for person-to-person transmission of VTEC O157:H7 (Su and Brandt, 1995).

Tarr and Hickman (1987) found that the highest annual incidence of HUS was in children less than 3 years of age. A study conducted in Washington State in 1987 by Ostroff *et al.* (1989) on VTEC O157:H7 infections revealed that the highest age specific incidence rate was among children younger than 5 years (6.1 cases per 100,000) and lowest for adults 50-59 years (0.5 cases per 100,000). Variation of incidence rates by age may be related to either greater likelihood of children being brought to medical attention or frequent exposure to the agent (in case of adults) e.g. in swimming pool waters. The young and the elderly are more often affected by the serious complications of *E. coli* infections namely, HUS and TTP. Consequently, the young and the elderly have the highest morbidity and mortality rates from VTEC O157:H7 infection (Su and Brandt, 1995). The prolonged use of antimotility or antidiarrheal agents in humans has also been proposed as risk factor in HUS (Su and Brandt, 1995).

2.12.3 Other risk factors associated with VTEC O157 infections

Other risk factors associated with VTEC O157:H7 infections were consumption of ground beef in a non-commercial setting such as picnic or special events. Drinking of well water, swimming, handling animal faeces, close contact with a person with diarrhoea and failure to wash one's hands after handling raw ground infected beef (Armstrong *et al.*, 1996). Cutting of raw meat or chicken on the same cutting board without cleaning it and other food preparation behaviours increase the risks of food borne illnesses (Klontz *et al.*, 1995).

Exposure to the dairy farm environment may have great health significance for urban residents and specific subgroups within the rural community than those people who have direct contact with cattle or consume unpasteurized milk (Reymond *et al.* 1996). Reymond *et al.*, (1996) used assays to detect antibodies to VTEC O157 lipopolysaccharide (LPS), and Vero toxin 1 (VT 1) to determine and compare exposure of dairy farm residents in Southern Ontario and in urban residents of Toronto, Canada. It was found that the frequency of O157 LPS antibodies was significantly higher in dairy farm residents (12.5%) than in urban residents (4.7%). The difference between the groups was even greater for VT 1 neutralizing antibodies with detection in 42% of dairy farm residents and only 7.7% in urban residents. In addition, children with declining maternal immunity, the elderly and other immunocompromised individuals who live on dairy farms have increased risk of infection and VTEC associated disease (Wilson *et al.*, 1996; Reymond *et al.*, 1996).

2.13 Epidemiology and transmission of VTEC O157

2.13.1 Reservoirs of VTEC O157 in cattle

Cattle are considered major reservoirs of Shiga-toxin producing *E. coli*. The bacteria generally inhabit the intestines without causing illness in the animals (Park *et al.*, 1996). Both healthy and sick cattle are major reservoirs for VTEC strains and several outbreaks have been associated with consumption of meat and meat products (Olsvik, 1990a). VTEC O157 causes no disease in cattle and has been found in all age groups. Gut colonization is transient with a median shedding duration of less than 30 days (Hancock *et al.*, 1997).

Most VTEC O157 infected cattle remain free of disease and are tolerant to VTEC O157 because cattle lack Gb_3 receptors (Cray and Moon, 1995). When Ingrid and co-workers (2000) conducted studies to examine cattle tissues for the receptor Gb_3 for receptivity to SLT binding in vitro and for susceptibility to enterotoxin effects of SLT in vivo, Gb_3 was not detected in gastrointestinal tract tissues of cattle, and SLT did not bind to blood vessels in any gastrointestinal and five extra intestinal organs examined. It was concluded therefore that cattle tissues lack Gb_3 receptors as well as vascular receptors for SLT receptivity. However, VTEC O157 causes fatal ileocolitis in newborn calves because newborn calves were found to have Gb_3 receptors for SLT that are confined to the intestines (Obrig, 1998). SLT 1 and SLT 2 produced by VTEC O157 in calves inhibit protein synthesis, leading to necrosis

or/and apoptosis of receptor bearing, susceptible, micro vascular endothelial cells. Ingrid *et al.* (2000), found that those Gb_3 receptors disappear with age.

Species differences in the tissue and level of Gb_3 expression and SLT binding explain in part why cattle are tolerant reservoir hosts for VTEC O157 infection and remain asymptomatic reservoir hosts. Humans, rabbits and pigs have vascular receptors for SLT and as result they are highly susceptible to infection by VTEC O157 (Ingrid *et al.*, 2000).

2.13.2 VTEC O157 in cattle meat (beef)

Just before slaughter, tissues of healthy and physiologically normal animals having no direct communication with the environment are regarded as sterile (Jespen, 1957; Gracey, 1982). At the beginning of the slaughter process of cattle, the hide is removed and the surface of the carcass previously protected from the environment by the hide is now exposed to contamination. Unless there is a cut in the meat, the meat below the surface is normally sterile. The inner tissues of the carcass may sometimes become contaminated either by the sticking knife introducing bacteria into the blood stream or by the bacteria penetrating from the intestinal tract in case of delayed evisceration (Maeda, 1984). This kind of contamination is however minor as compared to the surface contamination of the carcass.

According to Lawrie (1974), the main sources of surface contamination of carcasses are the hides, the knives, the hands and clothing of personnel. Faecal matter from the alimentary tract, soil, washing water and any other contaminated object coming in contact with the carcass during processing greatly contribute to carcass contamination (Lawrie, 1974; Niskannen and Pohja, 1977; Ingram and Simonsen, 1980). If some meat happens to be contaminated with the rare *E. coli* strain O157:H7, it will be on the surface of the meat only and not down inside the fibres of the meat. But as soon as the meat is cut or punctured, bacterial cells will enter into the cut or puncture.

2.13.3 VTEC O157 in cattle faeces

A study conducted by Chapman *et al.* (1993) at slaughterhouse in South Yorkshire England, found that 4% of rectal faecal swabs were positive for VTEC O157. From cattle presented for slaughter in the United Kingdom 0.83% of 6,495 bovine faecal samples were positive for VTEC O157 (Richards *et al.*, 1998). In the above studies, it was found that of 23 animals with positive rectal swabs, 30% also tested positive for EHEC O157 on carcasses by sampling neck trimmings and swabbing an adjacent area. Chapman and colleagues (1993) also reported that 8% of adjacent carcasses also tested positive, suggesting that there was another source of carcass contamination. However, Elder and colleagues (2000) suggested that cross-contamination of carcasses may be occurring in meat processing plants, and finding VTEC O157 at these levels during processing is not completely unexpected. According to Elder *et al.* (2000) carcass contamination may also occur through direct

contact with personnel, knives or other equipment. Carcasses may be directly in contact with each other during processing with the potential to transfer microorganism. In addition, air and/or water-borne contamination is possible.

Armstrong *et al.* (1996) estimated the prevalence of VTEC O157 in North America and European cattle to be in the range from 0.1 to 10%. According to Heuvelink *et al.* (1998), the isolation rate is greatly influenced by factors such as the target population, the sampling strategy and the screening method used.

2.13.4 Other animal sources of VTEC O157 than cattle

Animals other than cattle and humans that have been observed to shed *E.coli* O157 include sheep, horses, deer dogs, and birds (Kudva *et al.* 1996; Rice and Hancock, 1995; Hancock *et al.*, 1998). Long-term carriers have not been reported in any species although cattle, sheep, and humans have been sampled with sufficient intensity to assess duration of carriage (Hancock, *et al.*, 1998).

Sheep have been documented to naturally shed VTEC O157:H7. Researchers in Britain surveyed 700 sheep at slaughter (100 a month for 7 months) and isolated VTEC O157:H7 from rectal swabs of 18 (2.6%) sheep (Chapman *et al.*, 1996). Other domestic and wild animals may also harbour VTEC O157:H7. Hancock *et al.* (1997) examined the occurrence of non-bovine sources of VTEC O157:H7 on the farm in the Pacific North-west. Samples that were culture positive for VTEC

O157:H7 included one horse, two dogs, one pooled bird sample and two pooled flytrap samples. All rodent samples were negative. VTEC O157:H7 has also been isolated from 2 (0.4%) of 538 deer samples that shared ranches with cattle in Oregon, South Dakota, Texas and Washington (Rice and Hancock, 1995).

2.13.5 VTEC O157 in foods

Shiga-toxin producing *E.coli* are ingested usually from contaminated food or objects. Food is an important source of VTEC O157 infections. In several outbreaks of HC due to VTEC O157 in the USA, Canada, UK, Netherlands and Japan, the causative organisms have been isolated from products as diverse as hamburger meat, unspasteurized apple juice and unpasteurized milk (Strobaugh, 1997; Chapman *et al.*, 1993; Heuvelink *et al.*, 1996 and Izumiya *et al.*, 1997). In an outbreak of infection in Lanarkshire Scotland in which a number of elderly people died, cooked meat from an award-winning butcher was implicated indicating that the bacterium was difficult to control (Greenwood *et al.*, 1997). VTEC O157:H7 has also been isolated from healthy heifers on the farms associated with the milk borne incidents further indicating that cattle are a major reservoir of VTEC O157:H7 (Tarr, 1995).

2.13.6 Food Sources other than beef and milk

In addition to the more common highly publicised outbreaks involving hamburger, there have been infections associated with consumption of contaminated chicken, apple cider, unpasturised milk and water (Griffin and Tauxe, 1991). Sometimes,

other foods may be contaminated when they come into contact with surfaces such as plates, cutting boards or utensils that had previously been in contact with raw meat.

Although the proportion of outbreaks linked to beef products were the ones frequently reported in the U.S., 12.5% of the outbreaks were linked to other sources than beef products (USDA/APHIS: VS, 1997). Some of these unexpected food vehicles of transmission are acidic foods, salad, vegetables, lettuce and venison. The acidic foods confirmed as sources of outbreaks include unpasteurized apple juice and apple cider, mayonnaise and yoghurt (Feng, 1995).

Among other known sources of infection are poultry meat, venison, water, sandwiches, lettuce, dry cured salami, produce from manure fertilized gardens, handled potatoes, radish sprouts, alfalfa sprouts and yoghurt (Buchanan and Doyle, 1997).

2.13.7 Prevalence of VTEC O157 with season

Studies conducted by Hancock *et al.* (1997) and Van Donkersgoed *et al.* (1999) on North American cattle demonstrated that the peak prevalence of VTEC O157 occurs in late summer and early fall. This is also the time when most human outbreaks occur in North America i.e. in July through August (Armstrong *et al.*, 1996). Studies conducted by Heuvelink *et al.* (1998) have confirmed that excretion rate of VTEC O157 in cattle is frequently intermittent and seasonal, with a peak in the summer months.

2.13.8 Prevalence of VTEC O157 with age

When Heuvelink *et al.*, (1998) determined the prevalence of VTEC O157 on 10 dairy farms in Netherlands, it was found that the proportion of cattle infected varied from 0.8% to 22.4% on the seven farms that were positive for VTEC O157. The excretion rate was highest in calves of ages 4 to 12 months (21.2%). The differences in excretion rates between age groups may be attributed to age-related differences in rumen function. Adult cattle have a fully developed rumen and there are higher concentrations of volatile fatty acids and lower pH than in calves and these two factors inhibit the growth of VTEC O157:H7 (Rasmussen *et al.*, 1993).

2.14. Mechanisms of transmission of VTEC 0157

2.14.1 Raw meat (beef)

For infection to occur, both contamination of raw meat and inadequate cooking are prerequisites (Johnson and Maloney, 1996). According to Zhao *et al.* (1995), mincing or grinding of raw meat for use in burgers is thought to transfer pathogens from the surface to the interior of the burger and inadequate cooking may allow the survival of pathogens.

2.14.2. Raw cow milk and milk products

Raw cow's milk has often been associated with human VTEC O157 infections (Borczyk *et al.*, 1987; Chapman *et al.*, 1993; Keene *et al.*, 1997). The organisms have been isolated from samples of raw milk both from individual cattle (Machie *et*

al., 1997) and from bulk tanks (Padhye and Doyle, 1991). Heuvelink *et al.* (1998) suggested that the organisms are not being excreted in the milk but contamination probably results from faecal contamination of milk as it is collected. Pasteurisation kills VTEC O157:H7 and is an effective way to prevent milk-borne transmission (Kirk *et al.*, 1997).

2.14.3 Manure

It was hypothesised that plant foods can become contaminated from fertilization with raw manure, irrigation with contaminated water or contamination by human contact (Patriquin *et al.*, 2000). However, when Hancock *et al.* (1997) examined 36 dairy herds in the Pacific Northwest to asses the effect of manure handling practices such as application of manure to cattle forage crops and cattle housing, it was found that application of manure to crops was not associated with the prevalence of VTEC O157. This was because composting of fresh manure before application greatly reduces VTEC O157.

2.14.4 Feed and Water

Although, VTEC O157:H7 has not been detected in commercially purchased cattle feed, it is however, able to survive in dry feeds and multiply upon addition of moisture (Hanckock, 1998). According to Shere *et al.* (1998), drinking water for livestock could be the main conduit for transmission of O157 from one animal to another and it appears that water can be contaminated by oral contact alone.

Hancock *et al.* (1998) reported that survival and multiplication of O157 in feed and water troughs are major factors affecting levels of VTEC O157 on the farm.

Faith *et al.* (1996) identified animal drinking water troughs as a source of VTEC O157:H7 in their study of dairy farms in Wisconsin. In the Pacific Northwest Hancock *et al.* (1997) sampled trough water and associated bio films and found VTEC O157:H7 in four trough water samples and six water trough bio films. Waterborne transmission has been reported both from contaminated drinking water (Swerdlow *et al*, 1992) and recreational waters (Keene *et al.*, 1994). An unchlorinated municipal water source and deficiencies in the water distribution system were implicated as the probable source of contamination (Swerdlow *et al.*, 1992; Rice *et al.*, 1992a).

2.14.5 Person to person transmission

Person-to-person contact is an important mode of transmission through the oral-faecal route. An asymptomatic carrier state has been reported where individuals show no clinical signs of disease but are capable of infecting others. The duration of excretion of VTEC is about one week in adults, but can be longer in children. Reported person to person spread has resulted in several outbreaks in hospitals, child-care centers, nursing homes and other institutions (Kohli *et al.*, 1993).

2.15. Diseases caused by VTEC O157:H7 infection in humans (Zoonoses)

2.15.1 General clinical signs of VTEC infections in human

Gastrointestinal tract infections with VTEC O157:H7 commonly begin with crampy, watery diarrhoea, after an incubation period of 3-4 days (Griffin Tauxe, 1991). The incubation period for VTEC O157:H7 is estimated to range from 12 hours to 9 days, with median of 48 hours. Infection may also progress to bloody diarrhoea with stools containing amounts of blood ranging from streaks to closely visible blood (Ostroff *et al.*, 1989). Half the patients vomit but only about one third develop fever. More severe manifestations of this illness include intussusceptions, rectal prolapsed, and haemolytic anaemia (Griffin and Tauxe, 1991).

Infection with verocytotoxin producing *E.coli* in particular with VTEC O157:H7 has been associated with serious and potentially life threatening diseases. The diseases are haemorrhagic colitis (HC), Haemolytic Uraemic Syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) which is a manifestation of HUS (Karmali *et al.*, 1983b; O'Brien and Holmes, 1987).

