

Detection of *E. coli* O157:H7 in Meat Using Polymerase Chain Reaction Method and Culture Method

Mozhgan Bahadoripour, Mohammad Hassan Jahandar*

Department of Agriculture, Bam Branch, Islamic Azad University, Bam, Iran

***Correspondence to**

Mohammad Hassan Jahandar
Tel: +989155005780;
Email: Jahandar@iaubam.ac.ir

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Abstract

Introduction: *Escherichia coli* O157:H7, as a pathogenic agent, can be transmitted through the foods including meat, meat products, dairy products, vegetables and water. The World Health Organization has recommended that all countries in the world, especially developing countries, should consider the investigation of *E. coli* O157:H7 as a research priority. The aim of this study was to determine the frequency of *E. coli* O157:H7 in meat of cow, sheep, goat, and camel in Kerman province of Iran using culture and polymerase chain reaction (PCR) methods.

Methods: In this study, 280 meat samples consisting of sheep (90 specimens), cow (80 specimens), goat (60 specimens) and camel (50 specimens) meats were randomly separated from carcasses from April to July 2018. After the sampling, microbial culture was performed on the samples. Then, suspected *E. coli* O157:H7 colonies were evaluated by PCR assay.

Results: Out of the 280 samples, 73 samples (26%) were contaminated with *E. coli*. based on bacteriological tests, and 28 samples were identified as suspected *E. coli* O157:H7 serotype based on the lack of sorbitol fermentation. Subsequently, sorbitol-negative samples were tested by PCR procedure using specific primers. The results revealed that out of 28 cases, 21 cases (7.5%) were *E. coli* O157:H7.

Conclusion: As can be deduced from the observations of this study, to detect the *E. coli* O157, PCR as an accurate, fast, and reliable procedure can be used along with the culture method.

Keywords: *E. coli* O157:H7, PCR, Culture, Meat contamination

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Introduction

Meat is one of the most important sources of protein. It contains valuable proteins, essential amino acids, minerals (especially iron and zinc), and vitamins, which make it one of the most valuable foods.¹ The main source of meat contamination is livestock. Carcass contamination can be caused by microbial diseases of the livestock during slaughter or due to the non-compliance with the sanitation procedures during the slaughter process.² Wool, skin, moist parts of the body such as muzzle, mouth, eyelid, ear, anus, external genitalia, and breast are contaminated with various types of microorganisms which cause infection and food poisoning.³ In addition, there are some dangerous intestinal microbes in intestinal tract such as *Escherichia coli*. The most common serotype of this bacterium is O157:H7. In recent years, *Escherichia coli* O157:H7 caused epidemics in some

parts of the world and has attracted the attention of researchers and health care practitioners. *Escherichia coli* O157:H7 is considered as the most important serotype of enterohemorrhagic *Escherichia coli* (EHEC) which plays an important role in the incidence of Ulcerative colitis, Thrombotic thrombocytopenic purpura, and Hemolytic uremic syndrome.⁴ Hemolytic uremic syndrome has occurred in 2%-7% of patients and caused death in 3%-5% of cases.⁵ The World Health Organization (WHO) has recommended that all countries in the world, especially developing countries, should consider the investigation of *E. coli* O157:H7 as a research priority.

The isolation and identification of this bacterium using the conventional methods of microbiology are major challenges. Typically, MacConkey Sorbitol agar or antibody-based methods such as serological

