Detection of *Escherichia coli* O157 and *Escherichia coli* O157:H7 by the immunomagnetic separation technique and *stx1* and *stx2* genes by multiplex PCR in slaughtered cattle in Samsun Province, Turkey

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This study was conducted to investigate the presence of *Escherichia* (*E.*) *coli* O157 and *E. coli* O157:H7 and *stx1* and *stx2* genes on cattle carcasses and in rectal samples collected from Samsun Province of Turkey. A total of 200 samples collected from cattle carcasses and the rectal contents of 100 slaughtered cattle from two commercial abattoirs were tested using the immunomagnetic separation technique and multiplex PCR methods. *E. coli* O157 and *E. coli* O157:H7 were detected in 52 of the 200 samples (26%) tested. Of the positive samples, 49 were *E. coli* O157 and three were *E. coli* O157:H7. The *E. coli* O157 strain was isolated from 24 carcasses and 25 rectal samples, while *E. coli* O157:H7 was isolated from two carcasses and one rectal sample. Of the 49 samples positive for *E. coli* O157, 32 were from the rectal and carcass samples of the same animal, while two *E. coli* O157:H7 isolates were obtained from rectal swabs and carcasses of the same animal. The *stx1* and *stx2* genes were both detected in 35 *E. coli* O157 isolates and one *E. coli* O157:H7 isolate, but the *stx2* gene was only detected alone in two *E. coli* O157 isolates. Overall, 16 carcasses tested positive for *E. coli* O157 and one carcass tested positive for *E. coli* O157:H7 based on both carcass and rectal samples. Overall, the results of this study indicate that cattle carcasses pose a potential risk to human health due to contamination by *E. coli* O157 and *E. coli* O157:H7 in the feces.

**Keywords:** carcass, cattle, *E. coli* O157, *E. coli* O157:H7, rectum, *stx1*, *stx2*

**Introduction**

Enterohemorrhagic *Escherichia* (*E.*) *coli* strains are a subset of the Shiga toxin-producing *E. coli* (STEC) that cause diseases in humans and pose a threat to public health worldwide [19,20]. Many environmental and food sources have caused *E. coli* O157 or *E. coli* O157:H7 infections, but they are primarily attributed to consumption of food of animal origin, especially cattle [36], or to direct or indirect contact with cattle or other farm animals [24]. Human infection by *E. coli* O157:H7 has been reported in over 30 countries, and cattle appear to be the chief source of infection. Indeed, many outbreaks have been linked to beef consumption [8,10,26]. Cattle and other ruminants have been established as major natural reservoirs of *E. coli* O157 [32] and play a significant role in the epidemiology of human infections [20]. Specifically, between 1% and 35.8% of cattle in the United Kingdom, and the United States were estimated to be contaminated with *E. coli* O157 [8,15,25,34].

*E. coli* O157 and *E. coli* O157:H7 are present in the intestines of cattle as a component of the native microbiota and they can contaminate both the meat and the slaughterhouse environment. As a result, contamination of both carcasses and the environment by *E. coli* O157 and O157:H7 from the intestinal contents of cattle during slaughter is one of the most significant risk factors in transmission to humans [26,31]. Hide removal operations potentially constitute another very important source of cattle carcass contamination. Therefore, feces and hide removal are considered to be the main sources of *E. coli* O157 and *E. coli* O157:H7 contamination of carcasses during slaughter [12,25]. Contamination of carcass meat with *E. coli* O157 and *E. coli* O157:H7 can occur during dressing, primarily during the skinning, but also during the evisceration phase. Once the *E. coli* is transferred to the carcass surface, handling and trimming operations can spread the pathogen to the beef trimmings [17].

The pathogenicity of *E. coli* O157 and *E. coli* O157:H7, including STEC, is associated with several virulence factors. The main factor contributing to their pathogenicity is their capacity to produce two potent phage-encoded...
cytotoxins called Shiga-toxins (namely, Stx1 and Stx2). Shiga toxins cause diseases such as hemorrhagic colitis and hemolytic uremic syndrome through cytopathic effects on the vascular endothelial cells of the kidneys, intestines, central nervous system and other organs [16]. In addition to the production of toxins, another virulence-associated factor expressed by STEC is a protein called intimin, which is encoded by the eae gene and responsible for the intimate attachment of STEC to the intestinal epithelial cells. The role of other virulence genes through the production of enzymes such as enterohemolysin, an extracellular serine protease, and a catalase/peroxidase in causing infection appears to be minor [23].

