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PCR detection of *Escherichia coli* O157:H7 directed from slaughtered cattle in Shiraz, Iran

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ABSTRACT

Escherichia coli O157:H7 lives in the intestines of healthy cattle, and can contaminate meat during slaughtering practices. Detection of the low infectious dosage of bacterium requires a sensitive method. We developed polymerase chain reaction (PCR) assays to detect the gene Stx_2 irrespective of the bacterial serotype. In this study, the detection limit of the PCR protocol in detecting Stx_2 in *E. coli* O157:H7 without prior enrichment was 10^3 CFU/ml, and with 4 h prior enrichment in modified tryptic soy broth was 10^2 CFU/ml. During a period of 7 months (December 2004 to June 2005), 154 slaughtered cattle at Shiraz slaughterhouse, were randomly selected and examined for surface carriage of *E. coli* O157:H7 via detection the Stx_2 gene of verotoxigenic *E. coli* by PCR technique. *E. coli* O157:H7 was found in 14 of 145 (9.65%) feedlot cattle and in 1 of 9 (11.1%) dairy cull cows. This is the first report of the presence of *E. coli* O157:H7 in cattle from Iran.

***Keywords*:** *Escherichia coli* O157: H7, Stx_2, Cattle, PCR

INTRODUCTION

E. coli O157:H7 is a worldwide threat to public health is implicated in hemorrhagic colitis, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura (Wells *et al* 1991, Currie *et al* 1999, Blackburn *et al* 2000, McCarthy *et al* 1998 & Nicole *et al* 2001). Approximately 52% of outbreaks have been associated with bovine products (Chapman *et al* 2001, Chinen *et al* 2001). Most illness has been associated with eating undercooked, contaminated ground beef (Spika *et al* 1986, Carter *et al* 1987), suggesting that cattle are one of the most important sources of *E. coli* O157:H7 infection. An estimated 73,000 cases of infection and 61 deaths occur in the United States each year (Hancock *et al* 1994, Faith *et al* 1996). In the United States, the prevalence in cattle is reported to range from below 2% to around 45%, depending on the used culture techniques, the age of the animals, and the season in which samples are collected (Wells *et al* 1991, Hancock *et al* 1994, Zhao *et al* 1995, Faith *et al* 1996, Elder *et al* 2000). Animal prevalence between less than 1% and 13% have been reported in European countries

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(Garber *et al* 1995, Albihn *et al* 1997, Heuvelink *et al* 1999, Bonardia *et al* 1999, Andersen *et al* 2000). Isolation rates of bacterium from ground beef in Asia have so far been reported in Japan, India and China. Other Asian countries (like Iran) do not seem to be exempt from the *E. coli* O157:H7 infection (Son *et al* 1998, Durso *et al* 2004).

Most detection methods for verotoxigenic *E. coli* (VTEC) are based on the cultivation of serotype O157 (Gilgen *et al* 1998). The low infectious dosage of *E. coli* O157:H7 requires a sensitive detection method (Armstrong *et al* 1996). *E. coli* O157:H7 have two verotoxin genes (VT), shiga toxins (Stx_1 and Stx_2). Polymerase chain reaction (PCR) technique not only allows detection of VTECs irrespective of their serotype, but also detects all different variants of VT (Gilgen *et al* 1998).

The aim of the present study was to identification of *E. coli* O157:H7 from slaughtered cattle at Shiraz slaughterhouse, via the detection of Stx_2 gene using PCR. We were carried out this research for the first time in Iran.

MATERIALS AND METHODS

Sample collection. During a period of 7 months (December 2004 to June 2005), 145 feedlot cattle (reared intensively inside lots, weight 400-700 kg) and 9 dairy cull cows, slaughtered at Shiraz slaughterhouse, were selected randomly and examined for *E. coli* O157:H7. At the end of slaughtering practice, surface swabs were taken from the abdominal and lower parts of the carcasses with sterile wet cotton swabs. The samples were placed in sterile universal bottle and were transferred on ice to the laboratory within less than 2 h of collection. In the laboratory, 20 ml of tryptic soy broth (TSB, Oxoid) plus cefexime and tellurite (modified TSB) was added to each sample, which was then agitated vigorously by hand for 30 s. The suspensions were incubated over night at 37 ºC prior to DNA extraction.

