AN INVESTIGATION ASSETS OF OFFERED FOR SALE RAW MILK AND KASHAR CHEESE OF *ENTEROHAEMMORRHAGIC ESCHERICHIA COLI O157:H7* IN TRNC

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ABSTRACT

An ınvestıgatıon assets of e-offered for sale raw mılk and kashar cheese of *Enterohaemmorrhagıc Esherıchıa coli O157:H7* in North Cyprus.

In this study, presence of *E. coli O157:H7* has been examine in cheese that sold in market and raw milk bought from producers in Nicosia, North Cyprus. For this purpose, 40 different brand of raw cheese samples and 60 raw milk samples taken from different producers have been analized. *E. coli O157:H7* was not detected in total of 60 raw milk sample. However, *E. coli O157:H7* detected in 8 out of 40 pieces (%20) of cheese.

Research result showed that *E. coli O157:H7* minimal infective dose (10-100 cfu / g) was very low. Therefore it may cause illness. All hygine and technological rules should be followed during the production and consumption of raw milk and cheese. We found out that consumer should be trained about raw and under cooked milk and cheese consumption.

Keywords: E.coli o157 h:7, Raw Milk, Kashar Cheese, CHROMagar O157, North Cyprus

ÖZET

Kuzey Kıbrıs Türk Cumhuriyetinde ‘tinde çiğ sütlerde ve satışa sunulan kaşar peynirlerinin *Enterohemojenik Escherıchıa coli O157:H7* şuşunun araştırılması**.**

Bu çalışmada Kuzey Kıbrıs Lefkoşa ilçesinde marketlerde satışa sunulan Kaşar peynirlerinde ve üreticilerden temin edilen çiğ sütlerde *E. coli O157:H7* mevcudiyeti yönünden araştırıldı. Bu amaçla Lefkoşa ilçesinde marketlerde satışa sunulan farklı markalarda Kaşar peynirlerinden 40 numune ve farklı çiğ süt üreticisinden 60 ad çiğ süt analiz edilmiştir. İncelenen 60 ad çiğ süt örneğinde *E. coli O157:H7* gözlenmedi ve 40 adet kaşar peynirinin 8 adedinde (%20) *E .coli O157:H7* olduğu tespit edildi.

E. coli O157:H7’nin minimal enfektif dozunun (10-100 kob/g) çok düşük olması nedeni ile araştırmada bulunan numunelerin sonuçları itibariyle hastalık oluşturabilecek düzeydedir. Bulgular neticesinde, çiğ süt , kaşar peynirinin üretiminden tüketilmesine kadar geçen safhalarda tüm hijyenik ve teknolojik kurallara uyulması ile tüketicilerin Çiğ süt ve kaşar peynirinin az pişmiş veya çiğ tüketilmesiyle ilişkili riskler konusunda uyarılması gerektiği sonucuna varılmıştır.

Anahtar Kelimeler**:** *E. coli O157:h7*, Çiğ Süt, Kaşar Peyniri, CHROMagar O157, Kuzey ‘ Kıbrıs

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABREVATIONS

aw: Aactivity valume

˚C: Centigrate heat unit

cm: Cantimetre

E.coli: Escherichia coli

E.coli o157:H7: Escherichia coli o157:H7

HC: Haemorrhagic Colitis

HUS: Hemolytic Uremic Syndrome

TTP: Thrombotic Thrombocytopenic Purpura

VTEC: Verotoxigenic Escherichia coli

STEC: Shiga-toxin-producing Escherichia coli

EHEC: Enteohaemorrhagic Escherichia coli

ETEC: Enterotoxigenic Escherichia coli

EIEC: Enteroinvasive Escherichia coli

EPEC: Enteropathogenic Escherichia coli

EaggEC: Enteroaggregatif Escherichia coli

OAEC: Diffuser-adherent Escherichia coli

FEEC: Facultive Enteropathogenic Escherichia coli

LT: Heat-labile toxin

ST: Heat-Stable

SLT: Shiga-like toxin

Md: Megadalto

EASTI: Heat-stable enterotoxin

VT: verotoxin

pH: Power of Hydrogen

Kg: Kilogram

Mg: Milligram

g: Gram

L: Litre

g/L: Gram/Litre

mL: Millilitre

min: Minute

mm: Millimetre

µl: Microlitre

µm: Micrometre

kob/L: Coloni/Litre

kob/g: Coloni/Gram

NaCl: Sodium Clorine

H_2O_2: Hydrogen Peroxide

TSE: Turkısh Standards Institute

TRNC: Turkish Republic of Northern Cyprus

°SH: The Total Aciditiy Type Soxhelet-Henkel

S: Sample

sec: second

CHAPTER 1

INTRODUCTION

Milk and cheese of staple food are good environments for various microorganisms to grow including pathogenic microorganisms. If no attention is paid to the hygenic conditions during the production and preservation processes, they not only threaten the people's health but also may cause to economical losses because of some physical and chemical changes. The coliform group bacteria which affect the quality of the food in a negative way, are accepted as the indicator of the hygenic quality of the food. Among this group of bacteria, *E. coli* and recently more accentuated *Escherichia coli O157:H7* are important bacterial food infection factors. While minced meat is the main source of infections caused by *E. coli O157:H7*, it is stated that contaminated milk and milk products are also important factors (Baz et al., 2003). *E. coli O157:H7* is one of hundreds of serotypes of *E.coli* bacterium. It was reported that this bacterium was first insulated back in 1975 from a Californian woman patient that had a severe hemorrhagic diarrhea. The identification of *E. coli O157:H7* as a food pathogens was realised after two big epidemia seen in Oregon and Michigan in early 1982. It was determined that the reason of these epidemia was undercooked contaminated hamburger meatballs. In the infections caused by *E. coli O157:H7;* it is stated that abdominal crampings, bloody diarrhea, haemorrhagic colitis (HC), hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP) and sometimes death could be seen (Ünsal, 2007).

Food poisoning is one of the most common diseases in the world and in our country. While the most important infection is felcal-oral, the primary infection sources are human and animal feces and contaminated water, milk and milk products, poultry meat and meat products, marine and freshwater crustanceans. One of the most frequently isolated factors of food poisoning is Verotoxigenic E. Coli (VTEC).The concept VTEC was used for the first time in 1977 for toxin-producing E. Coli strains which is a cytotoxic for Vero cells. Another synonymous naming is Shiga-toxin-producing E. Coli (STEC) definition. Terms VTEC and STEC are used for E. Coli strains from the Shiga-toxin family that produce one or more toxins. "Enteohaemorrhagic E. Coli (EHEC)" defining is used for strains which are contained within VTEC (STEC) strains and factors of neamoltic uremic syndorme (HUS) and hemorrhagic colitics (HC). In this group, the most common serotype is 0157. First EHEC strain that was identified as HC and HUS factor in 1982 was 0157 (Tolun et

al., 2011). It was reported that *E. coli O157:H7* known as Verotoxin 1 and 2 produce 2 shigatoxins and the strains can produce one or both of them (Robinson et al., 2000).

E. coli O157:H7 infections and other STEC infections are currently seen in more than 30 countries (5), and the minimal infective dose of factor is at such low levels; <10 and <100 (Coia et al., 1998).

Various farm animals, especially cattles are the natural reservoir for this bacterium. In the most important ways of infections are contaminated food especially including meat and dairy products (Tolun et al., 2011).

Due to the complex biochemical structure and high water activity, raw milk is extremely a nutrient environment for pathogenic microorganisms. Fresh milk aseptically milked from a healthy animal contains few bacteria. Microbial contamination to milk starts by milking. The most important sources of contaminations are animal's breast, skin, hair, human hand, milking machines, milk containers and coolants. Generally, spread borne microorganisms such as air, dust, soil, water and fertilizer from this environment infect milk. Furthermore, various dairy products made from milk, in addition to the microorganisms previously contaminated to milk, they can be contaminated by microorganisms coming from human hand, water, instruments and equipment, addititives and packaging materials (Kılıç, 2010).

After determining the factor as raw or undercooked milk and milk products in some outbreaks linked to the *E . coli O157:H7* infections, such products have been recognised as risky food in terms of *E. coli O157:H7* (8,9). It is stated that the 2,9% of the source of the outbreaks occured in the United States of America is due to the raw milk (Meichtri et al., 2004).

In our studies, it is aimed to find the frequency level of *Escherichia coli O157:H7* strain from the 40 pieces fresh kashar cheese and 60 raw milk samples of the fresh cheese (obtained from raw milk producers) that are being sold in North Cyprus.

1.1 Raw Milk

Milk is naturally synthesized in the mammary glands in the animal's breast. Milk is directly taken after the breast is abosrbed by the offspring. There is no possibility for milk to be contaminated. Milk is no longer in contact with the outside during milking of the extra milk from the breast in any other ways. There may be some changes in the physical, chemical and microbiological characteristics of the milk depending on the milking containers, transport conditions, the conversion to the product in business and the time elapsed short or long. Since the milk of infected animals is not healthy considering the chemical and physical as well as the microbiological structures, the dangerousness for the offspring as well as for the ones that use it is important depending on the state of the disease factor. On the one hand, the milk of diseased animals to be processed poses obstacle to obtain quality products while on the other hand it causes people to be sick. One of the main tasks of the microbiology of milk in terms of preventing this type of negativities is to prevent problems from the outset that may born after determining the detrimental milk in terms of health and which are harmful for people's health; the biggest help to the milk an milk products micriobiology can be provided by milk hygiene on the issues such as making animal's health control, the processing of the milk as it should be and providing healthy products to the consumers. Milk is recognized as an essential nutrient for animal and human nutrition. Milk and milk products can be more easily absorbed by the human body and can benfit from a higher level.