According to studies reported from various parts of the world, cattle carcases carry a potential risk of the presence of E. coli O157 and E. coli O157:H7 through fecal contamination [5,7,22,25,35,38,39]. There have been many studies conducted to determine E. coli O157 and E. coli O157:H7 in only carcass or rectal samples at abattoirs worldwide, including Turkey [5,7,22,41]. However, to the best of our knowledge, there has been no attempt to detect E. coli O157 and E. coli O157:H7 in both rectal and carcass samples from the same animal. Therefore, this study was conducted to investigate the presence of both E. coli O157 and E. coli O157:H7 and to detect the presence of the stx1 and stx2 genes in isolates from both cattle carcases and their rectal samples obtained from two commercial abattoirs located in the Samsun Province of Turkey.

Materials and Methods

In this study, a total of 200 swab samples obtained from 100 slaughtered cattle were tested to investigate the presence of E. coli O157 and E. coli O157:H7, as well as to detect the presence of stx1 and stx2 genes in the isolates. Swab samples were taken from 100 cattle carcases and their rectal contents at two commercial abattoirs located in Samsun Province, Turkey between December 2007 and March 2008. Samples were collected immediately after removal of the hide (dressing step) from the carcass. Each sample from the same animal was assumed to be exhibiting typical presumptive positive characteristics of E. coli O157 colonies were identified, subcultured onto yeast extract-tripticase soy agar (Oxoid-CM 131-L21; Basingstoke, UK), incubated for 24 h at 37°C and subjected to the confirmatory tests described below:

**Confirmatory testing for E. coli O157 and E. coli O157:H7**

The pinpoint indol test was conducted, after which indol test positive colonies were streaked onto 4-methylumbelliferly-β-D-glucuronide sorbitol MacConkey (MUG-SMAC) agar (Oxoid-CM 813, Supl.SR 172 E; Basingstoke, England) and incubated at 37°C for 24 h. Up to five colonies exhibiting typical presumptive positive characteristics of E. coli O157 colonies were identified, subcultured onto yeast extract-tripticase soy agar (Oxoid-CM 131-L21; Basingstoke, UK), incubated for 24 h at 37°C and subjected to the confirmatory tests described below:

| Target gene | Sequence (5'-3') | Amplification product size (bp) | References |
|-------------|------------------|---------------------------------|------------|
| stx1F       | ATAAATCGCCATTCGTTGACTAC | 180                             | [30]       |
| stx1R       | AGAACGCCCACTGAGATCATC   |                                 |            |
| stx2F       | GGCACCTGTCGAAACTGCTCC  | 255                             | [30]       |
| stx2R       | TCGCCAGTTATCTGACATTCTG  |                                 |            |
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**Determination of virulence genes (**stx1** and **stx2)**

Multiplex PCR was conducted to detect the presence of the shiga-toxin genes (**stx1** and **stx2**) in *E. coli* O157 and *E. coli* O157:H7 isolates and were trated according to a modified version by using multiplex PCR assays [14,30]. The primers and PCR conditions used in this study are shown in Table 1. *E. coli* O157:H7 ATCC 43895 was used as a reference strain.

DNA extractions of strains were conducted using the boiling method. Briefly, each positive colony was inoculated on TSA and incubated for 24 h at 37°C. Two colonies were then selected and suspended separately in 500 μL of sterile distilled water in microcentrifuge tubes, after which they were incubated at 95°C for 10 min in a water bath (Memert, Germany). The tubes were then centrifuged (Hettich-Universal-320R unit) at 9,503 × g for 10 min, after which the supernatant containing the DNA to be used as the template DNA was transferred into Dnase/Rnase-free microcentrifuge tubes. The extracted DNA samples were stored at −20°C until use.