Investigations by Tarr (1995) on the clinical manifestations of VTEC O157 infections revealed that bloody diarrhoea that accompanies HC is the most widely recognized symptom although in the first few days of illness the diarrhoea is frequently non-bloody. In about 10% of cases the diarrhoea is non- bloody throughout the course of the illness. Gastrointestinal complications include cholecystitis, colonic perforation, appendicitis, pancreatitis and cholelithiasis (Tarr,

1995; Brandt *et al.*, 1998). The most commonly reported gastrointestinal complications include thrombotic microangiopathic anaemia (TMA) manifesting as HUS or TTP, which occurs in about 10% of cases (Tarr, 1995). Risk factors for the development of TMA include extremes of age and high leucocyte count at presentation. In children, the mortality rate in HUS is 3-5%. There is also a significant incidence of post HUS sequalae involving the kidneys, gastrointestinal tract and central nervous system. (Brandt *et al.*, 1998).).

According to Nataro and Kaper, (1998), TTP is one of the conditions that constitute HUS, it is not a separate disease caused by *E.coli* O157:H7. TTP means bleeding from tiny blood vessels in the skin and mucous membranes (Purpura) occurring with deficiency of blood platelets (thrombocytes). The vascular tissue damage associated with TTP resembles the more extensive form that is produced by HUS. Many agents or conditions have been implicated as causing TTP including VTEC O157:H7 infection (Su and Brandt, 1995).

TTP primarily affects the elderly and is sometimes thought to be part of the manifestations of HUS. There is a difference however in that neurological involvement, acute renal failure and fever are more prominent in TTP (Karmali *et al.*, 1983b). Acute bloody diarrhoea and abdominal cramps with little or no fever usually lasts one week.

2.15.2 Haemolytic uraemic syndrome (HUS)

HUS occurs mainly in young children and the elderly and is characterized by the onset of gastrointestinal bleeding, bloody urine, haemolytic anaemia, acute renal failure and thrombocytopaenia, typically about a week after the onset of diarrhoea. HUS is now considered to be the most common cause of acute renal failure in children in the UK and USA. It may be fatal in up to 10% of cases. According to O'Flanagan (1997) children less than 5 years and the elderly are more likely to have complicated illness.

HUS appears five to nine days after the onset of gastrointestinal tract symptoms but can occur as early as one to two days after diarrhoea begins. This syndrome is characterized by microangliopathic haemolytic anaemia (intravascular destruction of red blood cells) thrombocytopaenia (depressed blood platelets counts) and acute renal failure plus fever and neurological symptoms (Su and Brandt, 1995). Several different pathogens are suspected of causing HUS but VTEC O157:H7 is the most common pathogen isolated from patients with HUS (Neil *et al.*, 1987).

In USA, nearly all cases of post-diarrhoeal HUS are caused by infection with enterohaemorrhagic *E.coli* (EHEC) (Mahon *et al.*, 1997). In a study conducted by Siegler *et al.* (1994) in Utah it was found that 140 (89%) of 157 HUS cases were post diarrhoeal, while Griffin (1995) estimates that VTEC O157:H7 is likely to be responsible for 85-95% of HUS cases in the U.S. HUS occurs most commonly in children under the age of 10 but it has also been reported in adults (Neil *et al.*, 1987).

Persons with HUS have kidney failure and often require dialysis and transfusion. Some develop chronic kidney failure or neurological impairment such as seizures and stroke. It is estimated that about 61 cases of HUS occur annually in USA (Neil *et al.*, 1987).

2.15.3. Geographic distribution of HUS

Kinney *et al.* (1988) demonstrated that the incidence of HUS is not unique to a specific geographical region. Within the U.S, sporadic cases of HUS are more frequently reported from Northern than Southern States (Griffin, 1995). Outside N. America, VTEC O157:H7 has been isolated from humans in Argentina, Australia, China, Chile, Czechoslovakia, France, Germany, India, Ireland, Italy, Japan, South Africa and the United Kingdom (Griffin, 1995).

The largest reported outbreak that caused thousands of illnesses occurred in Japan thus illustrating the global distribution of VTEC O157:H7 (Izumiya, 1997). This outbreak and another one a year later, were associated with radish sprouts (Buchanan and Doyle, 1997).

Genetically indistinguishable strains of VTEC O157:H7 are commonly found in cattle herds hundreds or thousands of kilometres apart and sub typing of VTEC O157:H7 isolates suggests some regional transmission unrelated to cattle movement (Hancock, 1998).

2.15.4 Haemorrhagic colitis (HC)

HC or bleeding of the colon affects mainly adults and is marked by severe abdominal cramps followed by bloody diarrhoea, oedema, erosion or haemorrhages of the mucosal lining of the colon and the absence of conventional enteric organisms in the stool (Su and Brand, 1995). Symptoms of HC generally persist for several days to a few weeks and most of the hospitalised patients recover within one week without specific therapy (Su and Brandt, 1995). However, complications such as upper-gastrointestinal bleeding and stroke from HC associated with VTEC O157:H7 have been reported (Su and Brandt, 1995). HC may be the only manifestation of VTEC O157:H7 or it may precede the development of HUS.

2.15.5 Health outcomes associated with VTEC O157:H7 infections

An important characteristic of VTEC O157:H7 that contributes to its public health significance is its very small infective dose. An exceptionally low dose of VTEC O157:H7 averaging only 10 bacterial cells with a range of 2 to 2000 cells can cause infection as compared to ten thousand to millions of most *Salmonellae* (Chalasami and Medeiros, 2000; Epstein, 1997). In regards to VTEC O157:H7, the total number of bacteria required for infection appears to be about ca 100-200 organisms (Nataro and Kaper, 1998), which makes handling of contaminated materials particularly hazardous. Once introduced into a family or community, person to person transmission can occur especially through children who are not toilet trained.

2.15.6 Antibiotic treatment in VTEC O157 infections

Treatment for VTEC O157:H7 infection is primarily supportive including management of dehydration and complications such as anaemia and renal failure (Su and Brandt, 1995). Antiperistalsis agents should not be given. The strain is however sensitive to many antibiotics but treatment with antibiotics are not usually done because the symptoms and signs are self limiting. Symptoms usually resolve within two weeks (Su and Brandt, 1995 and Griffin, 1995).

CHAPTER THREE

0 MATERIALS AND METHODS

3.1 Study design

A cross-sectional study on the occurrence of EHEC 0157:H7 in beef was conducted in two slaughterhouses in the eastern zone of Tanzania.

3.2. Study area

The present study was carried out in two chosen slaughterhouse namely; the state owned Morogoro Municipal slaughterhouse, Morogoro municipality and the privately owned Ukonga-Mombasa slaughterhouse in Ilala municipality, Dar es Salaam region. Morogoro municipality is located in the mid-eastern part of Tanzania Mainland and lies between latitude $5^0$58' and 10' South of equator and between longitude $35^0$30' and $38^0$30' East Greenwich. Ilala municipality lies between longitude 39^0 E and $39^0$30' and between $06^0$40E and latitude $39^0$30 and 07^0 South of the Equator.

The two slaughterhouses were purposely selected because they receive cattle for slaughter from Shinyanga, Mwanza, Dodoma, Singida and Arusha regions (mostly Kiteto district) as well as from Usangu plains, in Mbeya region. In addition, cattle are also received from the different districts Morogoro region. The two slaughterhouses therefore, receive slaughter cattle from almost all over the country

due to high demand for cattle meat as well as due to easy accessibility by railway lines and good roads.

3.3. Slaughter cattle

According to 1994/95 livestock survey, Tanzania has 15.6 million cattle and over 60% of all the livestock are located in the regions mentioned in section 3.2 (Melewas, 1999). Cattle brought for slaughter to Morogoro and Dar-es-Salaam slaughterhouses are mostly indigenous Tanzania Shorthorn Zebus (TSZ). These animals are either trekked or loaded into railway wagons via the Tanzania Railway Lines and the Tanzania Zambia Railway (TAZARA) lines in case of Mbeya region.

In Tanzania, cattle are auctioned in livestock markets and then taken to holding grounds and often trekked for long distances to the slaughterhouses (personal communication with the meat inspectors of Morogoro and Ukonga-Mombasa slaughterhouses and personal observation).

3.4. General slaughterhouse conditions, meat hygiene and season

During this survey, the hygiene of meat and the slaughterhouses was studied. Although the Morogoro and Ukonga-Mombasa slaughterhouses are different in layout and design, they are generally similar in construction. These two slaughterhouses were designed and constructed as community concrete based

slaughter slabs under a roof supported by pillars. The hygiene of the slaughterhouses and meat were far from satisfactory.

The study area has a bimodal type of rainfall: the short rain season that begins in mid October and ends in December and then followed by a dry spell period from January to the beginning of March (Directorate of Meteorology, Morogoro station). The long rain season starts in early March and ends in mid June. It was therefore planned to obtain samples weekly in order to compare the occurrence of VTEC O157 in the different seasons of the year.

3.5. Sample size determination

A sample size to estimate the prevalence of verocytotoxigenic *E. coli* was calculated according to the formula of Martin et al. (1987).

The formula is: -

$n = Z^2 \times P \times Q/L^2$;

where:

n = required sample size

Z = value for a given confidence level

P = known or estimated prevalence of that population

Q = (1-P)=the proportion of negative

L = allowable error/the required precision i.e. the largest acceptable difference between the true and the estimated prevalence.

In this study, a confidence level was estimated at 95% with allowable error of estimation of 0.05. Since there was no known prevalence, P was estimated at 50% so as to give the maximum sample size. Therefore:

$n = 1.96^2 \times 0.5 \times 0.5 / 0.05^2 = 384.16$

The total number of beef swab samples to be taken was, therefore, three hundred and eighty four (384). Datas regarding age, sex, breed and geographical origin of each animal were not determined because no such records were kept at the slaughterhouses.

3.6. Swab samples and carcass sampling sites

A total of three hundred and eighty four (384) beef swab samples were collected randomly from different cattle carcasses slaughtered at the two slaughterhouses. Of these, two hundred beef swab samples were collected from Morogoro municipal slaughterhouse and one hundred and eighty four beef swab samples were collected from the privately owned Ukonga-Mombasa slaughterhouse in Ilala municipality, Dar-es- Salaam) between December 2000 and June 2001

Beef samples were taken at three different sites on the carcass (as recommended by the USDA Food Safety and Inspection Service (FSIS, 1996). The sites chosen were those immediately adjacent to the hide opening pattern marks as follows:

Brisket- anterior to the navel or ventral midline

Flank-posterior to the navel or the ventral midline and

Rump-the cushion of the round

In addition, beef swab samples were collected from any part of the carcass that seemed to be contaminated by faeces. It was not necessary to identify the carcasses sampled because all the carcasses were immediately taken away for sale after inspection was complete.

3.7. Sample collection and sampling procedure

An area of approximately 50 cm^2 was swabbed from each site. A sterile cotton wool swab moistened in normal saline was used to swab those sites on the beef carcass. The upper part of each swab was then suspended in a universal bottle containing 10 mls of transport medium (Stuarts, Oxoid Ltd, UK), sealed and then put into a cool box with ice. Each swab was completely covered by the transport medium. Between ten and twenty samples were collected per day (depending on the number of animals slaughtered) and then taken to the laboratory in the Department of Microbiology and Parasitology, Sokoine University of Agriculture, Morogoro.

3.8. Sample processing

Beef swab samples were processed on the same day for samples from Morogoro slaughterhouse or after one day in case of samples collected from Ukonga-Mombasa

slaughterhouse, Dar es Salaam. Beef swab samples that were not to be examined within two to three days after collection were immediately refrigerated at 4^0C. Each sample was given an identification number.

3.8.1. Pre-enrichment of beef swabs

In the laboratory, a sample swab from the transport medium was streaked on MacConkey agar and incubated overnight at 37^0C. Only those typical lactose fermenting pink colonies were picked. Each colony was then transferred into 10 ml of freshly prepared modified Tryptone Soya Broth (mTSB) (Oxoid Ltd., UK) as pre-enrichment media, and then incubated at 37^0C overnight (Dynal LTD, Oslo).

3.8.2. Preparation of CHROMagar ™ O157 plates

CHROMagar ™ O157 powder used in this study was supplied by the distributor Sotherncross Biotechnology (SA). The CHROMagar ™ O157 plates were prepared according to the manufacturer's recommendations (Appendix 3).

3.8.3. Dynabeads® anti-*E.coli* 0157

Aliquots of 20 µl of Dynabeads® anti-*E.coli* 0157:H7 were suspended into 1.5ml micro centrifuge tubes. A loopful of overnight pre-enriched broth culture was taken from each incubated sample and then transferred into the micro centrifuge tubes containing Dynabeads® anti-*E.coli* O157 ready for the IMS procedure. Each time,

six samples were picked because the Magnetic Particle Concentrator (MPC–S, Dynal, Oslo) could only handle six micro centrifuge tubes at a time (Appendix1).

3.8.4. Isolation of EHEC O157 by immunomagnetic separation (IMS) technique

First, the magnetic plate was removed from the Dynal MPC-S and six 1.5 ml micro centrifuge tubes were loaded into the Dynal MPC-S rack. Each pink coloured presumptive EHEC O157:H7 colony was picked from MacConkey agar plates and incubated overnight at 37^0C. One ml aliquot was then transferred to the micro centrifuge tube containing the immunomagnetic beads coated with Dynabeads *E.coli* O157:H7 antibody. The Dynal MPC-S rack was inverted a few times then incubated at room temperature for 30 min with continuous light agitation in a Dynal Sample Mixer instrument (Appendix 1). The magnetic plate was then inserted back into the Dynal MPC-S, inverted a few times and allowed to settle for 3 min. The micro centrifuge tube caps were carefully opened and the supernatant carefully aspirated using a clean pipette. The magnetic plate was removed, and 1 ml of wash buffer (PBS-Tween 20) was added to each tube. The above procedure was repeated twice. The bead-bacteria complexes were then re-suspended into 100µl of PBS-Tween 20 and whirl mixed using a vortex mixer, ready for culture on CHROMagar ™ 0157 plates. The bead bacteria complexes were spread over one half of the CHROMagar ™ 0157 plate with a sterile swab so as to ensure the break-up of the bead-bacteria complexes (Appendices 1 and 2).

The bead-bacteria complexes were further spread over one half of the agar plate by streaking with a sterile wire loop. The wire loop was always carried back into the

previously streaked quadrant several times to ensure that the beads are streaked into a fresh unstreaked quadrant. CHROMagar ™ 0157 plates were incubated at 37 ^{0}C for 24 hours. Only pink mauve coloured colonies, which are presumptive EHEC 0157:H7, were picked for further biochemical (Tables 4) and the latex agglutination test. Duplicate samples were stored at-70°C to await further studies such as biotyping and typing using DNA based methods.

3.9. Confirmation of EHEC O157 isolates

The presumptive EHEC O157:H7 isolates that showed the characteristic pink-mauve coloured colonies on CHROMagar ™ O157 culture media were characterized further using EHEC O157:H7 biochemical tests (Appendix 4) and the latex agglutination test (Appendix 5) respectively.