Such valuable nutrients are also indispensable for microorganisms; the presence of sufficient water and necessary nutrients create a hugw advatage for them. Microorganisms can be found in milk and its products vary a lot in terms of number and group. These are microorganisms mainly affecting human health and disease-creating (pathogens).

E. coli faecal is original. Therefore, they are used as faecal contamination indicator in nutrients. The presence of *E. coli* in food indicates that there is faecal contamination directly or indirectly since the faecal is original. Also it is a classic indicator that there may be enteric pathogenic bacteria. This bacterium are found in almost all kinds of cheese and raw milk. *E. coli* strains are generally found in intestines of human and warm-blooded animals as commensals. In addition, some strains are pathogenic and cause diarrhea (Kılıç, 2010).

Raw milk is rich in coliform bacteria group. This group of bacteria contamination of raw milk is due to the implementation of milking and milk collection operations under poor hygiene and sanitation conditions. The presence of coliform bacteria in raw milk and the number of it is important for reflecting the wrong way of doing the handling and storage operations as needed rather than drawing attention to faecal contamination or the presence of enteric pathogens. Faecal coliform, in terms of food security, has attracted more attention that others, and led to the fore as an indicator of food security. The presence of coliform in milk and milk products is accepted as an indicator of the presence of the enteric pathogens in the food.

Although *E. coli* is a typical *Enterobacter aerogenes, Klebsiella pneumoniae* and *Citrobacter freundii* are localized in plants and within the intestinal tract of animals in the nature. And this type of coliforms are more important as a sanitation indicator . The methods used in collection tanks and cooling tanks to cool down the milk in farm significantly affect the natural microflora of raw milk. During the period following the milking, lactic bacteria especially lactococcus lactis sub-cultures form the dominant flora. Cooling milk and storing in cold create an important opportunity in terms of psychrotroph microorganisms which are present in the ability to grow at temperatures below 7^0 C. This situation in a sense, lead to a selection of soil, water and weotkgancle microorganisms There are less that 500 pieces/ml microorganisms in milk that milked from a healthy animal. Basically, micrococci, lactic streptococci and lactobacilli are found in breast and milk ducts. Other microorganisms are present in the milk obtained from diseased animals. In general, these are pathogens and are dangerous in terms of health care and hygiene. During the milking, many different types and numbers of present microorganisms can be transmitted by milker, milking containers, animal hair and skin rash, barn air, litter and food, transportation, piping system with the tanks and drums during the storage in farm or in business. If no attention is paid to the hygenic conditions during the production and preservation processes, they not only threaten the people's health but also may cause to economical losses because of some physical and chemical changes (Kılıç, 2010).

1.2 Kashar Cheese

Milk and milk products have an important place in the food industry because of the importance of human life. Cheese, one of these products is a milk product which its history dates back to thousands of years and has the widest variety in dairy products (Öztek, 1991).

Cheese; is a nutritious dairy product consumed as fresh or ripened, obtained by heating the milk, adding starter culture, coagulating the proteolytic enzymes, filtrating the clot and seperating it from the whey and shaping it by salting and suppressing the curd (Yetişmeyen, 2001).

This is indicated that cheese has the 30% worldwide sales value of all dairy products. Cheese making is a porcess that has been continuing since a few thousand years. Documents related to the manufacture of cheese go back to 6000-7000 BC (Fox, 1999). It is thought that, cheese production was first made in Mesopotamia between Tigris and Euphrates rivers. Today, this region includes some particular parts of Turkey, Irag and Iran (Kosıkowskı,1997). For cheese, that has an important role for centuries in the nutrition of all the communities, it is estimated that there are 4000 different types in the world, today (Topal, 1996).

In Turkey, the most produced cheese types are mainly white cheese, kashar cheese, mihalic, tulum and herbed cheese. Kashar cheese that has an important place among our chese types is special cheese type of Balkan countries and Turkey with its rich component and loved taste (Topal, 1996).

According to Turkish Standards Institute “kashar cheese” is defined as: a hard structural milk product that has a specific aroma, taste, color, smell, consumed before curing or after cured, obtained by directly or after pasteurizing the cow, sheep or goat milk or their bellies according to the processing techniques and if necessary by adding additives. In the same standards, there are also definitions for “fresh kashar cheese” and “old kashar cheese”. Old kashar cheese “should be placed in the markets as ripened for 90 days after its production under certain circumstances to get distinctive qualities”. Fresh kashar cheese is defined as, “ a cheese made from pasteurized milk, not subject to the rippening process and marketed as fresh” (TSE, 1999). Since fresh kashar cheese is put on the market right after its

production and most of the kashar cheese products are fresh kashar cheese, this cheese has become increasingly important (Yaşar, 2007).

In recent years, the production of fresh kashar cheese has become widespread due the lack of a long process of maturation and therefore it reaches to the consumers economically more affordable. fresh kashar cheese is widely used in making toast, pizza, pita bread and different food production (Koca, 2004).

After the TSE 3272 kashar cheese revised, fresh kashar cheese has become legal and the production of this cheese has increased. In the standard, fresh kashar cheese as defined in "TSE" (TSE, 1999). While most of the the milk products produced and stored under appropriate conditions did not importantly alter during the storage process, cheese exhibits biologically a highly dynamic structure and depending on the time, some changes occur on its structure, component, microbiological and textural properties (Atasoy, 2003).

The raw material used in the formation of peculiar color, odor, flavor, texture, porosity and shell-like features of every kind of cheese plays an important role in the applied technique and maturation process (Karaca, 2007). Cheese ripening is defined as; the sum of complex biochemical events occured with physical, microbiological and enzymatic interactions to give the cheese specific flavor, odor and texture according to its type after kept waiting with different conditions and durations. It is necessary for biocehmical issues to realise suitably during the ripening process to obtain cheese with peculiar quality features (Kılıç, 2010).

Kashar cheese is from the sliceable semi hard cheeses and takes place in the "filet of cake" (plastic curd) group. The main feature of this group cheeses is that the curd is boiled in hot water and kneaded after being acidicated at a certain level.

1.2.1 Production Stages of Kashar Cheese

The milk to be processed into cheese is pasteurized at 72-74^{o}C for 15 seconds or at 65^{0}C for 30 minutes and then cooled to fermentation temperature (32-34^{0}C) and starter culture (0,01-0,015(10-15 g/100L)) and calcium chloride added.Milk is subjected to pre-cured for 30-35 minutes. And when the pH value reaches to 6.40-6.45, rennet is added until it gets maturity clot cuts in 45 minutes. The clot (Ph: 6,30-6,35) is cut in 1,5-2.0 cm sizes; they rest for 5-10 minutes and then get broken until it reaches to pea lentil-size. After leaving it

alone for about 5 minutes, it is ensured the collapse of the curd grains and curd juice is removed. Resudial clot is slowly mixed for 10 minutes. Meanwhile the clot is slowly increased to 36-38^0 C (and sometimes 40-42^{0}C) with the steam pipes located between the wall of vessel. After the temperature reached the specific level, stirring is continued for 15 minutes. During the said temperature there will be 1^{0}c heat increase and this process is applied as it completes in 30 minutes. With this process, the walls of the curd grains hardens, because of the contraction the whey seperation is faciliated and the acidity increase is promoted. The curd that released its water and matured at a sufficient level is transferred to the pressing unit with the aid of a suitable pump. Or it is pressed in the boat with their own private press. The pH is 5,9-6,15 at the beginning of the pressing. In the suppressing, one kilograms (kg) of weight is applied for 1 kg of curd at the beginning. Then, this value is gradually increased and reach up to 15 kg. The temperature of the place of pressing operation is 15-20^{0}C, the total pressing time is 1-2 hours. After the pressing, the pH value of curd reaches to 5,25-5,30. The pressed curd is cut into blocks for 25-30 cm length and 15-20 cm wide and by covering over it is allowed to fermantation for 15-20^{0}C. When the pH level of curd reaches 5,0-5,05 boiling phase begins. After the fermentation of curd completed, it is cut into thick slices for 3-5 mm with mechanical graters or rotary blade size reduction apparatus and transferred to metal baskets and dipped in the boiling boiler where the 5-6% saline hot brine lays in it. The acidity of the boiling water should be 10^{0}SH. If the acidity value exceeds the specfied level, losses from curd increases in the boiling process. The curd slices dipped in boiling boiler are kept here for 3-5 minutes. Meanwhile, it is converted into a homogeneous mass by mixing and inverting one or two times. The dough is cut appropriately for the size of the dies used and each piece is placed in the dies after binding process.

Molded cheeses are allowed to cool from 12-24 hours of rest and during the process they are inverted 5-10 minutes after placing in the molds. Inverting process is done 5-6 repeatedly in 1-2 hours and a smooth shape is obtained. Being sold as fresh kashar cheeses are packed and taken to cold storages after a one-week ripening process.Today especially in the businesses that have advanced technological infrastructure, boiling and mixing operations are not done as it is described above; it is utilized from special mechanisms that are developed for this purpose. The systems are used which defined as aggregate or kashkaval machines that are shredding curd blocks for about 0.5cm, boiling in their boilers,

giving plasticity, kneading and molding (Üçüncü, 2008). In the manufacture of the kashar cheese, curd is boiled and kneaded after its acidity reaches a certain level, this melting process applied in this modified product is not specific to the production of kashar cheese. It would be more appropriate to call the cheese as "Block Type Melted Cheese" which obtained by melting process. Usually mono, di and poli salts of citric acid and posphoric acid are used as emulsifying salts. Unfortunately, many businesses also add returned cheeses with the ratio ups to 15-20% in the production of this modified product. While some of the returned cheeses may be the cheeses that produced by the businesses themselves but has problems on the packaging, oppressed during the marketing and distorted, some of them may be cheeses that are rotten and moldy received from the market for cheap prices. Since it is economic and non-problematic this technology is prefered more (Gönç and Dinkçi, 2006).