To detect the **stx1** and **stx2** genes, 10 μL of extracted DNA was used as a template in a reaction mixture with a final volume of 50 μL that contained 200 mM of each deoxynucleoside triphosphate (dNTP), 250 nM **stx1** primer, 250 nM **stx2** primers, 1U of Taq DNA polymerase in 1× PCR buffer and 2 mM of MgCl2. The amplification of DNA (MJ Mini; BioRad, USA) was conducted as follows using a thermocycler (MJ Mini-PTC-1148; BioRad, USA): initial denaturation at 94°C for 10 min, 35 cycles of denaturation at 94°C for 1 min, 35 cycles of denaturation at 94°C for 1 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 7 min [14].

For gel electrophoresis, the 20-μL amplicon mixtures were supplemented with 4-μL of loading dye and loaded onto a 2.0% agarose gel containing ethidium bromide (Gene choice). Electrophoresis (Power Pac Basic; BioRad, USA) was then conducted at 90 V for 1.5 h. A 100∼1,000 bp molecular weight marker was used to identify the amplified products, which were visualized by UV illumination (WiseUV-Wuv-L50; Daihan Scientific, Korea). The nucleotide sequences and predicted product sizes of the primers are shown in Table 1.

**Results**

In the present study, *E. coli* O157 or O157:H7 was detected in 52 of 200 samples tested (49 *E. coli* O157 and 3 *E. coli* O157:H7). The *E. coli* O157 strain was isolated from 24 carcasses and 25 rectal samples, while the *E. coli* O157:H7 strain was isolated from two carcasses and one rectal sample.

Table 2. Isolation of *E. coli* O157 and *E. coli* O157:H7 strains from slaughtered cattle and detection of **stx1** and **stx2** genes

| Sampling site (No. of samples) | *E. coli* O157 n (%) | *E. coli* O157:H7 n (%) | **stx1** gene in *E. coli* O157 n (%) | **stx2** gene in *E. coli* O157 n (%) | Both **stx1** and **stx2** genes in same *E. coli* O157 n (%) | Both **stx1** and **stx2** genes in same *E. coli* O157:H7 n (%) |
|-------------------------------|----------------------|-------------------------|--------------------------------------|--------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|
| Carcass (n = 100)              | 8 (8.0)              | 1 (1.0)                 | 0 (0.0)                              | 2 (2.0)                              | 3 (3.0)                                                       | 1 (1.0)                                                       |
| Rectum (n = 100)              | 9 (9.0)              | 0 (0.0)                 | 0 (0.0)                              | 0 (0.0)                              | 3 (3.0)                                                       | 0 (0.0)                                                       |
| Carcass and rectum (n = 200)  | 16 (8.0)             | 1 (0.5)                 | 0 (0.0)                              | 0 (0.0)                              | 13 (7.5)                                                      | 0 (0.0)                                                       |
| Total cattle carcass samples  | 24 (24.0)            | 2 (2.0)                 | 0 (0.0)                              | 2 (2.0)                              | 17 (17.0)                                                     | 1 (1.0)                                                       |
| Total rectal samples (n = 100) | 25 (25.0)            | 1 (1.0)                 | 0 (0.0)                              | 0 (0.0)                              | 18 (18.0)                                                     | 0 (0.0)                                                       |
| Total number (n = 200)        | 49 (24.5)            | 3 (1.5)                 | 0 (0.0)                              | 2 (1.0)                              | 35 (17.5)                                                     | 1 (0.5)                                                       |

* **stx1** and **stx2** genes present in *E. coli* O157 isolated from carcass alone. † **stx1** and **stx2** genes present in *E. coli* O157 isolated from rectum alone.