3.9.1. Biochemical patterns

Biochemical procedures used were those described by the National Laboratory for Enteric Pathogens, National Microbiology Laboratory, Winnipeg, Manitoba (1986). In this study the biochemical profiles were determined for the 40 presumptive EHEC 0157 isolates obtained from the IMS procedure and culture on CHROMagar ™ 0157 above. The biochemical tests were carried out using the following reagents; sorbitol, triple sugar iron agar (TSI), Simmon's citrate agar and urea. Cellibiose and ONPG reagents were not available and hence the two tests could not be carried out. A portion of each colony obtained from the CHROMagar ™ 0157 media plates was used for the biochemical tests (Appendix 4).

3.9.2. *E.coli* O157 latex test (DR620M)

Each of the presumptive EHEC O157:H7 colony picked from CHROMAgar ™ 0157 was subjected to the latex agglutination test (LAT) so as to confirm that the isolates were EHEC O157:H7. The detailed LAT test procedures have been narrated in Appendix 5).

In brief, the latex reagents were brought to room temperature and vigorously shaken. One drop of the test latex reagent was dispensed onto a circle on the reaction card and a loopful of normal saline was placed on the other edge of the circle without mixing. Using an inoculating wire loop, a small amount of colony from the plate was transferred and emulsified first in the saline and then in the latex reagent. The resulting suspensions were turbid or milky in appearance. The reaction card was rocked in a circular motion for 1min while observing for agglutination (granular-like agglutination). A rapid agglutination (within one minute) in the antiserum-saline mixture was evidence of *E.coli* serotype O157 (Oxoid, UK). If no agglutination occurred within one minute, it was taken as a negative result indicating absence of EHEC O157. A portion of the colony that gave agglutination with the test reagent was further tested with the control latex reagent so as to ensure that the isolate is not an auto-agglutinating strain. A control latex reagent was supplied together with the CLST kit (Appendix 5).

3.9.3 Prevalence rate of EHEC O157.

The prevalence rate of EHEC O157:H7 obtained in this study were calculated following the formula by Martin *et al.*, (1987). Verocytotoxigenic EHEC O157:H7 prevalence was calculated as follows:

P= number of samples with EHEC O157:H7 x 100

Total number of samples collected

3.9.4 Statistical analysis

The correlation analysis tool by Green and D'Oliveira (1982) was used to compare the percent isolation rate of EHEC O157:H7 and the rainfall pattern in the study area.

This analysis tool and its formulae measure the relationship between two data sets that are scaled to be independent of the unit of measurement. The population correlation calculation returns and the covariance of two data sets divided by the product of their standard deviations: The formula used was:

$$r = \frac{N\sum a \times b - \sum a \times \sum b}{\sqrt{\left[N\sum a^2 - \left(\sum a\right)^2\right]\left[N\sum b^2 - \left(\sum b\right)^2\right]}}$$

Where: r = Correlation coefficient

N = Number of Subjects

$\sum a \times b$ = Total of a x b

$\sum a$ and $\sum b$ = Total for each variable

$(\sum a)^2$ and $(\sum b)^2$ = Total for each condition squared

$\sum a^2$ and $\sum b^2$ = Sum of squared individual scores.

The correlation tool is used to determine whether two ranges of data move together, that is, whether large values of one set are associated with large values of the other (positive correlation), whether small values of one set are associated with large values of the other (negative correlation), or whether values in both sets are unrelated (correlation near zero). In this study, the correlation tool was used to determine whether there is an association between the percent isolation rate of VTEC O157 and the rainfall pattern.

CHAPTER FOUR

4.0 RESULTS

4.1. Slaughterhouse and Meat hygiene

In the two slaughterhouses visited during this study it was observed that there was a single slaughter man stationed at each slaughterhouse. Consequently, one slaughtering knife was used to kill all animals, which was done on one killing floor. Flaying and evisceration processes were mostly carried out on the same floor and carcasses were hoisted on rails only during splitting and meat inspection. Same knives were also used to split several carcasses and to cut the offals.

One weighing scale was being used for all the carcasses belonging to one meat trader. Hooks used for hoisting carcasses during splitting and meat inspection procedures were simply rinsed in plain water and then stored for use on the following day.

It was further observed that slaughterhouse attendants often carry carcasses on their backs while putting on simple cotton coats that were used repeatedly before being washed could be easily washed or sterilised.

4.2. Presumptive EHEC O157:H7 isolates obtained on CHROMagar ™ O157 after IMS technique and rainfall pattern

Altogether 384 beef swab samples were collected for this study. Out of 200 samples collected from Morogoro slaughterhouse, 25 presented presumptive EHEC 0157:H7

(12.5%). At Ukonga-Mombasa slaughterhouse in Dar-es-Salaam, out of 184 presented 21 presumptive EHEC O157 out of 184 beef samples collected (11.4%). In total, therefore, there were 46 (12%) out of 384 that yielded presumptive EHEC O157:H7 colonies in the two slaughterhouses of Morogoro and Ukonga-Mombasa (Dar-es-Salaam). There were also 258, "other coliforms", and 17 *Proteus* species that showed steel blue and colourless colonies respectively on the same agar plates (Table 4). There were more EHEC O157:H7 isolates obtained during the months of December, March, April and May (Figure 1). On the other hand, fewer EHEC O157:H7 isolates were obtained during the months of January, February and June (the dry period) (Table 4 and Figure 1 and 2).

Table 4: Presumptive EHEC O157:H7 isolates on CHROMagar TM O157 media from Morogoro and Ukonga-Mombasa slaughterhouse by month of isolation

Month	Samples/ month	Presumptive EHEC O157:H7 isolates	Other coliform isolates	*Proteus* spp isolates	Rainfall (cm)*	% EHEC O157:H7 colonies
Dec.00	32	4	21	1	20.7	12.50%
Jan. 01	33	3	17	3	10.4	9.1%
Feb.	33	2	22	2	9.9	6.1%
March	36	6	18	2	17.1	16.7%
April	36	9	24	2	22.4	25.0%
May	105	15	99	1	9	14.3%
June	109	7	57	6	0.4	6.4%
TOTAL	384	46	258	17	89.9	11.98%

*Source : Rainfall data from the Directorate of Meteorology, Morogoro Station, (2002).

Figure 1: Percentage isolation of presumptive EHEC O157:H7 per month (Morogoro and Ukonga-Mombasa slaughterhouse)

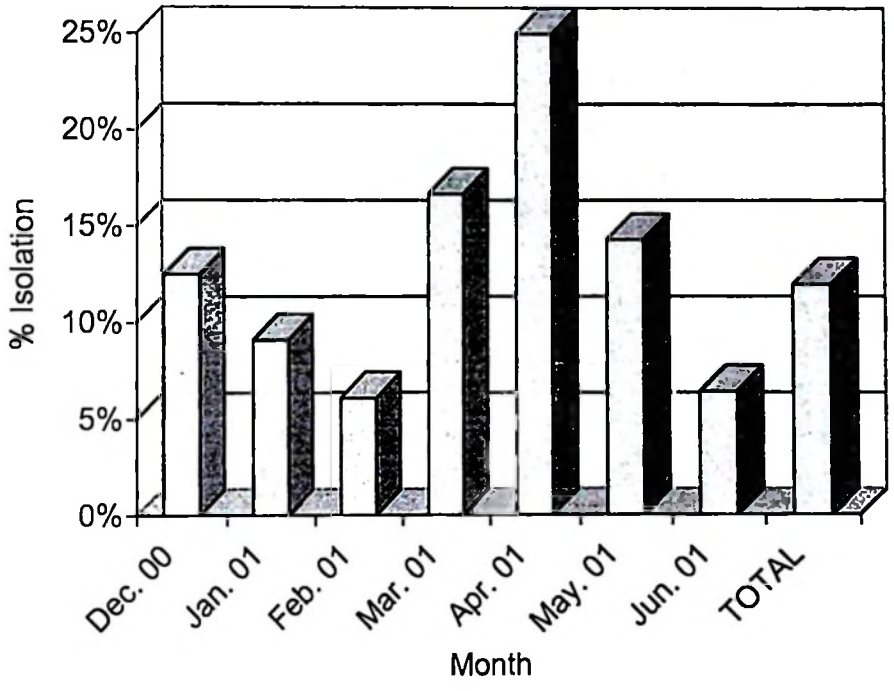
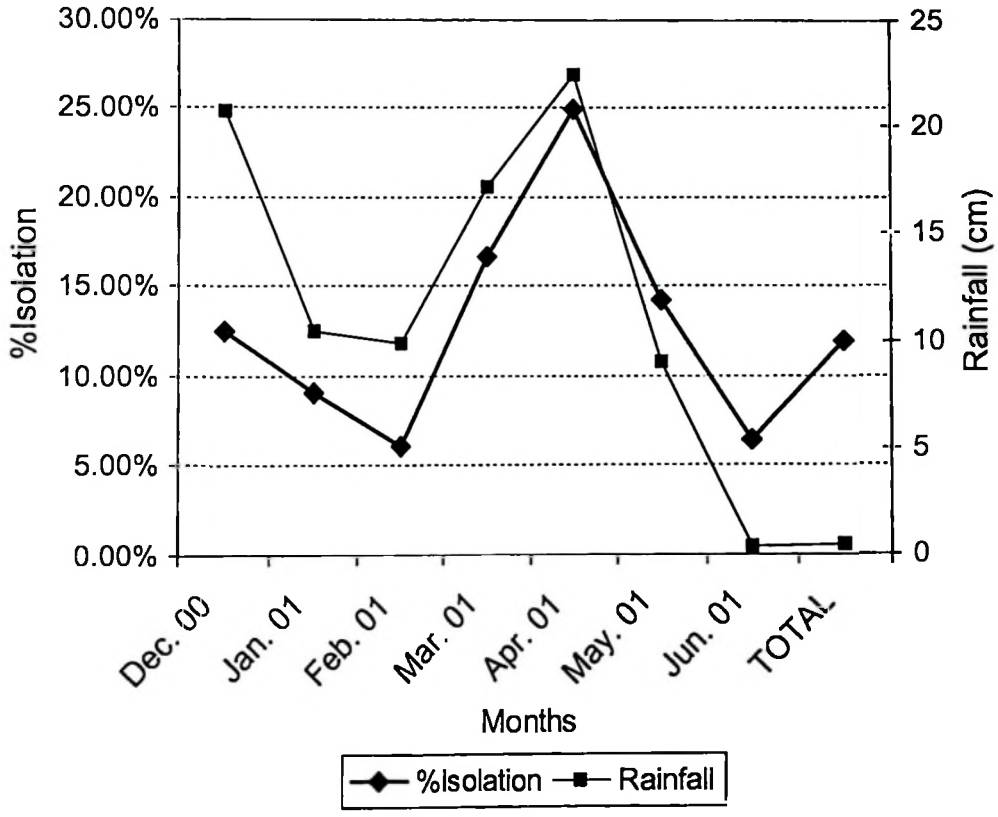


Figure 2: Percentage isolation of confirmed O157:H7 per month in relation to rainfall pattern (Morogoro and Ukonga-Mombasa slaughterhouse)



4.3. Confirmation of EHEC O157:H7 isolates

When the presumptive EHEC O157:H7 isolates (Table 4 above) were subjected to the biochemical tests and the latex agglutination test, 13 isolates from Morogoro Municipal slaughterhouse (Table 5) and 9 isolates from Ukonga-Mombasa slaughterhouse (Table 7) showed typical agglutination and biochemical patterns, characteristic of EHEC O157:H7. In total, therefore, 22 isolates out of 46 (5.72%) presumptive EHEC O157:H7 colonies from the two slaughterhouses were confirmed as EHEC O157:H7. The other 24 isolates did not show typical biochemical reactions or did not the agglutinate with the latex beads and were therefore considered to be non-negative (Tables 6 and 8).

Table 5: Morogoro Municipal slaughterhouse: Confirmed isolates showing typical biochemical patterns of EHEC O157:H7

Isolate Number	Sorbitol	Urea	Citrate	*T A/A	*S H_2S	*I Gas
2	-	-	-	A/A	-	+
3	-	-	-	A/A	-	+
4	-	-	-	A/A	-	+
5	-	-	-	A/A	-	+
7	-	-	-	A/A	-	+
10	-	-	-	A/A	-	+
13	-	-	-	A/A	-	+
14	-	-	-	A/A	-	+
15	-	-	-	A/A	-	+
17	-	-	-	A/A	-	+
20	-	-	-	A/A	-	+
21	-	-	-	A/A	-	+
22	-	-	-	A/A	-	+

*TSI= Triple Sugar Iron agar.

A/A=Acid/Acid

Acid/Alkaline

Table 6: Morogoro slaughterhouse: Confirmed isolates showing non-typical biochemical patterns of EHEC O157:H7

Isolate Number	Sorbitol	Urea	Citrate	T A/A	S H_2S	I Gas
1	-	+	+	A/Alk	-	+
6	+	-	-	A/Alk	-	-
8	+	-	-	A/Alk	-	-
9	+			A/Alk	-	
11	-	+	-	A/Alk	-	+
12	+	-	-	A/Alk	-	-
16	-	+	+	A/Alk	-	+
18	+	+	+	A/Alk	-	+
19	-	+	-	A/Alk	-	+
23	-	+	+	A/Alk	-	+
24	+	-	-	A/Alk	--	-
25	+	-	-	A/Alk	-	-

Table 7: Ukonga-Mombasa slaughterhouse: Isolates showing typical biochemical patterns of EHEC O157:H7

Isolate Number	Sorbitol	Urea	Citrate	T A/A	S H_2S	I Gas
1	-	-	-	A/A	-	+
3	-	-	-	A/A	-	+
4	-	-	-	A/A	-	+
5	-	-	-	A/A	-	+
7	-	-	-	A/A	-	+
11	-	-	-	A/A	-	+
13	-	-	-	A/A	-	+
14	-	-	-	A/A	-	+
15	-	-	-	A/A	-	+

Table 8: Ukonga-Mombasa slaughterhouse: Isolates showing non-typical biochemical patterns of EHEC O157:H7

Isolate Number	Sorbitol	Urea	Citrate	T A/A	S H_2S	I Gas
1	-	+	+	A/Alk	-	+
6	+	-	-	A/Alk	-	-
8	+	-	-	A/Alk	-	-
9	+	-	-	A/Alk	-	-
11	-	+	-	A/Alk	-	+
12	+	-	-	A/Alk	-	-
16	-	+	+	A/Alk	-	+
18	+	+	+	A/Alk	-	+
19	-	+	-	A/Alk	-	+
23	-	+	+	A/Alk	-	+
24	+	-	-	A/Alk	--	-
25	+	-	-	A/Alk	-	-

4.4. Prevalence rate of EHEC O157:H7

The overall prevalence rate of EHEC O157:H7 in cattle meat was calculated as follows:

$$P = \frac{\text{Number of samples with EHEC O157:H7} \times 100}{\text{Total number of samples collected}} =$$

$$P = \frac{22 \times 100}{384} = 5.72\%$$

The prevalence rate of EHEC O157:H7 in cattle meat in the two slaughterhouses of Tanzania during this study was 5.72%.

During this study, the percent isolation rates of EHEC O157:H7 were higher during the months of December (12.5%), March (16.7%) and May (14.3%) and were highest in April (25%) (Table 4).