Table 1.2: Fresh Kashar Cheese production Flow (Üçüncü, 2008)

Raw milk
Fat: 3,4-3,7%
pH:6,60-6,75

↓

Pre-pasteurization

↓

Fat standardization
Fat: 3,2-3,4%

↓

Pasteurization
72-74^{0}C/15 seconds

↓

Heating
32-34^{0}C

↓

Adding Calcium chloride
10-15 g/100 L Milk

↓

Adding starter culture
0,5%

↓

Leavening
Heat: 32-34^{0}C
pH: 6,40-6,45
Duration: 45 minutes

↓

Breaking Clot and cutting curd
Ph: 6,30-6,35

↓

Largeness of cutting:15-20 mm
Largeness of breaking: 6-7 mm

↓

Taking whey
1/3

↓

Whey+heating curd
36-38^{0}C(sometimes 40-42^{0}C)

↓

Mixing and Aciditing
pH: 5,90-6,0

↓

Curd pressing
1...15 kg/1 kg curd
1-2 hours
15-20^{0}C
At the beginning of the pressing pH: 5,9-6,0
At the end of the pressing pH: 5,25-5,30

↓

Curd fermentation
At the end of the fermentation pH: 5,0-5,05

↓

Portioning
(At the desired weight with the photocell system)

↓

Filling into cups

↓

Resting and Singe
12-24 hours

↓

Removing from the cup-turning

↓

Packaging
Packaging-vacuuming-Shrinking

↓

Storaging
+4/+10^{0}C

The quality of the milk that will be cheese and the unwanted saprophytic and pathogenic microrganisms and contamination during the production of the cheese affect the quality of the cheese significantly. If no strong meaures are not taken, the unwanted micoorganisms, at least partially, can be dangerous for the health of the consumers and lower the quality of the cheese by multiplaying the various stages of the production. In developed countries, in

addition the to some measures, a heating process is applied to the milk that will be cheese and starters are used to obtain good quality cheese. (good quality raw milk, having hygienic requirements during making process, active packaging system). The applied heat process (65-75^0 C/15 sec.) causes destruction of some microorganisms (especially base Lactobacillaceae and Streptococcoceae) that is necessary for cheese making with the unwanted organisms at the milk. Hence, pure cultures of the certain bacterial flora that plays an important role at the all stages of cheese making is added to the milk as "starter" after heating process (especially to provide the necessary conditions at growth and prevent the reproduction and activeness of some harmful microorganisms which cannot be degradated during the heating process and/or contaminated after the heating process). Since it is uneconomical, when considering our kashar cheeses to be served to the consumer without maturating, it is clear to see why the product threatens the health of public (Gönç and Dinkçi, 2006).

1.3 E. coli

This microorganism was defined in 1185 by Theodor Escheric for the first time, and previously was known as Bacterium coli commune and then as Escherichia coli (Chen and Frankel, 2005). E. coli had been recognised as a microorganism which is non-pathogenic and found in the human and animal intestinal tract of normal flora until 1950 (Münnich and Lübke-Becker, 2004). Later, E. coli which was evaluated as an indicator of fecal contamination and accepted as a indicator microorganism at food hygiene had been identified as a potential pathogen after discovering the some serotypes as the causes of some diseases (Uğur et al., 1998).

E. coli is a negative (-) bacterium that takes place in Enterobacteriaceae family, belongs to Escherichia type and easily dyed to bacteriological dyes. The length of this plain-looking bacilli is 2-6 µm. However, *E. coli* strains may give polymorphism as short and small coccoid-looking in some cultures, too long in some other cultures and sometimes as filamentous shapes that are branching. Most of them have peritrichous lashes and move (Natora and Kaper, 1998).

E. coli is a facultative anaerob bacterium that is expressed as hours in the warm-blooded animals digestive tracts after the birth and colonized in a short period of time (30). This

bacterium directly may pass to other people from the ones who do not obey the contaminated food and hygıenic rules effectively (Leclerc, 2002).

This microorganism continues to live as permanent flora members of this region, after being colonized to human gut. It is identified that E.coli; has more than 700 serotypes according to O (somatic), H (flagella) and K (capsule) antigens. Serotyping is used for distinguishing disease-causing strains (Natora and Kaper, 1998).

E. coli serotypes that causing to intestinal diseases leading to death of humans and animals are in the enterovirulent E. coli group seperate from the *E. coli* which is mainly flora of guts. Within this group, there are serotypes defined as 7 sub-groups which have different virulence factors and affect intestinal system differently. These are; Enterohaemorrhagic Escherichia coli (EHEC), enterotoxigenic Escherichia coli (ETEC), Enteroinvasive Escherichia coli (EIEC), enteropathogenic Escherichia coli (EPEC), Enteroaggregatif Escherichia coli (EaggEC), diffuser-adherent Escherichia coli (OAEC) and facultive enteropathogenic Escherichia coli (FEEC) (Tunail, 1999).

1.3.1 Enterohaemorrhagic Escherichia coli (EHEC)

Even though it is rarely seen in the E. coli members that are transmitted to human bodies through the food, (EHEC) constitutes the most important group due to the high rate of death (Atasever, 2007). EHEC, which causes serious diseases in pathogenic E. coli groups causes three main syndromes. These are; hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) (Gönül, 1994).

EHEC strains are also called as Verotoxigenic Escherichia coli (VTEC) due to their ability to produce verotoxin. Since the disease symptomps are the same with symptomps caused by Shigella dysenteriae type 1, they are also called as Shiga-like toxin-producing (SLTEC). Up to now, at least 8 toxin variants have been identified and and these are named under two groups (VT 1 or SLT 1 and VT 2 or SLT 2). EHEC strains, besides their verotoxin producing (Siga-like toxin) ability, they also have other virulence factors. In EHEC group, the most important serotypes are O157: H7 and O157: H-. It is stated that, the minimum infection doze of these serotypes is less than 10^2 cells. O26: H11, O26: H-, O111: H8, O111: H and possibly O22: H8, O91: H and O113: H21 serotypes are included in EHEC group (Atasever, 2007).

1.3.2 Enterotoxigenic Escherichia coli (ETEC)

ETEC group, is responsible for the 60-70% of the diseases named as tourist diarrheal disease which is seen in the people that go to especially from countries that have good hygienic conditions to countries which have lower hygienic standards and warm climate (Thapar, 2004). In addition, it is an important reason of infants and children diarrhea in developing countries (Karapınar, 1998). Bacterium is taken with contaminated milk and food. It is reported that the minimum infection doze is 10^8 bacteria. First, bacterium is colonized, then secretes the toxins (Atasever, 2007). The incubation time changes between 8-44 hours and is approximately 26 hours. The disease time is 24-30 hours and the infection causes rice water-like watery diarrhea and dehydration . The most important pathogenicity factor of ETEC is that it has an ability to produce enterotoxins which are heat-labile (LT, heat-labile) and/or heat stable (ST, heat-stable) (Vicente et al., 2005). ETEC strains can produce only one toxin or the both toxins. Heat-labile toxin (LT) is in the form of protein and gets inactivated at 60^0C in 30 minutes. LT is similar to Vibrio cholerae toxin immunologically and can be neuralized with cholerae antitoxin (Kırkpınar, 1998).

Heat-stable toxin (ST), besides its heat-stable ability, is stable to proteolytic enzymes, nucleases, lipases and organic solvents. The toxin consists of 18 amino acids. Colonization factors play important roles for ETEC strains to show its toxic effects. When ETEC reac hes the intestine, it is colonized to epithelial surface of small intestine and secretes one or more toxins that affect the small intestine. ETEC cells do not pentrate to the epithelial layer of small intestine and while it does not harm, it is colonized only on surface. Special attachment fimbriae plays a role in colonization.Fimbriae is in the filamentous stucture of protein and is specific to the species. Colonization factors are controlled by gens linked to the plasmid and plays a role at ETEC pathogenity with enterotoxins (Gönül,1994). O6, O8, O15, O20, O25, O63, O78, O85, O115, O128 AC, O148, O159, O167 serotypes are included in this group(34). It is stated that, feaceal contaminated food and drinking water play important roles for the transportation of ETEC (Atasever, 2007).

1.3.3 Enteroinvasive Escherichia. coli (EIEC)

The disease table EIEC serotypes is similar to Shigella and follows watery, bloody and mostly mucosal diarrhea, respectively. They are settled in the large intestine, proliferate in epithelial cells by entering here and kill the cells (Tunali, 1999).

Infection doze is high and between 10^6-10^8 cells. Incubation time is generally between 8-24 hours and approximately 11 hours. Contaminated water, cheese, potato salad, canned and salmon are among the source of nutrients that cause the occuring diseases. Especially E. coli O:124 is the most common one. In other serotypes, O28, O29, O136, O143, O152, O164 and O167 can be included (Karapınar, 1998).

It is stated that the virulence genes that cause to spread by invasive way are on the plasmin sized 120-140 Md (megadalto, the molecular weight in terms of dalto) (Atasever, 2007).

1.3.4 Enteropathogenic Escherichia coli (EPEC)

EPEC strains cause severe watery and bloody diarrhea. EPEC's which are the sources of disease common in infants and children cause the deaths of few hundred of children (Vicente et al.,2005). EPEC's show their effects by clinging to the intestinal epithelium. EPEC and intima have been identified as virulence factors. The gen that determines the intimin has been found in the plasmid in size of 50-70 Md (34). When it is present at contaminated water, milk, cheese, red and white meat for 10^5-10^7 levels, it may cause poisoning (Atasever, 2007).