tests are used to detect this bacterium in diagnostic laboratories. However, mistakes may occur in the final identification of the pathogen due to some defects in these methods. For example, sorbitol fermentation may fail to detect some strains of *E. coli* O157,⁶ or identification by serological methods may not be performed due to losing their outer membrane antigen through the mutation or because of cross-reactivity with other bacteria.⁷ It should be noted that neither of these methods is capable to identify the toxin-producing strains.⁸ Therefore, using the molecular techniques for identifying *E. coli* O157:H7 is essential. In *E. coli* O157 strain, O157 antigen is coded by a 12-gene cluster. Out of these genes, 6 genes are responsible for the sugar-base biosynthesis, 4 genes are responsible for transferring the sugar, and the 2 remaining genes encode the flippase protein and the O antigen polymerase. The protein which is encoded by the *rfbE* gene is located in the group of proteins involved in sugar-free biosynthesis and plays a major role in the synthesis of LPS bacteria.^{9,10} As soon as the bacterium binds to the intestinal mucosa, this pathogen begins to grow and secrete a toxin which is called Shiga toxin. Shiga toxin (Stx) is an important pathogenic factor in Shiga toxin producing *E. coli* (STEC), and Stx2 and Stx1 are two kinds of this toxin.^{1,12} Stx2 toxin is 1000 times more toxic for endothelial cells of the renal artery, compared to Stx1.¹² In this study, detection of *E. coli* O157:H7 by polymerase chain reaction (PCR) procedure was performed using two specific primers for the detection of *stx2b* and *rfbE* genes. The aim of this study was to determine the prevalence of *E. coli* O157:H7 infection in Kerman province using two methods of PCR and culture.

Materials and Methods

A total of 280 meat samples including 90 sheep, 80 cow, 60 goat and 50 camel specimens were prepared randomly from Kerman, Iran, from April to July 2018, according to the standard principles of sanitation. The samples were transferred to the laboratory on ice. Detection of *E. coli* O157:H7 and the isolation steps were carried out within 12 hours after sampling.

Microbial Culture

To find *E. coli* O157:H7 in the test samples, the Tryptic Soy Broth (Merck, Germany) containing 20 mg/L novobiocin was added to 10 g of homogenized tissue of each sample and incubated for 18-24 hours at 37°C. Then, 100 µL

of them was again incubated in Eosin Methylene Blue medium and MacConkey Sorbitol (Merck, Germany) containing cefixime and potassium tellurite for 24 hours at 37°C. In next step, the suspicious colonies of *E. coli* were traced using the Triple Sugar Iron (TSI) medium, IMViC test, and fermentation of sorbitol.¹³

DNA Extraction

Sorbitol-negative colonies of *E. coli* O157:H7 were selected for further investigation using the PCR procedure. For this purpose, DNA extraction was accomplished using the DNA extraction kit (Sinagen, Iran) based on the protocol provided by the company. The qualification and quantification of extracted DNA were examined by 1% agarose gel and Nanodrop device (Thermo Scientific, USA), respectively. The extracted DNA was stored at -20°C for PCR procedure.

PCR Procedure

The *stx2b* and *rfbE* genes with 300 and 126 bp, respectively, were amplified using one pair of specific primers (Table 1). PCR was carried out in 25 µL final volume containing approximately 50-100 ng template DNA, 2.5 µL of 10× PCR buffer, 2 µL dNTPs (2.5 mM), 2 µL MgCl₂ (10 mM), 1.5 µL of mix primer (5 pmol/µL), 0.125 U/µL of X' Taq DNA polymerase (Takara, Japan), and distilled deionized water up to a volume of 25 µL. To perform the PCR reaction, thermal cycling was carried out in AB 2720 thermal cycler (Applied Biosystem, USA) using a specific schedule with pre-denaturation at 95°C for 5 minutes, and 30 cycles of denaturation at 95°C for 30 seconds, annealing at 59°C and 62°C for 30 seconds for identifying *stx2b* and *rfbE* genes, respectively, and extension at 72°C for 30 seconds. The final extension step was completed at 72°C for 5 minutes.¹⁴ PCR products were checked by electrophoresis on 1.5% agarose gel in 1X TBE buffer with a 1 kb DNA size marker (Fermentas, Germany). Virtualization of PCR products was conducted using gel documentation system (BioRad, USA) (Figure 1).