Identification. We used modified in-house enrichment method for DNA extraction as described by Holland *et al* (2000). The PCR protocols used in this study are referred to as Gannon *et al* 1992 and Holland *et al* 2000. Oligonucleotides primers used for PCR were synthesized by Roche, Germany, based on published data (Gannon *et al* 1992, Meyer *et al* 1992).

Amplification of Stx_2 sequences, including genetic variants, was performed using the primer pairs II-F (CCATGACAACGGACAGCAGTT) and II-R (CCTGTCAACTGAGCACTTTG), producing an amplicon of 779 base pairs. PCR was done with some modifications to the protocol described by Holland *et al* (2000) and in 25 µl reactions containing: 0.2 µM of each primers Stx_2, 0.2 µM of each deoxynucleotide triphosphates, 1X PCR buffer (10 mM Tris-HCl [pH 8.3], 50 mM KCl, 0.001% gelatin), 1.5 mM $MgCl_2$, 2.5 U of Taq DNA polymerase (Roche Molecular system), and 2 µl DNA template. Cycling condition provided by Techne Thermocycler (England) was as follows: 94 ºC for 2 min, 30 cycle of 94 ºC for 30 s, 57.5 ºC for 45 s, and 72 ºC for 30 s and a final extension at 72 ºC for 10 min. PCR amplicons were run on 1% agarose gel as well ethidium bromide and visualized under UV illumination.

Sensitivity of the PCR. To assay the sensitivity of the PCR, 10-fold serial dilutions of *E. coli* O157:H7 (ATCC43895) in 0.1% peptone-water were prepared and the suspensions containing 10^1 to 10^8 colony forming unit (CFU)/ml were amplified without prior enrichment and also 4 h of enrichment. The PCR procedure was done as described by Uyttendaele *et al* (1999) and Holland *et al* (2000) and the PCR products were run on 1% agarose gel.

Specificity of the PCR. To determine the specificity of the PCR assay, 7 different bacterial strains included *E. coli* serotypes O1, O2, O6 and O55, *Salmonella* spp., *Klebsiella* spp. and *Listeria* spp. were tested by PCR with conditions describe above.

RESULTS

A total number of 154 samples obtained from 154 animals were analyzed by selective enrichment in mTSB followed by PCR with Stx_2-specific gene primers.

E. coli O157:H7 was detected in 15 of 154 (9.74%) samples. Figure 1 shows the PCR protocol for some isolated *E. coli* O157:H7.

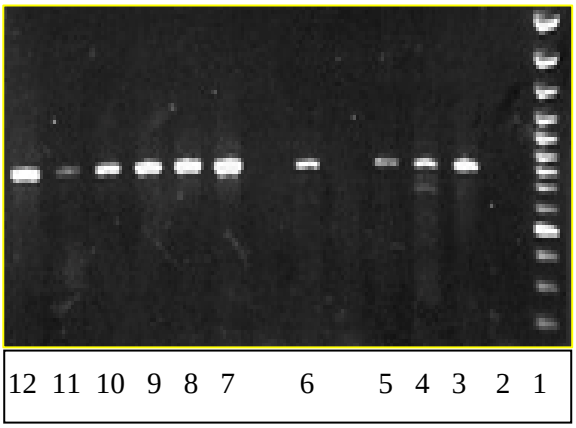

Figure 1. Products of PCR on some *E. coli* isolates. *Lane 1:* 100 bp ladder marker, *Lane 2:* Negative control, *Lane 3-12:* 779 bp positive samples.

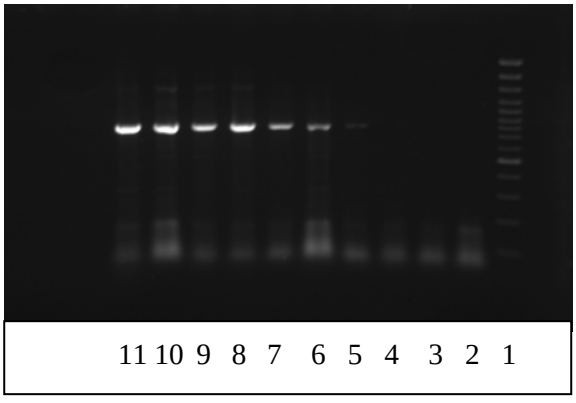

Figure 2. PCR products of a 10-fold serial dilution of *E. coli* O157:H7. *Lane 1:* 100 bp ladder marker, *Lane 2,3:* Negative control, *Lane 4-11:* Bacterial suspensions containing 10^1 to 10^9 CFU/ml, respectively.

