VEDİA ARTEMEL OYALTAN PREVALENCE OF *E. COLI O157:H7* IN RAW GROUND BEEF OFFERED FOR CONSUMPTION IN THE TURKISH REPUBLIC OF NORTHERN CYPRUS NEU 2017

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A THESIS SUBMITTED TO THE GRADUATE SCHOOL OF APPLIED SCIENCES OF NEAR EAST UNIVERSITY

By

VEDİA ARTEMEL OYALTAN

In Partial Fulfillment of the Requirements for the Degree of Master of Science in Food Engineering

NICOSIA, 2017

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last name:

Signature:

Date:

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ABSTRACT

The main reservoir of *E. coli O157:H7* serotype, which causes foodborne infections, has been accepted as raw ground beef. In this study, presence of *E. coli O157:H7* serotype has been searched in 70 samples of freshly prepared ground beef, which were taken from supermarkets and butchers in Nicosia and Kyrenia regions of TRNC.

VIDAS ECPT, Vitek 2 Compact and Lateks Agglutination methods have been used for the analysis of *E. coli O157:H7* serotype respectively. As a result of the analysis, *E. coli O157* serotype has been determined in three (4.28%) of the samples with VIDAS ECPT method. At the verification stage of these positive results, *E. coli O157* serotype has been found in two (2,85%) samples with Vitek 2 Compact; whereas, *E. coli O157* serotype has been found in two (2.85%) of the same samples and *E. coli O157:H7* serotype has been found in one (1.42%) sample with Lateks Agglutination test.

Keywords: TRNC; Ground Beef*; E. coli O157:H7;* VIDAS; Vitek 2 Compact; Lateks Agglutination Test

ÖZET

Gıda kaynaklı enfeksiyonlara neden olan *E. coli O157:H7* serotipinin ana rezervuarı çiğ sığır kıyması olarak kabul edilmektedir. Bu çalışma da KKTC'nin Lefkoşa ve Girne Bölgelerindeki market ve kasaplardan 70 adet taze kıyılmış sığır kıymasında *E. coli O157:H7*serotipi varlığı araştırılmıştır.

E. coli O157:H7 serotipi analizleri için sırasıyla VIDAS ECPT, Vitek 2 Compact ve Lateks Aglutinasyon metotları kullanılmıştır. Analizlerin sonucunda VIDAS ECPT ile üçünde (%4.28) *E. coli O157* serotipi tespit edilmiştir. Bu pozitif sonuçların doğrulama aşamasında ise Vitek 2 Compact ile numunelerin ikisinde (%2.85) *E. coli O157* serotipi, Lateks Aglutinasyon testiyle de aynı numunelerin ikisinde (%2.85) *E. coli O157* serotipi ve birinde (%1.42) *E. coli O157:H7* serotipi bulunmuştur.

Anahtar Kelimeler: KKTC; Sığır Kıyması; *E. coli O157:H7;* VIDAS; Vitek 2 Compact; Lateks Aglütinasyon Test

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LIST OF ABBREVIATIONS

A/E:	Attaching and Effacing
AMP:	Adenosine Mono-Phosphate
Aw:	Water Activity
BAM AOAC:	Bacteriological Analytical Manual
BSE:	Bovine Spongiform Encephalopathy
CDC:	Center of Disease Control and Prevention
DAEC:	*Diffuse- adhering E. coli*
DEC:	*Diarrheagenic E. coli*
E. coli:	*Escherichia coli*
EAggEC:	*Enteroaggregative E. coli*
EEB:	EHEC Enrichment Broth
EHEC:	*Enterohemorrhagic E. coli*
EIEC:	*Enteroinvasive E. coli*
EMB Agar:	Eosin Methylene Blue Agar
ETEC:	*Enterotoxigenic E. coli*
EPEC:	*Enteropathogenic E. coli*
FDA:	Food and Drug Administration
FSIS:	Food Safety and Inspection Service
GAP:	Good Agricultural Practices
GDP:	Good Distribution Practices
GHP:	Good Hygiene Practices
GKH:	Foodborne Diseases
GMP:	Guanosine Monophosphate
GPP:	Good Production Practices
GTP:	Good Trade Practices
GVP:	Good Veterinary Practices
HACCP:	Hazard Analysis and Critical Control Point
HC:	Hemorrhagic colitis
HUS:	Hemolytic Uremic Syndrome

H_2S:	Hydrogen Sulfide
ISO:	International Standards of Organisations
KCN:	Potassium Cyanide
LEE:	Locus of Enterocyte Effacement
LST:	Laurly Sulphate Triptose broth
LT:	Temperature Sensitive
mEC:	Modified EC (mEC) Broth
MNEC:	Menengitis – Sepsis- Associated E. coli
mTSB:	Modified Soy Broth
MUG:	4-methylumbelliferyl β- D-glucuronide
ONPG:	O-Nitrophenyl - Beta-D-Galactoside
PCR:	Polymerase Chain Reaction
SMAC:	Sorbitol MacConkey Agar
SLT:	Shiga Like Toxin
SS Agar:	*Salmonella – Shigella* Agar
ST:	Heat Resistant
STEC:	Shigatoxigenic *E. coli*
TIR:	Translocated Intimin Receptor
TN:	Trypticase Novobiocin
TTP:	Thrombotic Thrombocytopenic Purpura
UPEC:	Uropathogenic *E.coli*
UTI:	Urinary Tract Infections
WHO:	World Health Organization

CHAPTER I
INTRODUCTION

Food hygiene is the prevention of raw material and product to contact with physical (glass, metal, wood, mouse droppings, insects, etc.), chemical (washing agents, pesticides, etc.) and biological (microorganism, parasite, etc.) dangers during storage, process, preservation and sales stages. Products being exposed to the determined dangers threat food safety and therefore human health. Moreover, quality characteristics of products are affected negatively. Not paying attention to the cleaning of workspace and staff as well as carrying out the cleaning of equipment's and surfaces in a proper way cause disruption of food hygiene (Palandöken, 2017). Food safety can be defined as following necessary rules and taking measures at production, moving, storage, distribution and consumption stages of food in order to provide healthy and reliable food production (Erkmen, 2010). As long as food hygiene and food safety is not paid attention to, human health is under risk and workplaces are caused to suffer economic loss (Palandöken, 2017).

As a result of industrialization and urbanization, demand for ready and fast food increases; developments and innovations at control systems cannot reach this speed and eventually microbial diseases caused by food gradually increases (Halkman, Noveir, & Doğan, 1998). Furthermore, as industrialization and pollution increases, foods are exposed to transmission of non-edible chemicals. Parallel to the increase of population, more food is required; therefore the usage of additives is also increased. In order to ensure healthy and safe food production as well as ensuring competition and sustainability of competition, food safety management systems have been created (Erkmen, 2010).

The following efforts have importance as they may contribute on creating a healthy community with safe food production at production stage and at securing public health by ensuring food safety at the highest level from farm to fork among meat products (Yörük, 2013; Erkmen, 2010);

- Carrying out livestock fattening in a more scientific framework for obtaining high quality stock,

- Conducting ante-mortem and post-mortem examinations thoroughly,
- Completely ensuring hygiene and sanitation rules at production,
- Paying attention to choose qualified staff that can ensure food safety and to training staff,
- Applying effective heating processes,
- Cooling rapidly at appropriate time,
- Obeying hygiene rules at maximum level during slicing and packaging processes,
- Preventing cross contamination,
- Paying attention to storage temperatures and time,
- Applying ISO 22000 that contains Hazard Analysis and Critical Control Points (HACCP) at following food safety rules at food production places in a meticulous manner (Yörük, 2013; Erkmen, 2010).

Some new pathogens have been defined as foodborne pathogens in many parts of the world. Even though *E. coli O157:H7, Salmonella Typhimurium definitive type 104, Helicobacter pylori, Arcobacter butzleri spp., Bacillus cereus, Yersinia enterocolitica, Salmonella enteritiditis, Campylobacter jejuni, Vibrio vulnificus, Listeria monocytogenes, Staphylococcus aureus, Enterobacter sakazakii, Enterococci spp., Mycobacterium avium subsp. Paratuberculosis spp.* have been known as pathogens for many years, they have been determined as some of the 27 main food-borne pathogens among main foodborne infections within the past two decades (Güner, Atasever, & Aydemir Atasever , 2012; Food Safety and Inspection Service, 2016; Kartal, 2006; Sağlam & Şeker, 2016). Not only but also, these pathogens have been responsible for only 19% of total average number of food resourced infections. Thus, it is considered that there are many food resourced pathogens that haven't been defined yet (Kartal, 2006).

Changes in pathogens, dietary habits, increases at food, food of animal origins and animal trade, pollution, economic and technologic developments, structuring at health sector, demographical changes and increase of travel and migrations play an important role the epidemiology of food pathogens that have recently emerged or gained importance again (Güner, Atasever, & Aydemir Atasever , 2012). There are risk creating factors such as the lack of administrative determination, inadequacy of legal arrangements, applications and inspections, not carrying out pathogen

microorganism and chemical residue analysis and risk evaluation for food hazards and not training food producers and staff regarding personal hygiene (Erkmen, 2010). Bacterial pathogens follow various ways for the formation of infection. Adhesion on host cells, colonization on tissues, intra cell reproduction following invasion on cells in some cases and then spreading on other tissues or staying in cell are some of these ways. Enterotoxins, cytotoxins and neurotoxins that are produced by foodborne bacterial pathogens are important factors for the formation of clinical profiles. Having knowledge on pathogenicity factor of pathogen bacteria that cause foodborne diseases increases the efficiency of measures that will be taken against these bacteria. The developments that will be reached in consequence of that would reach in more correct results in the diagnosis, control and treatment of foodborne diseases (Telli & Doğruer, 2013). As well as bacterial pathogens, chemical infections also occur on food. Unplanned urbanization, usage of pesticides unconsciously, industrial establishments that do not have treatment systems discharging their waste to streams, soil, channel or atmosphere directly and usage of uncontrolled additives are among the important reasons of chemical infection. Manufacturers are required to be trained on the effects of additives that use in food production on public health and the amount of additives in food are absolutely required to be analysed during consumption stage. Residue analyses for chemical infections are required to be carried out and monitored particularly at sales points and the necessary legal regulations for carrying out these are required to be completed immediately (Erkmen, 2010).

1.1 Foodborne Diseases (FBD) are diseases that are formed when food, which contains pathogen bacteria or their spore forms (e.g.: infant botilismus) and contaminated water and various food or food that included toxigenic bacteria and toxins that are created by mould are consumed. While these diseases are clinical profiles that is seen with gastrointestinal symptoms mainly; they are examined in three main branches of intoxications, infections and toxi-infections (Akçelik et al., 2000; Alişarlı, 2013; Kartal, 2006). These 3 groups of infections are given in table 1.1 below as variation, effective bacteria, incubation period and disease dose.

1.1.1 Foodborne Infections are defined as foodborne infections that are diseases which take form as a result of consuming water and food that are contaminated with enteropathogenic bacteria or viruses (Akçelik et al., 2000; Alişarlı, 2013). Enteropathogenic microorganisms that are taken together with food are required to be alive during the consumption of food. Live

microorganisms that cause foodborne infection and that are taken with food settle in digestive system even though they have very little number in the food. These bacteria spread in the intestinal system by holding in it and cause inflammation. Whereas, some of them cause disease with the toxins they form at the intestinal system after they are taken in the body. There are some cases in which food has a role of only a passive carrier and transfer them without allowing pathogens to increase. These pathogens and infections that are caused by them for example *Mycobacterium tuberculosis* and tuberculosis disease are not within the scope of foodborne diseases. It should be noted that these kinds of pathogens usually cannot develop on food (Akçelik et al., 2000).

1.1.2 Foodborne Microbial Intoxications are named as disease profile intoxications that are shaped after pathogen bacteria or moulds are reproduce in food and the toxin created by them is taken through digestive system (Akçelik et al., 2000; Alişarlı, 2013). Pathogen microorganism is required to reproduce and release toxin in food. Intoxications take their form when toxins are consumed with food. In order for intoxication to take its form, it is not necessary to consume living pathogen microorganism with food. In other words, active toxin that cause intoxication is required to be taken together with food (Alişarlı, 2013). It is necessary to examine intoxications that are caused by bacteria in microorganisms and mycotoxicosis cases created by fungus toxins separately. While taking toxins produced by *Clostridium botulinum* and *Staphylococcus aureus* are fundamental in toxic poisonings caused by bacteria; many toxins such as particularly aflatoxins, ochratoxin A, patulin, rubrotoxin, izlanditoxin, zearalenon, T-2 toxin deoksinivalenol, stachybotrytoxin are taken as fungus toxins in mycotoxicosis cases (Akçelik et al., 2000).

1.1.3 Toxi-infections food poisonings, which are caused by toxins that are formed as a result of spore-creating bacteria creates spore in intestines or after many pathogen microorganisms that are taken with food and water are reproduced in intestines, their death and its cell lysis that takes its formed subsequent to their death are defined as toxiinfections. Their symptoms usually take their form due to toxins that are revealed as a result of bacterial cell colonization, sporing or their disruption (e.g.: *Clostridium perfringens* gastroenteritis) (Alişarlı, 2013).

Table 1.1: Some Characteristics of Foodborne Microbial Factors (Alişarlı, 2013)

Factors	Incubation period	Disease dose
A. Intoxications		
Basillus cereus (emetic form)	1-6 hours	NA
Clostridium botilium	12-72 hours	~1μg
Staphylococcus aureus	1-6 hours	100-200ng
B. Toxi-infections (Enterotoxin in intestine without infection)		
Basillus cereus (diarrea form)	6-12 hours	10^5-10^7
Clostridium perfringens	8-16 hours	10^7-10^8
C. Infections that are formed with the presence of enterotoxin due to bacterial adherence without invasion to intestine epithelium		
Aeromonas spp.	6-48 hours	10^3-10^8
Escherichia coli		
ETEC (ST)	16-48 hours	10^5-10^6
ETEC (LT)	16-48 hours	10^5-10^7
EHEC (O157:H7)	1-7 days	10
Vibrio cholerae	2-5 hours	10^6
Vibrio parahaemolyticus	3-76 hours	10^5-10^7
D. Infections that are formed due to bacterial invasion to intestinal immune system and epithelium cells		
Campylobacter jejuni	3-8 days	$\geq 10^3$
Salmomella spp. (non-thyphoidal)	6-72 days	10^3-10^6
Shigella spp.	1-7 days	10^3-10^4
Yersinia enterocolitica	3-5 days	10^3-10^7
E. Infections that cause organ invasion and systemic failures		
Listeria monocytogenes	Days, weeks	10^3-10^6
Salmonella typhi	10-21 days	1-10^2
Salmonella paratyphi	10-21 days	1-10^2

Although foodborne infections and intoxications are seen as an important problem throughout the world including the USA and Europe, they always possess a secondary importance when compared to respiratory tract infections. On the other hand, when infections and intoxications have not decreased and on the contrary have shown an increase recently despite the efforts given for minimizing these diseases, that makes these foodborne pathogen and toxins to be determined in food with more reliable and correct methods day by day (Akçelik et al., 2000).

When BSE and dioxin crisis that occurred particularly in Europe and *E. coli O157:H7* infections that are caused by beef emerged in the North America, the importance of safe food production and consumption has been understood and the necessity of constant improvements in food safety systems has been put forward (Yörük, 2013).

In order to prevent foodborne diseases, it is required to increase durability of food. For that purpose, it is required to kill microorganisms that cause disruption in food, stop or prevent their reproduction, protect food from external factors through various ways and make food enzymes inactive (Tayar & Hecer, 2013). Physical, chemical and biological methods are being used as main processes for increasing the durability of particularly meat and meat products (Tayar & Hecer, 2013; Öztürk, Gürbüz, & Çalım, 2006). The main objective for these fundamental preserving methods is to define internal and external factors as disruptor parameters for microbial development and reproduction and to take the necessary measures to reach the aimed product. Sensory, nutritive, toxicological quality and protection from economic characteristics as well as durability in meet are taken as the most fundamental and common point (Tayar & Hecer, 2013). Approaches such as Hazard Analysis and Critical Control Points (HACCP), Good Agricultural Practices (GAP), Good Veterinary Practices (GVP), Good Production Practices (GPP), Good Hygiene Practices (GHP), Good Distribution Practices (GDP) and Good Trading Practices (GTP) have importance in control and prevention of foodborne diseases (Güner, Atasever, & Aydemir Atasever , 2012).

Meat and meat products have an important place in foodborne diseases. Some part of microorganisms that can develop in meat and meat products can cause different forms of disruptions without affecting human health directly; whereas, other part cause diseases in human without creating any disruption in meat and meat products (Balpetek & Gürbüz , 2010).

Meat as human food is a product that is obtained by certain cutting, disintegrating and processing processes from skeletal muscles and internal organs of beef, sheep, goat, poultry animals, fisheries and various prey animals. When red meat is considered, meat that is made of striated muscle tissue that forms structure by folding skeletons of animals such as beef, sheep, goat and buffalo is understood (Gıda Teknolojisi Et ve Ürünleri Analizleri 1, 2016). The importance of meat in nutrition: as animal proteins (except for gelatin) contain essential amino acids with a

sufficient and balanced rate, they should absolutely be consumed by people. 50% of the daily protein need is recommended to be from animal origins. Among food of animal origins, meat is a food that is rich for vitamins, some minerals (particularly for P and Fe) and high quality proteins, it is also appetising, tasty, saturator and easy to produce (Arslan, 2002).

General chemical composition of fat-free striated muscle tissue that constitutes a large section of red meat compound is as follows: It includes 75% water, 20% crude protein, 3% fat, 1% mineral, 1% glycogen and various vitamins (Gıda Teknolojisi Et ve Ürünleri Analizleri 1, 2016; Arslan, 2002). The composition of fat-free striated muscle tissue shows differences naturally according to the strain of the animal, its type, way of nutrition, age, treatment to the animal before slaughtering and the region of the muscle (Gıda Teknolojisi Et ve Ürünleri Analizleri 1, 2016).

Ground meat: It is the red meat which is obtained by processing raw red meat that is disintegrated from the bones of butchery animals through mincing machine or mincing it with a knife or chopping knife. Raw red meat, which is obtained from skeleton muscles including only connective tissue, must be used when preparing ground meat. Ground meat cannot be prepared from meat obtained from sections that do not possess nutrition value such as sinew and tendon, mechanically separated meat, meat that contain bone pieces or skin, meat from head, pieces of linea alba that are not muscles, meat obtained from carpal and tarsal sections, scrapings of bones and diaphragm muscle (Gıda Teknolojisi Et ve Ürünleri Analizleri 1, 2016).

As case-ready ground meat might be produced from remaining meat, whose resource is unknown, or from products whose date of expiry is close and even from entrails and substances that are not supposed to be included in mince, its speed of spoilage will be increased as its surface area is increased; therefore it can be considered as a food whose safety is low and is risky in terms of health. Consequently its shelf life can be short (Şireli & Artık, 2014).

There are two criteria in determining the quality of ground meat; the rate of fat and colour. The colour of ground meat is required to be the same colour with the piece it is obtained from. The colour of ground meat to have a light red (pinkish) colour that is lighter than regular red indicated the increase of fat rate and consequently decrease of nutritional quality (Gıda

Teknolojisi Et ve Ürünleri Analizleri 1, 2016). The component of ground meat is given in the following table according to Turkish Food Codex.

Table 1.2: The component of ground meat according to Turkish Food Codex (Türk Gıda Kodeksi Et ve Et Ürünleri Tebliği , 2016)

Ground Meat	Fat percentage	Collagen/ Meat Protein Rate
Fat-free Ground Meat	≤ 7%	≤ 12
Full Fat Ground Meat	≤ 20%	≤ 15
Mixture ground meat that is allowed to be mixed with ground meat obtained from the meat of other animals	≤ 25%	≤ 15
Pork Ground Meat	≤ 30%	≤ 25

The criteria that are used in microbiologic quality control of food can be briefly defined as limits that set microbiologic characteristics of food. According to that microbiologic criteria determine the limits of microorganism that can be found in the food sample that is taken under analysis with standard methods or the levels of microorganism groups that are allowed to be contained of the food (Öztürk, Gürbüz , & Çalım, 2006). Microbiologic criteria of ground beef meat are given in Table 1.3 according to the Turkish Food Codex.

Table 1.3: Microbiologic criteria regulation of the Turkish Food Codex (Türk Gıda Kodeksi Mikrobiyolojik Kriterler Yönetmeliği, 2011)**.**

Food	Microorganisms/ toxins /metabolites	Sampling plan ([1])		Limits ([2])		Reference method ([3])
		N	C	M	M	
Ground Meat	Number of aerobic colonies	5	2	$5x10^5$	$5x10^6$	ISO 4833
	Salmonella	5	0	0/25 g-ml		EN/ISO 6579
	E. coli O157	5	0	0/25 g-ml		ISO 16654

In the study conducted by Başkaya and partners (2004) on case-ready ground meat, it was informed that as a result of microbiologic analysis determination of total aerobic mesophilic general count, coliform, *Escherichia coli*, Coagulase Positive *Staphylococcus aureus, Bacillus cereus*, yeast and mould numbers were $2.7x10^6$, $4.1x10^4$, $7.2x10^3$, $3.2x10^3$, $9.5x10^3$, $1.4x10^5$, $5.7x10^4$ kob/g respectively. In another study, the average numbers of aerobic mesophilic bacteria, total coliform bacteria, *Escherichia coli*, *Staphylococcus spp*., *Staphylococcus aureus*, yeast and mould were stated as $4.7x10^4$ kob/g, $6.0x10^2$ kob/g, $2.8x10^3$ kob/g, $3.2x10^5$ kob/g, $5.8x10^4$ kob/g, $4.8x10^4$ kob/g and $2.3x10^3$ kob/g respectively (Direkel et al., 2010). As a result of both studies, it was found that microbiologic criteria of ground meat were not at the desired quality.