1.3.5 Enteroaggregatif Escherichia coli (EAEC or EaggEC)

EAEC group mostly causes persistent diarrhea at the children of tropical countries. Its role in travelling diarrheas seen in adults is controversial. Heat-stable enterotoxin (EASTI) was found in the 50% of EaggEC's as virulence factor (Tunail, 1999).

1.3.6 Diffuser-adherent Escherichia coli (OAEC)

OAEC group also causes persistent diarrhea at children. They are diffused as they cling to the cell via adhesion. The existence of two seperate adgesin genes and as well as the presence of intimia were determined, lastly (Tunail, 1999).

1.4 The source and Epidemiology of *E. coli O157:H7*

There are different opinions for the source of *E. coli O157: H7* serotype. Results of several research show that this bacterium has spread to meat, milk, soil, water and thus into the environment via faeces of mammals and poultry slaughters defined as warm-blooded animals, especially cattle (Halkman, 2001).

Studies on the genetics of pathogenic bacteria are still intense. The theory on forming of the *E. coli O157:H7* serotype because of the genetic change on an enteric bacterium has been widely accepted after the genetic studies made on E. coli type (Park et al., 1999). It is accepted that, while the commensal E. colis choose the intentines of mammals, the pathogenic ones choose to localize to suitable places of circulatory system after overcoming intestinal epithelium. It is found in the chromosomal analysis made in the enteric pathogens that seperate DNA segments encode functional virulence characteristics and named as pathogen enclave (pathojenity island). More interestingly, these genes often arise as they are gained from other misroorganisms. Pathogen enclave gives the bacterium a complex virulence characteristic and prevents recombination with gene transfer. Pathogen enclave usually contains the genes responsible for cell surface proteins such as hemolysin and fimbriae. As this structure disclosed the evolution theories of *E. coli O157:H7* have got a new direction and some theories evolved, alongside the existing one, which support that at least particular pathogenic E. coli clones are included at different stages (Park, 1999). The localization of Locus of Enterocypte Effacement (LEE) to commensal bacterium chromosome is a main phase for forming of a EPEC-like clone (Jores et al., 2004). It is accepted that, the completion of evolution is by gaining of LEE before a *E. coli O157:H7*: -like ancestor, receiving STL-2 with transduction, gaining the EHEC plasmid which encodes the hemolysin and then gaining STL-1 and finally with the loss of ß -GUR activity with sorbitol fermentation (Park et al., 1999).

E. coli O157:H7 serotype is seen at dogs, birds, sheep, goats, pigs, chickens, rabbits, deer and human while the main source remains as cattle (Cleary, 2004). The reason for this is that the reason for many outbreaks caused by this bacterium is the meat and meat products and raw milk (Conedera et al., 2004). Even though, at first it was thought that the source of this bacterium was poultry slaughters, after determining that the 25 chicks which experimetally given *E. coli O157:H7* release this bacterium with their faeces eight months after the inoculation, the findings of the research made on 500 chicken faeces in 50 chicken

farm showed that the bacterium was not present and the poultry slaughters are not the potential source in this respect (Halkman et al., 2001).

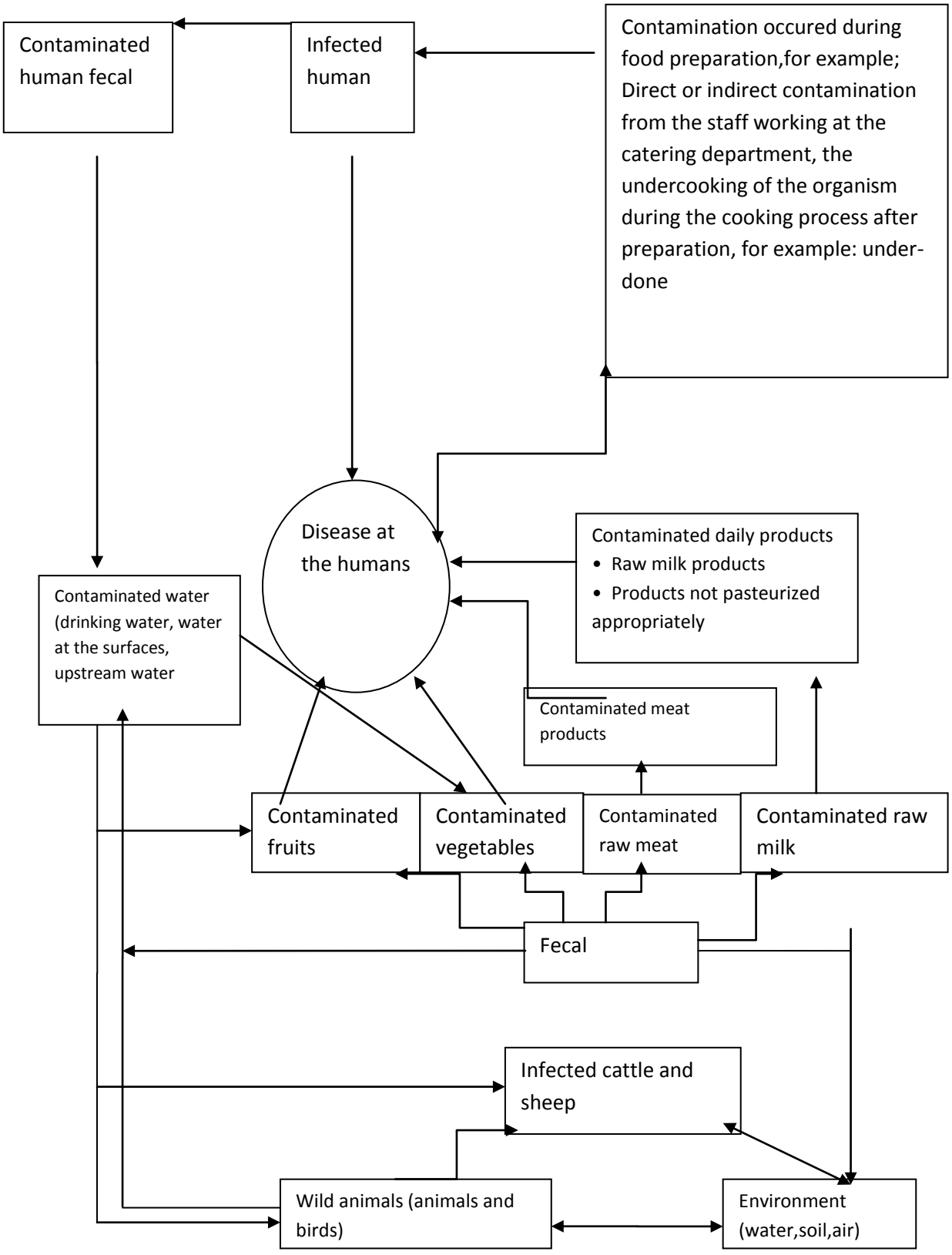
It is revealed that the soil and water which are contaminated with *E. coli O157:H7* and faeces of infected animals and human, have important roles in the transportation and spreading of this bacterium infections. Moreover, swimming in the water infected with the faeces may cause infections (Akçelik et al., 2000).

As the results of the studies it was concluded that spread of the infection via faece varies depending on age and season. In a study conducted on cattles in the UK, it was observed that the spread of bacterium via faece is low in winter season, while it is extremely increased during the summer season. (Similarly, Conedera and his colleagues (Conedere et al., 1997) reported that *E. coli O157:H7* serotype is more isolated at animals during summer season in their research made in Italy in slaughterhouses and farms.

It is reported that, especially baby and young cattles under six months are more resistant to *E. coli O157:H7*. It is said that these are infected and even though they do not show any disease symptoms, they spread a large number of bacteria to the environment with their fecal. (Yılmaz et al., 2002) have taken fecal sample from 330 cattles with systematic sampling procedure from five different slaughterhouses in Istanbul and even though they do not show any symptoms, they have isolated *E. coli O157:H7* in 14(4,2%) cattle fecal (most of them are under 2.) Similarly, in the researches made in America, Spain, Sweden, Germany, England and Canada, it was found that the spread of this bacterium to the environment via fecal is more with the animals under 24 months. Even though a serious decrease found at the number of bacteria at the fecal while passing through the rumen and intestines, experimentally, finding no clinical signs at the six-eight weeks old calves which had 107kob/g levels of *E. coli O157:H7* in their feeds showed that young cattles play important roles for spreading the *E. coli O157:H7* to the environment (Park et al., 1999).

The disease may easily pass to someone from another person who is infected because of cases occured by not paying enough attention to personal hygiene (Akçelik et al., 2000). It is known that, the infection may spread from person to person quickly especially at the places such as kindergarten and almshouses where there is not adequate personal hygiene (Doyle and Cliver, 1990). Figure 1 shows the distribution circle of *E. coli O157:H7* (Atasever, 2007).

Tables.1.4: The distribution circle of *E. coli O157:H7*



1.5 The Pathogenicity and Biochemical Properties of *E. coli O157:H7*

E. coli O157:H7 is an important pathogen at the EHEC group in Escherichia type within the Enterobacteriaceae (Yuk, 2006).

It is found that, the toxins within the *E. coli O157:H7* pathogen named as verotoxin (VT) or Shiga-like toxin (SLT) have important roles. These toxins have been previously named as verotoxins (VT) since they make cytotoxic effects to vero tissues which are obtained from the kidney of green African monkey (Bilgehan, 2004). However, later, due to a large similarity (99%) to shiga toxin which is created by Shigella dysenteriae type 1, it has been called as shiga-like toxin (SLT) (Uğur, 1998). It is found that all of the clinical isolates produce one and/or two toxins and these are named as SLT-1 ans SLT-2 (Gönül, 1994).