Results

Microbial Culture and PCR Procedure

Of the 280 samples, 73 samples (26.07%) were determined to be contaminated with *E. coli* based on bacteriological tests and 28 samples (10%) were identified as suspected *E. coli* O157:H7 serotype based on the lack of sorbitol fermentation. Subsequently, sorbitol-negative samples

Table 1. Specific Primers for Detection of *E. coli* O157:H7 Using PCR

Virulence Factor	Target Gene	Primer	Primer Sequences (5'-3')	Product Size (bp)
<i>rfbE</i>	<i>rfbE</i>	<i>rfbE</i> -F	TCAAAAGGAACTATATTTCAGAAAGTTTGA	126
		<i>rfbE</i> -R	CGATATACCTAACGCTAACAAAGCTAA	
Shiga toxin 2 subunit B	<i>stx2b</i>	<i>stx2b</i> -F	TATACGATGACACCGGAAGAAG	300
		<i>stx2b</i> -R	CCTGCCATTGAGAAAAGCAGC	

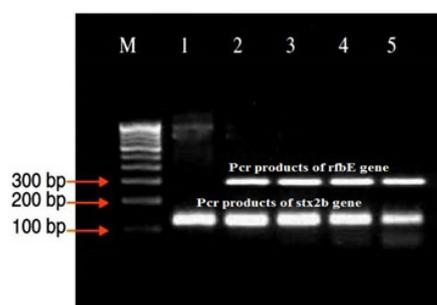


Figure 1. PCR Products of *stx2b* Gene (126 bp) and *rfbE* Gene (300 bp).

were selected for confirmation of the *E. coli* O157:H7 by PCR process and specific primers. The results of this study showed that out of 28 suspected cases, 21 cases (7.5%) were found to be *E. coli* O157:H7 and *E. coli* O157:H7 was not confirmed in 7 cases. The contamination frequency (out of 280 samples) of the cow, sheep, goat and camel meats were observed to be 2.14%, 3.57%, 1.42%, and 0.36%, respectively (Table 2). As it is noted, the incidence of infection in the warmer months of the year (June and July) was higher compared to colder months in both methods based on PCR and lack of sorbitol fermentation (Table 3).

Discussion

Escherichia coli O157:H7 is considered as the most

important serotype of the enterohemorrhagic *E. coli* and plays an important role in the incidence of ulcerative colitis, thrombotic thrombocytopenic purpura, and hemolytic uremic syndrome.⁴ Hemolytic uremic syndrome occurs in 2%-7% of patients and results in death in 3%-5% of cases.⁵ Although it has been proven that this bacterium is found in all geographical areas and can be isolated from different kinds of foods, its occurrence varies in different regions depending on the type of food, the sampling season and the methods of isolation.^{15,16}

Ruminants and their products are the main source of *E. coli* O157:H7 infection. The results of conducted research on camel meat in Iran showed that *E. coli* O157:H7 was detected in 1.1% of 94 camel carcass samples.¹⁷ However, in a study on camel stool which was conducted in the UAE, no *E. coli* O157:H7 was observed.¹⁸ In another research in 5 countries of East Africa on faecal and serum samples of 400 camels with STEC or anti-STX antibodies, no *E. coli* O157:H7 contamination was reported,¹⁹ which is not in accordance with the results of this study. The research results of Haji et al in 2011 showed that the prevalence of *E. coli* O157:H7 in 75 samples of camel meat was 1.7%,²⁰ using the PCR procedure. Rahimi et al in 2012 also reported that out of 50 camel meat samples, the frequency of *E. coli* O157:H7 was about 2% using PCR,¹⁷ which contradicted the results of the present research. This discrepancy may be due to sampling time, sampling

Table 2. Frequency of *E. coli* O157: H7 Contamination in Different Meats

Animal	No. of Samples	Subtracting the Positive Samples Based on the PCR Results of 2 Genes		Positive Samples for <i>E. coli</i> O157:H7 (% out of 280 Samples)	Samples Contaminated With <i>E. coli</i> Based on Culture Method (% out of 280 Samples)	Samples Positive for <i>E. coli</i> O157:H7 (% Per Each Animal Grope)	Samples Contaminated With <i>E. coli</i> Based on Culture Method (% Per Each Animal Grope)
		<i>stx2b</i> Gene	<i>rfbE</i> Gene	PCR	Culture	PCR	Culture
Cow	80	4	2	6 (2.14%)	22 (7.85%)	6 (7.5%)	22 (27.5%)
Sheep	90	6	4	10 (3.57%)	27 (9.64%)	10 (11.11%)	27 (30%)
Goat	60	3	1	4 (1.42%)	16 (5.71%)	4 (6.66%)	16 (26.66%)
Camel	50	1	0	1 (0.36%)	8 (2.85%)	1 (1.66%)	8 (16%)
Total	280	14	7	21 (7.5%)	73 (26.07%)	21	73