If ground meat is prepared from chilled red meat, the red meat is required to be processed in ground meat in maximum 6 days after the slaughter of animal or in maximum 15 days after the slaughter of animal if it is in a vacuum package (Gıda Teknolojisi Et ve Ürünleri Analizleri 1, 2016).

Bacteria multiply rapidly in temperatures between 40 and 140 ° F (4.4 and 60 ° C), which is called the “Danger Zone”. To keep bacteria at low level, beef ground meat should be kept at 40 °F (4.4 ° C) or at lower temperatures and it should be either used in 2 days or frozen. Thus, the ground meat will keep its freshness and the development of bacteria is slowed down. For storing in freezer for a long period, it can be wrapped in aluminium foil, freezer paper, or plastic bags made for freezing. Ground beef can stay safe without producing microorganism when it is frozen; however, it can lose quality over time. Therefore it should be used within 4 months. In order to destroy harmful bacteria, minced ground beef is required to be cooked to a safe minimum internal temperature of 160 ˚ F (71.1 °C) (Food Safety and Inspection Service, 2016).

E. coli O157:H7 bacteria can survive in refrigerator and freezer temperatures. For storing in freezer for a long period, it can be wrapped in aluminium foil, freezer paper, or plastic bags made for freezing. Ground beef can stay safe without producing microorganism when it is frozen; however, it can lose quality over time. Therefore it should be used within 4 months. While the actual infectious dose is unknown, most scientists believe that it takes only a small number of this strain of *E. coli* to cause serious illness and even death, especially in children

and older adults. Bacteria that are reproduced in ground beef meat are killed by thorough cooking, which is an internal temperature of should be 160 °F (71.1 °C) as measured by a food thermometer (Food Safety and Inspection Service, 2016).

Every microbiologically clean food is durable. Preventing factors show their activities and reproduction of microorganisms are ensured to be prevented. The abovementioned preventing factors are heating (F-value), cooling (t-value), water activity (Aw-value), concentration of hydrogen ion (pH-value) and conserving substances (such as Nitrite) (Tayar & Hecer, 2013). Survival and growth potential of EHEC can be affected from various parameters such as the presence of heat, content of food, concentration of salt and other preservative substances, the atmosphere where the meat is stored and the presence of other microorganisms (Batt & Tortorella, 2014). Ground meat and ground meat products are associated with EHEC infection at significant amount. Muscle tissues generally do not contain microorganism; however, surfaces that are open can be contaminated with EHEC (Batt & Tortorella, 2014). During the mincing of meat, its surface area increases and any pathogen organism that is on its surface can spread all over it (Batt & Tortorella, 2014; Food Safety and Inspection Service, 2016). The risk assessment carried out by FSIS has been started according to the increase of public awareness on determining *E. coli O157:H7* in beef, carcases and ground beef meat and on the association of *E. coli O157* in foodborne outbreaks that are resulted with serious diseases and death. The objective of this risk assessment is to evaluate and integrate present scientific data and information systematically.

1) Provide a comprehensive evaluation of the risk of illness from *E. coli O157:H7* in ground beef based on currently available data,
2) Estimate the likelihood of human morbidity and mortality associated with specific numbers of *E. coli O157:H7* in ground beef servings,
3) Estimate the occurrence and extent of *E. coli O157:H7* contamination at points along the farm-to-table continuum,
4) Provide a tool for analysing how to most effectively mitigate the risk of illness from *E. coli O157:H7* in ground beef, (Pathogen Reduction, HACCP applications can be beneficial),
5) Identify future food safety research needs (Brashears et al., 2002).

E. coli O157: H7 risk assessment is a fundamental risk assessment that shows present applications, behaviours and reflecting conditions at the farm-to-table process to applicable the extent (Food Safety and Inspection Service, 2001). The farm-to-table risk assessment model for ground beef should be carried out as it is stated in the figure below.

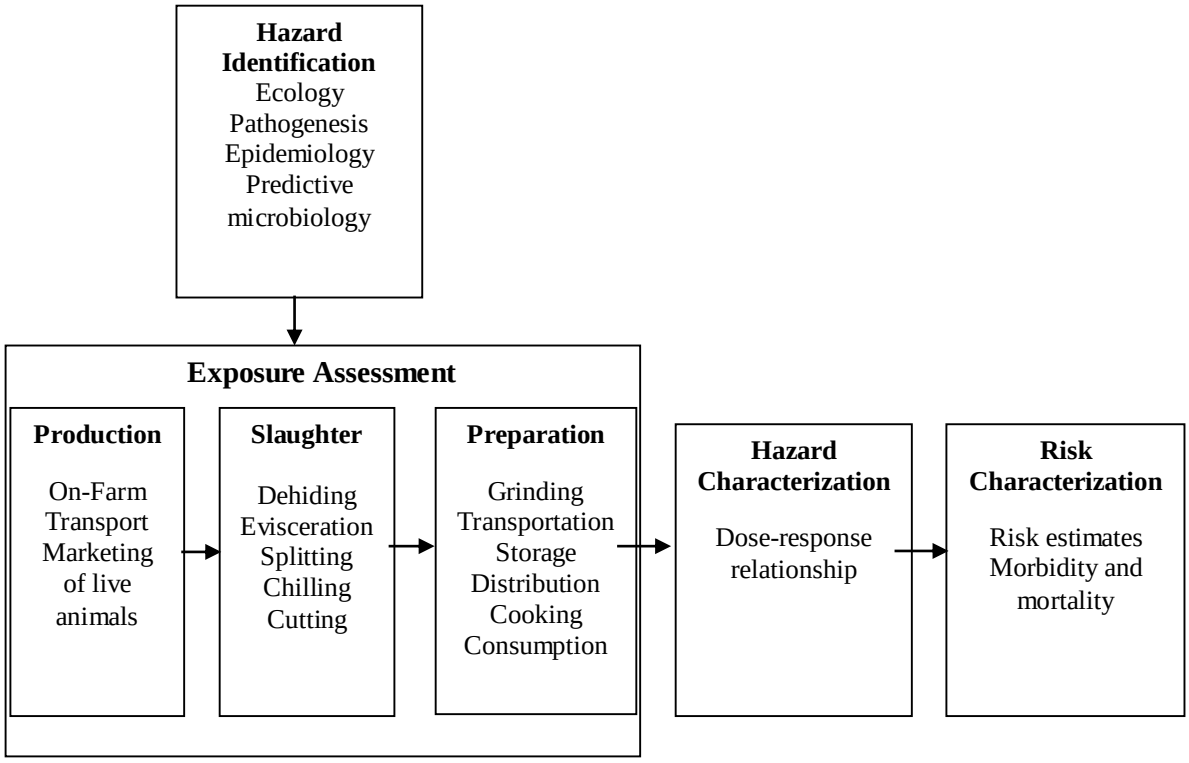

Figure 1.1: Farm-to-table risk assessment model for *E. coli O157:H7* in ground beef (Food Safety and Inspection Service, 2001). The outputs of *E. coli 0157:H7* risk assessment according to risk assessment model is given in Table 1.4 below.

Table 1.4: Outputs of the *E. coli* O157:H7 Risk Assessment
(Food Safety and Inspection Service, 2001)

Component	Module	Outputs
Hazard Identification		• Epidemiological information on human morbidity and mortality due to *E. coli O157:H7* • Microbiological information on the pathogenesis of *E. coli O157:H7* compared with other *E. coli* strains • Information on the source and transmission of *E. coli O157:H7* • Information on the environmental conditions that influence survival and growth (predictive microbiology) of *E. coli O157:H7*
Exposure Assessment	Production	• Herd and within-herd prevalence rates for infected live cattle prior to slaughter for ground beef • Prevalence of contaminated carcasses • Number of *E. coli O157:H7* organisms on contaminated carcasses
	Slaughter	• Prevalence of contaminated combo bins of trim • Number of *E. coli O157:H7* organisms in combo bins of contaminated trim • Prevalence of contaminated grinder loads of ground product • Number of *E. coli O157:H7* organisms in contaminated grinder loads of ground product
	Preparation	• Prevalence of contaminated cooked ground beef servings • Number of *E. coli O157:H7* organisms in contaminated cooked ground beef servings
Hazard Characterization		• Number of *E. coli O157:H7* diseases associated with cooked ground beef consumption • Annual number of hospitalizations due to *E. coli O157:H7* in cooked ground beef • Annual number of cases of HUS/TTP due to *E. coli O157:H7* in cooked ground beef • Annual number of deaths due to *E. coli O157:H7* in cooked ground beef
Risk Characterization		• Annual risk of disease from *E. coli* O157:H7 in cooked ground beef • Annual risk of disease from *E. coli* O157:H7 in cooked ground beef by seasonal exposure and age of the consumer • Identification of important variables that influence the risk of illness from *E. coli O157:H7* in ground beef • Identification of important food safety research areas

CHAPTER 2

ESCHERICHIA COLI

2.1 Definition

Escherichia coli is one of the most important members that are within Escherichia genus at Enterobacteriaceae family (Baysal, 2004; Akçelik et al., 2000; Stockbine et al., 2015; Batt & Tortorella, 2014; Adams & Moss, 2008). It is probably one of the most understood and worked on living organism throughout the world (Baysal, 2004; Tayar & Hecer, 2013; Stockbine et. al., 2015). *E. coli* was isolated from a child's faeces by German bacteriologist Thedor Escherich in 1885 for the first time and it was named as Bacterium coli commune. Then this bacterium was named as *E. coli* (Tayar & Hecer, 2013; Halkman, 2013; Stockbine et al., 2015; Adams & Moss, 2008; Demir, 2006). The bacterium is naturally found in normal intestine flora of warm-blooded animals and human and due to this characteristic; it is only accepted as faecal contamination index (Baysal, 2004; Tayar & Hecer, 2013; Özgül, 2014; Akçelik et al., 2000; Halkman, 2013; Halkman, 2005; Stockbine et al., 2015; Batt & Tortorella, 2014; Feng, 2012). At first *E. coli* was seemed to be harmless and only some of its enteropathogenic strains were mentioned. Then certain serotypes of the bacterium was found to show both pathogenic and enterotoxigenic characteristics and to contain various virulence factors (Akçelik et al., 2000). The presence of *E. coli* serotypes that cause diarrhea were found near the end of 1940s (Halkman A. K., 2013; Adams & Moss, 2008). When toxins similar with Vibrio cholera toxin were found in the middle of 1950s, that caused the perspective for *E. coli* to be changed (Halkman, 2013). All strains of *E. coli* may not cause diseases and consequently even though the presence of *E. coli* in food may pose a potential threat, there is no clear opinion that it causes sickness when it is consumed as food. However, it was stated that O157:H7 serotype was one of the serotypes of *E. coli* that is required to be paid attention to (Batt & Tortorella, 2014; Demir, 2006). *E. coli* bacteria are not found in nature unlike other coliform bacteria (Özkuyumcu, 2009).

2.2 Morphology

E. coli bacteria are not found in nature unlike other coliform bacteria under natural conditions (Özkuyumcu, 2009). However isolation of *E. coli* in samples taken from the environment such as water and food is an indicator that the tested substances were contaminated with faeces (Baysal, 2004; Özkuyumcu, 2009; Batt & Tortorella, 2014).

E. coli settles in gastrointestinal tract of warm-blooded animals within a few hours and a few days following birth (Özkuyumcu, 2009). *E. coli* are gram negative, oxidase negative, asporogenic, motile, facultative anaerobe basils (Tayar & Hecer, 2013; Özgül, 2014; Akçelik et al., 2000; Stockbine et al., 2015; Batt & Tortorella, 2014; Demir, 2006). They are bacteria with a width of 1-1.5μm and length of 2-6 μm, straight; their ends are round similar to a rod shape. It can be found as small and short similar to cocci in some cultures; whereas it can also be found as longer than normal and even filament shapes that divaricate like letter Y. It is possible for the both shapes to exist together. Although it moves through its lashes that exists around itself, its movements are slow (Baysal, 2004). They can even seem motionless (Baysal, 2004; Batt & Tortorella, 2014). Strains of *E. coli* usually create fimbria. Fimbriae play a role of assisting virulence factor with their characteristic to hold on cells (Özkuyumcu, 2009).

E. coli easily reproduce in general mediums such as bouillon and gelose. They create homogenous blur at bouillon; whereas at gelose they create slightly puffy, round, smooth S-type colonies with a diameter of 1-2 mm. The colonies reproduce at gelatine as small, transparent first, then white. They do not melt gelatine and serum coagulant. Some origins and those that are abstracted from urinary tract infections particularly may cause hemolysis at bloody gelose (Baysal, 2004).

2.3 Biochemical Chacteristic

E. coli is a typical mesophile that can grow from 7 ° C to 50 ° C; however despite the fact that some ETEC strains are reported to grow at temperatures as low as 4 °C, their optimum temperature is around approximately 37 ° C (Tayar & Hecer, 2013; Batt & Tortorella, 2014). It has a distinctive characteristic from some similar bacteria particularly at 44 °C (Baysal, 2004; Akçelik et al., 2000). It shows a significant heat resistance, with a D value at 60 C of the order

of 0.1 min and can survive refrigerated or frozen storage for extended periods. A pH value closer to neutral is ideal for growth; however it is possible to grow under good circumstances down to pH 4.4. Minimum aw value for growth is 0.95 (Akçelik et al., 2000; Adams & Moss, 2008).

E. coli bacilli take many sugars to pieces by creating acid and gas. Their ability to ferment lactose at 44 ^{o}C and their fermentation for different sugars are distinctive characteristics from other intestinal bacteria, particularly *Salmonella spp.* and *Shigella spp.* (Baysal, 2004; Stockbine et al., 2015; Batt & Tortorella, 2014; Adams & Moss, 2008). Therefore many media that contain lactose and an indicator are used. EMB medium is one of them and it contains lactose and eosin methylene blue. *E. coli* bacteria take lactose to pieces in this medium and create acid; thus their colonies are blue-black shine and the colonies of bacteria whose lactose is not taken to pieces are colourless. In media such as SS agar, McConkey gelose agar and etc., coli bacilli create red colonies (Baysal, 2004; Stockbine et al., 2015).

As more than 95% of *E. coli* strains can create acid and gas from glucose, they cannot perform inositol, adonitol, cellobiose and arabitol fermentation while they ferment lactose, mannitol, sorbitol, maltose, xylose, trehalose, arabinose, mukat and mannose (Baysal, 2004; Akçelik et al., 2000). They never create gas out of starch (Baysal, 2004).

While lysine-decarboxylase is ONPG and mobile positive at *E. coli* strains, reproduction and malonate usage at H_2S formation, urea and gelatine hydrolysis, phenylalanine deaminase, lipase, DNAse, KCN are negative (Özkuyumcu, 2009; Akçelik et al., 2000; Batt & Tortorella, 2014). The sole bacterium that is indol positive among β.-D-glucuronidase (MUGase, β-GUR) positive bacteria is *E. coli* (Akçelik et al., 2000; Halkman A. , 2005). *E. coli* plasmids were analyzed in details. It is known that enterotoxigenic strains carry five or more plasmids that include antibiotic resistance, enterotoxin production and cohering on antigens characteristics (Batt & Tortorella, 2014). Moreover, they present indol positive, methyl red positive, Voges Proskauer negative and citrate negative reactions at IMVIC tests (Baysal, 2004; Özkuyumcu, 2009).

2.4 Antigens

Coli bacillus has complex; but well antigen structure and different antigen types as they are similar with all bowel movements (Batt & Tortorella, 2014).

In 1940s' a serotypology diagram for *E. coli* that is based on lipopolysaccaride somatic O, flagellar H and polysaccharide capsular K antigen was suggested by Kauffman (Baysal, 2004; Halkman, 2013; Batt & Tortorella, 2014; Adams & Moss, 2008; Nataro & Kaper, 1998; Winn et al., 2006). O antigen represents the main group; whereas H represents the serovars at the currently applied O:H system (Batt & Tortorella, 2014; Adams & Moss, 2008). The first antigen groups that were also discovered by Kauffman were composed of 25 O, 55 K and 20 H antigens (Batt & Tortorella, 2014).

2.4.1 O Antigens: They are antigens with somatic, heat resistant lipopolysaccaride structure. They are resistant to boiling and alcohol and not resistant to formol. 171 separate choline O antigen have been found (Baysal, 2004; Batt & Tortorella, 2014; Feng et al., 2002). O antigens have cross reaction with other related microorganisms. For example, O antigens of *E. coli* make cross reaction with some O antigens on *Shigella spp*. and *Salmonella spp*. Particularly all O antigens (except for O antigens at some *Shigella sonnei*) make cross reaction with *Shigella spp*. As a result of this cross reaction, many antibody based tests which determine *E. coli* extensively cause incorrect positive results to be reached as it makes cross reaction with O antigens of other microorganisms (Baysal, 2004; Batt & Tortorella, 2014).

Coli bacilli are divided into serovars with their H and K antigens according to their O antigens serologically (Baysal, 2004).

2.4.2 H Antigens: *E. coli* lash antigens whose amount is few and that are monophasic are found in mobile origins, they have protein structure and thermolabile. They are destroyed with heating at 100 °C and alcohol and proteolytic ferments and they are resistant to formol. Only about 20 of them are used for identification (Baysal, 2004). More than 50 H antigens have been found until today (Batt & Tortorella, 2014). H antigens do not make cross reaction with each other and with H antigens of other bacteria (Baysal, 2004; Halkman, 2013).

2.4.3 K Antigens: K antigens are a piece of polysaccharide and cell capsules found in nature (Baysal, 2004; Batt & Tortorella, 2014). *E. coli* bacteria that include these antigens do not agglutinate with O antiserums. K antigens that were analysed according to their agglutination characteristics are named according to the difference their structure show. Approximately 80 kinds of K antigens that are named as K1, K2 were found. They are polysaccharide structured

antigens. They are resistant to heat and can be eliminated by boiling for a couple hours at 100 and sometimes 120 degrees (Baysal, 2004). K88 and K99 antigens cause diarrhea at pigs. Moreover, K99 antigen is related with diarrhea at calves and lambs (Batt & Tortorella, 2014).

2.4.4 Fimbria Antigens: Special fimbria antigens are found in *E. coli* bacteria that contain MR (mannose-resistant) fimbrias. Being named as F1, F2, F3… these antigens also contain some antigens that used to be considered as K antigens before (such as K88 =F4, K99 = F5) (Baysal, 2004).

2.5 Patogenesis

The number of virulence factors that are found in *E. coli* is very high. General virulence factors that enterobacteriaceae members have are also found in *E. coli*.

As well as having general virulence factors that the enterobacteriaceae family have, it has distinctive virulence factors (Özkuyumcu, 2009).

Table 2.1: Virulence Factors Specific to *E. coli* (Özkuyumcu, 2009)

Virulence Factor	Strain	Effect
P Fimbria, AFAI and AFAII, Dradesin, Type 1 battery	Uropathogenic strains	Holding on target cell
S battery	Strains that cause meningitis	Holding on target cell
EspA	EPEC strains	Holding on intestinal epithelial
CFA/I and CFA/II	ETEC strains	Bonding on small intestinal microvillus
Hemolysin	ETEC strains	Lysis of erythrocytes
Intimin	EPEC and some other strains	Triggering of disruption of absorption at intestine
LT	ETEC	Development of diarrhea as a result of CcAMP formation
ST	ETEC	Development of diarrhea as a result of CcMP formation
Shiga Toxin (verotoxin)	EHEC strains	Inhibition of protein synthesis

2.6 Clinic

Pathogenic *E. coli* strains are found in two categories as creating enteral and parenteral diseases. These are extra intestinal pathogenic *E. coli* and intestinal (diarrheagenic) *E. coli* (Özkuyumcu, 2009; Akçelik et al., 2000; Halkman, 2005; Murray et al., 2007).

2.6.1 *Extraintestinal Pathogenic E. coli*

E. coli strains have strains that cause diseases out of the gastrointestinal system as well as important intestinal infections and these strains are named as *Extraintestinal pathogenic E. coli* (ExPEC) (Halkman, 2005). The most commonly seen infection is the urinary system infection. The others are pneumonia, cholecystitis, peritonitis, osteomyelitis, sepsis, newborn meningitis, perineal abscess and cholangitis and they are not limited with these (Baysal, 2004; Özkuyumcu, 2009; Halkman, 2005).

Two important pathogenic groups (pathotypes) are found in this group. These are: Uropathogenic *E. coli* (UPEC) and Meningitis/Sepsis related *E. coli* (MNEC) (Akçelik et al., 2000; Halkman, 2005; Stockbine et al., 2014)

2.6.1.1 *Uropathogenic E. coli* (UPEC)

UPEC is related with urinary tract infections (UTI), which is the most common bacterial infection in humans (Özkuyumcu, 2009; Stockbine et al., 2015; Batt & Tortorella, 2014). In the U.S of America, UPEC strains cause 70–90% and 50% of community acquired and nosocomial UTI's, respectively in the U.S.A (Özkuyumcu, 2009; Batt & Tortorella, 2014). *E. coli* strains that cause urinary system infections are called uropathogen. For a strain to be uropathogen depends on some virulent factors. *E. coli* strains that belong to O1, O2, O4, O6, O7 and O75 serogroups frequently cause urinary system infection (Özkuyumcu, 2009). UTI which is associated with UPEC that doesn't have a single phenotypic profile was claimed to be related with various virulent factors including different types of toxins and adhesins, have been claimed to be involved in the pathogenesis of UPEC. These factors have been found in different percentages among subgroups of UPEC (Batt & Tortorella, 2014).