It is reported that, Shiga like toxin 1 (SLT) stops the protein synthesis by inactivating the 60 S ribosomal units of cells. It is found that the molecular weight and isoelectric points of this toxin called as SLT-1 A and B are 5000-7000 Md, and 7.1 respectively. Although, the other toxin SLT-2 is 55-60% similar to the SLT-1, it is called Shiga-like toxin. hela and vero tissue culture is toxic to its cells. The isoelectric point is 5.2 and do not neutralize with anti-shiga toxin .It is not fully clear whether *E .coli O157:H7* produce SLT in the food or the people get sick or not when they consume the food that already have toxins (Doyle, 1997).

The pathogenicity of the EHEC strains depends on the presence of other virulence factors, besides shiga-like toxin production. The most important one among these factors is membrane protein called as intima. It is known that the related gene (E. coli attaching and effecting, eae) is induced by chromosome. Intima provides a strong colonization with intestinal epithelial cells of bacterium. Besides Eae gene of *E. coli O157:H7* and *E. coli O157:H7* serotypes, there are some other serotypes. However, it is stated that this gene is not present in the small number of STEC which cause diseases in human, and probably there are other colonization factors in this strains (White, 2002).

The plasmid borne enterohemolis is defined as another virulence factor in the STEC strains. However, the importance of the enterohemolis gene in the plasmid was not explained sufficiently as virulence factor (Tunali, 1999).

The main causes of diarrhea are E. coli serotypes which are *E. coli O157:H7* and O126: H11. From these serotypes which cause same kind of diseases, O126. H11 was not found

in the food. In addition, while the different strains of O157: H7 serotypes produce VT-1 and/or VT-2 toxins, all of the strains of E. coli O126. H11 serotype produce only VT-1. *E.coli O157:H7* serotype takes place in the most dangerous food-borne pathogens as accepted today. Besides, it is stated that STEC serotypes such as O111 (unlike O157) are seen frequently in the food and they cause clinical diseases (Gönül and Kırkpınar,1994).

E. coli O157:H7 serotype differs from other E. colis: can not develop at 44.5 ^{O}C and above, can not ferment the sorbitol, do not have β -glucuronidase enzyme, against this, it has the eae gene, have 60 Md plasmid and produce OMP ecpression and enterohemoli not commonly at 5000-8000 Md weights. While the 95% of the sorbitol can be fermented in 24 hours, *E. coli O157:H7* sorbitol is fermentated in 48 hours. In addition, sorbitol positive ones are also found among SLTEC O157 strains (Özbaş and Aytaç, 1995).

While the 97% of the E. colis contain β-glucuronidase enzyme, *E. coli O157:H7* serotype β- glucuronidase is negative. While interohemolit which is accepted as a new type of hemolysin is produced by verotoxin positive *E. coli O157:H7* and *E. coli O157:H7*strains, this characteristic is not seen in other E. colis. Apart from these, *E. coli O157: H7* serotype is less resistant to bibile salts comparing to other E. colis. Its antigenic structure provides a clear distinction from other E. coli .

The fluorogenic MUG indicator of E. coli depends on the β-glucuronidase enzyme's activity which encoded by uida gene. While the presence of the gene was shown in the *E. coli O157: H7* in EHEC group, the sequence anaysis showed that there are some base mutation in this uidA gene of the serotype. Therefore, MUG reaction which is typical in *E. coli 0157:H7* serotype and other E. colis, is negative (Krishanan et al., 1987).

The serological connection among the E. coli strains were determined in 1921 for the first time. In 1937 Lowel asserted that, E. coli has two antigens called capsule and somatic, Kauffman also showed the flagella antigen in 1943. Accordingly, in E. coli, 165 somatic O antigens between O1-O171, 90 capsule K antigens between K1-K90 and 56 flagella H antigens between H1-H56 were found. Although 171 O antigens were determined in various researches, when the numbers 31, 47, 67, 72, 94 and 122 were eleminated, 165 O antigens left. According to recent studies, there are 174 O, 56 H and 80 K antigens (Doyle et al, 1997).

There are significantly cross-reactions between E. coli somatic O antigens and Salmonella, Shigella, Citrobacter and Providencia type bacteria. 25 of the most common O antigens with thermostable property are antigens. Capsule K antigens that are found in cell membrane, sheath or capsule are in L, B and A groups. L and B group is superficial somatic antigens and A group is capsule antigens. K antigens also show thermostable property. There are also Vi, a, β, F antigens in capsule antigens. The monophasic H antigen is found only in movable type and sensitive to heat. Flagellar H antigen do no cross-react with each other and with H antigens of other bacteria (Özbaş ve Aytaç, 1995).

1.6 The factors that affect the Reproduction and Viability of *E. coli O157:H7*

The growth in food and viability of *E. coli O157:H7* depending on some various internal and external factors especially such as heat, pH and water activity (Atasever, 2007).

1.6.1 The content of nutrient

The content of nutrient, is importantly effective on the viability of *E. coli O157:H7*. Hudson et al., 1997 reported that, the *E. coli O157:H7*level falls below the detection limit on the 27th day at the Colby and Feta cheeses and on the 30th day at the Romano cheese. Reitsma and Henning., 1996, reported that this pathogen can be alive for 158 days at Cheddar cheese. Ingham and his colleagues (Ingham et al, 2005) found that both of the pathogens had stayed alive for weeks at the salamura pickled specimens which were pended at 4 and 13^{O}C for 35 days by inoculating *E. coli O157:H7* at 10^{6}kob/ml level.

1.6.2 Heat

The optimum growth temperature of *E. coli O157:H7* is 37^{O}C. and growth temperature range changes bewteen 8-45 ^{O}C (Lechowich, 1998).It was reported that the temperature range should be between 19.3-41 ^{O}C for gas production and growth in 48 hours at *E. coli 0157:H7* EC broth. In addition, it was reported that it could grow rapidly between 30-42 ^{O}C at Tripticas Soy Broth and the generation time is 0.49 hour at 37^{O}C and 0.64 hour at 42^{O}C (Doyle, 1987).

It was determined that *E. coli O157:H7* serotype shows a better resistance against high temperature at higher temperatures that normal growth temperature by synthesizing a new group of proteins. In the same study, it was seen that heat-shock treatment to *E. coli*

O157:H7 that can grow at aerobic and anaerobic conditions increases the number of alive bacteria, and the applied heat-shock increases the ability of staying alive at processing temperature for bacterium inhibition. It was reported that *E. coli O157:H7* serotype is more sensitive to high temperature than Salmonella and *E. coli O157:H7* can be eleminated completely by pasteurization process applied to milk at a level higher than 10^4kob/ml. It was reported that *E. coli O157:H7* is highly resistant to freezing degrees and can stay alive in ground beef at -20^{O}C in 9 months.D'aoust and colleagues found that, the heat treatment causes a decrease at *E. coli O157:H7* inoculated raw milk at 1x10^5kob/ml level applied at 60-72 ^{O}C in 16.2 seconds for 2 log-unit at *E. coli O157:H7* level and the bacterium loses its viability at >64.5^{O}C. Massa et al., reported that the high temperature and short-time (72 ^{O}C in 15 seconds) process that is used widely at the pasteurization of milk is rather effective in *E. coli O157:H7* inhibition at 1x10^5kob/ml level. In addition, it was stated that this pathogen loses its ability to grow and verotoxin creating at raw milk which is maintaned in the refrigerator (5^{O}C) (Özbaş, 1995).

1.6.3 pH

The other important factor that is effective on the reproduction and viability of *E. coli O157:H7* is pH factor. It was expressed that since pathogen E. coli can not reproduce under pH 5.4, *E. coli O157:H7* serotype is also resistant to the low pH environments and the reason of this is the acid variation in the environment. It was reported that the resistance of the factor against the acid depends on the growth phase with the pH environment, the acid tolerance level is rather high for EHEC strains and *E. coli O157:H7* can stay alive for at least five hours at the 3.0 and 2.5 pH environments (Arocha, 1992).

Since *E. coli O157:H7* is resistant to acid, it facilitates the transition from the stomach to the intestines. Therefore, infection doze at people is rather low (Park, 1999). In a study conducted on the staying alive ability of *E. coli O157:H7* at extreme conditions, it was reported that, various acid resistance systems are effective for E. coli pathogen to keep its viability at acid stresses in stomach (ph 1-3) and intestines (pH 4.5-7), and once it is inducted, the acid resistance system can remain stable actively in cold (4^{O}C) storage(Lin et al., 1996).

1.6.4 Water Activit

The optimum water activity value (aw) of *E. coli O157:H7* was determined as 0.98. It was reported that when this value drops below 0.95 the growth of factor is blocked (Kırkpınar and Gönül, 1998). In the experimental studies and food-borne outbreaks, it was reported that *E. coli O157:H7* keeps its viability at a significant level in dry conditions (Park et al., 1999).

1.6.5 Salt

In a study that worked on the effect of the heat and high salt concentration on the growth of *E. coli O157:H7,* it was reported that this bacterium was completely inhibited at chicken extract broth at 37^{O}C for 8% and at 10^{O}C for 6% of NaCI concentration. This ratio was determined as 4% at TS broth. (Abdullah and Davies, 1999) found in their studies that, *E. coli O157:H7* reaches to 10^{8}kob/ml at mTSB liquid medium for 3.5% and 6.5% in NaCI concentration after a 30-40 hours of incubation period. It was reported that similarly to these findings, *E. coli O157:H7* can grow in the environments that have 6.5% NaCI and can keep its viability at high salt concentration (Özbaş and Aytaç, 1995).