The detection limit of the PCR protocol for Stx_2 in *E. coli* O157:H7 without prior enrichment was 10^3 CFU/ml, and with 4 h prior enrichment in mTSB, was 10^2 CFU/ml (Figure 2).

Using primer set Stx_2 for amplification of the 779 bp, amplification products were observed for standard strain of *E. coli* O157:H7 but not observed for the other *E. coli* serotypes (4 strains), *Salmonella*, *Klebsialla* and *Listeria* (Figure 3).

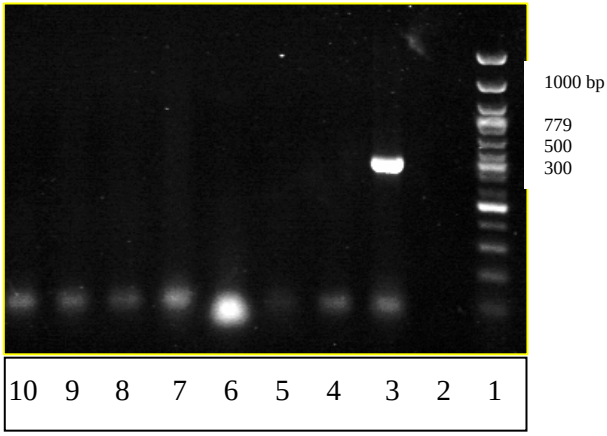

Figure 3. PCR products of 8 bacterial strains. *Lane 1:* 100 bp ladder marker, *Lane 2:* Negative control, *Lane 3: E. coli* O157:H7, *lanes 4-7: E. coli* serotype O1, O2, O6 and O55, *lane 8: Salmonella spp*, *Lane 9: Klebsiella spp*, *Lane 10*: *Listeria spp*.

DISCUSSION

This study was designed to identify and to estimate the prevalence of *E. coli* O157: H7 in slaughtered cattle at Shiraz slaughterhouse, and to establish the sensitivity and specificity of PCR protocol. The PCR protocol, that we have described here, permits rapid, accurate, and inexpensive detection of these important toxin genes in clinical materials and possibly in contaminated foods. We identified *E. coli* O157:H7 on 9.74% of the examined cattle. We recovered the organism from 11.11% of healthy dairy cow and 9.65% of healthy feedlot carcasses. Other studies have shown similar rates of isolation. Wells *et al* (1991) presented Shiga-like-toxin-producing *E. coli* (SLTEC) in 8.4% of the adult cows in Wisconsin and Washington states. Similar results were reported by Canadian study of randomly selected cattle at slaughter. SLTEC was isolated

from 10.5% of beef cattle and 19.5% of dairy cows (Clarke *et al* 1998). In a study on healthy cattle in Germany, SLTEC was recovered from 17% of dairy cows and 9.4% of bulls (Montenegro *et al* 1990, Wells *et al* 1991). The prevalence rates of VTEC in cattle reported from other European countries were from less than 1% to 13% (Blanco *et al* 1996, Albihn *et al* 1997, Elder *et al* 2000, Andersen *et al* 2000). Some studies from the United States indicate that more than 45% of the cattle are carriers of *E. coli* O157:H7 (Chapman *et al* 2001, Dargatz *et al* 1997, Heuvelink *et al* 1999, Bonardi *et al* 1999, Elder *et al* 2000).

The rates of VTEC isolation in our study are very similar to those reported by Son *et al* (1998) in Malaysia, Rivas *et al* (2001) in Argentina and Terajima *et al* (2000) in Japan. For detection of lower numbers of the pathogen, prior enrichment will be necessary (Uyttendaele *et al* 1999). In this study, we could easily detect the Stx_2 genes in as little as 10^3 CFU/ml without prior enrichment and 10^2 CFU/ml after enrichment of bacterial suspension. This result is in consistent with Pollard *et al* (1990). Results for positive controls of the above described experiment confirmed this detection limit.

In conclusion, this investigation, that was the first epidemiological survey on the occurrence of *E. coli* O157:H7 in healthy cattle in Iran, confirms that in Iran, as well as in other countries, cattle represent an important reservoir of this pathogen and the prevalence of the bacterium appears to be similar to those to the other parts of Asia (Albihn *et al* 1997, Gro *et al* 2001). Further studies are now needed to identify the vehicles of infection most relevant to our epidemiologic situation and the most appropriate measures to prevent the spread of this life-threatening food borne disease.

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