2.6.1.2 *Meningitis-Sepsis-Associated E. coli* (MNEC)

MNEC infection can cause severe neurological lesions that cause 20-40% death in newborns (Özkuyumcu, 2009; Batt & Tortorella, 2014). More than 50% of neonatal meningitis cases in the U.S.A are caused by MNEC strains which is a of K1 capsule antigen type (Özkuyumcu, 2009; Stockbine et al., 2015; Batt & Tortorella, 2014; Murray et al., 2007; Brooks et al., 2013). The polysialic K-1 antigen gains MNEC resistance against serum and phagocytic killing. Strains containing K1 capsule antigen at pregnant women are colonized in the gastrointestinal system and they are considered to pass from the mother to baby vertically. The source of infection is usually urinary tracts at the meningitis seen at adults and the strain rarely contain K1 antigen (Özkuyumcu, 2009). Most of the virulent factors of both UPEC and MNEC pathogenesis are encoded by genes located on pathogenicity islands (Batt & Tortorella, 2014).

2.6.2 *Intestinal (Diarrheagenic) E. coli*

E. coli serotypes that cause diarrhea at human today are called as pathogenic, entopathogenic, enterovirulent, diarrheagenic serotypes. These serotypes are categorized under six main groups of enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enterohemorrhagic (EHEC), difuse- adhering (DAEC), antero-agregative (EAggEC) according totheir virulent characteristics, pathogenity mechanism, clinical syndromes and O:H serotypes (Özkuyumcu, 2009; Halkman, 2013; Batt & Tortorella, 2014; Feng, 2012; Food Safety and Inspection Service, 2001; Winn et al., 2006; Feng et al., 2002; Murray et al., 2007). Apart from these groups, there is facultative enteropathogenic (FEEC) group which is rarely seen (Halkman, 2013).

2.6.2.1 *Enterotoxigenic E. coli* (ETEC)

The association of ETEC with diarrhea was accepted at the end of 1960's and beginning of 1970's for the first time (Batt & Tortorella, 2014). It is the most common cause of traveler's diarrhea that is seen at people who travel from regions with high hygienic conditions to regions with lower hygienic conditions and with hot climates (Tayar & Hecer, 2013; Özgül, 2014; Halkman, 2013; Batt & Tortorella, 2014; Winn et al., 2006; Feng et al., 2002; Murray et al., 2007; Brooks et al., 2013). It occurs when food and water contaminated with faeces is orally

taken (Özkuyumcu, 2009; Tayar & Hecer, 2013; Özgül, 2014). It is not informed to spread from people (Özgül, 2014; Özkuyumcu, 2009; Batt & Tortorella, 2014).

Two important virulent factors play role at the pathogenesis of infection that develops with ETEC strains; namely adhesion and enterotoxin. Adhesion molecules that are coded by plasmide and connected to special receptors at microvilluses at the small intestine. Adhesion molecules that are called colonization factor antigens (CFA/I, CFA/II) are merely found in ETEC strains (Özkuyumcu, 2009). The other virulent factor that play a role at pathogenesis are two enterotoxins that are coded at plasmid by ETEC strains. These are LT (labil toxin) and ST (stabil toxin) (Özkuyumcu, 2009; Tayar & Hecer, 2013; Akçelik et al., 2000; Halkman, 2013; Batt & Tortorella, 2014; Feng, 2012; Karmali, 1989; Winn et al., 2006; Feng et al., 2002). ST and LT are divided into two groups namely Group I and Group II (Akçelik et al., 2000; Özkuyumcu, 2009). Determinated genes of these toxins are coded at 30 MDa plasmid (Akçelik et al., 2000). It is understood that those that cause illness at human are ST I and LT I that are in Group 1 (Özkuyumcu, 2009; Akçelik et al., 2000). The structure and function of LT are similar to cholera toxin (Özkuyumcu, 2009). Adenylate cyclase activity is stimulated and cause anions (chlorine) to exit from cells, taking sodium inside decreases and consequently diarrhea occurs when excessive fluid is excreted to intestine (Özkuyumcu, 2009; Karmali, 1989). ST 1 causes loss of fluid by causing cyclic guanosine monophosphate formation (Özkuyumcu, 2009). Adenylote cyclose of LT intestinal cell stimulate guanylotecylose of ST and eventually accumulation of cyclic AMP (adenosine mono-phosphate) and GMP (guanosine monophosphate). Consequently these cyclic nucleotides cause juicy circle (Tayar & Hecer, 2013). In order for the agent to cause poisoning at adults, it is required to be 10^8 rate per gram of the food. However, young, old and disabled people may be sensitive to lower levels (Tayar & Hecer, 2013; Halkman, 2013; Batt & Tortorella, 2014; Feng et al., 2002). At infections that develop with ETEC strains, watery defecation together with cramps and stomach ache is seen. No blood or mucus is found at faeces. Vomiting and fever are not seen or they can rarely be seen (Özkuyumcu, 2009; Özgül, 2014; Murray et al., 2007). The disease limits itself within approximately 3-5 days (Özkuyumcu, 2009; Özgül, 2014). Due to its highly contagious dose, analysis is not carried out for ETEC unless high levels of *E. coli* are found in a food (Feng et al., 2002). At the same time ETEC causes watery diarrhea at newborn and young domestic animals including calves, lambs and pigs;

however it does not infect grown up animals (Batt & Tortorella, 2014). O6, O8,O15, O20, O25, O63, O78, O85, O115, O128ac, O148, O159, O167 serotypes are included in this group (Akçelik et al., 2000; Halkman, 2013; Nataro & Kaper, 1998).

2.6.2.2 *Enteropathogenic E. coli* (EPEC)

EPEC strain is the first *E. coli* that was accused of being associated with diarrhea at children in 1945 in the United Kingdom (Batt & Tortorella, 2014). It is the factor of diarrhea at babies particularly younger than six months in poor and developing countries throughout the world (Baysal, 2004; Özkuyumcu, 2009; Tayar & Hecer, 2013; Özgül, 2014; Akçelik et al., 2000; Batt & Tortorella, 2014; Karmali, 1989). Human is its main reserve (Tayar & Hecer, 2013; Halkman, 2013). People working at food industry and sewage water play role in food contamination (Tayar & Hecer, 2013; Feng et al.,2002). It can be contagious from people (Özgül, 2014). Pathogenity of EEC has not been determined thoroughly (Baysal, 2004; Tayar & Hecer, 2013). These serotypes typically show a different model of local adhesion (local adherence) on HeLa and HEp-2 cells (Murray et al., 2007). They produce attaching and effacing lesion at EPEC microvillus membrane. Attachment and effacement process is carried out by eaeA gene, which is chromosomally coded (Batt & Tortorella, 2014). These strains generally do not produce enterotoxin; however they can cause diarrhea (Baysal, 2004; Batt & Tortorella, 2014). EPEC strains hold on to intestinal cells with EspA filaments and inject intimin receptor molecule to epithelial cells by using type III secretion system (Özkuyumcu, 2009; Akçelik et al., 2000). For intimin to be hold, which is accepted as an important virulent factor, triggers a series of incidents to begin in the cell. Calcium concentration in the cell increases, cell proteins become phosphorylated and consequently tyrosine protein kinase activity is triggered and calcium oscillates. As a result of that, diarrhea occurs when microvillus is destructed and absorption at intestine is disrupted (Özkuyumcu, 2009). Genes that determinate intimins are found in plasmide with a magnitude of 50-70 MDa (Akçelik et al., 2000; Karmali, 1989). EPEC group contains multiple serovars that are resistant against most of the antibiotics (Baysal, 2004).

Diarrhea together with vomiting can be seen at diarrhea that develops with EPEC strains (Özkuyumcu, 2009; Halkman, 2013; Batt& Tortorella, 2014; Murray et al., 2007). Either fever does not increase to too high levels or high fever doesn’t exist (Özkuyumcu, 2009; Özgül, 2014).

It is assumed that 10^6 organism is the contagious dose of EPEC (Halkman, 2013; Feng et al., 2002). Blood and mucus in faeces is rare. The onset of the disease can be seen in a short time such as 4 hours (Batt & Tortorella, 2014). It is an infection that limits itself generally within a week (Özkuyumcu, 2009).

EPEC infections are associated with chronic diarrhea; however sequel malabsorption, malnutrition, loss of weight and growth failure can also be seen (Murray et al., 2007). Infections that are caused particularly with O111 serogroup can result in death at children, people suffering from malnutrition and babies within first month (Özkuyumcu, 2009).

In addition to human, EPEC can be contagious for animals including livestock, dogs, cats and rabbits (Batt & Tortorella, 2014).

026: H11, 026: NM, 055: NM, 055: H6, 055: H7, 086: NM, 086: H34, 086: H2, 0111: NM, 0111: H2, 111 H12, 0111: H21, 0114: H2, 0119: H6, 0125ac: H21, 0126: H27, 0127: H21, 0127: NM, 0127: H6, 0128ab: H2, 0142: H6 and 0158: H23 are found in traditional EPEC O: H serotips that were informed (Nataro & Kaper, 1998; Karmali, 1989).

2.6.2.3 *Enteroinvasive E. coli* (EIEC)

EIEC bacteria cause dysenteri form growth mainly at children as well as adults. The table of the disease shows similarity with *Shigella spp*. and it causes ulcerous and purulent distorted lesions and diarrhea with colitis format with the same characteristics by spreading into intestinal mucosa (Baysal, 2004; Tayar & Hecer, 2013; Özgül, 2014; Akçelik et al., 2000; Batt & Tortorella, 2014; Karmali, 1989; Feng et al., 2002; Murray et al., 2007; Brooks et al., 2013). They generally do not produce enterotoxin; however they carry a wide plasma with regards to their enteroinvasive characteristics (Baysal, 2004; Batt & Tortorella, 2014; Adams & Moss, 2008). Chill, trembling, fever, abdominal cramps and dysentery (with blood and mucus) are seen among its main symptoms (Tayar & Hecer, 2013; Özgül, 2014; Batt & Tortorella, 2014). Unlike other *E. coli*, these strains are immobile and lactose negative similar to *Shigella spp.* (Özkuyumcu, 2009; Feng et al., 2002; Brooks et al., 2013). Its incubation period is among 8-44 hours with an average of 26 hours (Tayar & Hecer, 2013). Infectious dose is approximately 10^6 bacteria (Özkuyumcu, 2009). Its contagion happens when contaminated water and food is taken. Infection from human

to human is uncommon (Özkuyumcu, 2009; Tayar & Hecer, 2013). As infective dose of EIEC seems much higher when compared to *Shigella spp.*, the organism is considered to be more sensitive against gastritis acidity (Adams & Moss, 2008). It was determined that virulent genes that cause invasive expansion are located on plasmid with a magnitude of 120- 140 MDa (Akçelik et al., 2000; Adams & Moss, 2008). EIEC is rarely found in the United States and it is also less common in the developing countries in comparison with ETEC or EPEC (Murray et al., 2007).

The most common serovars are O28a, p28e, OU2a, O112c, O124, O136, O143, O144, O152, O159, O164 (Baysal, 2004; Akçelik et al., 2000; Nataro & Kaper, 1998). HUS can be seen particularly at children due to the progress of the disease (Akçelik et al., 2000).

2.6.2.4 *Enteroaggregative E. coli* (EAEC or EAggEC)

EAEC strains can be seen many places in the world at all ages and it can cause chronic, persistent children diarrhea (Özkuyumcu, 2009; Özgül, 2014; Murray et al., 2007). Mild inflammation symptoms (stomach ache and fever) accompany with diarrhea; however, blood or faecal leucocytes are not found in faeces. Diarrhea can permanently last for 14 days (Batt & Tortorella, 2014). EAEC strains show adherence to HEp-2 and HeLa cells. Their surface is associated with a plasmid of 90 kb, a specific external membrane protein production and fimbria production (Özkuyumcu, 2009; Batt & Tortorella, 2014). These fimbriae cause the bacterium to create clusters during bacteria reproduction. Therefore they are called aggressive (Özkuyumcu, 2009). In addition, some strains produce plasmid coded EAST1 at the same time (Batt & Tortorella, 2014).

The term of "Typical EAEC" defines organisms which contain virulent genes that are under the control of global EAEC regulator AggR. Typical EAEC can be a common cause for pediatric diarrhea at babies in the USA and it is considered that food sourced outbreaks and diarrhea can be a potential cause at human immunodeficiency virus / AIDS patients (Murray et al., 2007).

The most commons are O3, O15,O44, O86, O77, O111, O127 serotypes (Nataro & Kaper, 1998).

2.6.2.5 *Difusely- adherent E. coli* (DAEC)

An important amount of association between DAEC infections and juicy diarrhea at children at 1-5 ages. Recently, DAEC strains showing diffusely adherent pattern at HEp-2 cells are shown as the cause of diarrhea in some epidemiologic studies (Özkuyumcu, 2009; Akçelik et al., 2000). The presence of two separate adhesion genes and eventually the presence of intimin have been found (Akçelik et al., 2000). Pathogenesis and clinic haven't been explained completely for DAEC (Özkuyumcu, 2009).

2.6.2.6 *Enterohemorrhagic E. coli* (EHEC)

Even though, *E. coli* is ordinarily a harmless bacteria found in the gut, in themid-1900s, scientists began uncovering strains of *E. coli* that could cause life-threatening diarrhea (Batt & Tortorella, 2014).

EHEC bacterium was found by Konowalchuk et al in 1977 for the first time and it was also found to show cytotoxic effect on Vero (African green monkey) cells and produce a toxin that is called verotoxin (VT) as it caused the death of these cells. Therefore, these pathogens were called verotoxigenic *E. coli* (VTEC) (Konowalchuk, Speirs, & Stavric , 1977).

Verotoxin, which plays a role in the infection to be formed, shows exactly the same similarities with the toxin that is caused by *Shigella dysenteriaetype 1*; therefore it is also called shiga-like toxin (Özkuyumcu, 2009; Winn et al., 2006). Consequently it is also alternatively named as *E. coli* that produces Shiga toxin (STEC) (Özkuyumcu, 2009; Food Safety and Inspection Service, 2016; Winn et al., 2006; Food Safety and Inspection Service, 2016). The studies revealed that there are at least two toxins VTI and VTII; however, due to their similarly to shiga toxin have also been called shiga-like toxin, SLT1 and SLT2 (Adams & Moss, 2008; Karmali, 1989; Brooks et al., 2013). It has been proposed that the nomenclature for these toxins be rationalised as shiga family toxins so that the prototype toxin shiga toxin is designated as STX, and SLT1 and SLT2 become stx1 and stx2 respectively (Adams & Moss, 2008).

As a result of two key epidemiologic observations, EHEC has been defined as a separate class than pathogenic *E. coli*. The first of these observations has been reported by Riley et al in 1982

for the first time (Nataro & Kaper, 1998; Food Safety and Inspection Service, 2001; Feng et al., 2002). These scientists researched two outbreaks, which caused a different digestive system disease that was characterized with intense stomach aches with cramps and bloody diarrhea following watery diarrhea. The disease was called Hemorrhagic Colitis (HC) and it was associated with taken uncooked hamburgers at a fast food restaurant in the body. O157:H7, which is *E. coli* serotype, was rarely isolated in faeces samples taken from the patients. The second key observation has been conducted by Karmali et al in 1983. These scientists reported the relation of rarely seen conditions of HUS, which is associated with *E. coli* that produces cytotoxin and fecal cytotoxin in the faeces. It is known that HUS (which is characterized with kidney failure, microangiopathic anemia and thrombocytopenia and it is defined as a triple syndrome) is typically formed before bloody diarrhea diseases and it is important to be distinguished from (HC). These two key clinical microbiological observations, one of which is based on *E. coli* serotype and the other on a specific cytotoxin, increase the importance of intestinal pathogen class that cause intestinal and renal diseases (Nataro & Kaper, 1998).

STEC-hemolysin, which is coded at 60 MDa plasmid is found in EHEC; whereas STEC-intimin that causes accumulation on intestinal epithelium cells is found (Akçelik et al., 2000; Brashears et al., 2002; Karmali, 1989). Furthermore, heat resistant enterotoxin (EAST1) and serine proteases are among virulent factors (Akçelik et al., 2000).

E. coli strains (Serotype *O157:H7* and serotype *O157:H*$^{-}$), which are particularly in O157 in EHEC epidemiology, have more significance as they show very high virulent effect (Akçelik et al., 2000; Murray et al., 2007).

EHEC may transmit to human from food, water and human (Nataro & Kaper, 1998; Food Safety and Inspection Service, 2001). The natural reservoir of EHEC strains are beefs (Özkuyumcu, 2009; Özgül, 2014; Kılıç, 2011; Nguyen & Sperandio, 2012). Consuming undercooked beef meat that was contaminated with the bacteria is one of the most important way of transmission (Tayar & Hecer, 2013; Brashears et al., 2002). In addition, the other ways are unpasteurized milk, unpasteurized juice, lettuce, spinach, sprouts, frozen cookie dough that has been commercially produced lately, sausages, hamburger, ground beef, apple juice, mayonnaise and

contaminated water (Stockbine et al., 2015; Feng, 2012; Adams & Moss, 2008; Brashears et al., 2002; Feng et al., 2002).

When EHEC strains that are resistant to gastric acid produce verotoxin, the infection dose is quite low (Özkuyumcu, 2009). Unlike other *E. coli* infections, taking 100 or less bacteria through mouth is sufficient for the infection to be formed in EHEC (Özkuyumcu, 2009; Özgül, 2014; Stockbine et al., 2015; Eblen, 2007; Feng et al., 2002). EHEC strains are usually a contributing cause for hemorrhagic (bleeding) colitis. Usually blood is seen in faeces in the disease. Nevertheless, only diarrhea is seen among some patients. Stomach ache and vomiting is seen, there is no fever or fever is very little (Halkman, 2013).

01:NM, 02:H5, 02:H7, 04:NM, 04:H10, 05:NM, 05:H16,06:H1, 018:NM, 018:H7, 025:NM, 026:NM, 026:H11, 026:H32, 038:H21, 039:H4, 045:H2, 050:H7, 055:H7, 055:H10, 082:H8, 084:H2, 091:NM, 091:H21, 0103:H2, 0111:NM, 0111:H8, 0111:H30, 0111:H34, 0113:H7, 0113:H21, 0114:H48, 0115:H1O, 0117:H4, 0118:H12, 0118:H30, 0121:NM, 0121:H19, 0125:NM, 0125:H8, 0126:NM, 0126:H8, 0128:NM, 0128:H2, 0128:H8, 0128:H12, 0128:H25, 0145:NM, 0145:H25, 0146:H21, 0153:H25, 0157:NM, 0157:H7, 0163:H19, 0165:NM, 0165:H19, and 0165:H25 are found among EHEC serotypes (Karmali, 1989).

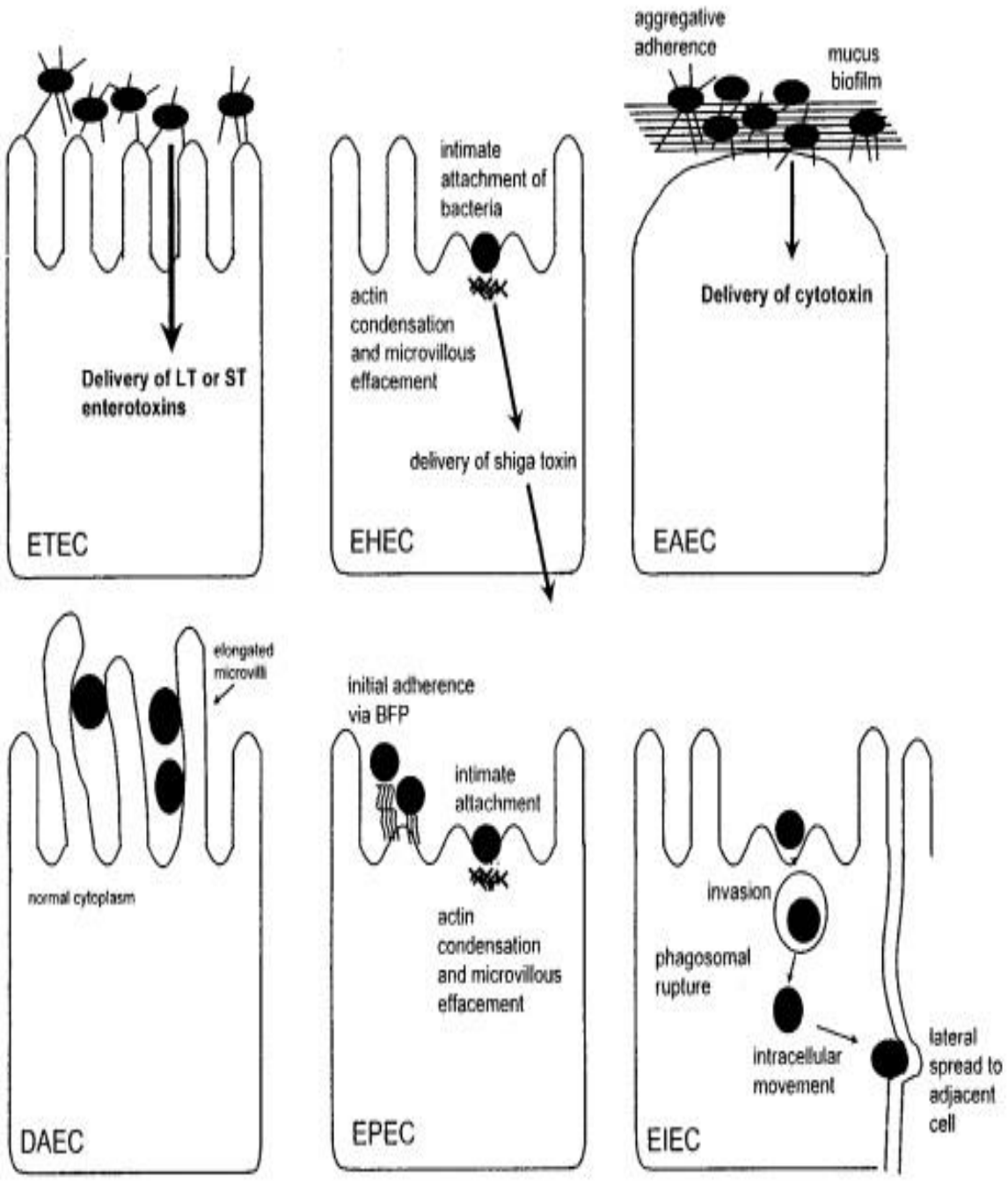

Figure 2.1: Pathogenesis of pathogenic *E. coli* groups (Nataro and Kaper, 1998)

Table 2.2: Major characteristics of the intestinal pathogenic *E. coli* pathotypes (Batt & Tortorella, 2014)

Pathotype	Clinical symptoms	Infections dose (cfu)	Main virulence factors	Location of main virulence genes
ETEC	Travellers' diarrhea, and profuse diarrhea in babies	10^8	Heat- labile and/or heat-stable enterotoxins	Plasmid
EIEC	Dysentery	$>10^6$	Invasion of colonic epithelial cells	Plasmid
EPEC	Diarrhea	10^8 -10^{10}	Locus of enterocyte effacement	Pathogenicity island on choromosome
EAEC	Diarrhea	10^{10}	Biofilm formation, secretory enterotoxins and cytoxins	Plasmid and chromosome
EHEC	Diarrhea, hemolytic colitis and hemolytic uremic syndrome	<50-100	Verotoxin and/or locus of/or locus of enterocyte effacement	Pathogenicity island and integrated lambda phage on choromosome
DAEC	Diaarrhea and extraintestinal infections	N/A	Induction of cellular projections from small intestine enterocytes	Chromosome or plasmid

2.7 *E. coli O157:H7* Serotype

Since 1982, *E. coli O157:H7* has been known as a type that produces enterohaemorrhagic, verotoxin or shigatoxin and it is the most common among the pathogenic *E. coli* group (Food Safety and Inspection Service, 2001; Kılıç, 2011). It is called *E. coli O157:H7* serotype as it represents 157. Somatic (O) antigens and 7. Flagellar (H) antigens (Batt & Tortorella, 2014; Winn, et al., 2006). *E. coli O157:H7* serotype that is associated with EHEC is accepted as the factor of a range of haemorrhagic colitis and haemolytic uremic syndromes where foods such as undercooked ground meat, raw milk and fresh produce have been implicated (Adams & Moss, 2008; Kılıç, 2011; Nguyen & Sperandio, 2012; Murray et al., 2007). An exponential rise in isolations of O157:H7 was reported in Canada between 1982 and 1986 and a study in the UK

between 1985 and 1988 suggested that the increased reporting of isolations there represented a real increase. The number of cases in the UK continued to increase until 1977 and has fluctuated between 600 and 1000 isolations per year since then (Adams & Moss, 2008).