In a study conducted on the effect of incubation degree and salinity on the growth of *E. coli O157:H7*, it was determined that the factor makes reproduction on the first day in TSB medium that has 6% NaCI at 7^{O}C and no reproduction was seen on the 38th day (Conner, 1992).

1.6.6 Competitive Flora

Lactic acid bacteria may cause the death or suppression to the growth of other microorganisms at the same environment with inhibitory substances that they produce (for example H_2O_2) and with the environment conditions that they change. It was reported that these bacteria have antagonistic effect on *E. coli O157:H7*. Lactic acid bacteria have also inhibitory effect on the psychrophilic microorganisms that can grow at refrigerator temperature (Park et al., 1999).

Palumba et al. (1997). in a study that they worked on the effect of the competitive flora and ambient temperature, they reported that this pathogen can grow when there is low number of competitive flora (8O ℃) while there was no growth when the number of competitive flora increases at the same conditions, but the factor keeps it viability and the

nutrients which are maintained on this heat degrees may pose a risk in the direction of *E. coli O157:H7* (Palamba et al., 1997).

Duffy et al , in a study that they worked on the effect of the competitive flora, heat and pH on the growth of the bacterium which *E. coli O157:H7* (3 log kob/ml) and mixed lactic culture (4 log kob/ml) inoculated at liquied culture (BHI), they reported that these factors are rather important on the growth of this pathogen and the best inhibition can be obtained when mixed culture is used and when the incubation is provided at neutral pH and at $37^{O}C$ (Duffyet al., 1999).

In another study which the effect of competitive flora on the growth of *E. coli O157:H7* investigated, it was reported that the bacterium would grow slower at raw milk than pesteurized milk and this is mostly caused by the antogonistic effect of the competitive flora (Wang et al., 1997).

1.6.7 Antibiotic Susceptibility Characteristics

*E. coli O157:H7*serotype is resistant to antibiotics and/or becoming increasingly resistant. It was reported that *E. coli O157:H7* serotype is resistant to cephalothin and colistin. Therefore, the use of antibiotic and anticoagulant in diseases is discussed. In scotland it was reported on the subject that it increases the risk of catching HUS/TPP for the patients who use antibiotics randomly and stomach acid-lowering pills (Doyle et al.,1997). It was reported that in an outbreak seen in Japan in 1996 where a total of 6000 people affected and the majority of the people were school-age children, the antidiarrheal medications which taken on the 7th day had started the disease table grow (Park et al., 1999).

1.7 The Nutrients Mediate the *E. coli O157:H7* Infection

E. coli O157:H7 was defined for the first in 1982 as food-borne pathogen microorganism Ağaoğlu et al., 2000). All kind of nutrients which are contaminated with animal (especially cattle feces) feces directly or indirectly, have the potential danger in terms of *E. coli O157:H7* infection. All over the world, a large part of these infections occured by this microorganisms are beef-borne nutrients which are primarily undercooked meat and products and unpasteurized milk (Irino et al., 2005). In addition, it is known that it plays an important role in the spread of soil and water contaminated with animal feces and mediately the transmission and spread of the disease (Göktan, 1990).

Initial studies showed that, the most important source of the transmission of *E. coli 0157:H7* is animal products. *E. coli O157:H7* is isolated from the meat and meat products such as ground beef, pork chops, turkey, chicken, lamb meat (Lindqvist et al., 1998). Also, it was demonstrated with the studies that *E. coli O157:H7* can be transmitted from apple juice, mayonnaise, raw or unpasteurized milk, non-chlorinated drinking water and vegetables (Eatreda-Munoz, 1998).

1.8 The Syndroms Caused By *E. coli O157:H7*

The minimal infection doze of *E. coli O157:H7* is at very low levels, such as (MID) 10-100 kob/g. While this level is reported as 2-2000 kob/g in some sources, it was reported that infection may ocur when the bacterial number is 10-100 kob/g (Reitsma and Henning, 1996).

E. coli O157:H7 which is included in EHEC group may cause three main syndromes; hemorrhagic colitis, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura (Stampi et al., 2004). Besides these, septicemia, meningitis and urinary tract infections may occur. *E.coli O157:H7* infections may be more effective on elder people and children (Vold et al., 2000).

According to Centers for Disease Control and Prevention, it was reported that in USA the number of cases caused by food-borne bacterial diseases is approximately 76 millions in a year and while 325.000 of them took treatment at hospital, 5.000 cases were resulted in death. It was reported that 20.000 of them were infection cases caused by *E. coli O157:H7* and approximately 250 of them were resulted in death. While the disease incidence is 2.1 per 100.000 people in the USA, it was reported that this rate was 3-5.3 between the years 1991-1996 in Canada (Ozer et al., 2006).

1.8.1 Haemorrhagic colitis (HC)

Incubation time is 3-9 days in this kind of infections. The severe abdominal pain and watery diarrhea occuring withing the first 24 hours and in the following days, not having the diarrhea turning into blood without faeces and heat are the most prominent clinical findings. The disease usally heals spontaneously within 2-9 days. Death may rarely occur on the situations that serious complications occured (Özbaş and Aytaç, 1995).

1.8.2 Hemolytic Uremic Syndrome (HUS)

The hemolytic uremic syndrome (HUS) which was defined for the first time in 1955 is the disease that causes the deaths the most. Elder people and infants are the most important risk groups in the disease table which occurs as a serious complication of hemorrhagic colitis. While the death rate is 10% in children, this rate is 50% in elder people. It was reported that the pathogen of the disease is related to the toxin which damages the endothelial cells and disrupts the clotting mechanism and the microthrombus cause the accumulation of waste products in the blood by blocking capillaries in the kidney or other organs. While it is seen in all age groups, it is known as the major cause of kidney failure especially in infants and children. Hemolytic anemia, thrombocytopenia, acute nephropathy and bloody diarrhea are the main clinical findings of the disease. Also, hepatitis, high blood pressure and heart failure can also be seen (Park et al., 1999).

1.8.3 Thrombotic Thrombocytopenic Purpura (TTP)

Clinical symptoms are usually similar to HUS in this disease table. However, central nervous system is affected more in TTP. Heat, hemolytic anemia, thrombocytopenia and neurological symptoms are typical clinical findings. The risk of death is rather high because of the blood clut occured in the brain (Park et al., 1999).

1.9 Protecting From *E. coli O157: H7* Infection

To prevent *E. coli O157:H7* infection, control measurements must be taken at every step of the production phases from food acquisition to processing and preparation (Atasever, 2007).

It is very important for the personnel who work in the farms, food processing places, nurseries and nursing homes to be trained about hygienic food providing techniques, the direct or indirect contaminations caused by raw or cooked nutrients and personal hygiene to reduce the bacteria transmission to people at minimum level (Peacock et al., 2001).

To prevent the infection to spread, it is especially provided for children to wash their hands with soap properly after using bathrooms, before eating food, and after contacting with farm animals and raw food (Atasever, 2007).

Carcass feces and contamination risks can be reduced with hygienic slaughter practices. In addition, as a preventive measure, the meat must be cooled under 7^{O}C rapidly after its cut and the milk must be cooled under 5^{O}C (Hudson et al., 1997). Since undercooked meat and products, unpasteurized milk products and fruit juices have risks for this bacterium, especially the heat treatment applied to this kind of nutrients must be all over the product (center included) and at 70^{O}C and above (Temelli, 2002).

Non-chlorinated water should not be drunk or used for surface cleaning of equipments in food processing places and water which chlorine or other effective disinfectants applied should be consumed (Doğruer, 2004).

It is suggested for the waste water to undergo certain processes which is used for irrigation of cereal, fruits and vegetables (Atasever, 2007). It was reported that, in an outbreak in East Massachusetts which 18 people affected by consuming unpasteurized fresh squeezed apple juice and HUS was found in four children, the reason was the apples which were exposured to fecal contamination from the ground and not washed. Similarly, it was stated that such an out break was observed in Canada in 1980 (Beser et al., 1993).

Today, there is no effective vaccine to be protected from diseases caused by EHEC but experimental studies on animals have been continuing (Temell, 2002).

1.10 The History of *E. coli O157:H7*

Food poisonings are one of the most common infectious diseases in our country and in the world. One of the other factors that often isolated in food poisonings is Verotoxigenic E. coli (VTEC). The concept Verotoxigenic (VTEC) was used for the first time in 1977 for E. coli strains which produce toxin that is cytotoxin for Vero cells. Another synonymous naming is "Shiga-toxin-producing E. coli (STEC)". VTEC and STEC terms are used for E. coli strains which produce one or more toxins from higa-toxin family. It is a serotype within the *E. coli O157:H7* EHEC group and it was identified in 1982 as food-borne pathogen (Tolun et al., 2011).

E. coli was isolated for the first time in 1885 by Theodor Escherich from a child's faeces and named as Bacterium coli commune and later "Escherichia coli" name was given. Initially, while this bacterium was only accepted as fecal contamination index since it was in the normal intestinal flora of warm-blooded animals, the perspective for the E. coli

changed because the presence of E. coli serotypes which cause diarrhaea was found through the end of 1940's, toxins similar to Vibrio cholerae toxin were found in mid 1950's and finally pathogen types which may lead to death in humans and animals were found. Today, one of the most important food-borne pathogens that is known is *E. coli O157:H7* which is a special serotype of this bacterium (Halkman,1997).

In Turkey, from the researches on the presence of *E. coli O157:H7* in milk and milk products, Levent Akaya et al, found *E. coli O157:H7* in 3 of (3%) 100 raw milk samples and 1 of (1%) 100 cheese samples which taken for analysis to determine the presence of *E. coli O157:H7* in the raw milk and cheese consumed in Afyonkarahisar.