4.5. Statistical analysis

Beef swab samples were collected during the months of December 2000 and between January and June 2001. There were more presumptive EHEC O157:H7 isolates obtained during the months of December, March, April and May than the months of January and February (Figure 1 and 2). On the other hand, there were fewer presumptive EHEC O157:H7 isolates during the months of January, February and June (the dry period) compared to the months of December, March, April and May

(Table 4). To determine the correlation between prevalence and rainfall pattern, the following equation was used:

$$r = \frac{n\sum a \times b - \sum a \times \sum b}{\sqrt{\left[n\sum a^2 - \left(\sum a\right)^2\right]\left[n\sum b^2 - \left(\sum b\right)^2\right]}}$$

Where: r = Correlation coefficient

n = number of subjects =2

a = 11.98 (isolation rate of *E. coli* O157:H7)

b = 0.5 (rainfall)

$\sum a \times b = \sum 11.98 \times 0.5$

$\sum a$ and $\sum b = \sum 11.98$ and $\sum 0.5$

$(\sum a)^2$ and $(\sum b)^2 = (\sum 11.98)^2 \times (\sum 0.5)^2$

$\sum a^2$ and $\sum b^2 = \sum 11.98^2$ and $\sum 0.5^2$

$\sqrt{[2 \sum 11.98^2 - (\sum 0.5)^2 2 \mid \sum 0.5^2 - (\sum 0.5)^2]}$

$\sum 11.98 \times 0.5 - \sum 11.98 \times \sum 0.5 = 0.758676$

From the calculations made above, the correlation coefficient (r) was found to be 0.76 and therefore $P \leq 0.05$, suggesting a strong positive correlation between the high percent of isolation of presumptive EHEC O157:H7 obtained and amount of rainfall.

CHAPTER FIVE

5.0 DISCUSSION, CONLUSION AND RECOMMENDATIONS

5.1 DISCUSSION

In this, a total, 22 isolates out of 384 beef swab samples collected from the two slaughterhouses were characterized as typical EHEC O157:H7 isolates (Tables 5 and 7). The overall prevalence of EHEC O157:H7 in the two slaughterhouses was therefore 5.72%. 24 other isolates that were suspected to be presumptive EHEC O157 on CHROMAgar ™ O157 media (Tables 6 and 8) did not show typical biochemical reactions or agglutination reaction with the latex agglutination test and were therefore considered to be negative. The results obtained from this study have indicated that cattle meat in Tanzania may be contaminated with the rare EHEC O157:H7 microorganisms.

Given the general poor hygiene of most of Tanzanian slaughterhouses, and the fact that these animals originated from various mainland districts, it can be assumed that EHEC O157:H7 is widespread in Tanzania.

Meat inspection procedures in the two slaughterhouses visited during this study, were based mostly on organoleptic methods; which rely on detecting changes in the sight, smell or texture of the tissues. According to the US National Academy of Sciences this procedure does not detect food borne microorganisms. Many contaminants could be present in numbers high enough to cause disease without

altering the sight, smell or feel of the carcass, or even without causing visible disease in living cattle, pigs or chickens.

The prevalence rate of EHEC O157:H7 in this study was found to be 5.7% and is nearly similar to the findings obtained by Doyle and Schoeni (1987) who tested US supermarket meats for the presence of these organisms. According to Doyle and Schoeni (1987), this bacterium was found in 3.7% of all beef samples analysed. Generally, the prevalence of EHEC O157:H7 is estimated to be 5% in cattle at the time of slaughter in UK (Chapman *et al.*, 1997). Other published research on EHEC O157:H7 in cattle has reported prevalence rates of 1.8% (Hancock *et al.*, 1997), 3.4 % (Rice *et al.*, 1997), and 7.5 % (Van Donkersqoed *et al.*, 1999).

From what has been cited above therefore, our findings have shown either a similar prevalence rate e.g. Chapman *et al.*, (1997), (5%) or a higher EHEC O157:H7 prevalence rate than what has been reported by some researchers e.g. Hancock *et al.* (1997) (1.8%). Findings in this study have also a lower prevalence rate than what was reported by other researchers e.g. Armstrong *et al.* (1996) reported a prevalence rate of 10 % in North America and European cattle. There are several reasons for the differences between the findings in our study and the findings by other researchers elsewhere. Some of the reasons for those differences are:

Lower prevalence rate of EHEC O157:H7 could be expected in Tanzania beef due to the fact that:

Cattle brought for slaughter in slaughterhouses were mostly indigenous Tanzania Shorthorn Zebus (TSZ) that came from pastoral areas. Cattle management systems in those areas involve grazing on natural grass throughout the year (Melewas, 1999). There is no supplementary feeding of any sort. According to Van Donkersgoed *et al.* (1999), Diez-Gonzalez *et al.* (1998) and Russell *et al.* (1998), feeding of hay to cattle and even greener pastures such as alfalfa, have lesser risks of cattle shedding EHEC O157:H7. The carbohydrates of hay are not easily fermented and hay does not promote the growth of acid resistant *E. coli* such as EHEC O157:H7157 (Russell *et al.*, 1998).

It is nevertheless noted that the prevalence reported here was higher than that of 1.8% and 3.4 % reported by Hancock *et al.* (1997) and Rice *et al.* (1997) respectively. There was an increased isolation rate of EHEC O157:H7 during the rainy season probably because cattle brought for slaughter are usually kept in crowded wet dirty pens where they could pick EHEC O157:H7 shed in faeces and transmit to others through the faeces. Because the animals are not washed or sprayed with water before they enter the killing floor, chances are that EHEC O157:H7 is carried in the hooves and skin (Hancock *et al.*, 1997, and Rice *et al.*, 1997).

Some cattle traders trek the animals for long distances to the slaughterhouses in Morogoro and Dar-es- Salaam slaughterhouses, and even when those animals are ferried into railway wagons or trucks, they receive only limited of hay and water. It follows that by the time they arrive at the slaughterhouses, the animals will be very

much stressed. According to Russell *et al.* (1998), dietary stress and feed type may alter *E. coli* shedding in cattle through their effects on the ruminal environment. These factors alter the concentration of volatile fatty acids and pH in the rumen, which in turn influence bacterial growth (Kudva *et al.*, 1995; Armstrong *et al.*, 1996). Cray *et al.* (1995) found that four calves fasted for 48 hours prior to oral inoculation with EHEC O157:H7 shed the organism for a significantly longer period compared to four non-fasted controls.

Furthermore, application of microbial control systems such as carcass washing with organic acids or thermal processing are usually not carried out. This could account for the relatively higher prevalence observed in this study compared to those slaughterhouses in developed countries. Elder *et al.* (2000) observed a reduction in carcass EHEC O157:H7 prevalence following washing from pre-evisceration to post processing. The study by Elder *et al.* (2000) has shown that the number of positive carcass samples was reduced by about 50% after carcasses were eviscerated and split and dropped significantly when carcass samples were taken after final washing strategies were executed.

This study has also shown a strong correlation between the isolation of presumptive EHEC O157:H7 and the rainfall pattern (Figure 1). Morogoro and Dar-es Salaam regions lie on the coastal hinterlands and experience a bimodal type of rainfall (Directorate of Meteorology, Morogoro Station, 2000). The short rain season begins in mid October and ends in late December and the long rain season starts in March

and ends in early June. During this study, it was observed that the isolation rate of EHEC O157:H7 was higher during the rainy season as compared to the dry season. There were more presumptive EHEC O157:H7 colonies isolated during the rainy months of December, March, April and May as compared to the dry spell period months of January, February and June (Figure 2). These findings are similar to those by Heuvelink *et al.* (1998) on Dutch Dairy farms have confirmed that excretion rate of EHEC 0157:H7 in cattle is frequently intermittent and seasonal, with a peak in the summer months. In this study there was a positive correlation ($P \leq 0.76$) between the percentage isolation rate of presumptive EHEC O157:H7 and the rainfall pattern in both slaughterhouses. Other studies conducted by Hancock *et al.* (1997) and Van Donkersgoed *et al.* (1999) on North American cattle have shown that peak prevalence occurs in the late summer and early fall. This is also the time in which most human outbreaks occur in North America i.e. in July through August (Armstrong *et al.* 1996). The findings in this study therefore agree with those findings by Heuvelink *et al.* (1998), Hancock *et al.* (1997) and Van Donkersgoed *et al.* (1999) suggesting a seasonal influence on the isolation rate of EHEC O157. There was increased isolation rate of presumptive EHEC O157:H7 in Tanzania during the rainy months of December (12.5%), March (16.7%), April (25%) and May (14.3%) (Table 4 and Figures 1 and 2). Studies conducted by Hancock *et al.*, (1997) and Van Donkersgoed *et al.*, (1999) on North American cattle meat have also reported a peak excretion rate of EHEC O157:H7 to be higher in summer months (section 2.13.7).

After the IMS technique, the bead-bacterial complexes were then cultured on CHROMagar ™ O157 media. In this study, only pink mauve coloured colonies were picked from CHROMAgar ™ O157 and were subjected to IMS. All SLT-producing strains of VTEC O157 (H7 and H-) give characteristic pink to purple colonies, which was the case here. According to the findings by Bettleheim (1998a), virtually all SLT-producing strains of *E. coli* O157:H7 (both H7 and H-) give characteristic pink to purple (mauve) colonies on CHROMagar ™ O157 and that is what was observed here. After plating on CHROMagar™ O157, the 46 isolates obtained were subjected to the Commercial Latex Agglutination Test (Oxoid DR 620) and the Biochemical tests so as to confirm that the isolates were EHEC O157:H7. Because of possibilities of serological cross-reactions between the O157 antigen and other *E. coli* serotypes as well as other members of the *Enterobacteriaceae*, the 46 EHEC O157:H7 isolates were again subjected to standard EHEC O157:H7 biochemical tests. According to Rice *et al.* (1992); Park *et al.* (1998a) and Bettleheim *et al.* 1998a), biochemical confirmation of the isolates is mandatory. In total, 22 isolates showed typical biochemical patterns.

EHEC O157:H7 strains do not ferment sorbitol while the majority of *E.coli* serotypes do ferment sorbitol. According to Bettleheim *et al.* (1998a), non-sorbitol fermenting colonies have to be tested with the Commercial Latex Slide Test (CLST) (Oxoid Ltd, UK. Product No. DR620M) in order to find out whether the isolate belong to the O157 serogroup and therefore a potential VT-producing strain. When the 46 presumptive EHEC O157:H7 isolates picked on CHROMagar™ O157 were

subjected to the CLST, only twenty-two isolates (22/46) showed typical agglutination reactions and these were the same ones that showed typical biochemical patterns. The two methods therefore confirmed the 22 to be EHEC O157:H7.

Confirmation of EHEC O157:H7 also requires identification of the H7 flagella antigen. Testing for the H7 antigen as well as for the production of SLTs that are associated with pathogenic strains are available through reference laboratories because EHEC O157:H7 isolates often require multiple passages before the flagella antigen is detected (CDC, 1994). Isolates that are non-motile or that are negative for the H7 antigen should be tested for the production of the SLT to identify pathogenic strains. According to CDC (1994), toxin testing of EHEC O157:H7 strains that have the H7 antigen is sometimes not necessary because virtually all these strains produce the SLTs. Some strains of EHEC O157:H7 have other H types and do not produce SLT, but that is rare and such strains are not recognized pathogens (CDC, 1994). Testing for H7 was however not done in this study because of lack of reagents.

The commercial latex slide test (CLST) offers a rapid and economical alternative to tube agglutination test for the identification of EHEC O157:H7 (Chapman *et al.*, 1989). Previous studies conducted by Sowers *et al.* (1996) to evaluate the performance of the commercial latex reagents for identification of O157 and H antigens of *E.coli* had also shown that CLSTs were good alternatives to standard serological methods or the biochemical tests. The CLST will detect by agglutination

E.coli strains possessing the O157-serogroup antigen. There is no doubt, therefore, that in this study EHEC O157:H7 was isolated.

The 24 presumptive EHEC O157:H7 obtained during isolation on CHROMagar ™ O157 that did not show typical biochemical patterns and agglutination reaction on CLST could have been picked because of their close similarity to EHEC O157:H7. For example, the colonial appearance of VTEC serotype 0111 can easily be mistaken with EHEC O157 on this media (Rice *et al.*, 1992b). Cross-reactions between the O157 antigen and other *E. coli* serotypes as well as other members of the *Enterobacteriaceae* could have also accounted for the false positives (Rice *et al.*, 1992b).

EHEC O157:H7 organisms have not been reported in Tanzania perhaps because the dangers associated with these microorganisms are unknown to some medical practitioners. Furthermore, there are no diagnostic facilities for EHEC O157:H7 in many laboratories. There is also a possibility that the cooking habits in most Tanzanian's families destroy EHEC O157:H7. Most foods are usually thoroughly boiled or fried before being eaten. As suggested by US-FDA, (1992), cooking meat to the temperatures exceeding 70 ^{0}C efficiently destroys EHEC O157:H7. However, there is still a danger of acquiring EHEC O157:H7 infections if the washing plates, and the cooking utensils are not washed properly or not washed with boiled water; which is the case in many Tanzania's situation. The food could also become

contaminated if the kitchen environment is not kept clean because domestic flies have been implicated to carry VTEC 0157 (Hancock *et al.* (1997).

At the Morogoro Regional hospital for example, routine testing for EHEC O157:H7 in patients and especially children with cases of diarrhoea are usually not carried out (Dr. Massi, personal communication) because there were no facilities for carrying out such tests.

In this study, SLT 1 and SLT 2 were not determined, however, it is important to know which SLT genes are present in the foods because these toxins are known to play a major role in human disease. Further studies are therefore needed to confirm that the EHEC O157:H7 isolates obtained are pathogenic or non-pathogenic strains by testing for SLT1 and SLT2 genes.

5.2 CONCLUSION AND RECOMMENDATIONS

Findings from this study have shown that beef in Tanzania may be contaminated with EHEC O157:H7, suggesting the risk of EHEC O157:H7 infection and possibilities of acquiring outbreaks of diseases such as HUS and HC by consumers of infected meat. The risks of contamination with EHEC O157:H7 are high and hence important that the authorities concerned in Tanzania take heed and address this potential health problem. Several conclusions can be drawn from data obtained in this study.

In this study it was observed that there was a positive correlation between the isolation of presumptive EHEC O157:H7 and the amount of rainfall. Since the isolation rate of presumptive EHEC O157:H7 was higher during the rainy season as compared to the dry season, consumers need to be educated on this danger. Health authorities in any case handle all situations of diarrhoea carefully having in mind that these could be caused by this pathogen.

Since the overall prevalence of EHEC O157:H7 in the two slaughterhouses was found to be 5.72%, it gives sufficient reasons for concern that cattle meat in Tanzania may be contaminated with EHEC O157:H7.

No cases of EHEC O157:H7 have been reported in Tanzania, and to the author's knowledge this is the first report on the occurrence of EHEC O157:H7. There is therefore every reason to carry out more research on this potential zoonosis in Tanzania.

From these results recommendations can be made with the aim of reducing the risk of food borne illness by intervening at stages of processing that pose a risk of carcass contamination, thus:

Health professionals should become familiar with EHEC O157:H7 and the illness it can cause, and should test for the organism in all persons with acute bloody diarrhoea. The potentially serious consequences of EHEC O157:H7 infections in the hospitals have not been addressed before; and therefore, the results reported here should form the basis for instituting concerted efforts to control this and other zoonotic diseases.