The outbreak, which occurred in Japan in summer 1996, is assumed to be resourced from a food that is called 'daikon sprouts' (radish sprouts), which is added in salads and food as garniture and even being consumed directly. It was also found in Japan in a desert, which is handmade and which is similar to Turkish delight and in a food, which is called 'O-benta' and which is prepared with rice, raw fish, salad and chicken or pork meat (Akçelik et al., 2000).

The resources of *E. coli O157:H7* that were seen in the USA between 1982 and 1994 were stated to be ground meat, human carrying the disease factor, vegetables, salads consumed at fast food restaurants, drinking and pool waters, steaks, raw milk and apple juice respectively; however the source of only 27,9% of the outbreaks has been determined (Akçelik et al., 2000). *E. coli O157: H7* was determined as a pathogen when the outbreaks continued in a widespread state and when the prevalence of the notified cases increased (Food Safety and Inspection Service, 2001).

According to up to date data, the most common outbreak that was seen after the EHEC outbreak in Japan in 1996 was in the outbreak in Germany (Kuşoğlu & Yaman, 2011). *E. coli O157:H7* serotype was known as the only foodborne VTEC before 2011 (Halkman, 2013). While the pathogen that caused the outbreak in Japan was the O157:H7 serotype, the serotype that caused the outbreak in Germany was first considered to be EHEC as it had the ability to produce Shiga toxin; however then it was understood that it was the rarely seen *E. coli O104:H4* serotype (Kuşoğlu & Yaman, 2011; Chattaway et al., 2011). As a result of genetic analysis, it was shown that the pathogen had 93% genetic homology with EAEC strain which rarely affects in foodborne serious incidents. Therefore, it was found that the cause of the outbreak was an EAEC strain, which gained the ability to produce Shiga toxin, and which is a type of O104: H4 (Feng, 2012).

Moreover, although *E. coli O157:H7* is the most dominant strain right now and despite the fact that it constitutes 75% of EHEC infections throughout the world, other non-O157 EHEC

serotypes also occur as the factor of foodborne diseases (Feng, 2012; Brashears et al., 2002; Murray et al., 2007).

2.8 Non-O157 Serotype

E. coli (*non-O157 STEC*) that produces non-O157 Shiga toxin emerges as an important public health problem (Eblen, 2007). More than 150 *non-O157 STEC* serotypes were isolated from people that had diarrhea or HUS (Murray et al., 2007). Some *non-O157 STEC* have the same virulent factors with *E. coli O157: H7* including locus of enterocyte effacement (LEE), Shiga toxin production and other plasmid mediated factors and can cause serious diseases or death (Eblen, 2007).

Non- O157 STEC has been found in ground beef meat and skin and faeces of cattle at levels that can be compared with *E. coli O157* (Eblen, 2007).

At least two additional virulent factors, which are used in the distinction of pathogen and non pathogen EHEC serotypes today, exist;

1. 'A/E phenotype' that is coded by eae gene

2. p0157 plasmid that can express EHEC hemolysin and various adherence factors.

E. coli strains of approximately 200 serotypes can express Stx. However, Stx- positive and Stx-negative strains can be found in most of these serotypes. More than 50 of these serotypes are associated with bloody diarrhea or HUS that is seen among people (Nataro & Kaper, 1998). As six *E. coli* serotypes that are O26, 045, O103, O111, 0121 and O145, are called the first non-0157 STEC as they cause more than 70% non-O157 STEC infections in the USA (Batt & Tortorella, 2014; Murray et al., 2007). The latest outbreaks which have been emerged due to these organisms have been reported in Japan, Germany, Italy, Australia, Czech Republic and the U.S.A. Between 5 to 234 organisms can be sufficient for these outbreaks to emerge. The source of infection couldn't be found in most of these infections. It is considered that 20-25% of HUS incidents that were seen in the North America occurred because *of non-O157:H7 EHEC*. In some countries such as Chile, Argentina and Australia, *non-O157:H7 EHEC* serotypes are the cause of the most of the HUS incidents. Non-O157:H7 EHEC strains are usually isolated

from patients that suffer from non-bloody diarrhea. In a study conducted in Belgium, more than 62% of Stx-producing *E. coli* strains that were isolated from faeces were found to be non-O157; whereas, only 32% were found to be O157:H7. In Seattle, *E. coli* strains that produce non-O157:H7 Stx are found in 1.1% of routine faeces samples. The isolation rate is higher than *Shigella spp*. and *Yersinia spp*. (0,2%); whereas lower than *Campylobacter* (2,5%) and *Salmonella spp.* (3,4%) or *E. coli O157:H7* (2,9%). Approximately the half of *E. coli* isolates that produce Stx that were isolated from patients in Boston and Virginia are non-O157:H7 serotypes (Nataro & Kaper, 1998). Non- O157 food outbreaks have been given in Table 2.3

Table 2.3: Some selected foodborne outbreaks of non-O157 EHEC serogroups (Batt & Tortorella, 2014)

Food	Country	Serogroup	No. of cases	No. of deaths
Fenugreek seeds	Germany	O104	3816	54
Ice cream	Belgium	O145:H28 and O26:H11	12	0
Fermented beef Sausage	Denmark	O26:H11	20	0
Cured mutton Sausage	Norway	O103:H25	17	1
Venison	United States	O103:H2 and O145:NM	29	0
Milk	United States	O111	24	0
Restaurant cross contamination	United States	O111:NM	341	1
Romaine lettuce	United States	O145	58	0
Raw clover sprouts	United States	O26	29	0

2.9 *E. coli O157:H7* Intestinal Colonization

Diseases related to *E. coli* infections are considered to involve colonization of the intestine and damage due to toxins. Infection of EHEC begins with entry of the bacteria through food or water taken in the mouth. Acid resistance of EHEC facilitates their survival through the low pH of the stomach. The bacteria pass through the small intestine, and virulence genes are turned on by environmental signals in the colon. The EHEC adhere to the enterocytes of the colon in a

characteristic intimate adherence and cause effacement of the microvilli. If sufficient Stx is produced, local damage to blood vessels in the colon results in bloody diarrhea. If sufficient Stx is absorbed into the circulation, vascular endothelial sites rich in the toxin receptor are damaged, leading to impaired function. The kidneys and central nervous system are sites that frequently are affected and HUS may develop (Batt & Tortorella, 2014).

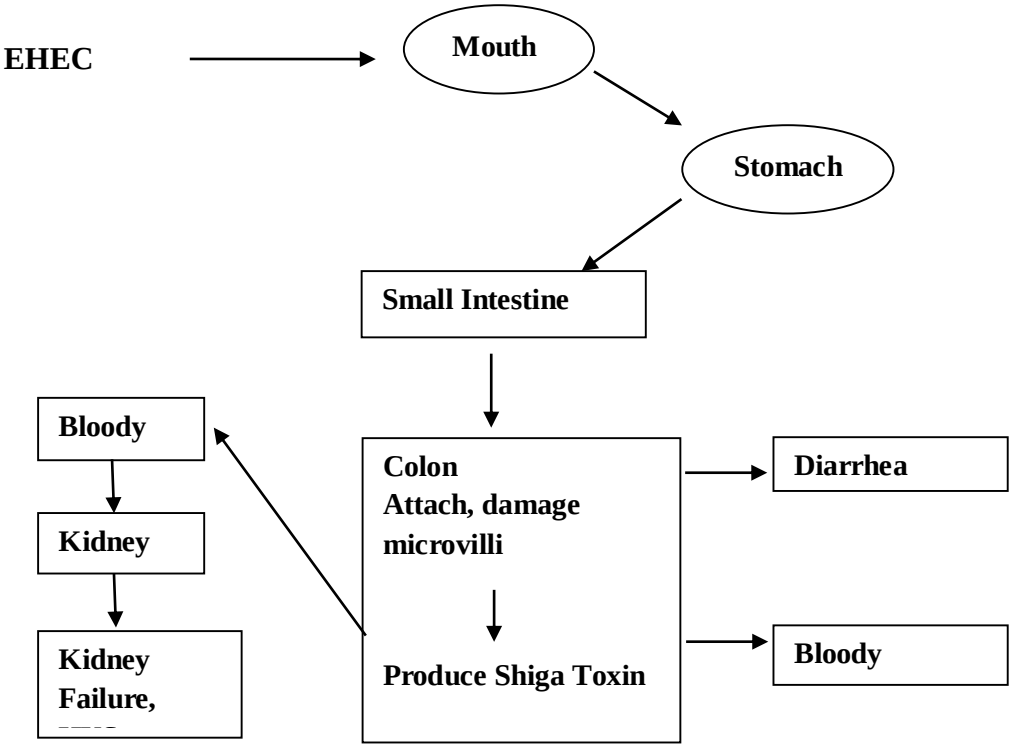

Figure 2.2 : Overview of disease in humans due to EHEC (Batt & Tortorella, 2014)

Adherence to intestinal epithelium cells is the most important characteristics of STEC infection. The interaction between STEC and epithelium cells has a difference between eae positive and eae negative STEC strains. Eae-positive STEC strains are found on characteristic AE lesions on intestinal epithelium cells. Even though AE lesions are not an important factor for bloody diarrhea and HUS that are seen in people, most of the strains that are responsible for these syndromes have eae gene. Thus, most of the EHEC strains are defined as eae-positive and eae gene is stated as an important risk factor at haemolytic uremic syndrome cases (Batt & Tortorella, 2014).

STEC strains have a pathogenicity island (PAI) that is named as locus of enterocyte effacement (LEE) and that codes the bacterial proteins that are necessary for the formation of AE lesion (Batt & Tortorella, 2014; Feng, 2012; Pihkala et al., 2012; Winn et al., 2006; Halkman, Noveir, & Doğan, 2001).

The similarities between the development of the pathogenicity island that is called *Enteropathogenic E. coli* (EPEC) LEE and AE lesions that are the response of EPEC infection enable similar cases of STEC to be understood. LEE is composed of 5 major polycistronic operons that are named as LEE1, LEE2, LEE3, LEE4 and LEE5. The products of LEE operations are effector proteins that are composed of type III secretion apparatus (LEE1, LEE2 and LEE3), protein translocation system (LEE4) and adherence system consisting of an outer membrane protein called intimin or eae and its receptor, translocated intimin receptor (TIR), LEE5 that codes these receptors and that are translocated by the secretion systems (Batt & Tortorella, 2014).

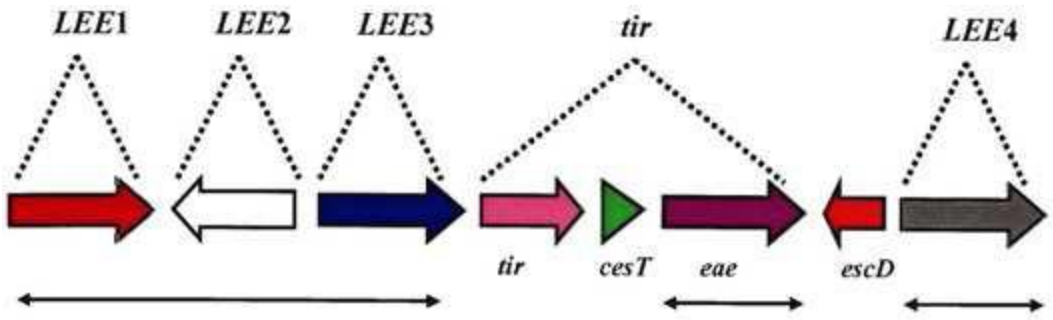

Figure 2.3: Tir operon structure of LEE locus of STEC from LEE1 to LEE4 (Ramachandran, 2002)

The secretion apparatus is a molecular syringe structure, which begins inside the bacterial cytoplasm, extends through the inner and outer membranes and passes through the host cell membrane. Secreted proteins are transferred from the bacterial cytoplasm to the host cell through this structure. The secreted proteins that are encoded by LEE include TIR,

mitochondrion-associated protein, EspF *(E. coli* secreted protein F), EspG, EspH and EspZ. Most of the proteins that code non LEE are settled by LEE secretion apparatus. TIR protein is settled inside the cell membrane of the host cell and acts like a receptor at the bacterial level for the intimin gene. TIR and other secreted proteins activate a number of stimuli and play a role in reconstruction of intestinal epithelium cells in the cell physiology. EspJ, which is a secretion protein coded by non LEE, is expressed as an antivirulent factor. Erasing this protein indicated that survival period is longer experimentally in lambs and mice and an association was found between the pathogen passage and host life (Batt & Tortorella, 2014).

2.10 Virulens factor STEC:

Among the most important virulence characteristics of *E. coli O157* is its ability to produce one or more Shiga Toxin (also called verocytotoxins, and formerly known as Shiga-like toxins). Shiga toxin is the critical virulence factor in Shiga toxin–producing *E. coli* (STEC) diseases (Batt & Tortorella, 2014). The Shiga toxin that was found first is Stx 1, verotoksin 1 (VT1) and it is the same with the Stx that was produced by *S. dysenteriae type 1* (Halkman, Noveir, & Doğan, 2001; Stockbine et al., 2015; Batt & Tortorella, 2014; Feng, 2012; Winn et al., 2006; Wılson, 2007; Nataro & Kaper, 1998). The second one, Shiga toxin 2, represent a family of Shiga toxin (Wılson, 2007) and is a more divergent molecule, with only 56% amino-acid homology with ST 1 (Halkman, Noveir, & Doğan, 2001; Batt & Tortorella, 2014). Recently, a variant of Shiga toxin (Stx1), called Stx1c, was reported, and this variant is most commonly found in strains of ovine origin and may be found as the only Stx subtype or in combination with other subtypes (Batt & Tortorella, 2014). This toxin type was not found in eae-positive STEC and has been associated with mild or no disease in humans. In contrast, there are several antigenic variants of Stx2, named Stx2c, Stx2d, Stx2d activatable, Stx2e, and Stx2f that differ in their biological activity and association with disease (Table 2.4). Stx2 is most often associated with severe sequelae, such as hemolytic uremic syndrome, which is characterized by acute renal failure (Feng, 2012). Other variants of Stx have been reported, but there is no information on their clinical significance (Batt & Tortorella, 2014). The Stx toxins are composed of five B subunits (7.7 kDa each) and a single A subunit (32 kDa) (Halkman, Noveir, & Doğan, 2001; Batt & Tortorella, 2014; Wılson, 2007; Nataro & Kaper, 1998; Karmali, 1989; Winn et al., 2006). A subunit binds to disulside link A1 (28 kDa) and A2 (4 kDa) link (16). The B subunit

binds to globotriaosylceramide (Gb3), a glycolipid of unknown function found to varying degrees in membranes of eukaryotic cells (Batt & Tortorella, 2014; Nataro & Kaper, 1998; Winn et al., 2006). While Gb3 is the main receptor for Stx, the Stx2e variant uses GB4 as the receptor. Stx2e is classically associated with pig edema disease rather than human disease, but occasional strains that express only this variant are isolated from patients with HUS or diarrhea (Nataro & Kaper, 1998). The A subunit enzymatically inactivates the 60S ribosomal subunit, thus blocking protein synthesis. Although they possess the same mechanism of action, there is only 55% identity in amino-acid sequence between the B subunits of Stx1 and Stx2. The B subunit possesses enzymatic activity that enables the toxin to cleave a specific adenine base from the 28 S rRNA and thereby prevent protein synthesis (Batt & Tortorella, 2014). The resulting disruption of protein synthesis leads to the death of renal endothelial cells, intestinal epithelial cells, Vero or Hela cells, or any cells which possess the Gb3 receptor (Nataro & Kaper, 1998).

Table 2.4 : Virulens factors of STEC (Batt & Tortorella, 2014)

Virulence factor	Characteristics
Shiga toxins	Cytotoxic proteins that are the principal virulence factor of STEC
Stx1	Shiga toxin produced by STEC and almost identical to Stx produced by *Shigella dysenteriae* serotype 1
Stx1c	Variant of Stx1 that is found in some eae-negative STEC; associated with no symptoms or mild diarrhea in humans
Stx2	Prototype of nonStx1 toxins; associated with severe disease in humans
Stx2c	Associated with diarrhea and HUS in humans; common in ovine STEC
Stx2d	Associated with eae-negative STEC and mild disease in humans
Stx2dact	Vero cell cytotoxicity is increased 10- to 1000-fold by elastase in intestinal mucus; strains with this toxin are highly virulent
Stx2e	A variant responsible for edema disease of pigs; rare in human disease and associated with mild diarrhea or asymptomatic infections in humans
Stx2f	A variant frequently isolated from pigeon droppings; rare in human disease
Adherence	LEE-encoded intimate adherence system; induces AE lesion formation. Includes genes for TTSS; intimin; translocated intimin receptor; Esp B, F, G, H, Z; non-LEE-encoded effectors.

Yılmaz et al. (2003) carried out a study to detect VT1, VT2 and eaeA genes and to determine the frequency of these genes in *E. coli* O157:H7 strains isolates from cattle, cattle carcasses and abottoir environment. As a result of multiplex- PCR investigation that was conducted with DNA

samples extracted from O157:H7 strains, 5 strains were found to be positive for VT1 – VT2 – eaeA genes, 18 strains VT2 – eaeA and 3 strains for only eaeA gene. Therefore, it was stated that preventive measurements are necessary to prevent contamination of *E. coli* O157:H7 to carcasses at slaughterhouses.

2.11 Biochemical and Antigenic Characteristics:

E. coli O157:H7 serotype is distinguished from other *E. coli* for being 44.5 ^{o}C, MUG negative, not having β – glucuronidase enzyme, but having eae gene, carrying 60 mDa plasmid and not commonly seen OMP expression at 5000-8000 Dalton molecule weight and enterohemolysin production (Akçelik et al., 2000; Halkman A. K., 2013; Halkman, Noveir, & Doğan, 2001). *E. coli O157: H7* is easily distinguished from other enteric *E. coli'*s biochemically; because while sorbitol is slowly fermented, usually other *E. coli* ferments sorbitol easily (Food Safety and Inspection Service, 2001). Formation of enterohemolysin is only created by verotoxin positive *E. coli* O157:H7 and *E. coli* O157:H^{-} serotyoes (Halkman, Noveir, & Doğan, 2001; Akçelik et al., 2000). Together with these differences, the serotype shows more resistant to acid in comparison with other serotypes; thus its importance increases (Halkman, 2013). According to WHO, sorbitol positives are also found among STEC O157 strains. *E. coli O157:H*$^{-}$ is positive (Halkman, Noveir, & Doğan, 2001). Except for these facts, *E. coli O157:H7* serotype is less resistant to bile salts than other *E. coli* (Halkman, Noveir, & Doğan, 2001; Akçelik et al., 2000). Biochemical characteristics of *E. coli O157:H7* strains are given in Table 2.5.