It was determined that the presence of Verotoxigenic Escheria coli in milk and milk products sold in Istanbul was being investigated and results showed that E. coli was present in 9 of 74 cheese samples (Tolun et al., 2011).

In North Cyprus, no written record has been found on the existence of *E. coli O157:H7* in raw milk and kashar cheese.

CHAPTER 2

MATERIALS AND METHODS

2.1 Material

Samples: In TRNC, 40 kashar cheeses from the cheeses sold in markets in Nicosia between the dates September-December 2013, and 60 raw milk samples from different parts of raw milk carriers formed the materials of research.Kashar cheese samples were used as they were taken from the store. Raw milk samples were placed in steril sample containers as they were taken from raw milk jugs for 100ml. These taken samples were analyzed whether they contain *E. coli O157:H7* or not.Since it is an important food-borne pathogen, various commercial chomogenic and fluorogenic media were developed as an alternative to reference methods for finding *E. coli O157:H7* in food.

These media; Rainbow agar 0157 (Biolog, Hayward, USA), BCM O157:H7 (Biosynth AG, Staad, Switzerland), CHROMagar O157 (CHROMagar, Paris, France), and Fluorocult O157: H7 (merck, Darmstadt, Germany) (Maryland, 2001).

In our study, we used CHROMagar O157 dust medium by considering its accuracy, specificity, sensitivity criteria because of the performance that chromogenic and fluorogenic media showed, since they are in the reference methods and also since they reduce the cost and work force in laboratories and since the obtained data give absolute scores(Halkman, 2005).

2.2 Media

2.2.1 CHROMagar O157 (chromogenic and fluorogenic media)

Compund

Total..... 29.2 g/L

Agar.....15.0

Pepton & Yeast extract.....13.0

Chromogenic mix.....1.2

15/30^{O}C-pH: 7.0±0.2

It was heated by stirring in purified water for 29.2 g/L taken from media which counted as commercial and it was kept at boiling temperature for two more minutes after the boiling had started. The prepared medium was kept in hot water bath ($47^{O}C$) during the analysis for use. The medium was prepared according to its procedure.

2.2.2 Mtsb-Broth with Novobiocin (Merck 1.09205)

Compound g/L
Soy Peptone 3.0

NaCI 5.0

Bile Salts No 3. 1.5

D (+) Glucose 2.5

K2HPO4 4.0

Novobiocin 0.02

The medium was prepared in distilled water by dissolving at 33 g/L concentration from commercially sold medium, distributed to flasks for 225 ml and then sterilized at $121^{O}C$ for 15 minutes.

Its pH at $25^{O}C$ is 7.3± 0.2 after sterilization.

The prepared broth is clear and yellowish colour (Ozer and Demirci,2006).

2.2.3 Tryptone Water (Merck, 8.03057)

Compound g/L
Peptone from casein 10.0

Sodium chloride 5.0

After weighing for 1.5 g, the commercially sold medium was dissolved in 100 ml distilled water. After completing the homogenized process, it was distributed into tupes for 5 ml. It was sterilized at 121 ^{O}C for 15 minutes at the end of the processs (Halkman, 2005).

2.3 Method

In TRNC, 40 samples were taken from markets in Nicosia, and 60 raw milk samples were taken from raw milk producers between the dates September-December 2013. The taken samples were analyzed in terms of *E.coli O157:H7*. The samples were put in sterile

containers after obtaining from raw milk producers, kashar cheeses were taken from different companies found in the markets for 250gr, 500gr and 1kg and they were analyzed under cold chain conditions in a short time.

2.3.1 The Preparation of the Samples

After being brought to the laboratories, the samples were weighed into sterile stomacher bags for 25 g for microbiologic analysis and then homogenization process was started.
Two samples for 25 gr were taken from the inside of each kashar cheese and samples were taken for 25 ml from raw milk samples. Double analysis were made for each sample to increase the reliability of microbiological analysis and to minimize the error rate (Anderzant, 1992).

2.3.2 Homogenization

25 g of each raw milk and Kashar cheese samples were weighed into sterile stomacher bags for *E. coli O157:H7* analysis. 225 ml mTSB-Broth with Novobiocin (merck 1.09205) was added to the 25 g sample weighed for *E. coli O157:H7* and it was homogenized with stomacher for three minutes (Koneman et al., 1997).

2.3.3 Enrichment

After being brought to the laboratory, raw milk and Kashar cheese samples media were homogenized with Mtsb-Broth with Novobicin (Merck. 1.09025), incubated at 37^{O}C for 16-20 hours and subjected to the enrichment process(Koneman et al., 1997).

2.3.4 The Isolation of *E. coli 0157: H7* on Solid Media

It was transferred to petri dish from enriched medium for 1 ml and then 20ml CROMagar medium was added into it. After mixing the media and sample containers thoroughly by drawing eight, it was left for incubation at 37^{O}C for 24-48 hours. After mixing petri dishes thoroughly they were left to dry, and the dried petri dishes were left for incubation after turning down at oven for 37^{O}C in 24-48 hours (Anderzant and Splittstoesser, 1992).

Metalic blue and lilac coloured growths were observed in the petri dishes at the end of incubation. The metalic blue coloured colonies were observed as E. coli and the lilac

coloured ones were observed as E. coli O157: H7 and the petri dishes were subjected to the verification test after the observed growth.

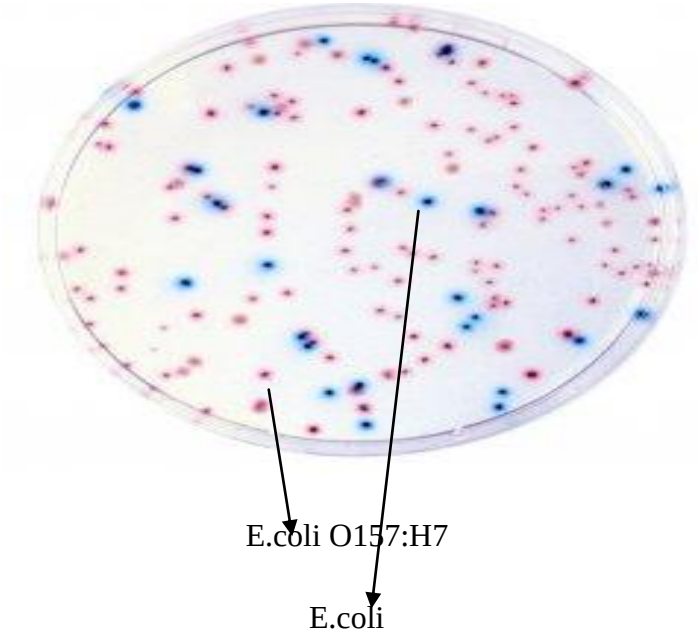


Figure 2.3: Petri dish sample observed in the samples

2.4 Serological Tests

2.4.1 *E. coli O157:H7* Test

For *E. coli O157:H7* test, a drop of sterile physiological saline water was added on a clean lame and it was stirred until obtaining a homogeneous and cloudy suspension after taking from fresh culture of suspicious bacterium, carefully.

Then a drop of *E. coli O157:H7* antiserum (Denka-Seiken) was added to prepared suspension and stirred and formation of agglutination *E. coli O157:H7* was considered positive within one minute.

In the preparation of medium, 10 g peptone, 3 g meat extract, 5 g NaCI and 3 g agar agar were weighed and dissolved in 1000 ml distile water. After adjusting the pH level of prepared mixture to 7, they were dispensed to 13x100 mm glass tubes for 6.3 ml. After cooling the medium to 45^{O}C which was sterilized in autoclave at 121^{O}C for 15 minutes, the dilutions of antisera (Denka-Seiken) 1/64 – 1/65 536 were prepared. After stirring the tubes, they were put in the fridge for medium to solidify. Some of the prepared tubes were used without adding antiserum for control. The medium was removed from refrigerator before the study and they were allowed to reach room temperature. Then, fresh culture of

suspicious bacterium was soped in to niddle-tipped essence and to test which contained antiserum and to control tubes which do not contain antiserum for 0.3 cm and it was inoculated. At the end of an incubation period of 24 hours at 37℃, reproduction until the bottom was considered in the control tubes and 1/65 536 tubes, and the reproduction which occured only in the inoculation place was considered as H7 positive in the 1/64 tube. The reproduction until the bottom was considered as negative in each of the 12 tubes (Farmer and Davis, 1985).

CHAPTER 3

RESULT AND DISCUSSION

3.1 Test Results

The isolation of *E. coli O157:H7* strain: lilac coloured loleni were not observed in the 60 samples of the seedings made with raw milk after incubation and lilac coloured colonies were found only in 8 samples of the seedings made with kashar cheese samples.