The difference in the data shown in columns 5 and 6 compared to the next two columns 7 and 8 is % of positive samples out of total (280) samples and % of positive samples within each animal group, respectively.

Table 3. Frequency of *E. coli* O157: H7 Contamination in Meat, in Different Months of Sampling

Month	No. of Samples	Samples Contaminated With <i>E. coli</i> O157:H7 Based on PCR	Samples Suspicious to <i>E. coli</i> O157:H7 Based on Lack of Sorbitol Fermentation (% out of 280 Samples)	Samples Contaminated With <i>E. coli</i> Based On Culture Method (% out of 280 Samples)	Samples Suspicious to <i>E. coli</i> O157:H7 Based on lack of Sorbitol Fermentation (% Per Each Month)	Samples Contaminated With <i>E. coli</i> Based on Culture Method (% Per Each Month)
April	65	1.07	5 (1.78%)	12 (4.28%)	5 (7.69%)	12 (18.46%)
May	65	1.07	4 (1.42%)	14 (5%)	4 (6.15%)	14 (21.535)
June	76	2.14	8 (2.85%)	25 (8.92%)	8 (10.52%)	25 (31.64%)
July	74	3.21	11 (3.92%)	22 (7.85%)	11(14.685)	22 (29.72%)
Total	280	7.5	28 (10%)	73 (26.05)	28	73

The difference in the data shown in columns 4 and 5 compared to the next two columns 6 and 7 is related to % of suspicious samples based on lack of sorbitol fermentation out of total (280) samples and % of contaminated samples based on lack of sorbitol within each month, respectively.

method, number of samples, sex, and age of the studied animals.

Studies have shown that the prevalence of this pathogen in beef and its products is between 0% and 8.27%.^{16,21,22} Rahimi et al examined 203 carcasses of cows, of which 42.4% were positive for *E. coli* and 6.4% showed *E. coli* O157:H7 infection, which was higher than the report of the present study.²³ In a study on veal in an industrial slaughterhouse, it was found that slaughtered animals were contaminated with *E. coli* O157:H7, ranging from 0 to 8%.²⁴ Tests for detection of *E. coli* O157:H7 are not carried out in slaughterhouses and factories in Iran; therefore, the possibility of contamination of meat products with this pathogen is increased. In another study, Hajian et al demonstrated that the prevalence of *E. coli* O157:H7 in beef was 2.2%, which was lower than results of this study.²⁰ Furthermore, researches stated that contamination of cow carcasses usually occurs directly and indirectly during slaughtering, transporting, peeling, discharging the viscous material, and transferring to refrigerator.²⁵

Blanco et al reported that the rate of contamination with verotoxin-producing *E. coli* varies from 0 to 60% and the prevalence of O157:H7 has been estimated to be 5% in cows at the time of slaughter.²⁶ Turkish scholars in 2006 reported that *E. coli* O157:H7 was isolated from 77 samples (13.6%) of slaughtered cattle, 66 samples (11.7%) of which belonged to the O157:H7 serotype and 11 samples (1.9%) were non-motile strains (O157:NM).²⁷ In a conducted study in Bangladesh in 2008, the researchers showed that 14.4% of buffalos (n=174), 7.2% of cows (n=139) and 9.1% of goats (n=110) were infected with STEC O157:H7.²⁸ Samples of the rectal contents were collected immediately after the animals were slaughtered.²⁸ The rate of contamination of beef with *E. coli* O157:H7 in Netherlands, England and the United States were 10.4%, 13.4%, and 28%, respectively, which were higher compared to the results obtained in this study.^{15,29,30} Moreover, the frequency of *E. coli* O157:H7 contamination in beef in this study was higher than the reported value in Ireland (0.6%), Italy (6.3%), Switzerland (3.2%), and Argentina (3.8%).^{16,31-33}