Tablo 2.5 : Biochemical Characteristics of *E. coli O157:H7* (Winn et al., 2006)

Biochemical Test	***E. coli O157:H7***
Indole	+
Methyl red	+
Voges- Proskauer	-
Citrate	-
Lysine decarboxylase	+
Argininine dihydrolase	V (17)
Ornithine decarboxylase	V (65)
ONPG	+
Fermentation of :	
Lactose	+
Sorbitol	-
Mannitol	+
Adonitol	-
Cellobiose	-
Yellow pigment	-

+, 90% or more strains positive; -, 90% or more strains are negative; V, 11-89% of strains are positive.

O157 antigenic determinant of *E. coli O157:H7* is found in polysaccharide section of cellular lipopolysaccharide of the bacterium. As a result of the analyses, the determinant was defined as linear polymer of tetrasaccharide units that are composed of D-glucose, L-fucose (6-deoxi- L-galactose), 2-acetamido-2-deoxi-D-galactose, 4-acetamido-4, 6-dideoxi-D mannose (1:1:1:1) and that are recurred (Halkman, Noveir, & Doğan, 2001).

MUG reaction, which is typical in other *E. coli*, is negative in *E. coli O157:H7* serotype. It can be said that MUG negative *E. coli O157:H7* isolates are verotoxin positive (Halkman, Noveir, & Doğan, 2001).

Serologic association among *E. coli* strains was first determined by Dodgeon et al. in 1921 for the first time and in 1937 Lowel claimed *E. coli* had 2 types of antigens that are capsule and somatic. Then in 1943, Kauffman showed the flagellar antigen. According to that, 165 somatic O antigens that are demonstrated among O1-O171, 90 capsule K antigens that are demonstrated among K1-K90 and 56 flagellar H antigens that are demonstrated among H1-H56 have been determined in *E. coli*. In accordance with the latest studies, 174 O, 56 H and 80 K antigens are found today. There are a significant amount of cross reactions among O antigens of *E. coli* and

Salmonella spp., Shigella spp., Citrobacter and *Providencia type* bacteria (Halkman, Noveir, & Doğan, 2001).

2.12 Development and Survival:

E. coli O157:H7 serotype develops at optimum 37 ºC and pH 7,2 like other *E. coli*. Most of the studies carried out for the development of *E. coli O157:H7* serotype, are for the isolation studies of this serotype (Halkman, Noveir, & Doğan, 2001).

Various studies show that *E. coli O157:H7* that plays a key role in foodborne hemorrhagic colitis cases is acid-resistant and the tolerance helps it pass through the strong acid environment of stomach easily (Halkman, 2013; Halkman, Noveir, & Doğan, 2001; Tosun & Gönül, 2003). This is accepted as a factor that affects the infection dose of this bacterium to be very low at people having acid-resistance. Unlike *Salmonella spp.*, it stays alive during digestion that lasts for approximately 3 hours in human stomach that has 1-2 pH value and it passes to the intestine from the stomach and thus the relation is explained (Halkman, Noveir, & Doğan, 2001). Even though growing characteristics of *E. coli O157* generally look similar with *E. coli* types, *O157:H7* serotype has an atypical tolerance for acid. The acid tolerance helps *E. coli O157: H7* to survive from traditional fermentation process for fermented dried meat and sausages (Batt & Tortorella, 2014).

Acidic food such as mayonnaise, unpasteurized apple juice, fermented rigid salami and cheese show an important characteristic of *O157*:H7; which is the characteristic of the ability of *E. coli O157:H7* to grow in low pH conditions in food, in which other pathogens cannot survive. The organism can adapt to acidic conditions and thus, it was found that *E. coli O157:H7*, which can stay alive for 31 days at 8 ºC in cider, can maintain its liveliness at meat salad that contains more than 40% mayonnaise and whose pH value is 5,40 - 6,07, it can also stay alive for a couple of weeks at pH 4,2 and even it can develop in characteristic pH of meat salad and acetic, lactic and citric acids are efficient respectively (Halkman, 2013; Halkman, Noveir, & Doğan, 2001).

E. coli O157:H7 was aimed to be adapted to acid by exposing to 1, 2, 3, 4 hours (acid shock method) and 18 hours (adapting to acid method) at pH 4.5, 5.0, 5.5 in the study conducted by

Tosun (2003). As a result of the study, cells that were adopted to acid did not gain acid tolerance; whereas cells that were exposed to acid shock gained acid tolerance according to the control culture and it was found that the culture, which was adapted to acid at pH 4,5, gained acid tolerance at maximum level ($p<0,05$). In another study, *E. coli O157:H7* was adapted to acid by enculturation once and twice at pH 5.0. Cells that were adapted with acid showed an increased resistant against lactic acid and they were informed that they survived for a longer period during the fermentation of sausage according to the cells that were not adopted and moreover, the survival was increased at minced dry salami (pH 5.0) and cider (pH 3.4) (Leyer, Wang, & Johnson, 1995).

In the study carried out by Yapar, microbial infections, which are resourced from insufficient and faulty implementations during slaughter, breaking into pieces, processing and storing stages, were aimed to be reduced by using acid extract made of plum (plum extract), pomegranate syrup and citric acid, whose antibacterial affects are known. In ground meat control samples, Enterobactericeae count was found between $10,5\times10^3$ - 25×10^3 kob/g gap, coagulase between *Staphylococcus* $4,2\times10^2$ - $7,1\times10^2$ kob/g and the total number of aerobic mesophilic microorganism was between the gap of $10,4\times10^5$ – $11,9\times10^5$ kob/g and microorganisms of coliform group were between the gap of 21-460 EMS/g, *E. coli* 15-240 EMS/g. In the ground meat samples, which were proceeded with natural pomegranate syrup, acid extract made of plum (plum extract) and citric acid, a reduction in the numbers of total aerobic mesophilic organism *E. coli, Enterobactericeae,* Coagulase negative *Staphylococcus* and it was stated that antibacterial effects of pomegranate syrup, acid extract made of plum (plum extract) and citric acid were seen (Yapar, 2006).

E. coli O157:H7 serotype also shows resistance to high salt concentration (Halkman, Noveir, & Doğan, 2001). *E. coli O157:H7* can also develop at 6.5% NaCl according to the study conducted by Glass et al. The incubation effect of NaCl begin at 8.5% concentration and it can develop in fluid media that contain 200 ppm nitrite and 4,0% NaCl and that have pH 5,6 level. It was found that it was reduced in fermented sausages that had 3,5% NaCl and 69 ppm sodium nitrite with pH 4.8; yet it was not inhibited totally (Glass et al., 1992). Another study indicated that *E. coli O157:H7* serotype reached 10^8 kob/ml level at 3,5% and 6,5 NaCl % concentrations that were

added to mTSB broth medium as a result of an incubation period of 30-40 hours (Halkman, Noveir, & Doğan, 2001).

Enterohemorrhagic E. coli can develop in milk and ground meat with the presence of a low number of accompanying flora at 8 ºC; however, no development occurs at the presence of high amount of accompanying flora at this temperature. The presence of high amount of accompanying flora prevent *Enterohemorrhagic E. coli* serotypes to develop, the presence of high amount of accompanying flora keep staying alive even though it doesn't show any development at 5 ºC temperature, risk continues among the food that is kept at 5 ºC temperature and the risk increases for temperatures at 8 ºC or higher temperatures (Halkman, 2013).

E. coli O157:H7 is not resistant to heat. It is killed at pasteurization conditions that are carried out at suitable conditions. Therefore, products that will be consumed without applying heating process are required to be implemented another process that will eliminate *E. coli O157:H7* (Tosun & Gönül, 2003).

Methods to eliminate *E. coli O157:H7* in fresh meat, dairy products, products that are consumed raw and fermented products should be developed. Furthermore, routine methods that ensure the determination of all EHEC types are required to be developed and the roles of other strains which are not O157:H7 and which do not produce verotoxin in foodborne poisoning cases should be determined as well. This probability should be taken into consideration and reconsidered among the standards that were prepared for the food, which is possible to be infected with *E. coli O157:H7* (Tosun & Gönül, 2003).

E. coli O157:H7 which is transmitted on the meat during slaughter can pose a risk for fermented meat products, which are not processed with any heat and whose microbiological safety depends on only fermentation and drying. In the studies that were conducted on the behaviour of *E. coli O157:H7* on fermented meat products, it was found that fermentation and drying processes cause a 1-2 log unit reduction on the number of pathogens and it can survive for 2-3 months while being stored at +4 ºC. It was determined that pH, NaCl concentration, fermentation and

storing temperature, type of storage, amount of humidity and starter usage are effective on the liveliness of the organism on fermented meat products (Çoşansu & Ayhan, 2000).

Gama radiation as an alternative to the usage of chemical and preservatives for reducing the number of *E. coli O157:H7* in food was assessed to be effective. Gamma beam is an electromagnetic radiation that beams from the reaction of cores of elements such as Co60 and Cs137 that are side products of atomic fusion. It was stated that gamma radiation, which is applied as a dose between 1.5 and 3.0 kGy for the elimination of *E. coli O157:H7* in chicken meat and ground beef meat that is detached mechanically is very effective. Gamma radiation D10 value for *E. coli O157:H7* that is exposed to radiation at fixed phase under 0°C under vacuum for both meats is 0.27 kGy (the dose that is necessary for 90% of the present organism or decimal decrease to be eliminated). This serotype showed a bigger resistance to the effects of gamma radiation at temperatures that are below freezing. D10 value at chicken meat, which is obtained mechanically at –5°C, is 0.44 kGy (Thayer & Boyd, 1993).

In another study, Doğan Halkman analyzed the death kinetics of *E. coli O157:H7* and *E. coli* type 1 after irradiation doses of ground beef ranging from 0.0 kGy to 1.5 kGy and during storage conditions -18 ^{0}C for 30 days. D_{10} values of *E. coli O157:H7*, *E. coli* type 1, and natural contaminant coliforms were 0.245 kGy, 0.552 kGy and 0.293 kGy, respectively. Furthermore, an irradiation dose of 1.5 kGy was shown to inactivate 10^5 MPN/g of serotype O157:H7 and 10^3 MPN/g of *E. coli* type 1. This inactivation level might be considered safe for the consumption of ground meat and is a suitable irradiation indicator (Doğan Halkman, 2003).

2.13 Source and Transmission

There are different opinions on the source of *E. coli O157:H7* serotype. EHEC is usually accepted as zoonotic origin (Batt & Tortorella, 2014; Aslantaş, Erdoğan, & Bulut, 2004). Firstly main source of this bacterium was considered to be dairy cows and its transmission to human was through dairy products; however, no finding that would confirm this hypothesis was found in the conducted studies (Halkman, Noveir, & Doğan, 2001). *E. coli O157:H7* serotype is found that it is transmitted to meat, milk, soil, water and therefore to the whole environment through

the faeces of warm-blooded animals mainly ruminants (beef and sheep), dog and birds (Batt & Tortorella, 2014; Aslantaş, Erdoğan, & Bulut, 2004; Halkman, Noveir, & Doğan, 2001). The reason for that is the proof that uncooked beef meat and in many cases and raw milk in rare cases were caused by this serotype (Adams & Moss, 2008; Halkman, Noveir, & Doğan, 2001). Briefly, all food substances, drinking and domestic water that are contaminated with faeces directly or indirectly are defined as medium in *E. coli O157:H7* infections (Akçelik et al., 2000; Stockbine et al., 2015; Batt & Tortorella, 2014; Adams & Moss, 2008; Nataro & Kaper, 1998; Kılıç, 2011; Halkman., 2013; Halkman, Noveir, & Doğan, 2001). Except for meat products, cheese, unpasteurized milk and dairy products, salads and salad dressings, homemade sandwich, radish sprouts, unpasteurized cider and fresh squeezed apple juice, mayonnaise, orange juice, clover and turnip cabbage, lettuce, spinach, fruit, nuts, strawberry, pizza and cookie dough are food sources that cause *E. coli* outbreaks as well (Batt & Tortorella, 2014; Adams & Moss, 2008; Winn et al., 2006; Halkman, Noveir, & Doğan, 2001).

It is known that the most important transmitter in outbreaks is human and human to human transmission of an outbreak is seen at fast rate at places, where personal hygiene is not sufficient such as preschools and flophouses particularly (Halkman, Noveir, & Doğan, 2001). Moreover, transmission of harmful bacteria that are found in raw ground meat or in their beef broth by contacting on other food through cutting boards, kitchen utensils and similar utensils is an important cause for the transmission of diseases (Brashears, 2002).

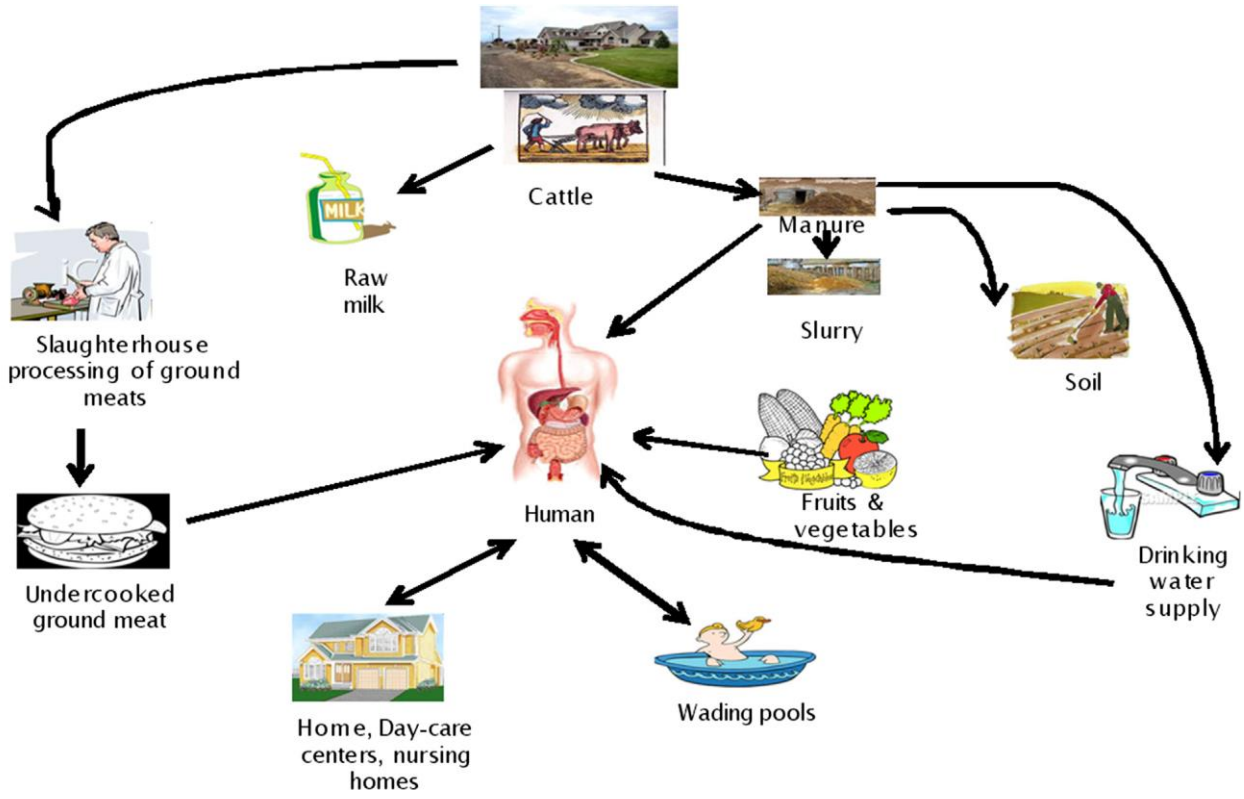

Figure 2.4: Sources of *E. coli* infection (Batt & Tortorella, 2014)

Studies on the evolution of pathogen bacteria continue intensively. Genetic analyses that are carried out on *Escherichia coli*, *Salmonella spp.* and *Shigella spp.* species show that *E. coli O157:H7* serotype is not an individual pathogen and the theory that it was evolved from an enteric bacterium is widely adopted. While Commensal *E. coli* choose the intestines of mammals, it is accepted that pathogen *E. coli* exceed intestinal epithelium and localized in the circulatory system where it deems suitable (Halkman, Noveir, & Doğan, 2001). Understanding critical factors in survival of EHEC and colonization in cattle would assist developing alternative strategies for preventing EHEC's transmission on environment and eventually preventing human infection (Nguyen & Sperandio, 2012).

EHEC contamination may occur during the slaughter and stripping the skin of carcass and it is mainly resourced from animal skin, faeces or gastrointestinal contents (Batt & Tortorella, 2014; Gün et al., 2001). Therefore, it is important in terms of transmission of this pathogen with

excrement to know the frequency of infection with this factor. Its reason is that cattle carry the factor in their gastrointestinal in an asymptomatic manner and they contaminate the environment and food with their excrement (Aslantaş, Erdoğan, & Bulut, 2004).

According to FSIS, the main focus of some farm slaughter is that *E. coli O157:H7* prevalence in cattle herds in farms are assessed before they are send to slaughter and in farms, *E. coli O157:H7* is generally tested to determine whether they exist in the herds or not. A faeces test which is used to determine the presence of colonized cattle whose intestinal tracts are infected with *O157:H7*. Cattle, whose tests made for *E. coli O157:H7* in their skin, hair or hooves are resulted positive, are marked as infected with *E. coli O157:H7* (Brashears, 2002). Cleaning visible sections of carcasses, washing carcass (with hot water) and pasteurizing with steam are included in the process of decreasing EHEC contamination risk during skipping process of the skin of carcass (Batt & Tortorella, 2014).

In the study carried out by Gün et al. (2001), they conducted the search of *E. coli O157:H7* frequency in cattle carcasses and in the excrement of cattle that are brought to slaughterhouses in Istanbul for slaughter. *E. coli O157* was isolated in 14 (4.2%) of the excrement of cattle and 12 (3.6%) of these isolates reacted with H7 antiserum. Moreover, 12 of cattle carcasses were isolated with O157 and 8 of them were informed to show reaction with H7 antiserum (Gün et al., 2001). In another study conducted in Hatay, *E. coli O157* was isolated and identified in 77 of 565 analyzed cattle. It was also informed that 77 *E. coli O157* strains (%85.7) that were isolated were determined as *E. coli O157:H7* (Aslantaş et al., 2004).

In another study, enterohemolysin *(EhlyA)* and intimin *(eaeA)* virulence genes of 14 *E. coli O157:H7* strains, which were isolated from 457 fecal samples (237 calves and 220 cattle), were determined with PCR method. While *EhlyA* gene was determined in 13 (92.8%) strains, the *eaeA* gene was positive in 8 (57.1%) strains. Of the 8 *eaeA* genes, 4 (50.0%) were obtained from diarrheic calves, 2 (25.0%) from non-diarrheic calves, and 2 (25.0%) from healthy cattle. With this study, it was confirmed that especially diarrheic calves and cattle are a reservoir off *E. coli 0157:H7* strains that may be pathogenic for human (Kuyucuoğlu et al., 2011).

2.14 *E. coli O157:H7* Ways of Spread

EHEC usually spread through consumption of contaminated food and water, human-to-human transmission, contact with animals or through contact with media that are contaminated with faeces (Stockbine et al., 2015; Batt & Tortorella, 2014; Feng, 2012; Nataro & Kaper, 1998).

The transmission of *E. coli* O157:H7 on human can be explained under 4 main titles (Batt & Tortorella, 2014).

1) Food-to-Human,
2) Human-to-Human,
3) Water-to-Human,
4) Animal-to-Human.

2.14.1 Food-to-Human Transmission:

75% of *E. coli O157:H7* infections are considered to be foodborne (Batt & Tortorella, 2014). Any kind of food, drinking and domestic water that are contaminated with animal faeces and mainly cattle faeces directly and/or indirectly pose a threat in terms of *E. coli O157:H7* (Akçelik et al., 2000; Stockbine et al., 2015; Batt & Tortorella, 2014; Adams & Moss, 2008; Nataro & Kaper, 1998; Halkman, Noveir, & Doğan, 2001; Halkman, 2013).

Most of the infections throughout the world are caused by mainly cattle sourced food such as raw or not inadequately cooked meat and meat products (hamburger being in the first place) and unpasteurized milk. Ruminants other than cattle and other animals are informed to be *E. coli O157:H7* source or vector as well (Akçelik et al., 2000; Stockbine et al., 2015; Batt & Tortorella, 2014; Feng, 2012; Adams & Moss, 2008; Demir, 2006; Halkman, Noveir, & Doğan, 2001; Halkman, 2013).

The most important food that mediates on the transmission of *E. coli O157:H7* beef ground meat (Akçelik et al., 2000; Feng, 2012; Wılson, 2007). Furthermore, it is isolated from meat products that are prepared with ground meat, pork, sheep, chicken meat and hamburger and meatballs being in the first place (Nataro & Kaper, 1998; Winn et al., 2006; Halkman, Noveir, & Doğan, 2001). *E. coli O157:H7* outbreak was determined to be caused by a hamburger in

fast food restaurant in 1982 for the first time (Batt & Tortorella, 2014; Winn et al., 2006; Feng, 2012). Its biggest outbreak, which was reported in the North America in December 1992 and January 1993, was resourced from hamburgers that were eaten from a fast food chain. In a study, it was determined that homemade hamburgers were an important source of O157:H7 as well and in another study, it was informed that infections were resourced from uncooked hamburgers being exposed to cross contamination by those who prepare the food instead of being directly taken in the body (Nataro & Kaper, 1998). Nevertheless, it was stated that uncooked hamburgers caused 600 people to got sick and death of 4 children in the outbreak that was seen in the US in 1993 (Adams & Moss, 2008). In addition, food such as deer meat, sausages, dried (not heat processed) salami are determined as the resource of contamination (Batt & Tortorella, 2014).