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Table 1. Primers and PCR conditions

| Primers | PCR conditions |
|---------|----------------|
| **stx1** primer | 200 mM of each deoxynucleoside triphosphate (dNTP), 250 nM **stx1** primer, 250 nM **stx2** primers, 1U of Taq DNA polymerase in 1× PCR buffer and 2 mM of MgCl2. |
| **stx2** primer | 200 mM of each deoxynucleoside triphosphate (dNTP), 250 nM **stx1** primer, 250 nM **stx2** primers, 1U of Taq DNA polymerase in 1× PCR buffer and 2 mM of MgCl2. |

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**Fig. 1.** Amplification products of **stx1** and **stx2** genes in *E. coli* O157 and O157:H7 identified by multiplex PCR. Lane M: Marker (100 ∼ 1,000 bp).
Of the 49 samples that contained *E. coli* O157, 32 were from the rectal and carcass samples of the same animal, while the other 17 isolates were all from different cattle. Specifically, eight were from carcasses and the remaining nine were from rectal samples. Two of the *E. coli* O157:H7 isolates were obtained from the carcass and rectal swabs of the same animal, while the remaining isolate was obtained from a carcass sample (Table 2).

The results of multiplex PCR for the detection of stx1 and stx2 genes are shown in Fig. 1. While both the stx1 and stx2 genes were detected in 35 *E. coli* O157 isolates from 17 carcass and 18 rectal samples, they were detected in only one *E. coli* O157:H7 isolate from a carcass sample. The stx2 gene alone was detected in only two *E. coli* O157 isolates and the stx1 gene was not detected alone in any isolates. Neither of these genes was detected in the 12 *E. coli* O157 and two *E. coli* O157:H7 isolates from carcass and rectal samples (Figs. 1 and 2).

**Discussion**

In Turkey and other parts of the world, although there have been many studies conducted to determine the presence of *E. coli* O157 and *E. coli* O157:H7 in either carcass or rectal samples at abattoirs, the authors of the present study are unaware of any attempts to detect *E. coli* O157 and *E. coli* O157:H7 in both the rectal and carcass samples of the same animal. Therefore, the present study was conducted to investigate the contamination of carcasses and their rectal contents with *E. coli* O157 and *E. coli* O157:H7 at two abattoirs in Samsun Province of Turkey because the majority of food-borne *E. coli* O157 and *E. coli* O157:H7 infections in humans occur after the consumption of contaminated beef and cattle products [31].

Isolation rates of *E. coli* O157 and *E. coli* O157:H7 from bovine carcasses and feces ranging from low (0.39%) to high (17.0%) have been reported for Mexico, Ireland, Belgium, England, France, Poland, Germany, the United States, Turkey and other countries. *E. coli* O157 or *E. coli* O157:H7 isolates obtained from cattle carcasses or feces have also been found to contain at least one of the stx1, stx2, eaeA, hylA and fliC genes [5,7,22,25,29,35,38,39,41]. In contrast to other countries, there has been only one study of carcasses reported in Turkey [21]. The results of that study revealed that 3.9% and 2.4% of the bovine carcasses were contaminated by *E. coli* O157 and *E. coli* O157:H7, respectively. There have been a few studies of cattle feces conducted in Turkey [1,3,9,41], the results of which revealed that *E. coli* O157 or *E. coli* O157:H7 were present in 1.28% and 13.6% of the samples, respectively. It has also been reported that at least one virulence gene (stx1, stx2 or eae genes) was detected in the *E. coli* O157 or *E. coli* O157:H7 isolates from feces samples [1,3,41].