Table 3.1a: Finding *E. coli O157:H7* strain in the raw milk - the results of analysis

The number of raw milk samples	**Observed methalic blue colonies (E. coli)**	**Observed lilac coloured colonies (*E.coli O157:H7*)**	**No reproduction observed**
60	60	0	None

Table 3.1b: Finding *E. coli O157:H7* strain in the kashar cheese the results of analysis

The number of kashar cheese samples	**Observed methalic blue colonies (E. coli)**	**Observed lilac coloured colonies (*E.coli O157:H7*)**	**No reproduction observed**
40	Reproduction in 32 of them	Reproduction in 8 of them	8

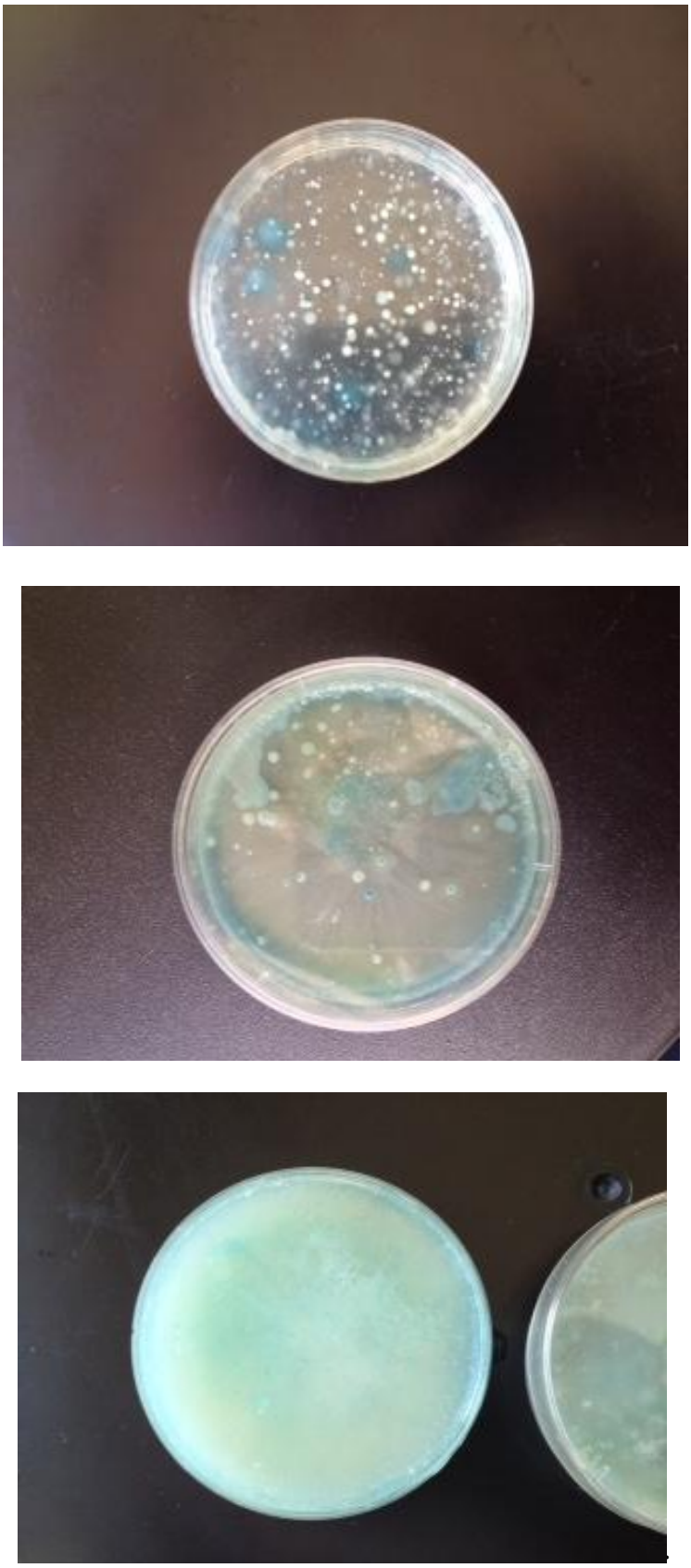


Figure 3.1: Microbiological analaysis of the kashar cheeses and Raw milk dishes and of ‘ the incubation

3.2 Assessment

The colonies which are reproduced by showing typical E. coli morphology at CT-SMAC medium were considered as suspicious. *E. coli O157:H7* Antiserum (Denka-Seiken) and lame agglutination test was performed to cultures which have typical biochemical properties (+,+,-,-) at IMVIC tests, which have negative (-) test results for β-glucuronidase activity, oxidase and hydrogen sulfide (H2S) and positive (+) test results for lysine decarboxylase, glucose, lactose and sucrose. *E. coli O157:H7* antiserum (Denka-Seiken) and mobility tests were performed to isolates that give agglutination and the positive isolates were evaluated as *E. coli O157:H7* (Farmer and Davis, 1985).

Standard strain: *E. coli O157:H7* (NCTC12900) was used as control strain at all stages of isolation and identification.

3.3 Statistical Analysis :

Kashar cheese was observed in %20 of the reproductive, Kashar cheese was the growth of %80 provided

H0: Is irrelevant in terms of bacteria in dairy products.

H1: In terms of interest between bacteria has kashar cheese products.

Test:Two proportions of inclepenclent samples.

Kasar cheese statıstıcally

Sisnificance (p:0.0203)

3.4 Results and Evaluations

Milk is an excellent culture medium for particular microorganisms especially for pathogens depending mostly on heat, microorganisms which compete with one another and their metabolic products . Raw milk is the second nutrient that causes *E. coli O157:H7* infection right after hamburger. First *E. coli O157:H7* infection was found in 1986 in USA with raw milk. Food poisoning is one of the infection diseases which keeps its importance both in the world and in our country. One of the most important ones is *E .coli O157:H7* strain and its importance has been increasing in recent years. It is known that milk and milk products play important roles in the transmission of pathogens.

Since *E. coli O157:H7* strain can be found widely in the milk and dairy products industry, especially in farm environments, it is accepted as one of the most important ones among the pathogens which are transmitted to humans with food and threatening the human health. This importance of it is not because it has more pathogens comparing to others, but at the same time, because it causes outbreaks by reinfecting to food after bad sanitary conditions, insufficient pasteurizations, cooking and pasteurizations even though it is rather resistless to heat process. Many researchers have reported that, E. coli factors have been isolated from different milk products such as Proteus mirabilis, Proteus vulgaris, Hafnia alvei, Bacillus spp, Staphylococcus spp, Morgenalla morganii, Klebsiella. Pneumoniae (Güneşen and Büyükyörük, 2003).

The *E. coli O157:H7* Hemorrhagic colitis and gastro-enteritis factor was first isolated from hamburgers in USA . From these years, there have been studies which reporting that *E. coli O157:H7* is a factor for food poisoning in USA, Canada and England (Öztürk, 1993).

Contaminated foods are not limited with beef and raw milk in the food-borne infections caused by *E. coli O157:H7*, and the variety of contaminated food is increasing. *E. coli O157:H7* can stay alive in the food and beverages which have pH lower than 4: in fact, an outbreak caused by unpasteurized apple juices was reported (Üçüncü, 2003).

It was reported that *E. coli O157:H7* type bacterial were isolated from unpasteurized milk samples between the years 1996-1997 in England (Kılıç, 2010).

The outbreaks caused by o157 and non O157 serotypes are increasing. Since all of the VTEC serotypes produce Shiga toxin, methods have been developed based on the detection of genes which are responsible for/to Shiga toxin production. One of them determine the stx 1 and stx 2 genes which are responsible for shiga toxin production with PRC methd.

Govaris et al., (2002). found *E. coli O157:H7* strain in both cheese types in the study on feta and curd cheeses(Govaris et al., 2002).

A higher number of bacteria were detected in feta cheeses comparing to curd cheeses (Aksu,1999). In a study that they worked on 50 white cheese samples, they isolated E. coli strain from one sample. Dayıcı found that 4 types of mihalic cheeses did not contain *E.*

coli O157:H7 which were prepared by cow, sheep and goat milk mixture without pasteurization (Dayıcı, 2000).

In Turkey, Istanbul, in a study which Tolun at all participated, the presence of first isolation Verotoxicologic Escherichia coli (VTEC) in the sold milk and milk products was investigated, 87 milk and 74 cheese samples were examined and microorganism was monitored in milk for 24% and 1.3% in cheese at the end of the study (Tolun et al., 2011).

CHAPTER 4

CONCUSION AND RECOMEMMENDATION

With this thesis, related to the *E. coli O157:H7* in milk and milk products which has risk for public health, it was aimed to collect data on the contamination level and its importance on public health by examining presence of *E. coli O157:H7* in milk and milk products in Nicosia district of TRNC.

The results of the study; It was concluded that from the raw milk and kashar cheese samples, no determined contamination rate was seen in raw milk and although it was seen for 33.3% in kashar cheese samples, it was at substantial levels.

Results show that, no enough attention has been paid to the hygienic rules in these businesses and it is at such levels that it is threatening the public health.

In addition to cross-contamination risk, *E. coli O157:H7* which is an organism that its disease-causing capacity is high since its minimal infective dose is low, it shows a rapidly increasing poisioning trend. While the infections caused by *E. coli O157:H7* have a broad spectrum such as abdominal cramps, sometimes bloody diarrhea and in further levels hemorrhagic colitis (HC), haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura(TTP), some cases may result in deaths.

According to Turkish Food Codex Communiqué on Microbiological Criteria, *E. coli O157:H7* should not be found in cheese products.

As a result; even if the contamination rate is low in food, because the minimal infective dose (10-100 kob7g) is low and fatal cases are frequent, it is thought that providing the application of HACCP (Hazard Analysis and Critical Control Point) approach and existing food laws would bring permanent and robust solution.

The collection of these samples from different sources and the anonymity of the application of pasteurization heat to the milk used in the cheese making are among the factors that affect the isolation rate in the study.

It was observed that the levels of E. coli hygiene indicator microorganisms and contamination of sold cheeses were higher than the levels reported in the Turkish Food

Codex Communiqué on Microbiological Criteria, and therefore the fresh cheeses are above the limit in terms of risk for consumer health.

Therefore, companies and producers should be informed about hygiene-sanitation which produce this kind of food that its consumption is rapidly increasing today, especially.

We reach to a conclusion that, the levels of hygiene indicator microorganisms and contamination are above the levels reported in Turkish Food Codex Communiqué on Microbiological Criteria and they may pose risk in terms of consumer health.

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