The Veterinarians, meat inspectors and the public health personnel who are responsible for inspecting carcasses and production of wholesome meat for public consumption, should take stern measures to ensure that carcass contamination by faeces and ruminal contents is minimal and that dirty carcasses are washed with portable water.

Since there was a strong correlation between the isolation rate of presumptive EHEC O157:H7 and the rainfall season, it is recommended that cattle brought for slaughter during the rainy months should not be housed in dirty, open muddy pens. Furthermore, skin and hooves must be washed with clean water before entering the killing floor.

Diagnostic facilities such as IMS kit and special media e.g. CHROMagar ^{TM}O157 for the detection of EHEC O157:H7 should be provided in the slaughterhouses so as to screen carcasses contaminated with this pathogen. A trained laboratory technician

may assist the meat inspectors in screening the carcasses before being taken to the butcheries.

There are currently no effective control measures of reducing carcass contamination with EHEC O157:H7 such as cattle washing before slaughter (personal observation). There is a need to develop and reinforce such control methods in the slaughterhouses.

Slaughtering, flaying and inspection knives as well as hooks must be sterilised after work. Other utensils and cutting boards that have come into contact with raw meat should be washed using hot soapy water.

It is important to caution the Tanzanian public of dangers of consuming meat not thoroughly cooked. Consumers need to take more responsibility. Preparation and sanitation methods are key to prevent food borne illness in the homes as in other areas of food handling (Collins, 1997).

REFERENCES

Abramov, V. (1996). *Escherichia coli* O157:H7 Fact Sheet No. 125. *Health communications and Public Relations*; Food Safety Unit; World Health organization (WHO) 1211 Geneva 27, Switzerland.

Acheson, D.W.K. (1996). *Escherichia coli* O157:H7 and other Shiga-toxin producing bacteria "Are these important pathogens?" *Division of Geographic Medicine and Infectious Disease*, New England Medical Center, Boston, Ma.

Acres, S.D. (1985). Enterotoxigenic *Escherichia coli* infections in newborn calves: A Review. *Journal of Dairy Science* 68:229-256.

Armstrong, G.L., Hollingsworth, J. and Morris, J.G. (1996). Emerging food borne pathogen: *Escherichia coli* O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. *Epidemiological Review.***18:** 29-51.

Armstrong, J.L., Shigeno, D.D., Calomiris, J.J. and Seidler, R.J. (1981). Antibiotic-resistant bacteria in drinking water. *Applied Environmental Microbiology.* **2:**277-283.

Bacon, R.T., Belk, K.E., Sofos, J.N. and Smith, G.C. (1998). Incidences of *Escherichia coli* O157:H7 on hide, carcass, and beef trimmings samples collected from United States Packing Plants. *Center for Red Meat Safety, Department of animal Sciences, Colorado State University, Fort Collins, Co.* pp 523-1171.

Bean, N.H. and Griffin, P.M. (1990). Food borne disease outbreaks in the United States between 1973 and 1987. Pathogens, Vehicles, and Trends. *Journal of Food Protection* **53**(9): 804-817.

Benjamin, M. M. and A. R. Datta. (1995). Prevalence of enterohemorrhagic *Escherichia coli*. *Applied Environmental Microbiology* **61**:1669-1672.

Bettelheim, K.A. (1995). Identification of Enterohaemorrhagic *Escherichia coli* by means of their production of enterohaemolysin. *Journal of Applied Bacteriology* **79**: 176-180.

Bettelheim, K.A. (1998a). Reliability of CHROMagar™ O157 for the detection of enterohaemorrhagic *Escherichia coli* (EHEC) O157 but not EHEC belonging to other serogroups. *Journal of Applied Microbiology* **85**:425-428.

Bettelheim, K.A. (1998b). Studies of *Escherichia coli* cultured on Rainbow™ Agar O157 with particular reference to Enterohaemorrhagic *Escherichia coli* (EHEC). *Microbiology and Immunolology* **42**:265-269.

Bettelheim, K.A. and Thompson, C.J. (1987). New methods of serotyping *Escherichia coli*: implementation and verification. *Journal of Clinical Microbiology* **25**: 781-786.

Blanco, M., Blanco, J.E., Gonzalez, E.A., Mora, A., Prado, C., Fernandez, L., Rio, M., Ramos, J., Alonso, M. P. (1996). Prevalence and characteristics of *Escherichia coli* serotype O157:H7 and other producing *Escherichia coli* in Healthy Dairy Cattle. *Epidemiology and Infection* **117**: 251-257.

Boerlin, P., McEwen, S. A., Boerlin-Petzold, F., Wilson, J. B., Johnson, R.P. and Gyles, C. L. (1999). Association between virulence factors of Shiga toxin-toxin-producing *Escherichia coli* and disease in humans. *Journal of Clinical Microbiology* **37**: 497-503

Borczyk, A.A., Karmali, M.A. and Lior, H. (1987). Bovine reservoir for Vero toxin producing *Escherichia coli* O157:H7. *Lancet* **1**: 98.

Brandt, J.R., Joseph, M.W., Fouser, L.S., Tarr, P.I., Zelikovic, I. and McDonald, R.A. (1998). Cholelithiasis following *Escherichia coli* O157:H7-associated Haemolytic Uraemic Syndrome. *Paediatric Nephrology* **12**: 222-225.

Brunder, W., Schmidt, H. and Karch, H. (1997). EspP, a novel extra cellular serine protease of enterohaemorrhagic *Escherichia coli* O157 cleaves human coagulation factor V. *Molecular Microbiology* **24**:767-778.

Buchanan, R.L. and Doyle, M.P. (1997). Food borne disease significance of *Escherichia coli* O157:H7 and other enterohaemorrhagic *Escherichia coli. Food Technology* **51**: 69-75.

Buchko, S.J., Holley, R.A., Buchko, S. J,. Olson, W. O., Gannon, V.P.J. and Veira. D.M. (2000). The Effect of different grain diets on faecal shedding of *Escherichia coli* O157:H7 by Steers. *Journal of Food Protection* **63**(11): 1467-1474.

Buxton, A. and Frazer, G. (1977). Escherichia. *Animal Microbiology Vol. 1* Immunology, Bacteriology, Mycology, Diseases of Fish and Laboratory Methods. Blackwell Scientific Publications, Edinburgh pp. 92-102.

Calderwood, S. B., Acheson, D. W. K,. Keusch, G. T,. Barrett, T. J. and Griffin, P. M. (199). Proposed new nomenclature for SLT (VT) family. *ASM News* **62**:118-119.

Cameron, S., Walker, C., Beers, M., Rose, N. and Anear, E. (1995). Community outbreak of Haemolytic Uraemic Syndrome attributed to *Escherichia coli* O111:NM. *Morbidity and Mortality Weekly Report* **44**: 550-551, 557-558.

Caprioli A., Luzzi I., Rosmini F., Resti C., Edefonti A., Perfumo F. Farina, C., Goglioo, A., Gianviti, A. and Rizzoni, G. (1994). Community-wide outbreak of Haemolytic Uraemia Syndrome associated with non-0157 producing-producing *Escherichia coli. Journal of Infectious Diseases* **169**:208-11.

Carbonetti, N.H. and Williams, P.H. (1984). A cluster of five genes specifying the aerobactin iron-uptake system of plasmid Col V–K30. *Infection and Immunity.* **46**: 7-12.

Centre for Disease Control and Prevention (CDC) (1994). *Escherichia coli* O157:H7. *Procedure for Isolation and Identification from stool specimens.* Food borne and Diarrhoeal Disease Branch, Division of Bacterial and Mycotic Diseases, National Centre for Infections Diseases, Centre for Disease Control and Prevention, MS C-09 1600 Clifton Road, Atlanta, Ga 30333. 158pp.

Chalasani, S. and Medeiros, L. (2000). What you should know about *Escherichia coli* O157:H7. Ohio State University Fact Sheet HYG-5561-98 Posted at http:/Ohioline. Ag, Ohio-state. Edu/hyg-fact/5000/5561. Html site visited on 9/10/2001.

Chapman, P.A., Siddons, C.A., Cerdan Malo, A.T. and Harkin, M.A. (1997). A One-year study of *Escherichia coli* O157 in Cattle, Sheep, Pigs and Poultry. *Epidemiology and Infection* **119**: 245-250.

Chapman, P.A., Siddons, C.A., Harkin, M.A. (1996). Sheep as a potential source of verocytotoxin-producing *Escherichia coli* 0157. *Veterinary Record* (letter to Editor).

Chapman, P.A., Siddons, C.A., Wright, D.J., (1992). Cattle as a source of verotoxigenic *Escherichia coli* O157. *Veterinary Record* 131: 323-324.

Chapman, P.A., Siddons, C.A., Wright, D.J., Norman P. (1989). Verotoxin-producing *Escherichia coli* infection in Sheffield: cattle as a possible source. *Epidemiology and Infection* 102: 439-445.

Chapman, P.A., Siddons, C.A., Wright, D.J., Norman P., Fox J., and Crick, E. (1993). Cattle as a possible source of Verocytotoxin-producing *Escherichia coli* O157 infections in man. *NEWSFLASH. Ontario,* Canada. 123pp.

Chapman, P.A., Siddons, C.A., Zaddik P.M., and Jewes, L. (1991). An improved selective medium for the isolation of *Escherichia coli* O157. *Journal of Medical Microbiology* **35** (2): 107-110.

Collins, J. E. (1997). Importance of changing Consumer lifestyles on the Emergence/Reemergency of foodborne pathogens. *Emerging infections Diseases* 3(4): n.pag. On line. Site visited on 9/10/2001.

Collwell, R. (1997). Presentation at "Protecting the public against foodborne-pathogens: *Escherichia coli*". September 25-26, Washington, DC.

Conner, D.E. and Kotrola, J.S. (1995). Growth and survival of *Escherichia coli* 0157:H7 under acidic conditions. *Applied Environmental Microbiology* **35**: 382-385.

Cray, W.C. and Moon, H.W. (1995). Experimental infection of calves and adult cattle with *Escherichia coli* O157:H7. *Applied Environmental Microbiology* 61: 1586-1590.

Cray, W.C., Casey, T.A., Bosworth, B.T. and Rasmussen, M.A. (1995). Effects of dietary stress on infection of calves by *Escherichia coli* O157:H7. Epidemiology. *Conference of Research workers in Animal Disease*: Chicago, IL.111pp.

Deneke, C.F., McGowan, K. and Gorbach, S.L. (1984). Attachment of human and pig (K88) Entgerotoxigenic *Escherichia coli* strains to either human or porcine small intestinal cells. *Infection and Immunity* **45**(2): 522-524.

Deng, M. and Fratmico, P. A. (1996). Multiplex PCR for rapid identification of Shiga-like toxin-producing *Escherichia coli* O157:H7 isolated from foods. *Journal of Food Protection* **59**:570-576.

Diez-Gonzalez, F., Todd R., Callaway, M. G., Kizoulis, J. B. and Russell, J. (1998). Grain feeding and the dissemination of acid-resistance *Escherichia coli* from cattle. *Elsevier Science* **5383**(281): 1666-1668.

Donahue-Rolfe, A., Jacewicz M. and Keusch G.T. (1989). Isolation and characterization of functional Shiga toxin subunits and denatured halotoxin. *Molecular Microbiology***3**:1231-1236.

Doyle, M P. and Schoeni, J.L. (1987). Isolation of *Escherichia coli* O157:H7 from retail fresh meats and poultry. *Applied and Environmental Microbiology* **53**(10):2394-2396.

Doyle, M. P. Schoeni, J. L. (1984). Survival and growth characteristics of *Escherichia coli* associated with haemorrhagic colitis. *Applied Environmental Microbiology* **48:** (4): 855-856.

Doyle, M. P., Zhao, T., Meng, J., and Zhao, S. (Schoeni, J.L. (1997). *Escherichia coli* O157:H7. In. Doyle, M. P., Beuchat, L. R., and Montville, T. J. (Eds). Food Microbiology: fundamentals and frontiers. Washington *American Society for Microbiology* pp.171-191.

Doyle, M.P. and Padhye, V.V. (1989). *Escherichia coli* O157. In: food borne Bacterial Pathogen, (Edited by Doyle, M.P.). Marcel Dekker, New York. pp. 235-281.

Duffy, G. (1999). Methods for Verocytotoxigenic *Escherichia coli* (F-FE 344/99). Teagasc, *The National Food Centre; Dunsinea, Castle knock,* Dublin 15, Ireland.115pp.

Elder, R.O., Keen, J.E., Siragusa, G.R., Barkocy-Gallagher, G.A., Koohmaraie, M. and Laegreid, W.W. (2000). *Proceedings of the National Academy of Science* **97**: 2959-2961.

Elwell, L.P. and Shipley, P.L. (1980). Plasmid mediated factors associated with virulence of bacteria to animals. *Annual Review Microbiology* **34:** 465-496.

Endo, Y, Tsurugi, K., Yutsudo, T., Takeda, Y., Ogasawara, K. and Igarashi, K. (1988). Site of the action of a verotoxin (VT2) from *Escherichia coli* O157:H7 and a Shiga toxin on eukaryotic ribosome. *European Journal Biochemistry* **171**:45-50.

Epstein, E. (1997). The Science of Composting. Technomic Publishing Co., Lancaster-Basel. 258pp.

Evans, D. G. and Evans, D.J. Jr. (1978). New surface-associated heat-labile colonization factor antigen (CFA/II) produced by enterotoxigenic *Escherichia coli* of serogroup O6 and O8. *Infection and Immunity* **21**:638-648.

Evans, D.G. Jr. and Evans, D.G. (1990). Colonization factor antigens of human pathogens. *Current Topics Microbiology and Immunology* **151**: 129-131.

Evans, D.G., Evans, J. and Pierce, N.F. (1973): Differences in the response of rabbit small intestine to heat labile and heat stable enterotoxins of *Escherichia coli. Infection and Immunity* **7**: 873-880.

Faith, N, G., Shere, J. A., Brosch, R., Arnold, K. W., S. E., Ansay, Lee, M. S., Luchansky, J. B. and Kaspar C. W. (1996). Prevalence and clonal nature of *Escherichia coli* O157:H7 on dairy farms in Wisconsin. *Applied and Environmental Microbiology* **62**:1519-1525.

Feng, P. (1995). *Escherichia coli* serotype O157:H7: novel vehicles of infection and emergence of phenotypic variants. *Emerging Infectious Diseases* **1**: 1-9.

Feng, P., Lampel, K., Karch, H. and Whittam, T. (1998). Genotypic and phenotypic changes in the emergence of *Escherichia coli* O157:H7. *Journal of Infectious Disease* **177(6):** 1250-1753.

Fey, P. D., Wickert, R. S., Rupp, M. E., Safranek, T. J. and Henrichs, S. H. (2000). Prevalence of non-O157:H7 Shiga toxin-producing *Escherichia coli* in diarrhoeal stool samples from Nebraska. *Emerging Infectious Diseases* **5(6):** 529-532.

Food and Drug Administration (US-FDA) (1992). Preventing foodborne illness: *Escherichia coli O157:H7 Bad bug book.* Washington, DC.21pp.