Sheep, after the cattle, are considered as the second major ruminant in providing meat in Iran, and some researchers declared that the role of sheep as a reservoir of *E. coli* O157:H7 is the same as that of cattle.^{5,34} The rate of infection of lambs with this pathogen in Iran, Australia, the United States, Italy, and Egypt were reported to be 93.3%, 0.5%, 1.5%, 0.77%-1.7%, and 4%, respectively. A survey on 62 beef and 60 goat meats, using PCR process, demonstrated that the prevalence of *E. coli* O157:H7 was 4.8% and 1.7% for lamb and goat, respectively.¹⁷ Another study on 159 lambs showed that the prevalence of *E. coli* O157:H7 was 3.8% using PCR method.³⁵ In another study in Egypt, the prevalence of *E. coli* O157:H7 in lambs and goats were 2.5% and 2%, respectively.^{36,37} The infection

rates in lamb and goat meat in Italy were reported to be 7.3 and 0.7%, respectively.³⁸ Other researchers reported that the prevalence of *E. coli* O157:H7 was 1.2-5.5% in lambs in the United States²¹ and 0.3-1.5% in Australia.^{39,40} Differences in the prevalence of *E. coli* O157:H7 can be due to the sampling methods, the size of selected samples, and the method of isolation.²⁹

In this study, the highest rate of contamination was found in summer, which is consistent with Hancock et al and Elder et al studies.^{30,41} The prevalence *E. coli* O157:H7 is typically low in winter and increases in the spring with the peak levels during summer. This is because the cold kills and reduces the load of *E. coli* O157:H7.⁴²

An investigation was done on 145 feedlot cattle and 9 dairy cull cows in a slaughterhouse in Shiraz, Iran. *E. coli* O157:H7 was identified in cattle through detection of the *Stx2* gene using PCR procedure. The results showed that 14 cases out of 154 (9.65%) feedlot cattle and in 1 cases out of 9 (11.1%) dairy cattle were infected with *E. coli* O157:H7.²⁴ In detection of the *E. coli* O157 and O157:H7 strains using *Stx2* in Shiraz, Iran, *E. coli* O157:H7 was found in 6 (3.92%) of 153 sheep, from which 5 sheep were <2 years old, highlighting the possible effect of age and differences in diets on gastrointestinal flora.⁴³

In the present study, 28 samples (10%) were identified as *E. coli* O157:H7 based on the lack of sorbitol fermentation. The results of PCR procedure demonstrated that out of the 28 samples, 21 cases were identified as *E. coli* O157:H7. These results confirmed the importance of molecular methods in detecting this pathogen. This method not only can save time but also is very cost-effective. Therefore, it is suggested as a superior procedure in isolating *E. coli* O157:H7 from foods. In this regard, previous researches from Iran declared that PCR procedure is a fast method to detect the *E. coli* O157:H7 in the pharmaceutical, food, and water industries.⁴⁴ In another study in 2009 in Iran, researchers have proven that PCR-based methods are fast and reliable methods to detect and identify as few as 100 cells of *E. coli* O157:H7, *Vibrio cholerae*, and *Salmonella typhimurium*.⁴⁵

The present study is the first report on the *E. coli* O157:H7 contamination rate in meat in Kerman, Iran and suggests that the consumption of semi-cooked meat (especially beef and lamb) may contribute to the transmission of this pathogen to humans. Therefore, the precise monitoring and observance of health principles during slaughtering, transportation, and maintenance processes can reduce the transmission rate. More studies are recommended in other regions of Iran.

Ethical Approval

This article does not contain any studies with animals performed by any of the authors

Competing Interest

The authors declare there is no conflicts of interest.

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