In the present study, the *E. coli* O157 strain was isolated from 24 carcasses (24%) and 25 rectal samples (25%), while the *E. coli* O157:H7 strain was isolated from two carcasses (2%) and one rectal sample (1%). These values for *E. coli* O157 were higher than those of previously conducted studies. However, previously conducted studies show a wide range of isolation ratios for *E. coli* O157 and *E. coli* O157:H7. This variation may be due, at least in part, to the sensitivity of the method, diverse geographical
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origins of cattle, numbers of cattle, study design, number of herds and cattle, sex and age of cattle, season, abattoir conditions and treatment with antimicrobial substances during the process [6,7,39]. It has also been reported that the prevalence of *E. coli* O157 and *E. coli* O157:H7 varies with the seasons, generally increasing in the warm months of March-September in the northern hemisphere [4,6,7,25,39]. Another important factor influencing the identification of individual strains of *E. coli* is the isolation method. Indeed, the detection of *E. coli* O157:H7 from cattle fecal samples is known to be very difficult due to their low concentration. Therefore, direct inoculation of samples onto plates is not sensitive enough. Several enrichment culturing methods and isolation methods have been developed to counter this problem [38]. One of the more sensitive methods is the IMS technique [28,38,40], which is why enrichment/IMS procedures were employed in the present study. Another study found that the IMS technique was superior to the classic culture technique for *Salmonella* isolation (unpublished data). In the present study, swab samples were collected from the rectum of the cattle instead of direct feces samples, because the recto-anal junction mucosa has been identified as the primary site of *E. coli* O157:H7 colonization in cattle [18,33,35]. In attempting to manage *E. coli* O157 and *E. coli* O157:H7 contamination in abattoirs, it is crucial to consider cross contamination during slaughter. *E. coli* O157 and *E. coli* O157:H7 have been reported to spread easily onto carcass surfaces from the hide or during evisceration [2,12,21]. The results of the present study support that contention, with many rectal and carcass samples of the same animal being positive.

Epidemiological studies in cattle indicate that the horizontal transmission of *E. coli* O157 or *E. coli* O157:H7 occurs in groups of animals, and that contaminated water may facilitate its spread and persistence within herds [13,15]. Therefore, control of the spread of *E. coli* O157 and *E. coli* O157:H7 at the farm level becomes very important. Moreover, cross contamination may occur during the slaughter of cattle and other processes at abattoirs.

The results of the present study indicate that meat from cattle poses a risk to human health in Turkey because of potential *E. coli* O157 and *E. coli* O157:H7 contamination. To minimize the risk to public health, implementation of the HACCP system in abattoirs is recommended.

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**References**

1. Aksoy A, İstanbulluoğlu E, Aşan TZ, Yıldırım M, Özarslan B. Kırıkkaile ilinde sığır ve koyun dışkılarında *Escherichia coli* O157:H7 prevalansının belirlenmesi. Vet Hek Mikrobiol Derg 2005, 5, 3-8.

2. Arthur TM, Bosilevac JM, Brichita-Harhay DM, Guerini MN, Kalchayanand N, Shackelford SD, Wheeler TL, Koohmaraie M. Transportation and lairage environment effects on prevalence, numbers, and diversity of *Escherichia coli* O157:H7 on hides and carcasses of beef cattle at processing. J Food Prot 2007, 70, 280-286.

3. Aslantaş Ö, Erdoğan S, Cantekin Z, Gülaçtılı İ, Evrendilek GA. *Escherichia coli* O157:H7 on hides and non-O157 serotypes, and *Salmonella* in commercial beef processing plants. J Food Microbiol 2006, 106, 338-342.

4. Barkocy-Gallagher GA, Arthur TM, Rivera-Betancourt M, Rue M, Nou X, Shackelford SD, Wheeler TL, Koohmaraie M. Seasonal prevalence of *Shiga* toxin-producing *Escherichia coli*, including O157:H7 and non-O157 serotypes, and *Salmonella* in beef processing plants. J Food Prot 2003, 66, 1978-1986.

5. Carney E, O'Brien SB, Sheridan JJ, Mc dowell DA, Blair IS, Duffy G. Prevalence and level of *Escherichia coli* O157 on beef trimmings, carcasses and boned head meat at a beef slaughter plant. Food Microbiol 2006, 23, 52-59.

6. Centers for Disease Control and Prevention (CDC). Foodborne diseases active surveillance network, 1996. MMWR Morb Mortal Wkly Rep 1997, 46, 258-261.

7. Chapman PA, Cerdán Malo AT, Ellin M, Ashton R, Harkin MA. *Escherichia coli* O157 in cattle and sheep at slaughter, on beef and lamb carcasses and in raw beef and lamb products in South Yorkshire, UK. Int J Food Microbiol 2001, 64, 139-150.

8. Chapman PA, Wright DJ, Higgins R. Untreated milk as a source of verotoxigenic *E. coli* O157. Vet Rec 1993, 133, 171-172.