Food Safety and Inspection Service (FSIS) (1997). USDA's advice on consumers to use a thermometer when cooking hamburgers. [n.pag. online. Internet. 26 Feb.1997] site visited on 11.07.2001.

Food Safety and Inspection Service (FSIS). (1996). Pathogen Reduction; Hazard Analysis and Critical Control Point (HAACP) Systems. US Department of Agriculture. Washington, DC.120pp.

Fratamico, P. M., Strobaugh, J., Terence, P. (1997). Evaluation of an Enzyme-Linked Immunosorbent Assay (ELISA). *United States Department of Agriculture, Agriculture Research Service,* USA.

Fratamico, P.M., Strobaugh, J.R., Terence, P., Medina, M.B. and Gehring, A.G. (1996). Real-time Detection of *Escherichia coli* O157:H7 using a surface plasmon resonance biosensor. *Eastern Regional Research,* 600 East Mermaid Lane, Wyndmoor, P. A.15pp.

Frost, A.J. and Hill, A.W. (1982). Pathogenesis of experimental bovine mastitis following a small inoculum of *Escherichia coli. Research in Veterinary Science* **33**: 105-12.

Fukushima, H. and Comyoda, M. (1999). An effective, rapid and simple method for isolation of Shiga toxin-producing *Escherichia coli* O26, and O157 from faces and food samples. *Zentbl. Bakteriology* **289**:415-428.

Fukushima, H., Hoshina, K. and Comyoda, M. (2000). Selective isolation of *eae*-positive strains of shiga toxin –producing *Escherichia coli. Journal Clinical Microbiology* **38(4):** 1684-1687.

Gaastra, W. and De Graaf, F. (1982). Host-specific fimbrial adhesins of non-invasive enterotoxigeinic *Escherichia coli* strains *Microbiological Reviews.* **46**: 129-61.

Gibbons, R.A., Sellwood, R., Burrows, M. and Hunter, P.A. (1977). Inheritance to resistance neonatal *E.coli* diarrhoea in pig: Examination of the genetic system. *Theoretical Applied Genetics* **51**: 65-67.

Gilhuus Moe Afseth, C.C. and Olsvik, O. (1989). The use of immunomagnetic separation for selective enrichment of bacteria. Advanced technology for the clinical laboratory and biotechnology (ATB). *Abstracts of the 5th European Edition of the Oak Ridge Conference,* Milan, Italy.5pp.

Gracey, J.F. (1982). Chemical residues in meat, Chapter 10 and Meat microbiology and food poisoning, Chapter 11. In: Thornton's Meat Hygiene, 7th Ed. Bailliére Tindall, and London. pp. 158-198.

Green, J. and D'Oliveira (1982). *Learning to use Statistical Tests in Psychology.* Open University Press, Milton Keynes-Philadephia. pp.143.

Greenwood, D., Slack, R. and Peutherer, J. (Eds.) (1997). *Escherichia coli. Medical Microbiology.* Churchill Livingstone, UK. pp. 290-294.

Griffin, P.M. (1995). In: *Infections of the Gastrointestinal Tract*, eds, Blaser, M.J., smith, P.D., Ravdin, J.I., Greenberg, H.B. and Guerant, R.L.(Raven, New, York). pp.739-761.

Griffin, P.M. and Tauxe, R.V. (1991). The Epidemiology of infections caused by *Escherichia coli* O157:H7, other Enterohaemorrhagic *Escherichia coli* and associated Haemolytic Uraemic syndrome. *Epidemiological Review* **13**: 60-98.

Gross, R.J. (1991). The pathogenesis of *Escherichia coli* diarrhoea. *Reviews in Medical Microbiology* **2**: 37-44.

Guineé, P.A.M. and Van Leeuwen, W.J. (1976). Phage-typing of *Salmonella typhimurium* in the Netherlands. *Methods in Microbiology.* 11:157-191.

Hadad, J.J. and Gyles, C.L. (1982). Scanning and transmission electron microscope study of small intestine of colostrums-fed calves infected with selected strains of *Escherichia coli. American Journal of Veterinary Research* **43**:41-49.

Hancock, D. D., Besser, T. E., Gill, C. and Bohach, C. H. (1999). Cattle, hay and *Escherichia coli Science.* **284:** 561-562.

Hancock, D. D., Besser, T.E., Rice, D.H., Ebel, E.D., Herriot, D.E. and Carpenter, L.V. (1998). Multiple sources of *Escherichia coli* O57:H7 in feeds and dairy

farms in the North Western USA. *Preventive Veterinary Medicine* **16**(1): 11-19.

Hancock, D.D., Rice, D.H., Herriott, E.E., Besser, T. E., Ebel, E.D. and Carpenter, L. V. (1997). Effects of farm manure-handling practices on *Escherichia coli* O157 prevalence in cattle. *Journal of Food Protection* **60**:363-366.

Harnett, N.M. and Gyles, C.L. (1983). Enterotoxigenicity of bovine and porcine *Escherichia coli* of O groups 8, 9, 20, 64, 101 and X46. *American Journal of Veterinary Research* **44**:1210-2114.

Heuvelink, A. E., De-Boer, E. and Edel, W. (1996). *Escherichia coli* O157:H7. *Tijdschrift-voor-Diergenees kunde* **121**(11): 321-325.

Heuvelink, A. E., van den Biggelaar, F. L., de Boer, E., Herbes, R. G., Melchers, W. J., Huis, J. H. and Monnens, L. A. (1998). *Escherichia coli* O157:H7. *Journal Clinical Microbiology* **36**: 878-882.

Heuvelink, A.E., Kar, N.C., Meis, J.F.G. M., Monnens, L.A.H. and Melchers, W.J.G. (1995). Characterization of Verocytotoxin-producing *Escherichia coli* O157 isolates from patients with Human Uraemic Syndrome in Western Europe. *Epidemiology and Infection* **115**:1-4.

Heuvelink, A.E., Zwartkruis-Nahuis, J.T.M. and de Boer, E. (1997). Evaluation of media and test kits for the detection and isolation of *Escherichia coli* O157 from minced beef. *Journal Food Protetion* **60**: 817-824.

Hill, W.E. and Carlisle, C.L. (1981). Loss of plasmids during enrichment for *Escherichia coli*. *Applied Environmental Microbiology* **41**: 1046-1048.

Hummel, P.H. (1974): Isolation of *Salmonella* from cattle at Dar-es-Salaam. *Bulletin of Epizootic Diseases of Africa* **22**:109-119.

Ingram, M. and Simonsen, B. (1980). Meat and Meat products. In. Microbial Ecology of Foods, Vol. 2. *Food Commodities by the International Commission on Microbiological Specification for Foods.* Academic Press. pp. 333-407.

Ingrid, M.B., Morgan, T.W., Ackermann, M.R., Nystrom, E.D., Samuel, J.E., Cornick, N.A., and Moon, H.W.(2000). Cattle lack vascular receptors for *Escherichia coli* O157:H7 Shiga-toxin. *Proceedings of the National Academy of Sciences* 97(19): 10325-10329.

Izumiya, H., Terajima J., Wada A., Inagayaki Y., Itoh, K., Tamura K. and Watanabe, H. (1997). Molecular typing of enterohamorragic *Escherichia coli* O157:H7 isolated in Japan by using pulsed-field gel electrophoresis. *Journal of Clinical Microbiology* **35**:1675-1680.

Jacewicz, M., Clausen, H., Nudelman, E., Donahue-Rolfe, A. and Keusch, G.T. (1986). Pathogenesis of Shigella diarrhoea XI. Isolation of a shigella toxin-binding glycolipid from rapid jejunum and HeLa cells and its identification as globotriasylceramide. *Journal of Experimental Medicine* **163**: 1391-1404.

Jespen, Å. (1957). Application of bacteriological and biochemical tests in the hygiene judgement of meat and meat products. In: Meat Hygiene by FAO and WHO, Rome. FAO Agricultural studies No. 34, WHO Monograph Series No. 33.23pp.

Jiwa, S.F.H and Musses J. (1987). Enterotoxin, Cytotoxin and Haemaglutinin production by *Escherichia coli* isolated from diarrheic goats. *Proceedings of the 5th Tanzania Veterinary Association Scientific Conference,* Vol.5, Tanzania Veterinary Association, Tanzania. pp.218-219.

Jiwa, S.F.H., Kanuya, N. L. and Senyagwa, A .A., (1986). Enterotoxigenic colibacillosis as a constraint to livestock production. *Proceedings of the 4TH Tanzania Veterinary Association Scientific Conference,* Vol.4, Veterinary Association pp.37-139.

Johansson, L., Underdal, B., Grosland, K., Whelehans, O.P. and Roberts, T.A. (1983). A survey of hygienic quality of beef and pork carcasses in Norway. *Acta. Veterinary Scandinavica* **24**:1-13.

Johnson, J.R. (1991). Virulence factors in *Escherichia coli*. Urinary tract infection. *Clinical Microbiology* **2** : 37-44.

Johnson, RP., Durham, RJ., Johnson, ST., MacDonald, L.A., Jeffrey, S.R. and Butman, B.T. (1995). Detection of *Escherichia coli* O157:H7 in meat by an Enzyme-Linked Immunosorbent Assay, EHEC-Tek. *Applied Environmental Microbiology* **61**: 386-388.

Johnson, S.A., and Maloney, M.D. (1996): The emergency of verotoxin producing *Escherichia coli* O157:H7: Implications for health and research. *National Conference on Undergraduate Research*, Spelman College, NC.

Kanda, T., Sasa, Y., Kimura, H. and Nishina, T. (1997). Other VTEC non-O157 serotypes. *Journal of Japan Veterinary Medical Association* **50**(11): 663-668.

Karmali, M. A. (1989). Infection by verocytotoxin-producing *Escherichia coli. Clinical Microbiology Reviews* **2:**15-38.

Karmali, M. A., Steele, B.T., Petric M., Lim, C. (1983a). Sporadic cases of haemolytic- uraemic syndrome associated with faecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools. *Lancet* **I:**619-620.

Karmali, M.A., Petric, M., Lim, C, Arus, G.S., and Lior, H. (1985). The association between idiopathic Haemolytic Uraemic Syndrome and infection by producing-producing *Escherichia coli. Journal Infectious Disease* **151:**775-782.

Karmali, M.A., Steele, B.T., Petric, M., Lim, C. (1983b). Sporadic cases of Haemolytic Uraemic Syndrome associated with faecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools. Lancet 1: 619-620.

Kasai, K., Galton, J., Terasaki, P.L.(1985). Tissue distribution of the PK antigen as determined by a monoclonal antibody. *Journal Immunogenetic Separation* **12**: 213-220.

Kayihura, M. (1982). Salmonellosis reservoirs in animals as sources of human infection. Msc Thessis, University of Nairobi, Kenya.168pp.

Keene, W.E., McAnulty, J. M., Hoesly, F.C. (1994). A swimming-associated outbreak of hemorrhagic colitis caused by *Escherichia coli* 0157:H7 and *Shigella sonnei. New England Journal of Medicine* **331:**579-584.

Keene, W.E., Sazie, E., Kok, J., Rice, D.H., Hancock, D.D., Balan, V.K., Zhao, T., Doyle, MP. (1997). An outbreak of *E.coli* O157:H7 infections traced to jerky

made from deer meat. *Journal of the American Medical Association* **277**(15): 1229-1231.

Kehl, S.K., Havens, P., Behnke, C.E. and Acheson, D.W.K. (1997). Evaluation of the Premier EHEC assay for the detection of Shiga toxin-producing *Escherichia coli*. *Journal of clinical Microbiology* **35**: 2051-2054.

Kinney, J.T., Gross, C., Porter, C., Rogers, M., Schonberger, L. and Hurwitz, E. (1988). Haemolytic Uraemic Syndrome: a population based study in *American Journal of Public Health* **78**: 64-65.

Kirk, J.H., Price, S. and Wright, J.C. (1997). *Escherichia coli* O157:H7 in milk. *Large Animal Practice* **18**:16-19.

Klontz, K.C., Timbo, B., Fein, S. and Levy, A. (1995). Prevalence of selected food consumption and preparation behaviours associated with increased risks of food-borne disease. *Journal of Food Protection* **58**: 927-930.

Kohli, HS., Chanduri, A.K.R., Todd, W.T.A., Mitchell, A.A.B. and Liddeli, K.G. (1993). The Hartwoodhill Hospital *Escherichia coli* O157 outbreak. *Communicable Diseases and Environmental Health in Scotland Weekly Report* **27**(93/13): 8-11.

Konowalchuk, J., Dickie, N., Stavric, S. and Speirs, I. (1978): Comparative studies of five heat-labile toxic products of *Escherichia coli. Infection and Immunity* **22**: 644-648.

Konowalchuk, J., Speirs, J.L. and Stavric, S. (1997). Vero response to a cytotoxin of *Escherichia coli. Infection and Immunity* **18**:775-779.

Kudoh, Y., Obata, H., Kusonoki, J., Monma, C., Shingaki, M., Yangawa, Y., Yamada, S., Matsuhita, S., Itoh, T. and Otah, K. (1994). Epidemiological surveys on producing-producing *Escherichia coli* infections in Japan. In: *Recent Advances in Verocytotoxin-producing Escherichia coli Infections*, (Ed). Karmali, M.A. and Goglio, A.G. Amsterdam,: Elsevier Science, B.V. pp.53-56.

Kudva, I. T., Blanch, K. and Hovde, C. J. (1998). Analysis of *Escherichia coli* *Applied and Environmental Microbiology* **64**:3166-3174.

Kudva, I. T., Hunt, C. W., Williams, C. J., U. Nance, M. and Hovde, C. J. (1997). Evaluation of dietary influences on *Escherichia coli* 0157:H7 shedding by sheep. *Applied and Environmental Microbiology* **63**:3878-3886

Kudva, I.T., Hartield, P.G., Hovde, C. J. (1995). Effect of diet on the shedding of *Escherichia coli* 0157:H7 in a sheep model. *Applied Environmental Microbiology* **61**:1363-1370.

Kudva, I.T., Hatfield, P.G. and Hovde, C.J. (1996). *Escherichia coli* O157:H7 in microbial flora of sheep. *Journal of Clinical Microbiology* **63**: 431-433.

Kunkel, S.L. and Robertson, D.C. (1979). Factors affecting release of heat labile enterotoxin by enterotoxigenic *Escherichia coli.* *Infection and Immunity* **23**:652-659.

Larsen, J.L. (1976). Differences between enteropathogenic *Escherichia coli* strains isolated from Neonatal calf diarrhoea (NCD) and post weaning diarrhoea (PWD) in pigs. *Nordic Veterinary Medicine Journal* **28**: 417-429.

Lawrie, R.A. (1974). The spoilage of meat infecting organisms. Sources and nature of external contamination. In: Meat Science 2nd Ed. Pergemon Press Ltd., New York. pp 157-189.

Le Jeune, J., Hancock, D.D. and Besser, T.E. (1997). *Escherichia coli* O157:H7 in cattle water troughs: A possible on-farm reservoir. Abstracts of the 5th Annual Food Safety Farm to Table conference, Northwest Food Safety Consortium, Moscow, ID.123pp.

Levine, M.M. (1978). *Escherichia coli* strains that cause diarrhoea but do not produce heat- labile or heat–stable enterotoxins and are non-invasive. *Lancet* **64**:1 119-1122.