When outbreaks related with hamburgers were compared at the beginning, the diversity of media on illnesses caused by EHEC increase in time (Nataro & Kaper, 1998). The pathogen, which is also found apart from meat products and which causes outbreaks, has been seen as the cause of various infections that were resourced from various cheese, unpasteurized milk most commonly and even from dairy products such as yoghurt, salads and salad dressings, homemade sandwich, turnip sprouts, unpasteurized cider and fresh squeezed apple juice that were generally defined as safe food. Mayonnaise, orange juice, clover and turnip cabbage, lettuce, spinach, fruit, nuts, strawberry, pizza and cookie dough are determined as food sources that cause *E. coli O157:H7* outbreaks as well (Stockbine et al., 2015; Batt & Tortorella, 2014; Feng, 2012; Winn et al., 2006; Halkman, Noveir, & Doğan, 2001).

Unpasteurized fruit juice was the factor that causes the outbreak, which took place in mid 1990s. An outbreak occurred in Japan in 1996 which was sourced from turnip sprouts and affected 10000 people and it was recorded as the biggest outbreak that has occurred (Akçelik et al., 2000). Later numerous outbreaks that were caused by food such as lettuce, salads, various sprouts and spinach were informed. The outbreak which was seen in the U.S. in 2009 was informed to be caused by frozen cookie dough (Feng, 2012).

Generally food has been reported as infection medium for 131 separate outbreaks, in which 22600 people affected in total and 530 people developed HUS. It was also confirmed to cause the death of 90 people. 43 of 131 outbreaks were caused by cattle products; whereas 25 were

sourced from vegetables and fruits and 19 from milk and dairy products (Batt & Tortorella, 2014). According to the data obtained from CDC, foodborne outbreaks among 2006-2016 are given in the table below.

Table 2.6: Foodborne *E. coli O157:H7* Outbreaks among 2006 – 2016
(Reports of Selected E.coli Outbreak Investigations, 2017)

Year	Food	Syptom	Case Count	States	Hospitalization	Death
2006	Fresh Spinach	HUS and kidney failure	199	26	102	3
2006	Taco Bell	HUS and kidney failure	71	5	53	0
2007	Totino's and Jeno's Frozen Pizza	HUS and kidney failure	21	10	8	0
2008	Ground beef from Kroger/Nebraska ltd.	HUS and kidney failure	49	7	27	0
2009	Prepackaged Cookie Dough	HUS and kidney failure	72	28	34	0
2009	Beef from JBS Beef Company	HUS and kidney failure	23	9	12	0
2009	Beef from Fairbanks Farms	HUS and kidney failure	26	8	19	2
2010	Beef from National Steak and Poultry	HUS	21	16	9	0
2010	Cheese	HUS	38	5	15	0
2011	In-shell Hazelnuts	-	8	3	4	0
2011	Lebanon Bologna	-	14	5	3	0
2011	Romaine Lettuce	HUS	58	9	33	0
2012	Organic Spinach and Spring Mix Blend	HUS and kidney failure	33	5	15	0
2013	Ready- to- Eat Salads	HUS	33	4	7	0
2014	Ground beef	-	12	4	7	0
2015	Costco Rotisserie Chicken Salad	HUS and kidney failure	19	7	5	0
2016	Beef products Produced by Adams Farm	HUS and kidney failure	11	5	7	0

2.14.2 Human-to-Human Transmission

Even though consuming undercooked food products that are animal origin is seen as the probable way of transmission in most of the infections seen in human, there are increasing proofs that VTEC infection may affect people through human-to-human transmission (Karmali, 1989).

Human-to-human transmission can occur in daycare centers, hospitals, nursing homes, and private residences and can cause *E. coli O157: H7* outbreaks. Because the infectious dose is so small, it is very easy for the bacteria to be transmitted among people with close physical contact. The most important way of human-to-human transmission is the mode of transmission through the oral–fecal route. An asymptomatic carrier state has been reported, in which individuals show no clinical signs of disease but are capable of infecting others (Batt & Tortorella, 2014).

As patients that have clinical findings are frequently hospitalized, other patients and healthcare personnel including laboratory staff can be under the risk of EHEC infections. Occurrence of this kind of infection cases that were transmitted from hospital and laboratory show the rightness of this concern. Therefore hospital infection control teams are required to be aware of this potential threat and to review the working procedures so as not to be exposed to these kinds of infections (Coia, 1998).

In a conducted study, 50 outbreaks were determined to be transmitted through the oral–fecal route. 40 (80%) of them included day care centers, 5 (10%) included private residence, 3 (6%) included community, 1 (2%) included school and 1 (2%) included residential facility and it was informed that these outbreaks peaked during the summer (Rangel et al., 2005).

In an outbreak, which was among preschool children that were probably infected after drinking raw milk from a farm, secondary cases were seen among the family members of some children and that suggested that infection spreads in the family. Furthermore, a nurse, who worked at a pediatric nephrology unit, developed HUS in connection with *E. coli 0157: H7* infection. The infection was considered to be transmitted by contacting with a child that had HUS as a result of the studies that were carried out. The situation is revealed as the strain, which infected both the child and the nurse, had identical phage type and biotype pattern (Karmali, 1989).

Thus far, 23 outbreaks have been recorded. 943 were affected in these outbreaks in total and 1 person was confirmed dead. 67 people developed HUS. Most of the reported human-to-human *E. coli O157:H7* outbreaks have been observed in nursing home, day-care centers, or hospitals (Batt & Tortorella, 2014).

2.14.3 Water-to-Human Transmission

E. coli O157:H7 is associated with outbreaks that cause high morbidity and mortality throughout the world and that is transmitted through water. Water intended for recreation (e.g., pools, shallow lakes) and for human consumption also can become contaminated with faeces and can cause infection. When lakes become contaminated, several weeks or months can be required for water-quality conditions to improve or return to normal. EHEC also has been isolated from bodies of water (ponds, streams), wells, and water troughs, and has been found to survive for months. Waterborne transmission has been reported, both from contaminated drinking water and from recreational waters as well (Batt & Tortorella, 2014). Therefore, it becomes important to analyze *E. coli O157:H7* prevalence in water sources that are particularly used for drinking and to develop diagnostic methods for early detection (Saxena, Kaushik, & Mohan, 2015).

Water used for drinking or recreation has been reported as the vehicle of infection for 54 outbreaks: 7 outbreaks associated with water parks and pools; 23 with lakes, springs, canals, and streams; 10 with well water; 11 with ‘drinking water’; and 3 with tap water. Fecal material from ruminant animals, domestic or wild, is the probable source of *E. coli O157:H7* in lakes, streams, and wells and for some ‘drinking water’ outbreaks (Batt & Tortorella, 2014).

In summer 1991, bloody diarrhea and hemolytic uremic syndromes that were caused by *E. coli O157:H7* that was sourced from a park that was near a lake near Portland Oregon were seen. 21 people (all of them were children with an average age of six) were determined that had *E. coli 0157: H7* infection associated with the park. Their illness was not associated with food or drink consumption. All of the cases were associated with the swimming of children. Therefore lake water that is contaminated with *E. coli O157:H7* infection and that is used for swimming purposes is determined to be one of the most possible media for the transmission of the infection (Keene et al., 1994).

A major *E. coli O157:H7* infection took place in Alpin in Wyoming State in summer 1998. 157 patients were found in the illness, which was associated with drinking unchlorinated municipal water (Olsen et al., 2002).

In another conducted study, 31 outbreaks that was transmitted through water was informed and 21 of them was determined to be sourced from water intended for recreation and 10 from drinking water. Outbreaks that are caused from water intended for recreation were informed in 1991 for the first time; 14 (67%) of these outbreaks occurred in lakes or ponds and 7 (33%) in swimming pools. It has also been informed that outbreaks that are caused from contaminated drinking water occur more common than other type of outbreaks. Outbreaks that are sourced from contaminated drinking water constitute 3% of all of the outbreaks and 15% of cases that are linked with the outbreak. 4 of the outbreaks was considered to be from well water systems, 3 from municipal water supply system and 1 from cross contamination of spring water, residential tap water and ice. It was found that outbreaks were caused as 2 of the 3 municipality water supplier did not use chlorine and the other one used out of order chlorinator (Rangel et al., 2005).

2.14.4 Animal-to-Human Transmission

Outbreaks that are resourced from contamination with animals were reported in the United States in 1996 for the first time and they are one of the most recent recognized ways of transmission (Rangel et al., 2005).

E. coli O157:H7 infection can be transmitted to human from animals. Most of the animal resourced *E. coli O157:H7* outbreaks were noted to be observed in animals in farms, fairs or zoos. The way of transmission in visiting fairs that are held for agriculture, zoos or farms had been considered to be limited with the way of receiving from hand-to-mouth as a result of contact with contaminated surfaces or contact with animals; however according to the indicators that are seen lately, it was understood that inhaling dust particles also cause infection. Therefore, animal-to-human transmission has been determined as an important risk factor for EHEC infection for public. EHEC takes approximately a week to be eliminated from the body. Although it takes less time in adults, it can take longer time in children.

Among the 11 outbreaks that were informed in a study, it was stated that cow or calves were exposed directly or indirectly. 5 of these 11 outbreaks occurred in farms, 2 in district fairs, 2 in zoos, 1 in a stable and 1 in a camp (Rangel et al., 2005). According to another source, 26 outbreaks have been stated so far and 757 people were affected from these out breaks in total. It was confirmed that 65 people developed HUS and 1 person was killed (Batt & Tortorella, 2014).

The way of transmission, percentages, HUS development and death rates of *E. coli 0157:H7* outbreaks that occurred among 1982 – 2011 are given in Table 2.7 below.

Table 2.7: For the period of 1982–2011, there were 234 *E. coli O157:H7* outbreaks (27 564 cases) (Batt & Tortorella, 2014)

Mode of transmission	Outbreaks	Illness	Death	HUS
Foodborne	131 (56%)	75%	90	530
Waterborne	52 (22%)	18%	51	142
Animal or their environment	26 (11%)	3%	01	65
Person to person	23 (10%)	3%	06	67

2.15 Diseases Caused by the Bacteria

Foodborne diseases that are caused by *Diarrheagenic E. coli* (DEC) have clinical, public health and economic importance. Annual treatment costs and the cost of labor loss of illnesses that are caused only by *E. coli O157:H7* serotype is estimated at 229-610 million US$. According to the estimations of USA Centers for Disease Control and Prevention, the total of foodborne microbiologic diseases only in the USA are 76 million cases per year, 300,000 of them are treated; whereas 5,000 deaths are seen and *E. coli O157:H7* is regarded as responsible for 20,000 cases and 250 deaths.

Diseases that are caused by *E. coli O157:H7* create three main disease tables that are HUS and Trombotic Thrombocytopenic Purpura (TTP) whose courses are typical and quite strong (Tayar & Hecer, 2013; Halkman, 2013; Halkman, Noveir, & Doğan, 2001; Feng, 2012; Adams & Moss, 2008; Pihkala et al., 2012; Brashears et al., 2002; Karmali, 1989).

2.15.1 Hemorrhagic colitis

Hemorrhagic colitis was found in Michigan and Oregon in 1982 for the first time and 47 hemorrhagic colitis cases were determined. Case control studies show that the illness was associated with eating hamburgers from a well known fast-food restaurant chain (Karmali, 1989).

EHEC strains are bleeding intense intestinal inflammation factor for children under 5 years old. 1-2 days later consuming contaminated food, symptoms begin with abdominal cramps and watery diarrhea (Adams & Moss, 2008; Karmali, 1989). Severe bloody diarrhea is seen and all of the faeces keeps being composed of blood (Özkuyumcu, 2009; Adams & Moss, 2008; Brashears et al., 2002; Halkman A. K., 2013). Stomach ache and sometimes vomiting can be seen. There is no fever or it can be seen rarely (Halkman, 2013; Karmali, 1989). Hemorrhagic colitis is an acute disease that usually lasts for 4-10 days and that limits itself (Adams & Moss, 2008). The disease is separated from dysentery that is defined with shigellosis and gastroenteritis caused by invasive *E. coli* as there is no fever and it contains bloody faeces (Halkman, Noveir, & Doğan, 2001).

2.15.2 Hemolytic Uraemic Syndrome (HUS)

The connection between VTEC and HUS was found in 1985 for the first time (Karmali, 1989; Halkman, Noveir, & Doğan, 2001). It is seen in children under 10 years old that are infected with O157:H7 (Özkuyumcu, 2009). HUS is an disease which causes death. HUS shows three symptoms, acute renal failure (acute nephropathy), thrombocytopaenia (a drop in the number of blood platelets) and microangiopathic haemolytic anaemia (reduction in the number of red blood cells) (Özkuyumcu, 2009; Halkman, 2013; Adams & Moss, 2008; Karmali, 1989; Winn et al., 2006; Murray et al., 2007). It is estimated that 0-15% of hemorrhagic colitis cases develop HUS in average. HUS can cause permanent loss of renal function (Halkman, Noveir, & Doğan, 2001; Brashears et al., 2002). HUS has subgroups that are typical and begin with prodromal bloody diarrhea and that are atypical and contain phase with diarrhea (Halkman, Noveir, & Doğan, 2001; Karmali, 1989). Upper and lower respiratory tract symptoms, fever and vomiting can be seen (Karmali, 1989). Furthermore, icterus, frequently hypertension and heart failure can also be seen (Halkman, Noveir, & Doğan, 2001). It is most common in children among whom it is

the leading cause of acute renal failure. Approximately 10% of children under 10 with symptomatic *E. coli O157* infection go on to develop HUS and consequently they will require kidney dialysis and the mortality rate is generally 3–5% (Adams & Moss, 2008; Karmali, 1989). Even though 30% of the survivors may develop chronic renal failure, hypertension or long term permanent disability in a neurological disorder type, modern administration techniques reduced the mortality rate to 10% or less (Karmali, 1989). In 70 cases seen between 1980 and 1986 the fatality rate was 6%, with 13% of cases showing some long-term kidney-damage (Adams & Moss, 2008).

2.15.3 Trombotic Thrombocytopenic Purpura (TTP)

TTP was defined in 1924 for the first time and its clinical and pathologic characteristics are similar with HUS. 271 cases were analyzed, 75% of the patients died in 90 days; whereas other patients were found to have 5 separate clinical characteristics such as fever, thrombocytopenic purpura, microangiopathic haemolytic anaemia, neurogical findings that frequently change and renal function disorder (Karmali, 1989). Blood clot occurs in the brain of patients and consequently it is resulted with death (Brashears et al., 2002; Halkman, Noveir, & Doğan, 2001; Halkman, 2013). The incubation period of the disease is quite variable and it is seen in 2-8 days and sometimes in 12 days. Depending on medical problems, death is mainly seen in elder people (Halkman, 2013; Brashears et al., 2002). On the other hand, it is stated that TTP is rare in *E. coli O157:H7* infections (Halkman, Noveir, & Doğan, 2001).

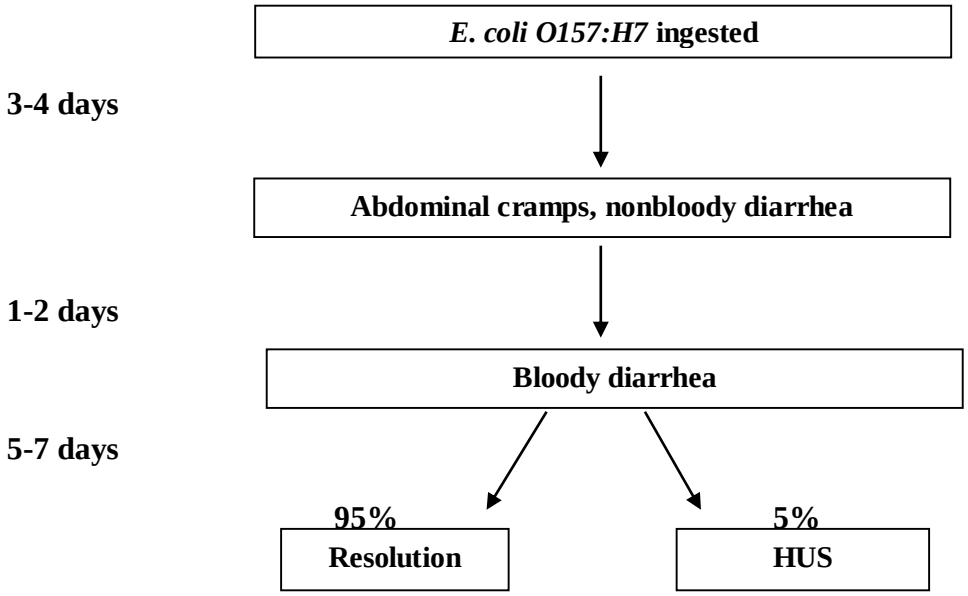

Figure 2.5: Natural history of Infection with *E. coli O157:H* 7 (Batt & Tortorella, 2014)

E. coli O157:H7 infections are more effective in the young people. In a study carried out in Japan, it was clearly showed that young people and children are more sensitive to the disease caused by *E. coli O157:H7* serotype. More than 80% of people under 20, in whose faeces the bacterium was found, showed typical symptoms; whereas 70% of people between 30-46, in whose faeces *E. coli* O157:H7 serotype was found again, did not show typical symptoms (Halkman, Noveir, & Doğan, 2001).

The clinical course among 5 adult patients that were seen in Philedelphia in the USA in 1971 couldn't be explained with known intestinal diseases and when similar diseases, in which no etiological factor was determined, were also reported in other states of USA as well as Europe and Japan, USA Center for Disease Control and Prevention serotyped 300 *E. coli* strains retrospectively in 1973-1982 and the strain, which was isolated from a 50 year old woman from California, who had severe bloody diarrhea, in 1975, was determined as *E. coli O157:H7* (Halkman, Noveir, & Doğan, 2001).

Apart from individual cases, *E. coli O157:H7* was seen in 47 cases, 26 in Oregon and 21 in Michigan USA in 1982 for the first time and both of them were seen in 2 outbreaks that do not

have resemblance with the previous outbreaks. While it was determined that eating meatball sandwiches caused the illness, *E. coli O157:H7* was found in frozen meatballs that had the same party no in one of the outbreaks. Homemade sandwiches were informed to cause an outbreak in Ottawa, Canada in the same year. Similar cases were seen in the USA, Canada and England right after that, then the same disease was found in Mexico, China, Argentina and Belgium and the factor of the outbreak, which caused 16 people to be killed in Japan in summer 1996 was shown as *E. coli O157:H7* (Halkman, Noveir, & Doğan, 2001).

The density of diseases that are caused by *E. coli O157:H7* is accepted as less than 10 of 100.000. In spite of that, patients spread *E. coli O157:H7* for 10 days after the illness occurs; approximately 5% develop HUS and blood in the faeces is seen among less than 50%. However, some resources state the probability of blood to be seen in faeces is 90% (Halkman, Noveir, & Doğan, 2001).

Molecular analyses in outbreaks that begin with 1996 in Japan and sporadic cases have shown that not a single strain but strains with different genotypes that had been spread throughout Japan were responsible. Analyses showed that more than 80% of EHEC strains that were isolated from patients were O157:H7 serotype and in addition, the number of O26 and O111 serotypes gradually increased. Similarly it was stated that VTEC strains, which are not *E. coli O157:H7*, were isolated more from patients with HUS and diarrhea and more than 100 serotypes were isolated from cattle that is the fundamental source in the studies conducted on the cattle population in various countries (Halkman, Noveir, & Doğan, 2001).

2.16 Treatment

Treatment of EHEC disease is limited largely to supportive care (Nataro & Kaper, 1998). In the treatment of EHEC infections, it is fundamental to support lost fluid and electrolytes orally or parenterally. Medicines that prevent or reduce bowel movements are not recommended as they increase toxin absorption (Özkuyumcu, 2009). Even though EHEC strains are generally susceptible to antibiotics, there are no prospective studies showing conclusively that the use of antibiotics alters the outcome of disease (Özkuyumcu, 2009; Nataro & Kaper, 1998).

In a prospective study conducted by Proulx et al., they demonstrated a trend toward a lower incidence of HUS in those receiving antibiotics. Consistent with this study, a retro-spective study conducted during the 1996 outbreak in Japan indicated that early treatment with one specific antibiotic, fosfomycin, was associated with a reduced risk of HUS (Nataro & Kaper, 1998).

The use of antibiotics may be harmful for two potential reasons; first, lysis of bacteria by some antibiotics leads to increased release of toxin, at least in vitro (Özkuyumcu, 2009; Nataro & Kaper, 1998). Second, antibiotic therapy could kill other intracolonic bacteria, thereby increasing the systemic absorption of toxin. Treatment of renal disease due to EHEC is primarily supported, except for some experimental therapies currently being evaluated in clinical trials. Current treatment regimens may include dialysis, hemofiltration, transfusion of packed erythrocytes, platelet infusions and other interventions as clinically indicated. Severe disease may require renal transplant (Nataro & Kaper, 1998).

2.17 Methods Being Used in the Search of *E. coli O157:H7*

Enterohemorrhagic E. coli O157:H7 serotype is one of the most dangerous foodborne pathogen bacteria which are known today. Therefore, it is not allowed to be found in any food or any food raw materials (Halkman, 2005).

There are various methods that can be categorized in two main groups briefly that are classic and developed for the search of *E. coli O157:H7* serotype in various food, clinical and environment samples. When classic and developed methods are considered for the search of *E. coli O157:H7* serotype as for the search of other bacteria, classic methods are advantageous for the cost of consumable materials; however cost of labour force, sensitivity of analysis and time are its disadvantages (Halkman, Noveir, & Doğan, 2001).

Various methods are being worked on for determining *E. coli O157:H7* in food, clinical samples and other materials. Those that are defined as classic among them are based on biochemical tests and they are commonly used in routine test laboratories. Developed tests and mainly serologic methods are usually applied in research laboratories primarily due to their cost and experience factors (Halkman, Noveir, & Doğan, 2001).