9. Çabalar M, Boyunkara B, Gülhan T, Ekin İN. Prevalence of rotavirus, *Escherichia coli* K99 and O157:H7 in healthy dairy cattle herds in Van, Turkey. Turk J Vet Anim Sci 2001, 25, 191-196.

10. de Boer E, Heuvelink AE. Foods as vehicles of VTEC infection. In: Duffy G, Garvey P, McDowell DA (eds.). *Verocytotoxigenic* *E. coli*. 1st ed. pp. 181-200, Food & Nutrition Press, Trumbull, 2001.

11. Eklund M. Enterohemorrhagic *Escherichia coli* (EHEC) Findings from Humans in Finland. Publications of the National Public Health Institute A. A23. National Institute for Health and Welfare, Helsinki, 2005.

12. Elder RO, Keen JE, Siragusa GR, Barkocy-Gallagher GA, Koohmaraie M, Laegreid WW. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. Proc Natl Acad Sci USA 2000, 97, 2999-3003.

13. Faith NG, Shere JA, Brosch R, Arnold KW, Ansai SE, Lee MS, Luchansky JB, Kaspar CW. Prevalence and
clonal nature of *Escherichia coli* O157:H7 on dairy farms in Wisconsin. Appl Environ Microbiol 1996, 62, 1519-1525.
14. Fitzmaurice J. Molecular diagnostic assay for *Escherichia coli* O157:H7. Ph.D. Thesis. National University of Ireland, Galway, 2003.
15. Garber L, Wells S, Schroeder-Tucker L, Ferris K. Factors associated with fecal shedding of verotoxin-producing *Escherichia coli* O157 on dairy farms. J Food Prot 1999, 62, 307-312.
16. Garrido P, Blanco M, Moreno-Paz M, Briones C, Dahbi G, Blanco J, Blanco J, Parro V. STEC-EPEC oligonucleotide microarray: a new tool for typing genetic variants of the LEE pathogenicity island of human and animal Shiga toxin-producing *Escherichia coli* (STEC) and enteropathogenic *E. coli* (EPEC) strains. Clin Chem 2006, 52, 192-201.
17. Gill CO, McGinnis JG. Contamination of beef trimmings with *Escherichia coli* during a carcass breaking process. Food Res Int 2000, 33, 125-130.
18. Greenquist MA, Drouillard JS, Sargeant JM, Depenbusch BE, Shi X, Lechtenberg KF, Nagaraja TG. Comparison of rectoanal mucosal swab cultures and fecal cultures for determining prevalence of *Escherichia coli* O157:H7 in feedlot cattle. Appl Environ Microbiol 2005, 71, 6431-6433.
19. Griffin PM. *Escherichia coli* O157:H7 and other enterohemorrhagic *Escherichia coli*. In: Blaser MJ, Smith PD, Ravdin JJ, Greenberg HB, Guerrant RL (eds.). *Principles of Infectious Diseases.* McGraw-Hill, New York, 1995.
20. Griffin PM, Tauxe RV. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. Epidemiol Rev 1991, 13, 60-98.
21. Gun H, Yilmaz A, Turker S, Tanlasi A, Yilmaz H. Contamination of bovine carcasses and abattoir environment by *Escherichia coli* O157:H7 in Istanbul. Int J Food Microbiol 2003, 84, 339-344.
22. Guyon R, Dorey F, Malas JP, Griment F, Foret J, Debyser V, Collodot JF. Superficial contamination of bovine carcasses by *Escherichia coli* O157 isolated from cattle, pigs and *S. enterica* at the bovine terminal rectal mucosa. Infect Immun 2006, 74, 4685-4693.
23. Law D. Virulence factors of *Escherichia coli* O157 and other shiga toxin-producing *E. coli*. J Appl Microbiol 2000, 88, 729-745.
24. Locking ME, O’Brien SJ, Reilly WJ, Wright EM, Campbell DM, Coia JE, Browning LM, Ramsay CN. Risk factors for sporadic cases of *Escherichia coli* O157 infection: the importance of contact with animal excreta. Epidemiol Infect 2001, 127, 215-220.