Levine, M.M. (1987): *Escherichia coli* strains that cause diarrhoea: enterotoxigenic, enteropatogenic enteroinvasive enterohaemorrhagic and enteroadherent. *Journal of Infectious Diseases* **155**: 377-389.

Linberg, A.A., Brown, J.E., Stromberg, N., Westling-Ryd, S. J.E. and Karlson, K. A. (1987). Identification of the carbohydrate receptor for Shiga toxin produced by *Shigella dysenteriae* type 1. *Journal of Biology and Chemistry* **262**:1779-1785.

Lingwood, C. (1994). Verotoxin-binding in human renal sections. *Nephron* **66**:21-28.

Lingwood, CA., Law, H., Richardson, S. (1987). Glycolipid binding of purified and recombinant *Escherichia coli* produced verotoxin in vitro. *Journal of Biology Chemistry* **262:** 8834 - 8839.

Lior, H, and Borczyk, A.A: (1987). False positive identifications of *Escherichia coli* O157. *Lancet* **I**:333-335.

MacDonald, K.W., O'Leary, M.J., Cohen, M.L., Norris, P., Wells, J.G., Noll, E., Kobayashi, J.M. and Blake, P.A. (1998). *Escherichia coli* O157:H7; an emerging gastrointestinal pathogen: Results of a one-year, prospective, population-based study. *Journal of the American Medical Association* **25** (24): 3567-3570.

Machie, S. C., Chapman, P. A. and Siddons, C. A. (1997). A fifteen month study of *Escherichia coli* O157:H7 in a dairy herd *Epidemiology and Infection* **118:**17-25.

Mackenzie, A.M.R., Lebel, P., Orrhine, E., Rowe, P.C., Hdyde, L. and Chan, F. (1998). Sensitivities and specificities of premier *Escherichia coli* O157 and Premier EHEC Enzyme immunoassays for diagnosis of infection with verotoxin (Shiga-like toxin)-producing *Escherichia coli. Journal of Clinical Microbiology* **36:**1608-1611.

Maeda, G.L.M. (1984). Bacteriological assessment of surface contamination of cattle carcasses in some slaughterhouses in Tanzania. MVM Dissertation, Sokoine University of Agriculture, Tanzania. pp. 4-9.

Mahon, B.E., Griffin, P.M., Mead, P.S. and Tauxe. (1997). Haemolytic uraemic syndrome. Surveillance to monitor trends in infection with *Escherichia coli* O157:H7 and other Shiga toxin-producing *Escherichia coli. Emerging Infectious Diseases* Letter 3(3): 409-412.

March, S.B. and Ratnam S. (1986). Sorbitol Mac Conkey medium for detection of *Escherichia coli* O157:H7 associated with Haemorrhagic Colitis. *Journal of Clinical Microbiology* **23:** 869-872.

Marques, L.RM., Moore, M.A., Wells, J.G., Wachmuth, I.K. and O'Brien, A.D. (1986). Production of Shiga-like toxin by *Escherichia coli. Journal of Infectious Diseases* **154**: 338-341.

Martin, S.W., Meek, A. H. and Willeberg, P. (1987). *Veterinary epidemiology: Principles and Methods.* Iowa State University Press, Ames pp. 23

Melewas, J.N. (1999). The history, spread and extent of CBPP and CCPP in Tanzania. The National Perspectives. *Proceedings on the CBPP/CCPP workshop* pp 4-5 held at the TANESCO Training Institute, Morogoro, Tanzania.

Miller, B.G., Newby, T.J., Stokes, C.R., Hampson, D.J., Brown, P.J. and Bourne, F.J. (1984). The importance of dietary antigen in the cause of post weaning diarrhoea in pigs. *American Journal of Veterinary Research* **45**: 1730-33.

Miller, L.G, Kaspar, CW. (1994). *Escherichia coli* O157:H7 acid tolerance and survival in apple cider, *Journal of Food Protection* **57**:460-464.

Minga, U. M. (1984): Animal Salmonellae in Tanzania. Cultural and Epidemiological studies on the occurrence of Salmonella in cattle and chicken. *PhD Thesis submitted to RVAU,* pp 4-28. Copenhagen, Denmark.

Minga, U.M. and Kikopa, R., (1983). Bacteria and Fungi of Veterinary Importance isolated in Tanzania between 1965-1982. *Tanzania Veterinary Bulletin* **5** (1): 1-4.

Moon, H. W. (1974). Pathogenesis of enteric diseases caused by *Escherichia coli. Veterinary Science Compedium of Medicine* **18:** 179-211.

Moon, H.W., Isaacson, R.E. and Pohlenz, J. (1979). Mechanisms of association of enteropathogenic *Escherichia coli* with intestinal epithelium. *American Journal of Clinical Nutrition* **32**:119-27.

Moore, K., Damrow, T., Abbott, D.O. and Jankowski, S. (1995). Outbreak of acute gastroenteritis attributable to *Escherichia coli* serotype O157:H21 Helena, Montana, *Morbidity and Mortality Weekly Report* **44:**501-503.

Nataro, J.P. and Kaper, J. B. (1998). Diarrhoeagenic *Escherichia coli Clinical Microbiology Review.* 11(1): 142-201.). [http:emr.asm.0rg.content. vol.11 issue] site visited on 23/8/2001.

Ndzinga, I.F., Mary Hall, M.L.M. and Mares, R.G. (1982). Isolation of Salmonella from meat and meat products in Botswana. *Tropical Animal Health and Production* **14:**89-90.

Neill, M.A., Tarr, P.I. and Clausen, C.R. (1987). *Escherichia coli* O157:H7 as the predominant pathogen associated with haemolytic uraemic syndrome: a prospective study in the pacific Northwest. *Paediatrics* **80:** 37-40.

Newburg, D.S., Chaturvedi, P., Lopez, E. (1993). Susceptibility to hemolytic-uraemic syndrome relates to erythrocyte glycosphingolipid patterns. *Journal of Infectious Disease* 168: 476-479.

Niskanen, A. and Pohja, M.S. (1977). Comparative studies on sampling and investigation of microbial contamination of surfaces by contact plate and swab methods. *Journal of Applied Bacteriology* **42**: 53-63.

O'Brien, A.D. and Holmes, R.K. (1987): Shiga and Shiga-like toxins. *Microbiological Review* **51**: 206-220.

O'Brien, A.D., Gentry, M.K., Thompson, M.R., Doctor, B.P., Gemski, P. and Formal, S.B. (1979): Shigellosis and *Escherichia coli* diarrhoea: relative importance of invasive and toxigenic mechanisms. *American Journal of Clinical Nutrition* **32**: 299-233.

O'Flanagan, D. (1997): Fatalities from *Escherichia coli* O157: Eastern Healthy Board, Dr. Stephen's Hospital, Dublin 8.4pp.

Obrig, T.G. (1998). In: *Escherchia coli* O157:H7 and other Shiga- Toxin-producing *E.coli* strains (eds). Kaper, J. B and O'Brien, A.D. *American Society of Microbiology,* Washington, DC pp. 303-311.

Olsvik, O. and Skjerve. E. (1989). Immunomagnetic enrichment of bacteria: a method for obtaining pure cultures. *Abstracts of the International Conference on Antimicrobiol Agents and Chemotherapy,* Houston, Texas.2pp.

Olsvik, O., Hornes, E., Wasteson, Y. and Lund, A. (1990a). Detection of virulence determinants in enteric *Escherichia coli* using nucleic acid probes and polymerase chain reaction. In. Wadstrom, T., Svennerholm, A. M., Wolf-Watz, H. and Makela, P. H. (Eds). Molecular pathogenesis of Gastrointestinal infections. *Academic Press,* New York, NY.10pp.

Olsvik, O., Wasteson, Y., Lund, A. and Hornes, E. (1991). Pathogenic found in Food. *International Journal of Food Microbiology* **12**:103-114.

Ørskov, E. and Ørskov, I. (1979). Special *Escherichia coli* serotypes from enteropathies in domestic animals and man. *Advances in Veterinary Medicine* **29**: 7-14.

Ørskov, I. and Ørskov, E. (1977). Serology, chemistry and genetics of O and K antigens of *Escherichia coli. Bacteriological Review* **41: 3**:667-710.

Ostroff, S.M., Kobayashi, J.M. and Lewis, J.H. (1989). Infections with *Escherichia coli* 0157:H7 in Washington State wide disease surveillance. *Journal American Medical Association* **262**:355-359.

Padhye, N.V. and Doyle, M.P. (1991). Rapid procedure for detecting enterohaemorrhagic *Escherichia coli* O157 in food. *Applied and Environmental Microbiology* **57: 2693-2698.**

Padhye, N.V. and Doyle, M.P. (1992). *Escherichia coli* O157:H7: epidemiology, pathogenesis and methods for detection in food. *Journal of Food Protection* **55**: 555-65.

Park, C.H., Martin, E.A., White, E.M. (1998). Isolation of a non-pathogenic strain of *Citrobacter sedlakii* which expresses *Escherichia coli* O157 antigen, *Journal of Clinical Microbiology* **36**:1408-1409.

Park, C.K., Gates, K.M., Vandl, N.M. and Hixon, D.L. (1996): Isolation of Shiga-like toxin producing *Escherichia coli* (O157 and non-O157) in a community hospital. *Diagnosis and Microbiology of Infectious Diseases* **26**: 69-72.

Patrquin, D.G. (2000). Reducing Risks from *Escherichia coli* O157: on the Organic Farm. *Eco-Farm and Garden Magazine of Canadian Organic Growers,* Winter Issue. Biology Department, Dalhousie University Halifax, NS, Canada.10pp.

Perera, J. l., Marques, L.R.M. and O'Brien, A.D. (1988). Isolation and characterization of monoclonal antibodies to Shiga-like toxin II of enterohaemorrhagic *Escherichia coli* and use of the monoclonal antibodies in colony Enzyme-Linked Immunosorbent Assay. *Journal of Clinical Microbiology* **26**: 2127-2131.

Pohl, P. (1991). Les *Escherichia coli* verotoxigenes isolates des bovines. *Annual Medicine Veterinaire.* **135**:569-576.

Pradel, N., Liverelli, V., Champs, C., Palcoux , J., Reynaud, A., Scheytz F., Sirot, J., Joly, B. and Forestier, C. (2000). Prevalence and characterization of Shiga toxin-producing *Escherichia coli* isolated from cattle, food, and children during a one-year prospective study in France. *Journal of Clinical Microbiology* **38**:1023-10311.

Raghubeer, E.V. and Matches, J.R. (1990). Temperature range for growth of *Escherichia coli* serotype O157:H7 and selected coliforms in *E. coli* medium. *Journal of Clinical Microbiology* **28**(4): 355-359.

Ramotar, K., Boyd, B.,Tyrell, G., Gariepy, J.,Lingwood, C.A. and Brunton, J. (1990). Characterization of Shiga-like toxin1 and VT1-B subunit purified from overproducing clones of the SLT1 cistron. *Biochemistry Journal* **272**:805-811.

Rasmussen, M. A., Cray, W. C., Cassey, T. A. and Whipp, S. C. (1993). Rumen contents as a reservoir of enterohaemorrhagic *Escherichia coli*. *FEMS Microbiology Letter*. **114:** 79-84 (Medline).

Reid, S.D., Herbelin, C.J., Bumbaugh, A.C., Selander, R.K. and Whittam, T. (2000). Parallel evolution of virulence in pathogenic *Escherichia coli Nature* **406** (6791): 64-67.

Reymond, D., Johnson, R.P. and Karmali, M.A. (1996). Neutralizing antibodies to *Escherichia coli* O157 verocytotoxin 1 and antibodies to O157 lipopolysaccharide in healthy farm family members and urban residents. *Journal of Clinical Microbiology* **34** (9):2053-2056).

Rice, D. H. and Hancock, D. (1995). Bovine sources of *Escherichia coli* 01157:H7. *Epidemiology. Conference of Research Workers in Animal Diseases* November. **13**:14. Chicago, IL.

Rice, D. H., Eric, D. E., Dale, D., Hancock, T.E., Besser, D. E., Herriott., Linda, V. C. (1997): *Escherichia coli* O157 in cull dairy cows on farm and at slaughter. *Journal of Food Protection* **60**(11):1386-1387.

Rice, E.W., Johnson, C.H., Wild, D.K. and Reasoner, D.J. (1992a). Survival of *Escherichia coli* O157:H7 in drinking water associated with a water borne disease outbreak of hemorrhagic colitis. *Letters in Applied Microbiology* **15**:38-40.

Rice, E.W., Sowers, E.G., Johnjson, C.H., Dunnigan, M.E. and Strockbine, N.A. (1992b). Serological cross-reaction between *Escherichia coli* O157 and other species of the genus Escherichia. *Journal of Clinical Microbiology* **30**:1315-1316.

Richards, M. S., Corkish, J.D., Sayers, A. R., McLaren, I. M., Evans, S.J. and Wray, C. (1998). Studies of the presence of *Escherichia coli* O157 in bovine faeces submitted for diagnostic purpose in England and Wales on beef carcasses in slaughterhouses in the UK. *Epidemiology and Infection* **120**: 187-192.

Riley L.W., Remis, R.S., Helgerson, S.D., McDee, H.B., Wells, J.G., Davis, B.R., Herbert, R. J., Olcott, E.S., Johnson, L.M. and Haegrett, N.T. (1983). Haemorrhagic colitis associated with a rare *Escherichia coli* serotype. *New England Journal of Medicine* **308**: 681-685.

Robertson, D .C., McDonel, J. L. and Dorner, F. (1985). *Escherichia coli* heat labile enterotoxin. *Pharmacology and Therapeutics* 28:303-339.

Russel, J., Callawy, T., Diez-Gonzales, F. and Kizoulis, M. (1998). Cattle diets and *Escherichia coli.* Cornell News. *US Food and Drug administration Center for Food Safety and Applied Nutrition.* [http://www.news.cornell.edu/release/Sept. 98.] site visited on 10/5/2001.

Russell, J.B., Rychlic, J.L. (2001). Factors that affect rumen microbial ecology. *Science* **292:** 1119-1122.

Samadpour, M. (1995). Molecular epidemiology of *Escherichia coli* by Restriction Fragment Length Polymorphism using Shiga-like toxin genes. *Journal of Clinical Microbiology* **33:** 2150-2154.

Saxena, S.K., O'Brien, A. D. and Ackerman, E. J. (1989). Shiga toxin Shiga-like toxin II variant, and ricin are all single-site RNA N-glycosidases of 28 SRNA when microinjected into Xenopus oocytes. *Journal of Biological Chemistry* **264:** 596-601.

Schimdt, H., Beutin, L. and Karch, H. (1995). Molecular analysis of the plasmid encoded haemolysin of *Escherichia coli* O157:H7 strain EDL. 933. *Infection and Immunity* **63:**1055-1061.

Scotland, S.M., Willshaw, G.A., Smith, H.R. (1987). Properties of Strains of *Escherichia coli* belonging to serogroup O157 with special reference to production of Verocytotoxins VT1 and VT2. *Epidemiology and Infection* **99:**613-624.