2.17.1 Classic Methods

According to the method recommended by the Food and Drug Administration (FDA) / Bacteriological Analytical Manual (BAM AOAC) / International Organization for Standardization (ISO), the search of *E. coli O157:H7* is composed of enrichment, isolation and verification phases (Akçelik et al., 2000). The presence of this bacterium is searched with these tests in a certain amount of sample that is analyzed. Accordingly, the presence of *E. coli O157:H7* in the material is seen respectively as enrichment in a selective liquid medium, cultivation in a selective and distinctive solid media, determining suspected colonies as *E. coli O157* with biochemical and/or latex agglutination tests and finally, determining whether the isolate contain H7 antigen or not (Akçelik et al., 2000; Halkman, Noveir, & Doğan, 2001; Halkman, 2005). In addition, verotoxin analysis are required to be made in isolates, which are found to be *E. coli* O157 (Halkman, Noveir, & Doğan, 2001; Halkman, 2005). *E. coli O157* or other pathogenic *E. coli* serotypes produce one of the two verotoxins that are called VT1 and VT2 or both of them. Instead of determining what the serotype is (whether it is H7 or not), it is required to examine whether a typical colony forms VT1 and/or VT2; as it is important in terms of public health (Halkman, 2005). One or more of the modified media such as modified EC (mEC) broth, modified Soy Broth (mTSB), Laurly Sulphate Triptose (LST) broth, EZ coli enrichment medium, EHEC Enrichment Broth (EEB) and Trypticase Novobiocin (TN) are used in selective media that are used for determining *E. coli O157:H7* serotype with classic methods (Akçelik et al., 2000; Halkman, 2005; Halkman, Noveir, & Doğan, 2001).

In isolating *E. coli O157:H7* by using solid media, sorbitol negative and β-D- glucuronidase negative characteristics of this serotype are being used (Akçelik et al., 2000). According to the standard method, usage of Sorbitol MacConkey Agar (SMAC), Hemorrhagic Colitis (HC) and various modifications of this medium is recommended for the isolation of *E. coli* O157:H7 (Akçelik et al., 2000; Halkman, Noveir, & Doğan, 2001). Selectivity against accompanying flora are tried to be gained with various selective additives such as SMAC Agar medium antiserum, 5-bromo-4-chloro-3-indoxyle-β-D-glucuronic acid cyclohexyl ammonium salt (BCIG), cefixime and tellurite, rhamnose and cefimixe and MUG (Halkman, Noveir, & Doğan, 2001).

The final stage is the definition of typical colonies that develop in the selective solid medium (Akçelik et al., 2000; Halkman, Noveir, & Doğan, 2001). The tests that are used for verification are Latex agglutination tests, Shiga-like toxin (SLTI) I and II tests colony hybridization and other valid tests that are also given in the table below.

Table 2.8: Test kits that are used for the search of *E. coli O157:H7* (Akçelik et al., 2000)

Name of the test	Principle
RIM	Latex agglutination
E.coli O157	Latex agglutination
Prolex	Latex agglutination
Ecolex O157	Latex agglutination
Petrifilm HEC	Immunobloting
EZ COLI	Tube- EIA
Dynabeads	Immunomagnetic separation
HEC O157	ELISA
EHEC- TEK	ELISA
Assurance	ELISA
TECRA	ELISA
E.coli O157	ELISA
VIP	Immunoprecipitation
Reveal	Immunoprecipitation
NOW	Immunoprecipitation
GLISA	Immune flow
Q-Trol *E.coli* O157	Sandwich- EIA

2.17.2 Other Classic Methods

2.17.2.1 H7 Antiserum- Sorbitol Fermentation Method:

Another characteristic that distinguishes *E. coli O157:H7* serotype from other *E. coli* serotypes is that it contains H7 antigen. While only 10% of *E. coli* type-1 strains have H7 antigen, all of *E. coli O157:H7* serotype has this antigen. Using this characteristic, analyses are made by developing H7 antiserum-sorbitol fermentation medium for searching *E. coli O157:H7* (Akçelik et al., 2000).

2.17.2.2 Hydrophobic Grid Membrane Filtration that Contains Enzyme Antibody

This method is successfully used in isolation from fresh meat and raw milk that are naturally contaminated. Samples are prepared as needed and left for incubation according to this method. Suitable distillations of the culture is prepared and passed through hydrophobic grid membrane. *E. coli O157:H7* antiserums, which are hold with immunoblot method on nitrocellulose membranes that are located on the selective solid medium filter. Immuno positive colonies are chosen and taken from the filter, isolates similar to Vero-Cytotoxic *E. coli O157:H7* are defined with biochemical, serological and Vero-cell cytotoxicity tests (Akçelik et al., 2000).

2.17.2.3 Antibody Direct Epifluorescence Technique (Ab-DEFT)

This is a modified method developed for defining *E. coli O157:H7* in milk and juices. In this method, the sample is treated with trypsin and Triton X-100 for 15 minutes after being directly homogenized, then it is passed through a prefilter with 5µm pore diameter, then through black polycarbonate filter with 0,2 µm pore diameter. The last filter is pained with anti-O-157 polyclonal antibody, which is directly marked with fluorescein, then washed and examined under the epifluorescence microscope. The sensitivity of Ab-DEFT method is the same with the standard method and it can be safely used at the presence of *E. coli O157:H7* at 16 kob/g level (Akçelik et al., 2000).

2.17.3 Developed and Fast Methods

Although many infections can be identified with classic methods, sometimes they can be insufficient. It is possible to have false negative results as particularly accompanying flora is masked. Therefore various fast and developed methods have been created (Halkman, Noveir, & Doğan, 2001).

2.17.3.1 GLISA (Gold Labelled Immuno Sorbent Assay) Fast Search and Verification Test:

GLISA test is an immunological screen test, which is developed for searching and verifying *E. coli* O157:H7 in food based on immune flow principle. This is a single stage test method, which is fast and can be easily applied by anyone, and which also has 99,9% sensitivity and 99,4% specifity and it is applied in 20 minutes after the enrichment phase (Akçelik et al., 2000).

2.17.3.2 EZ Coli Fast Search System

EZ Coli is fast immune analysis method for *E. coli O157* that is found standard micropipette. It is composed of two elements as single stage enrichment medium that is selective for other coliform bacteria and EZ coli detector tip for searching *E. coli O157* EZ coli detector tip is a microflament ELISA test in the shape of micro pipette tip that has a 6-month shelf life (Akçelik et al., 2000; Halkman, Noveir, & Doğan, 2001).

2.17.3.3 Immunomagnetic Separation Technique

Immunomagnetic separation (Immunomagnetic seperation) technique is used for searching microorganisms in food materials and in other samples, fast, has high specificity and easy to apply. The principle of this method is that it is based on determining the desired microorganism by using beads that can be magnetized and that are covered with specific immune chemical agents (monoclonal, polyclonal and recombinant antibodies (Akçelik et al., 2000).

In addition, various analyses methods are being used on mainly DNA based tests and immune enzymatic methods. These methods are Immunoassay, Radioimmunoassay (RAI), Fluorescent Immunoassay (FIA), Enzyme Immunoassay (EIA), Immunoperoxidase tests. However, these tests may be a little expensive. Despite being so sensitive, cross reactions and suspicious situations may emerge in some cases and diagnosis may delay. Immunological determination and identification systems provide a remarkable shortening in the analysis period. Latex agglutination, ELISA, colony immunblot analyses, direct immonofluorescence filter and immune catching techniques are used among them. Monoclonal and polyclonal antiserums are used against O and H antigens for that purpose (Halkman, Noveir, & Doğan, 2001).

Nucleic acid based analysis are mainly divided into two groups that are methods, which use DNA Stxs gene or eae gene specific DNA probe and PCR analysis. Stxs gene of these is specific to pathogens that produce only Stx pathogens; however as different *E. coli* serotype and even Citr. Freundii can produce Stx II variants; therefore these tests are not used for determining O157:H7 directly. Likewise, not only *E. coli O157:H7*, but also other EPEC serotypes give positive results for methods that are specific to the eae gene. However, there is no such restriction in analyses related with β-glucuronidase (uidA) gene. Lastly, it is stated that only *E. coli O157:H7* serotype

can use PF-27, which is oligonucleotide DNA probe with 18 bp that is sensitive to a specific region of uidA gene. While multiplex PCR analyses are sensitive, specific and able to determine phenotypic variants of O157:H7 serotype, it is stated that the system is very complex and too expensive to use in routine analyses of food and clinical samples (Halkman, Noveir, & Doğan, 2001).

CHAPTER 3

RELATED RESEARCH

Among food of animal origin, meat is a nutrition, which is rich in terms of vitamins, some minerals (particularly for P and Fe) and high quality proteins, it increases appetite, it is delicious, saturating and easy to produce. It is recommended that 50% of daily protein need should be food of animal origin (Arslan, 2002). Therefore, meat and meat products have a significant role for human nutrition.

Ground meat, which is among meat products, is red meat created by processing raw red mead of butchery animals that are separated from their bones through mincing machine or by manually mincing with a knife or a chopping knife. Only raw red meat, which is obtained from skeletal muscles including connective tissue, should be used when ground meat is being prepared. Ground meat cannot be prepared with any other parts of the animal except for these parts (Gıda Teknolojisi Et ve Ürünleri Analizleri 1, 2016).

Fat percentage and colour are two significant criteria that determine the quality of ground meat. The colour of ground meat should be the same with the colour of the meat, which is used to prepare the ground meat. At the same time when the colour of the meat has a pinkish structure, it caused the increase of fat percentage and reduction of nutrition quality of ground meat (Gıda Teknolojisi Et ve Ürünleri Analizleri 1, 2016).

According to the Turkish Food Codex, fat-free ground meat, ground beef, allowed mixture ground meat and ground pork meat should have fat percentage of $\leq 7\%$, $\leq 20\%, \leq 25\%$, $\leq 30\%$ respectively (Türk Gıda Kodeksi Et ve Et Ürünleri Tebliği , 2016).

Microbiologic criteria determine the level of microorganism or microorganism groups that are allowed to be present in a food sample. According to the Turkish Food Codex Regulation on Microbiological Criteria, *E. coli O157* and Salmonella are not allowed to be found in ground

meat samples; whereas aerobic colonies at $5x10^5$ - $5x10^6$ level are allowed (Türk Gıda Kodeksi Mikrobiyolojik Kriterler Yönetmeliği, 2011).

Başkaya et al. (2004) conducted a study on ready ground meat and informed that the total aerobic mesophilic general living, coliform, *E. coli* numbers are found as $2.7x10^6$, $4.1x10^4$, $7.2x10^3$ kob/g respectively. The average of total aerobic mesophilic bacteria, coliform bacteria, *E. coli* numbers are stated to be found in ground meat samples as $4.7x10^4$ kob/g, $6.0x10^2$ kob/g, $2.8x10^3$ kob/g respectively (Direkel et al., 2010). As a result of the both studies, it is found that the microbiologic criteria of ground meat are not at the desired quality.

In order for the freshness of ground meat to be preserved and slowing down the development of bacteria, ground meat should be kept at 4.4 °C or at lower temperatures and should be used or frozen in two days. Ground meat can stay safe without reproduction of microorganisms when frozen; however it can lose its quality in time. Therefore it should be used within 4 months. To destroy harmful bacteria, it is necessary to cook ground beef until it reaches an inner temperature of at least 71.1 °C (Food Safety and Inspection Service, 2016).

E. coli O157:H7 serotype is an important pathogen that may cause serious human diseases such as bloody diarrhea, HC and HUS (Ertaş et al., 2013). STEC is a significant problem of developed countries. General habitat of STEC strains are intestinal systems of ruminants such as beef and sheep in particular. Most of the food borne infections that are resourced from *E. coli O157:H7* are caused by beef origin food such as raw meat and raw milk (Ertaş et al., 2013).

Ground beef in particular is the main reservoir of *E. coli O157:H7* serotype. Food contaminated with faeces constitutes primary resource of infections at human (Biçer, 2012). In addition, bacteria that are transmitted to meat during slaughter, when the hygiene of slaughterhouse is insufficient, remain their liveliness in food if they are not applied necessary heat process and they are transmitted to consumers. Apart from these, transmitting to cooked food with cross contamination and transmission with milk and dairy products are significant as well (Ertaş et al., 2013).

E. coli O157:H7 is known as one of the most dangerous food borne pathogens. Therefore it is not allowed to be found in any food or food raw material of food (Halkman, 2005). The search

of serotype is divided into two main groups that are classic and developed methods in this framework (Halkman, Noveir, & Doğan, 2001).

70 raw ground beef samples that are obtained from markets and butchers have been searched for the presence of *E. coli* O157:H7 serotype in this study. First of all VIDAS ECPT has been used, then positive results have been verified with Vitek 2 Compact and Latex Agglutination tests.

As a result of the analysis made with VIDAS ECPT, 3 of 70 ground beef samples have been found as *E. coli O157* positive, two of the three positive results have been found as *E. coli O157* and one of the samples, which had been determined as *E. coli O157*, has been determined as *E. coli O157:H7* serotype.

Positive results that have been obtained with VIDAS ECPT have been verified with two different methods. Plantation has been made from the enrichment fluid to selective medium in the first method; whereas in the second method, the enrichment fluid has been immune-concentrated with VIDAS ICE and plantation has been made to selective medium. Reproductions that occurred in media as a result of incubation have been assessed. The presence of more pure colonies has been observed in plantations that were made after VIDAS ICE. Colonies, which were obtained from CT-SMAC Agar and which were purified in Bloody Agar, have been used at the verification of *E. coli* O157 serotype with Vitek 2 Compact. With the Latex Agglutination test, the presence of both *E. coli O157* and H7 serotypes have been made with colonies obtained from CT-SMAC again. Both two verification tests showed positive results in terms of *E. coli O157* serotype with the same samples. In consequence of these results, Vitek 2 Compact and Latex Agglutination tests have verified each other.

In the researches made in the ground beef samples;
Fantelli & Stephan (2001), made a study in ground beef and ground pork meat samples with VIDAS ECPT for the presence of *E. coli O157*; whereas Rozand, et al. (2002) searhced ground meat with VIDAS ECO. Moreover, Lu et al. (2012), carried out a study in which they reanalysed the samples, which they had contaminated, with VIDAS ECPT, VIDAS ECO and PCR- based BAX. As a result of these analyses, it was stated that VIDAS ECPT was more superior to other methods.

Balpetek & Gürbüz (2010) and Aydemir Atasever & Atasever (2015) made analyses on ground meat samples and made verification processes by using Vitek 2 Compact.

Noveir et al. (2002), Aslantaş & Yıldız (2002) and Keleş et al. (2006) determined *E. coli O157* serotype by using Latex Agglutination test in ground meat analyses; whereas Direkel et al. (2010) informed that they determined *E. coli O157:H7* serotype in ground meat analyses with Latex Agglutination test.

In other studies on this issue *E. coli O157:H7* serotypes have been found as follows, Abdol-Raouf et al. (1996), found three in 50 ground meat; Pahdye & Doyle (1991), found three in 107 fresh ground beef; Rozand et al. (2002), found four in 3450 French ground beef samples, Jamshidi et al. (2008), found one of 100 ground beef samples; Sezgin & Kök (2015), found four in 50 ground beef samples.

CHAPTER IV
MATERIAL AND METHOD

4.1 Material

4.1.1 Samples

This study carried out with 70 freshly prepared ground beef samples that were collected from various markets and butchers in Nicosia and Kyrenia, TRNC. Samples kept in the sterilized containers through the maintenance of cold chain system were taken to the laboratories.

Table 4.1: Sampling regions in Northern Cyprus and number of primary samples

Region	Market Type	Number of Samples
Nicosia	Supermarket	30
	Butcher	25
Kyrenia	Supermarket	8
	Butcher	7

4.1.2 Media

4.1.2.1 Liquid Media

4.1.2.1.1 Buffered Peptone Water (BPW) (Biomerieux 51094)

Compound g/L

Peptone	10 g
Sodium chloride	5 g
Disodium phosphate	3.5 g
Potassium dihydrogen phosphate	1.5 g

The pH level of the mixture is set for 7.2-7.4 and is poured into bottles of 225 ml. Schott bottles are kept at 121 ℃ for 15 minutes and at +4 ℃ after being sterilized in autoclave until being used.

4.1.2.2 Solid Media

4.1.2.2.1 ChromID™O157:H7 Agar (O157 H7 ID-F) (Biomerieux SA ref 42605)

Compound g/L	
Gelatin Peptone (cow or pig)	5.5 g
Yeast extract	6 g
Sodium chlorite	5 g
Sodium carbonate	0.13 g
Neutral red	0.01 g
Sodium deoxycholate (cow and pig)	1.5 g
Carbohydrate mixture (cow)	24 g
Activator mixture	0.25 g
Chromogenic substrate mixture	0.25g

O157:H7 ID agar was obtained in a frozen state in a bottle of 200 ml. It was kept at room temperature at first for 15 seconds before being placed in water bath, which was set for 45-50 ºC and controlled thermostatically. When the temperature of the media reached 45 ºC, it was poured into sterile petri dishes and the media was shared. Petri dishes that wouldn't be used immediately and that contained media were kept refrigerated at +4 ºC.

4.1.2.2.2 Sorbitol MacConkey Agar (SMAC) (LAB161)

Compound g/L	
Peptone	20.0 g
Sorbitol	10.0 g
Bile salt no:3	1.5 g
Sodium chloride	5.0 g
Neutral red	0.03 g
Crystal violet	0.001 g

Agar no:2	12.0 g

The pH level of the mixture is set for 7.1-7.3 and it was sterilized in autoclave for 15 minutes at 121 °C. Cefixime Tellurite Selective Supplement were added into the medium which was cooled to 50 ºC, it was poured into 9mm diameter petri dishes, which were sterile at aseptic conditions as 12,5–15 ml, and it was kept at + 4 °C until being used.

4.1.3 Supplement

4.1.3.1 Cefixime –Tellurite Selective Supplement (LAB X161)

Compound 500 ml/mg	
Cefixime	1.25 mg
Potassium Tellurite	0.05 mg

1 vial Cefixime Tellurite Selective Supplement was added in 500 ml SMAC medium as melted in 5 ml distilled water.

4.2 Method

4.2.1 Sampling

In the study 70 ground meat samples that were provided from various markets and butchers in the TRNC were taken into sterile dishes and brought to the laboratory by protecting the cold chain.

4.2.2 Sample Preparation and Homogenization

- The sample of 25 g was weighted by fulfilling hygiene conditions and taken into sterile bags that have a separator on the side.
- Stomacher was used after adding 225 ml of TPS fluid and it was made to be homogenized.
- Incubated at 41.5 ºC for 6-24 hours and pre-enrichment was materialized.

4.2.3 Screening the samples with VIDAS UP *E. coli O157:H7* ECPT in Mini VIDAS (BioMérieux SA ref 30122)

- After the incubation, samples in the Stomacher bag were shaken and homogenized again.
- They were taken into a sterile tube with a 10 ml pipette from the enrichment fluid in the bag with side separator. Then 0.5 ml was taken with micropipette and transferred into sample well in the Vidas ECPT strip.
- It was heated for 5± 1 minutes by using the Vidas Heat and Go device. Strip was taken and left for cooling. Then it was placed in the VIDAS device and the sample was screened.
- Unboiled enrichment fluid that was in the tube was kept at 2-8 ^{o}C for verification purposes to be used when it is necessary.

4.2.4 Mini VIDAS UP *E. coli O157:H7* ECPT Principle

VIDAS UP *E. coli* O157:H7 is a fluorescent test that is linked with enzymes that are used in automatic VIDAS device for detecting *E. coli O157:H7* (ELFA). Solid phase receptacle (SPR) serves both as pipette apparatus and as solid phase. Inner section of SPR is covered with recombinant phase proteins in order to catch *E. coli O157:H7*. Reactive that is for this test is ready to use and they were pre-distributed to closed strips.

All stages of the test are fulfilled by the device automatically. The mixture of the reaction is taken in and out of the SPR many times.

A part of enrichment liquid media is distributed in reactive strip. Existing *E. coli O157:H7* is caught by recombinant phage proteins that are coated in the inner section of SPR. Unbound compounds are eliminated during the washing steps. Then alkaline phosphatase conjugate approximately makes a circle in SPR and connects to *E. coli O157:H7's*, all of which is connected to phage proteins on the SPR wall spontaneously.

With a last washing step, unbound conjugate is cleaned. Substrate (4-methyl- umbelliferyl phosphate) is taken in SPR and left at the last detecting phase. Conjugate enzyme catalyses hydrolysis of a fluorescent product (4-methyl-umbelliferone) of this substrate, whose fluorescent is measured at 450 nm.

The results are automatically analyzed by the device at the end of the test. Then these values are compared with an inner reference and each sample is interpreted separately.

4.2.5 Verification of positive VIDAS UP *E. coli 0157:H7* results

Mini VIDAS shows the results of the screened samples as positive or negative. For the results that are negative, the process ends at that phase. Verification is required for positive results. Verification is required to be made by using unboiled enrichment medium that was kept at 2-8 ^{o}C and within approximately 48 hours after the end of incubation. 2 separate methods have been used for the verification of positive results.

4.2.5.1 Method 1

Loop was planted from unboiled enrichment medium that was kept in a tube at 2-8 °C on Cefimixe – Tellurite chromID O157:H7 ID Agar and CT- SMAC Agar and petri dishes were incubated for 18-24 hours at 37 ±1 °C.

4.2.5.2 Method 2

0.5 ml was transferred from unboiled enrichment media that was kept at 2-8 °C into VIDAS ICE strip. Mini VIDAS device was used again for immuno-concentration process. Plantation was made on Cefimixe – without Telluride O157:H7 ID Agar and CT-SMAC Agar from the sample liquid taken from here, which became selective. Petri dishes were incubated for 18-24 hours at 37 ±1 °C. 4 separate media that were obtained as a result of an incubation of 18-24 hours were individually assessed.