25. McEvoy JM, Doherty AM, Sheridan JJ, Thomson-Carter FM, Garvey P, Mcguire L, Blair IS, McDowell DA. The prevalence and spread of *Escherichia coli* O157:H7 at a commercial beef abattoir. J Appl Microbiol 2003, 95, 236-266.
26. Mead PS, Griffin PM. *Escherichia coli* O157:H7. Lancet 1998, 352, 1207-1212.
27. Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. Clin Microbiol Rev 1998, 11, 142-201.
28. Okrend AJG, Rose BE, Lattuada CP. Isolation of *Escherichia coli* O157:H7 using O157 specific antibody coated magnetic beads. J Food Prot 1992, 55, 214-217.
29. Osek J, Gallien P. Molecular analysis of *Escherichia coli* O157 strains isolated from cattle and pigs by the use of PCR and pulsed-field gel electrophoresis methods. Vet Med (Praha) 2002, 47, 149-158.
30. Paton AW, Paton JC. Detection and characterization of shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx1, stx2, *eaE*, enterohemorrhagic *E. coli* hlyA, *rfbO111*, and *rfbO157*. J Clin Microbiol 1998, 36, 598-602.
31. Philips CA. The epidemiology, detection and control of *Escherichia coli* O157. J Sci Food Agric 1999, 79, 1367-1381.
32. Rasmussen MA, Cray WC Jr, Casey TA, Whipp SC. Rumen contents as a reservoir of enterohemorrhagic *Escherichia coli*. FEMS Microbiol Lett 1993, 114, 79-84.
33. Rice DH, Sheng HQ, Wynia SA, Hovde CJ. Rectoanal mucosal swab culture is more sensitive than fecal culture and distinguishes *Escherichia coli* O157:H7-colonized cattle and those transiently shedding the same organism. J Clin Microbiol 2003, 41, 4924-4929.
34. Sargeant JM, Gillespie JC, Oberst RD, Phebus RK, Hyatt DR, Bohra IK, Galland JC. Results of a longitudinal study of the prevalence of *Escherichia coli* O157:H7 on cow-calf farms. Am J Vet Res 2000, 61, 1375-1379.
35. Sheng H, Lim YY, Knecht HJ, Li J, Hovde CJ. Role of *Escherichia coli* O157:H7 virulence factors in colonization at the bovine terminal rectal mucosa. Infect Immun 2006, 74, 4685-4693.
36. Slutsker L, Ries AA, Maloney K, Wells JJ, Greene KD, Griffin PM. A nationwide case-control study of *Escherichia coli* O157:H7 infection in the United States. J Infect Dis 1998, 177, 962-966.
37. Standing Committee on Agriculture and Resource Management. Australian Standard for Hygienic Production of Meat for Human Consumption. SCARM Report No. 54. AS 4461: 1997. CSIRO Publishing, Melbourne, 1997.
38. Tutenel AV, Pierard D, Van Hoof J, Cornelis M, De Zutter L. Isolation and molecular characterization of *Escherichia coli* O157 isolated from cattle, pigs and chickens at slaughter. Int J Food Microbiol 2003, 84, 63-69.
39. Varela-Hernández JJ, Cabrera-Díaz E, Cardona-López MA, Ibarra-Velázquez LM, Rangel-Villalobos H, Castillo A, Torres-Vitela MR, Ramírez-Alvarez A. Isolation and characterization of Shiga toxin-producing *Escherichia coli* O157:H7 and non-O157 from beef carcasses at a slaughter plant in Mexico. Int J Food Microbiol 2007, 113, 237-241.
40. Weagant SD, Bryant JL, Jinneman KG. An improved rapid technique for isolation of *Escherichia coli* O157:H7 from foods. J Food Prot 1995, 58, 7-12.
41. Yilmaz A, Gun H, Uğur M, Turan N, Yilmaz H. Detection and frequency of VT1, VT2 and *eaE* genes in *Escherichia coli* O157 and O157:H7 strains isolated from cattle, cattle carcasses and abattoir environment in Istanbul. Int J Food Microbiol 2006, 106, 213-217.