Senerwa, D., Olsvik, O., Mutanda, L.N., Lindqvist, K.J., Gathuman, J.M. and Wachsmuth, I.K. (1989a). Enteropathogenic *Escherichia coli* serotype 0111:HN isolated from pre term neonates in Nairobi, Kenya. *Journal of Clinical Microbiology* **27:**1307–1311.

Senerwa, D., Olsvik, O., Mutanda, L.N., Lindqvist, K.J., Gathuman, J.M. and Wachsmuth, I.K. (1989a). Colonisation of neonates in a nursery ward with

enteropathogenic *Escherichia coli* and correlation to the clinical histories of neonates. *Journal of Clinical Microbiology* **27:** 2539-2543.

Shere, J. A., Bertlett., K. J. and Kaspar, C. W. (1998). Longitudinal study of *Escherichia coli* O157:H7 dissemination on four diary farms in Wisconsin. *Applied and Environmental Microbiology* **64**: 1390-1399.

Shui, Tu., Deirdrel, P.L., Linda, Y.S. and Peter, L.I. (1999): Effects of membrane protonophreson the ATP content of immunomagnetic beads captured *Escherichia coli* O157.H7. Eastern Regional Research, 600 East Mermaid lane, Wyndmoor.56pp.

Siegler, R. L., Pavia, A. T., Christofferson, R.D. and Milligan, M.K. (1994). A 20-year population-based study of postdiaarrhoeal haemolytic uraemic syndrome in Utah. *Paediatrics* **94:** 35-40.

Smith, H.W. and Huggins, M.B. (1978). The influence of plasmid-determined and other characteristics of enteropathogenic *Escherichia coli* on their ability to proliferate in the alimentary tract of piglets, calves and lambs. *Journal of Medical Microbiology* **11**: 471-92.

Smith, H.W., Green, P. and Parsell, Z. (1983). Vero cell cytotoxin in *Escherichia coli* and related bacteria: Transfer by phage and conjugation and toxic action in laboratory animals, chickens and pigs. *Journal of General Microbiology* **129**: 3121-37.

Sowers, E. G., Wells, J. G. and Strockbine, N. A. (1996). Evaluation of commercial latex reagents for identification of O157 and H7 antigens of *Escherichia coli.* *Journal of Clinical Microbiology* **34(5):** 1286-1289.

Su, C. and Brandt, I.J. (1995). *Escherichia coli* O157:H7 infection in humans. *Annual International Medicine* **123**:698-714.

Sussman, M. (1985). *Escherichia coli* in human and animal disease. In: Sussman, M. (Ed). The virulence of *Escherichia coli*. Academic Press, London, pp. 86-90.

Svendsen, J., Risiing, H.J. and Christensen, S. (1977). Studies on the pathogenesis of enteric *Escherichia coli* infections in weaned pigs: Bacteriological and immunofluorescent studies. *Nordic Veterinary Medicine Journal* **29**:212-220.

Swerdlow, D.L., Woodruff, B. A., Brady, R. C. (1992). A waterborne outbreak in Missouri of *Escherichia coli* 0157:H7 associated with bloody diarrhea and death. *Annual Internal Medicine* **117**:812-9.

Tarr, P.I. (1995). *Escherichia coli* O157:H7. Clinical diagnostic and epidemiological aspects of human infection. *Clinical Infectious Diseases* **20:** 1-10.

Tarr, P.I. and Hickman. (1987). Haemolytic uraemic syndrome epidemiology: A population based study in King County, Washington, 1971-1980. *Paediatrics* **80:**41-45.

Tauxe, R.V. (1997). Emerging food borne disease: an evolving public health challenge. *Emerging Infectious Diseases* **3(4):** 425-34.

Taylor, C.M., Milford, D.V. and Rose, P.E. (1990). The expressions of blood group PI in post-enteropathic Haemolytic uraemic syndrome. *Paediatric Nephrology* **4:** 59-61.

Thompson, J.S., Hodge, D.S. and Borczyk, A.A. (1990). Rapid Biochemical test to identify verocytotoxin positive strains of *Escherichia coli* O157. *Clinical Microbiology* 28(2): 165-168.

Tzipori, S., Gibson, R. and Montanaro, J. (1989). Nature and distribution of mucosal lesions associated with enteropathogenic and enterohaemorrhagic *Escherichia coli* in piglets and the role of plasmid-mediated factors. *Infection and Immunity*. **57:** 1142-1150.

USDA/APHIS/VS, (1997). *Escherichia coli* 0157:H7 in raw milk: a review. *Animal Health Insight, Spring/Summer*: 7:1-8.

Van Donkersgoed, J., Graham, T. and Ganonn, V. (1999). The prevalence of verotoxins, *Escherichia coli* O157:H7 and *Salmonella* in the faeces and rumen of cattle at processing. *Canadian Veterinary Journal* 40: 332-338.

Vernozy-Rozand, C., Mazuy, C., Ray Gueniot, S., Boutrand-Loei S., Meryand, A. and Richard, V. (1997). *Escherichia coli. Revue-de Medicine- Veterinaire* **148** (11): 879-882.

Waddell, T., Head, S., Petruc, M. (1988). Globotriosyl ceramide is specifically recognized by the *Escherichia coli* verocytotoxin 2. *Biochemistry and Biophysics Research communication* **152:**674-679.

Wagner, A. (2000). Extension Food Technologist. *Bacterial Food Poisoning.* Texas Agricultural Extension Service. Hypertext mark-up by Gretchen Eagle and Dan Lineberger.130pp.

Wasteson, Y., Lund, A., and Olsvik, O., (1990). Enterotoxin and cytotoxin producing *Escherichia coli* . In: Olsvik, O. and Bukholm, G. (Eds), Application of molecular biology in diagnosis of infectious diseases. *Norwegian College of Veterinary Medicine*, Oslo. pp.5-15

Weber, G. (1990). The National Cattlemen's Beef Association, U.S. Department of Agriculture. USA.6pp.

Wells, J.G., Shipman, LD., Greene, K.D., Sowers, E.G., Green, J.H., Cameron, D.N., Downes, F.P., Martin, M.L., Griffin, P.M., Ostroff, S.M., Potter, M.E., Tauxe, R.V., and Wachsmuth, I.K. (1991). Isolation of *Escherichia coli* serotype O157:H7 and other Shiga-like toxin-producing *Escherichia coli* from dairy cattle. *Journal of Clinical Microbiology* **36**: 985-989.

Whittam, T.S. (1993). Evolution of *Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli* strains. In Kaper, J. B. and O'Brien A.D. (ed.) *Escherichia coli* O157:H7 and other Shiga-toxin-producing *Escherichia coli* strains. *American Society for Microbiology* **36**: 195-209.

Willshaw, G.A., Smith, H.R., Roberts, D., Thirlwell, J., Cheasty, T. and Rowe B. (1993). Examination of raw beef products for the presence of Vero cytotoxin producing *Escherichia coli*, particularly those of serogroup O157:H7. *Journal of Applied Bacteriology* **75**:420-426.

Wilson, J.B., Clarke, R.C. and Renwick, S.A. (1996). Verocytotoxigenic *Escherichia coli* infection in dairy farm families. *Journal of Infectious Disease* **174**: 1021-1027.

Wright, D.J., Chapman, P.A. and Siddons, C.A. (1994). Immunomagnetic Separations. A sensitive method for isolating *Escherichia coli* O157 from food samples. *Epidemiology and Infection* **113** (1): 31-39.

Yu, J. and Kaper, J. B. (1992). Cloning and characterization of the *eae* gene of enterohemorrhagic *Escherichia coli* O157:H7. *Molecular Microbiology* **6**: 411- 441.

Zhao, T. M., Doyle, M. P., Shere, J. and Garber, L. (1995). Prevalence of enterohaemorrhagic *Escherichia coli* O157:H7 in a survey of diary herds. *Applied Environmental Microbiology* **61**: 1290-1293.

Zhao, T., Doyle, M. P., Harmon, B. G., Brown, C. A., Mueller, E. and Parks A. H. (1998). Reduction of carriage of enterohemorrhagic *Escherichia coli* 0157:H7 in cattle by inoculation with probiotic bacteria. *Journal of Clinical Microbiology* **36**: 641-647.

APPENDICES

Appendix 1. Immunomagnetic separation technique (IMS)

Materials used

Dynabeads anti-*E. coli* O157:H7:

Dynabeads anti-*E. coli* O157:H7 are uniform, paramagnetic polystyrene microscopic beads with adsorbed and affinity purified antibodies against *E. coli* O157 covalently bound to the surface. The beads are supplied in a suspension of Phosphate Buffered Saline (PBS), pH 7.4 with 0.1% bovine serum albumin (BSA) and 0.02% sodium azide (NaN_3) (Dynal Biotech, Oslo).

Dynal Magnetic Particle Concentrator-S (MPC-S) for micro centrifuge tubes).

The Dynal MPC-S contains tube housing and a removable magnetic slide. It contains sintered rare-earth neodymium iron boron permanent magnets that will not diminish significantly during the lifetime of the product. The Dynal MPS-S is designed to hold six micro centrifuge tubes with working volumes ranging from 10 µl to 2ml.

Dynal Sample Mixer (MX-1)

The Dynal sample mixer is made of a powerful motor/gearbox offering rates of rotation up to more than 40 rpm, while the speed regulation circuitry gives a fixed setting of 18 rpm as well as a fully variable mode. The mixer operates on 12V DC power supplied by a wall- mounted transformer unit.

The Dynal sample mixer MX-1 is supplied with a turret to which a 12 tube-mixing wheel is attached. It allows mixing of up to 12 samples at a time in tube sizes ranging from 1.5 ml Eppendorf tubes up to 17 mm diameter tubes.

Wash buffers (PBS Tween)

Wash buffers (phosphate buffered saline) tween 20 contains 0.15M NaCl, 0.01M sodium phosphate buffer and pH 7.4 with 0.05% Tween 20.

Other materials used (but not provided with the IMS kit)

Micropipette (10-100µl)

1ml dispenser pipette.

Tryptone Soya Broth (TSB)-(Oxoid Ltd, UK).

Test tubes, glassware, loop swabs and pipettes.

Nutrient agar slant.

All reagents were of analytical grade.

Appendix 2. Procedure for immunomagnetic separation (IMS)

The protocol recommended by the manufacturer Dynal Biotech, (Dynal A/S, Oslo) for immunomagnetic separation was adopted in processing samples collected from cattle carcasses.

The IMS procedure was performed according to the manufacture's instructions as follows:

Remove the magnetic plate and load the necessary number of 1.5 ml micro centrifuge tubes into the Dynal MPC-s

Re-suspend Dynabeads anti-*E.coli* O157 until the pellet in the bottom disappears by using a vortex machine. Dispense 20 µl of Dynabeads anti-*E. coli* O157 into each tube. Add 1 ml of the pre-enriched sample aliquot and close the tube. Change to a new pipette for each new sample.

Invert the Dynal MPC-s rack a few times and then incubate at room temperature for 10 min with gentle continuous agitation to prevent the beads from settling (e.g. in a Dynal sample mixer, MX-1).

Insert the magnetic plate into the Dynal MPC-S and invert the rack several times to concentrate the beads into a pellet on the side of the tube. Allow 3 minutes for proper recovery.

Open the tube cap using the tube opener provided and carefully aspirate and discard the sample supernatant as well as the remaining liquid in the tube cap.

Remove the magnetic plate from the Dynal MPC-S.

Add 1 ml of wash buffer (PBS-Tween). Do not touch the tube with the pipette since this can cross-contaminate the samples as well as the wash buffer. Close the cap and invert the Dynal MPC-S a few times to re-suspend the beads.

Repeat steps 5-8

Repeat steps 5-7.

Re-suspend the Dynabeads-bacteria complex in 100µl of wash buffer (PBS-Tween 20). Mix briefly using a vortex mixer. The enriched bacteria on the beads are now ready for use in the detection step.

Appendix 3. Preparation of CHOMagar O157 plates

CHROMagar ™ O157 (Dynal Product No. 740.01

CHROMagar ™ O157 contains agar 15g/L, peptone, yeast extracts and salts 13g/L. Other ingredients include a special chromogenic mix 1g/L and pH 6.8.

The CHROMagar O157 ™ media was prepared according to manufacture's instruction (CHROMagar Microbiology, Paris,) as follows:

A pre-weighed CHROMagar ™ O157 powder supplied by the Sotherncross Biotechnology, SA, was mixed with 250 ml of purified water.

The powder was slowly dispersed in water by rotating until swelling of the agar. The mixture was then brought to a boil (100^0C) by repeated heating, swirling, or stirring regularly until complete fusion of agar grains (large grains replacing foam) has occurred. The mixture was then cooled in water bath at 48^0C. Freshly prepared medium may be kept for a day at room temperature or stored for several days in a refrigerator in dark.

Colony colour interpretation on CHROMagar ™ O157

Colony co lour	Micro organisms pre-identified.
Mauve	**E.coli O157**
Steel blue	**Coliforms**
Colourless to gray	**Proteus**

Source: Dynal Biotech, Oslo.

Appendix 4. Confirmatory biochemical tests

Pink mauve colonies were picked from CHROMagar™ O157 media plates and inoculated on blood agar to obtain pure culture. The following tests were set up: TSI slant, Simmon's citrate, and Sorbitol and Urea.

Typical EHEC O157:H7 give the following reactions:

Biochemical test	Reactions
TSI slant	A/A, H_2S-ve, Gas
Simmon's citrate	Positive
Sorbitol	Negative
Urea	Negative

Source: National laboratory for enteric pathogens, Winnipeg, Manitoba (1986).

Appendix 5. *E.coli* O157 latex test kit(DR620M)

A latex agglutination test for the identification of *E.coli* serogroup O157 has been adopted from Oxoid Limited, Basingstoke, Hampshire. England.

Components of the kit.

DR 621M Test latex.

Consist of blue latex particles bound with specific rabbit antibody to the somatic antigen.

DR 622M Control latex.

Consist of blue latex particles bound with pre-immune rabbit globulin.

DR 623M Positive control suspension

A suspension of inactivated *E.coli* O157 cells in buffer.

DR 624M Negative Control suspension

A suspension of inactivated *E.coli* O116 cells in buffer.

DR 500G Reaction cards

There are 35 disposable reaction cards provided in the kit.

Control Procedures

The positive control suspension must cause visible agglutination with the latex reagent within one minute but not the negative control.

Do not use the test if reactions with the control suspensions are incorrect.

Test method

Bring the latex reagents to room temperature. Make sure the latex suspension are mixed by vigorous shaking. Expel any latex from the dropper pipette for complete mixing.

Dispense one drop of the latex onto a circle on the reaction card. Place it close to the edge of the circle.

Add some loopfuls or a Pasteur pipette drop of saline to the circle. Ensure that the latex and saline do not mix at this stage.

Using a loop pick off a portion of the colony to be tested and carefully emulsify it in the saline drop. Ensure that the resulting suspension is smooth.

Mix the latex and suspension together and spread to cover the reaction area using the loop. Flame the loop.

Rock the card in a circular motion observing for agglutination. Do not rock the card for more than one minute and do not use a magnifying glass.

If no agglutination occurs then proceed to other Non-sorbitol fermenting (NSF) colonies if these are present.

If agglutination with the test reagent does occur, then it is necessary to test a portion of the colony with the control latex reagent to ensure that the isolate is not an auto-agglutinating strain. When finished dispose of the reaction card into disinfectant