4.2.6 Screening positive samples with Vidas I.C.E. coli O157 ICE (bioMérieux SA ref 30526)

- 0.5 ml of fluid medium is transferred to strip well number 4 of Vidas ICE strip with micro pipette.
- SPR and strips are inserted into the instrument. The assay code and colour labels on the SPRs are checked to match.

- The assay is initiated as directed in the Operator's Manual. All the assay steps are performed automatically by the instrument. The results are obtained within approximately 40 minutes.
- Immuno-concentration procedure is completed.

4.2.7 VIDAS I.C.E. coli O157 ICE principle

- VIDAS Immuno-Concentration *E. coli O157* (ICE) kit includes two ready-to-use components:
    - A pipette tip-like disposable device, the Solid Phase Receptacle (SPR), which serves as the solid phase as well as the pipetting device for the assay. The interior of the SPR is coated with anti-*E. coli O157* antibodies adsorbed on its surface.
    - A strip which contains all the wash and release solutions.

Part of the enrichment broth is dispensed into the reagent strip and the sample is cycled in and out of the SPR.
E. coli O157 present in the broth will bind to the anti- *E. coli O157* antibodies coating the interior of the SPR. Unbound sample components are then washed away.
A final enzymatic step releases the captured *E. coli O157* into one of the strip wells.
The entire Immuno-concentration process is performed automatically by the instrument.

4.2.8 Evaluation of ChromID™ O157:H7 Agar

Reproduction of bacteria has been observed after the incubation. Green or blue-green colonies are evaluated as characteristic colonies for *E. coli O157:H7*.

4.2.9 Evaluation of SMAC Agar

Colourless colonies were evaluated as sorbitol negative; whereas pink-red colonies were evaluated as sorbitol positive as a result of incubation.

4.2.10 Vitek 2 Compact GN (Biomeriux ref no 21314)

Prepare the inoculum from a pure culture, according to good laboratory practices. In case of mixed cultures, a re-isolation step is required. It is recommended that a purity check plate be done to ensure that a pure culture was used for testing.

1) Select isolated colonies from a primary plate, if culture requirements aremet, or
Subculture organism to be tested to appropriate agar medium andincubate accordingly.
2) Aseptically transfer 3.0 mL of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0) into a clear plastic (polystyrene) test tube (12 mm x 75 mm).
3) Use a sterile stick or swab to transfer a sufficient number of morphologically similar colonies to the saline tube prepared in step 2.
Prepare the homogenous organism suspension with a density equivalent to a McFarland No. 0.50 to 0.63 using a calibrated VITEK® 2 DensiCHEK™.
Note: Age of suspension must not exceed 30 minutes before inoculating card.
4) Place the suspension tube and GN card in the cassette.
5) Refer to the appropriate Instrument User Manual for instructions on data entry and how to load the cassette into the instrument.
6) Follow your local inspecting agency's guidelines for disposal of hazardous waste.

4.2.11 Latex Agglutination Test (Microgen M44)

- Dispense 1 drop (30 µL) of isotonic saline (M40) on two wells of a clean, dry Microgen agglutination slide.
- Using an inoculating loop, remove several suspected *E. coli* colonies from the Sorbitol MacConkey agar plate. Only select colourless colonies whose morphology resembles that of *E. coli*.
- Emulsify the colonies in two drops of saline on the test slide to produce a heavy, smooth suspension. Spread the suspension over the entire surface of the wells.
- Rock the slide gently for 30 seconds and observe for autoagglutination of clumping. If the suspensions remain smooth, proceed to section 5. If the suspension is 'stringy' or 'granular' the sample is unsuitable for testing with Microgen *E. coli* since. It may give a

falsely positive agglutination when latex is added. In this event, an alternative test method should be used.

- Gently shake each latex reagent to ensure a homogeneous suspension.
- Add 1 drop of Microgen *E. coli O157* Test latex to one of the bacterial suspensions, and one drop of Microgen *E. coli O157* Control Latex to the other. Do not allow the latex dropper to touch the bacterial suspensions.
- Mix the suspensions with a fresh mixing stick for each combination.
- Rock the slide gently for two minutes and observe for agglutination. An agglutination reaction is indicated by visible aggregation of the latex particles.
- Discard the used slides and mixing sticks into a suitable disinfectant.

CHAPTER V

RESULT AND DISCUSSION

5.1 Result

In this study, 70 raw ground beef have been obtained from markets and butchers located in Nicosia and Kyrenia districts of the TRNC. These samples have been searched for the presence of *E. coli O157:H7* serotype. Samples have been obtained from 30 markets and 25 butchers in Nicosia; whereas they have been taken from 8 markets and 7 butchers in Kyrenia. After isolation and confirmative analyses, *E. coli O157* serotype was found in two samples collected from Nicosia and Kyrenia markets. Then in a one of these samples, *E. coli O157:H7* serotype has been detected (Table 5.1). *E. coli O157* positive results have been found among the samples obtained from markets, one from Nicosia and one from Kyrenia. *E. coli O157:H7* serotype has been found in the sample taken from Nicosia. The distribution of the results of the samples according to regions and obtained locations has been given in the table below.

Table 5.1: Distribution of the results of the ground beef samples according to Districts, Markets and Butchers

	Region	**Obtained Location**	**Number**	***E. coli* O157 Positive**	***E. coli* O157:H7 Positive**
Ground beef	Nicosia	Market	30	1 (1.42%)	1 (1.42%)
Ground beef	Nicosia	Butcher	25	Not found	Not found
Ground beef	Kyrenia	Market	8	1 (1.42%)	Not found
Ground beef	Kyrenia	Butcher	7	Not found	Not found

Mini VIDAS, Vitek 2 Compact and Latex Agglutination tests have been applied for 70 ground beef samples.

Three (4.2%) positive results have been found in the result of VIDAS ECPT. Two methods have been applied for the verification phase of these positives.

1. method; enrichment fluid of the positive samples have been directly planted to CT- SMAC Agar and CT-Chrom-ID O157:H7 Agar.

2. Method; The enrichment fluid of the sample has been immuno-concentrated with VIDAS ICE and it has been planted in CT-SMAC and Chrom-ID O157:H7 Agar. Sorbitol negative (colourless colonies) in CT-SMAC agar and green or bluish-green colonies in Chrom-ID O157:H7 agar have been accepted as suspicious colonies for *E. coli O157:H7*. Suspicious colonies have been observed to be reproduced in the planted media. As VIDAS ICE eliminated most of the competitive enteric flora, the density of reproduction on plate has been seen to be less in the planting made afterwards. Reproduction in SMAC Agar and Chrom- ID O157 media has been given in the figure.

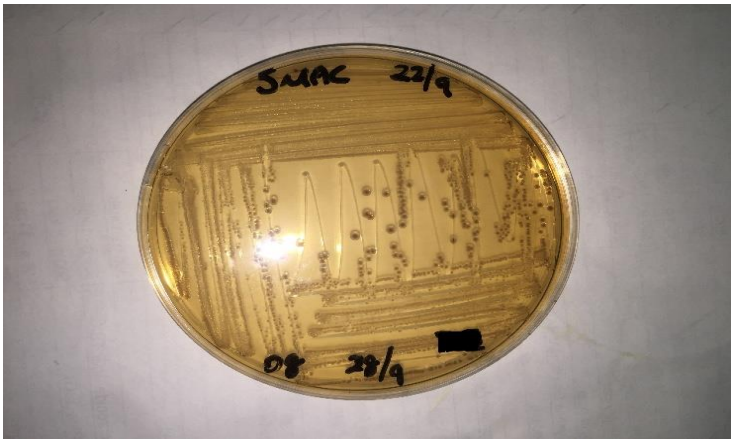

Figure 5.1: Sorbitol negative colourless colonies isolated in SMAC Agar

Figure 5.2: Green or bluish-green colonies isolated in Chrom – ID O157:H7 Agar

E. coli 0157 positive results given by VIDAS ECPT have been confirmed whether it is O157 or not. *E. coli* O157 serotype has been determined with Vitek 2 Compact; however H7 serotype could not be determined. At this stage, suspicious colonies, which have been obtained with methods 1 and 2 and whose isolation has also been obtained at CT-SMAC Agar, have been re-isolated by passing to Blood Agar. Two (2.85%) *E. coli* 0157 serotypes have been found among the samples that had been swiped with Vitek 2 Compact. In line with the data obtained as a result of Vitek 2 Compact, the colonies, which had been isolated according to method 2 after VIDAS ICE, have been evaluated as more pure colonies as they had been immune-concentrated.

Latex Agglutination test has been applied then. Latex Agglutination test is made with sorbitol negative colourless colonies that are isolated on SMAC agar. Its reason is to minimize false negativities that can occur during the test. It is possible to determine *E. coli O157* and H7 serotype with Latex Agglutination test. *E. coli O157* has been found in two samples (2.85%) and *E. coli O157:H7* serotype has been found in one (1.42%) of these two samples. The results of the agglutination test that was made on SMAC Agar has been given in the figure.

Figure 5.3: *E. coli O157* Control and O157 Test results that have been made with Latex Agglutination test

Figure 5.4: *E. coli O157:H7* serotype result made with Latex Agglutination test

Two *E. coli 0157* serotypes, which had been obtained as a result of Vitek 2 Compact and Latex Agglutination tests that were made at the verification phase of VIDAS ECPT, have shown that these two test verify each other. The results according to the methods that were used in the analysis methods are given in the table.

Table 5.2: Distribution of results according to methods

Positive Results Sample no	**VIDAS ECPT**	**Vitek 2 Compact *E. coli O157***	**Latex Test *E. coli O157***	**Latex Test *E. coli O157:H7***
1	Positive	Positive	Positive	Positive
2	Positive	Positive	Positive	Negative
3	Positive	Negative	Negative	Negative

5.2 Discussion

Meat and meat products have a risk in terms of foodborne pathogen bacteria when they are produced in conditions that are not in line with hygienic and technologic rules. One of the pathogens that may cause bacterial resourced food poisoning is *E. coli O157:H7*, whose significance has been increasing nowadays (Ünsal, 2007)

Being a microorganism with high level of disease creating ability, *E. coli O157:H7* also has the cross contamination risk and it shows an increasing poisoning trend. While infections resourced from *E. coli O157:H7* has a wide spectrum from abdominal cramps to bloody diarrhea from time to time, and HC, HUS and TTP at further stages; whereas some cases may be resulted with death.

Fantelli & Stephan (2001) have found 1 *E. coli O157* serotype in ground beef and 4 in ground pork in the analyses they conducted with VIDAS ECPT among 211 ground beef and 189 ground pork samples in their study. However, they stated that they did not find *E. coli O157:H7* serotype. Temelli et al. (2011) analysed 106 samples of meat and meat products with VIDAS ECPT. As a result of these analysis, 27 (37,50%) of 72 meat samples and three (8,82%) of 34 meat product samples showed positive results with VIDAS ECPT. As a result of the verification of these samples, it was stated that the samples have been found at 5,55% and 0,00% positive for *E. coli* O157 serotype (except for H7) respectively.

Rozand et al. (2002) stated that 175 (5.07%) of 3450 ground beef samples showed positive results and only 4 of them were isolated as *E. coli O157*.

Lu et al. (2012) expressed in their study that was carried out in 2012 that they had inoculated 18 *E. coli O157:H7* serotype to raw ground beef, raw milk, soy bean sprout, raw chicken and fresh papaya juice and they had these food samples contaminated. Contaminated samples had been reanalysed for the presence of O157:H7 with VIDAS ECPT, VIDAS ECO and PCR- based BAX. They had determined that VIDAS ECPT UP gave 18 positive results; whereas PCR system gave 6 positive results and VIDAS ECO gave negative results for all of them. When these three methods were compared thereon, it was stated that VIDAS ECPT was twice superior to the other methods. VIDAS ECPT, which is the same method, has been used in this study and

when the results are taken into consideration, it is assessed that they are in conformity with the opinion of Lu et al.

Vitek 2 Compact has been used at the verification phase of the plates, which were obtained from the positive results of VIDAS ECPT. Two studies, which made analysis on ground beef with Vitek 2 Compact has been found as a result of the researches. Balpetek & Gürbüz(2010) investigated the presence of *E. coli O157* in ground beef samples with Vitek 2 Compact and they have said that they had found *E. coli O157* serotype in 4 samples. Aydemir Atasever & Atasever (2015) searched the presence of *Listeria monocytogenes* and *Salmonella spp*. in 100 ground beef samples with Vitek 2 Compact; however, the presence of *E. coli O157:H7* was made with Latex Agglutination test. *E. coli O157:H7* has been reported to be isolated in three of the ground beef samples. In this study, two of the three samples, which were analysed with VIDAS ECPT have been determined as *E. coli O157* serotype with Vitek 2 Compact.

Contamination frequency, created risk level and public health safety of raw ground beef, which was obtained from various butchers and markets in TRNC, in terms of the presence of *E. coli O157:H7* serotype has been investigated with this study. VIDAS ECPT method has been used in this study and three positive results have been found in 70 ground beef samples as a result of the analyses. Fantelli & Stephan (2001) and Temelli et al. (2011) stated to find similar results.

In the studies made with latex agglutination test, Noveir et al. (2002) determined six isolate *E. coli O157* in 57 raw ground beef samples with biochemical tests; however only one of them was determined as *E. coli O157* serotype with the agglutination test that was made with latex.

Aslantaş & Yıldız (2002) informed that they have found one *E. coli O157* serotype as a result of the analyses made on 100 ground beef samples with Latex agglutination. Alişarlı & Akman (2004) have found *E. coli O157* in 10 of 300 ground beef samples in Van with Latex agglutination test. Keleş et al. (2006) expressed that they had isolated *E. coli O157* serotype in one of 41 ground beef samples as a result of latex agglutination.

Bayar (2007) conducted a study on meat and meat products and stated that the presence of *E. coli O157:H7* has been found in 10% of 100 samples with the Latex agglutination test.

Direkel et al. (2010) analysed 86 ground beef samples with latex agglutination test and they found *E. coli O157:H7* among 6 of them.

In addition, Latex agglutination has been used for determining *E. coli* 0157 and *E. coli O157:H7* serotypes in various foods.

Ulukanlı & Çavlı (2006) conducted a study on meat doner and isolated *E. coli O157:H7* serotype in nine of 80 samples with latex agglutination test.

Akkaya et al. (2007) carried out a study and stated that they had found *E. coli O157:H7* in three (3%) of 100 raw milk samples and one (1%) of 100 cheese samples that were analysed with latex agglutination test.

Temelli et al. (2011) noted that they found positive results for *E. coli O157* in two ground beef samples among 106 red meat and meat product samples with latex agglutination tests.

In our study, three positive *E. coli O157* serotypes that were obtained with VIDAS ECPT have been verified with latex agglutination and two *E. coli O157* serotypes and one *E. coli O157:H7* serotype have been found.

Foods have a significant role in the spread of infections resourced from *E. coli O157:H7*. Food of animal origin leads the first place among these foods. There are many studies on the isolation of *E. coli O157:H7* serotype in ground beef throughout the world and in Turkey.

Halkman et al. (1998) searched for the presence of Enterohemorrhagic *E. coli O157:H7* in 225 raw ground beef samples. *E. coli O157* serotype has been found in one of the raw ground beef sample. However, it was noted that *E. coli O157:H7* serotype hasn't been found.

Aslantaş & Yıldız (2002) carried out a study in Kars region on food of animal origin and stated that *E. coli O157:H7* hasn't been found among 200 meat and meat products; however *E. coli O157:H-* serotype has been found in a ground beef sample.

Noveir et al. (2002) isolated *E. coli O157* serotype in 57 ground beef samples with standard cultural method. Even though they have *E. coli O157* positive results in two of EZ Coli kit, it was found that there was no *E. coli O157* in the enrichment medium of these samples.

Alişarlı & Akman (2004) searched the presence of *E. coli O157* serotype in 300 ground beef samples that are sold by retail in Van. It was noted that *E. coli O157* serotype was found in 4.66% (7/150) rate in ground beef and 2% (3/150) rate in ground mutton.

Keleş et al. (2006) conducted a study, in which *E. coli O157* serotype was isolated with 2.43 % rate in 41 ground beef samples and they noted that H7 serotype could not be found.

Temelli et al. (2011) searched the presence of *E. coli O157:H7* in 106 red meat and meat products. Two ground beef samples gave positive results for *E. coli* O157; however it was noted that they didn't give positive results for *E. coli O157:H7* serotype.

Dontorou et al. (2003) searched the presence of *E. coli O157:H7* in 114 meat products (ground beef and uncooked frozen hamburger), which were obtained from different farms and markets in a study conducted in Greece. It was stated that *E. coli O157:H7* was not found in ground beef and hamburger products.

Abdol-Raouf et al. (1996) carried out a study in the Middle Egypt and found *E. coli O157:H7* in three (6%) of 50 ground beef samples obtained from slaughterhouses, supermarkets and farmhouses. In another study, three (2.8%) of 107 fresh ground beef samples were found positive for *E. coli O157:H7* (Pahdye & Doyle, 1991).

Baran & Gülmez (2000) conducted a study in Kars District and examined 100 animal origin products in total, as 50 ground beef and 50 chicken thigh samples that are being sold in this district for the presence of *E. coli O157: H7*. The bacteria could not be isolated in the chicken samples; whereas it was isolated in three (6%) of the ground beef samples. Rozand et al. (2002) carried out a study and reported that four *E. coli O157:H7* serotypes have been found in 3450 French ground beef samples.

Cagney et al. (2004) 1533 ground beef and meatballs samples that were taken from various supermarkets and butchers in Ireland have been examined for the presence of *E. coli O157:H7* and *E. coli O157:H7* was found positive in 43 samples. It was noted that 2.70% (32/1183) of the contaminated samples were taken from supermarkets and 3.14% (11/350) were taken from butchers; whereas 41 samples had verotoxin producing genes (VT1 – VT2) of *E. coli O157:H7*.

Ünsal (2007) 120 beef samples that were obtained from various butchers and markets in Erzurum District and Provinces have been analysed. It was found that 4 (3,3%) of 120 beef samples contained *E. coli* O157 and 2 (1,6%) of them were *E. coli O157: H7*.

Bayar (2007) found *E. coli O157:H7* at 20% (2/10) rate in ground lamb samples and at 40% (4/10) rate in ground beef samples.

Jamshidi et al. (2008) examined 100 ground beef samples in Ireland. It was noted that *O157:H7* was found in one of the samples as a result of 7 isolations of non-sorbitol fermenting (NSF) colonies that were obtained in this study.

In a study conducted in Mersin District, microbiological criteria of ground beef samples that were obtained from 86 different butchers were examined and as a result of the analysis, *E. coli O157:H7* was noted to be found in six ground beef samples (Direkel et al., 2010).

In another study, *E. coli 0157* was found in 4 ground meat samples among the samples of 173 meat and meat products (fermented sausage, salami, sausage, hamburger meatball, İnegöl meatballs, pastrami, ground meat and poultry products), which were provided for consumption in Konya District, and *E. coli O157:H7* was isolated in three of them (Balpetek & Gürbüz, 2010).

A study was conducted in Iran on beef, goat, hippopotamus, lamb and camel meat, and *E. coli 0157* was found in 14 of 295 meat products and *E. coli 0157:H7* in one of the samples (Rahimi, Kazemeini, & Salajegheh, 2012).

Kalın & Öngör (2014) carried out a study in 2014 and examined ground beef samples that are sold for consumption for the presence of *E. coli 0157:H7*. They stated that they found *E. coli 0157:H7* in six (7.5%) of ground beef samples.

Sezgin & Kök (2015) investigated the presence of *E. coli O157:H7* among 50 ground beef samples in a study they conducted in Aydın. *E. coli O157:H7* was stated to be isolated in four of the examined samples.

E. coli O157:H7 was stated to be found on three samples out of 100 ground beef samples that are sold for consumption in Erzurum District as a result of the analysis (Aydemir Atasever & Atasever, 2015).

In our study, *E. coli O157:H7* serotype was found in one (1.42%) of 70 raw ground beef samples. It is observed that our results are in line with the studies when studies conducted with ground beef samples are taken into consideration.

CHAPTER VI

CONCLUSION AND RECOMMENDATIONS

With regards to raw ground beef, which is the main reservoir of *E. coli O157:H7* strain, this thesis study is the first study, which examined ground beef for the presence of *E. coli O157:H7* among ground beef obtained from various butchers and markets in the Turkish Republic of Northern Cyprus and which studied on its importance in terms of public health. No other study has been found that used the methods, which were used in this study.

As a result of examining 70 ground beef samples in this study, 2.85% *E. coli O157* and 1.42% *E. coli O157:H7* serotypes have been determined.

According to the Turkish Food Codex Regulation on Microbiological Criteria, *E. coli O157* should not be found in ground meat. As ground beef is one of the most consumed meat products and as *E. coli O157:H7* is a bacterium, whose minimal infection dose is low, it is concluded that determining 1.42% *E. coli O157:H7* serotype is at substantial level and it can constitute risk for the public health.

The following suggestions should be taken into consideration in order to minimize the incidence of *E. coli O157: H7* poisonings from minced meat

- It should be kept at a temperature of 4 °C and below.
- The internal temperature should be cooked to a minimum of 70 °C.
- It shouldn't be consumed raw or undercooked.
- It shouldn't be bought pre-processed from the butchers or supermarkets. It would be better to buy freshly prepared ground beef.
- If it's possible it should be asked first to use the meat grinder empty.
- To avoid breaking the cold chain, it is suggested that meat and meat products should be bought at the end of the shopping.

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