Prevalence of *Escherichia coli* O157:H7 in bovine feces in North West of Iran

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Abstract

The objective of this study was to identify and estimate the prevalence of *Escherichia coli* O157:H7 in cattle fecal samples in northwest of Iran, using multiplex polymerase chain reaction (PCR). In this study, cattle fecal samples were collected from an abattoir in Tabriz, Iran. After enrichment, isolation was carried out on CT-SMAC culture and afterwards, the identification of *E. coli* O157 was achieved on colorless sorbitol negative colonies. Then, one of these colonies was analyzed by PCR to identify genes coding for verotoxin 1 and 2 (*vt1* and *vt2*), intimin (*eceAO157*), and H7 flagella antigen (*fliCh7*). Of 200 samples, 22 were positive by CT-SMAC culture. Of the 22 sorbitol-negative samples investigated by PCR, two isolates were identified as *E. coli* O157:H7. Three isolates carried *eaeAO157* specific *locus* (non-VTEC *E.coli* O157) and one or both the verotoxin genes (*vtx1* and *vtx2*) only (non-O157 VTEC). Low prevalence rates of *E. coli* O157:H7 in cattle feces as an important animal reservoir of the mentioned bacterium should be added to the variety of factors mentioned for the low prevalence of *E. coli* O157:H7 in Iran.

Introduction

Escherichia coli O157:H7 (*E. coli*) is the predominant strain of verotoxin-producing *E. coli* (VTEC) worldwide, which causes infectious diseases from simple diarrhea to hemorrhagic colitis (HS) and hemolytic-uremic syndrome (HUS).[1] This pathogenic strain produces verotoxin, intimin (encoded by the *eaeA* gene), and some other virulence factors that are required for their pathogenicity in disease induction.[2,3]

In the reports of many researches, human illnesses have been attributed to the consumption of food products contaminated with O157 and non-O157 strains of *E. coli* from animal origins.[4] On the other hand, animals and their feces have been considered as the main source of VTEC and reported incidence of E. *coli* O157. The various prevalence of E. *coli* O157 including E. *coli* O157:H7 in the feces of animals such as cattle depends on the geographical location and seasons of the year and this rate varies from 1 to 62% in cattle feces.[5]

Although *E. coli* O157:H7 strain has been isolated from different animal species, it has been shown to be more prevalent in ruminants than in the others.[4] In addition, human infectious diseases have been traced in most cases to cattle, originating from their feces and/or their food products.[4] Therefore, the cattle is the most important animal reservoir of the mentioned bacterium.[6] It is well documented that this harmful pathogen can be transferred to human by direct and indirect methods.[7]

Few studies have been done on Iranian animal feces on *E. coli* O157:H7 identification; Therefore, this is the first study in northwest of Iran. The objective of this study was to identify and estimate the prevalence of *E. coli* O157:H7 in cattle fecal samples using multiplex PCR in northwest of Iran.

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Materials and Methods

Sampling

This study was carried out in an abattoir in Tabriz (the biggest city in northwest of Iran), in summer 2009. The cattle were sent from different northwestern provinces of Iran to this abattoir to be slaughtered. About 200 fecal samples were collected and sent for microbiological studies. All samples were packed in sterile plastic boxes together with ice and transported to the laboratory immediately after sampling for laboratory analysis.

Enrichment and isolation of bacteria

Fecal samples were prepared by adding 25 g of feces to 225 mL buffered peptone (BPW) water. Then the samples were stored at 36°C overnight. Each enrichment culture was serially diluted in BPW. Next, the samples from 10^{-4} to 10^{-5} dilutions were plated to cefixime-tellurite sorbitol MacConkey (CT-SMAC) agar plates and screened for typical sorbitol-negative colonies. Mentioned colonies were selected and transferred to Brain Heart Infusion agar (BHI agar) and incubated for 24 h in 37°C.

Identification test

For each sample, up to 10 colonies per sample sorbitol non-fermenting colonies that were colorless were selected. After oxidase and catalase rapid test, all oxidase negative and catalase positive bacteria were identified as *E. coli* by IMV_iC test using Indole test in SIM medium (Merk, Germany), citrate utilization test in Simmons citrate agar (Merk, Germany), and the Methyl red and Voges-proskaure tests using MR-VP medium (Merk, Germany).

DNA extraction

A colony of BHI agar was transferred to a clean microtube and was added 500 mL lysis buffer (pH 8) containing 5 mol NaCl, 100 mmol Tris-base, 20 mmol EDTA-Na2, and CTAB 20%. It was incubated in 60-65°C for 10 min and then was centrifuged at 12,000 × g for 10 min. The pellet was resuspended in $CHCl_3$-isoamyl alcohol (24:1) and centrifuged at 12,000 × g for 1 min. Then, the pellet was resuspended in cold isopropanol and transferred to the refrigerator for 30 min. afterwards, ethanol 70% was added to the supernatant and centrifuged at 12,000 × g for 1 min. Finally, 50 µL TE-buffer was added to the pellet and stored as DNA template.

Detection of *E. coli* O157 by polymerase chain reaction

After DNA extraction, sorbitol-negative *E. coli* colonies were analyzed by PCR to identify genes coding for verotoxins 1 and 2 (*vtx1* and *vtx2*), intimin for *E. coli* O157 (*eaeA*O157), and H7 flagella antigen (*fliCh7*) with special primers according to the literature.[8]

The oligonucleotide primers (MWG, Germany) used in this study were designed according to Gannon, 1997.[8] Each 25 µL

Table 1. Number of sorbitol-negative *E.coli* isolates in which verotoxin-positive O157 was isolated from a total of 200 samples tested, and their corresponding virulence genes as determined by polymerase chain reaction.

Number of sorbitol-negative *E.coli* isolates	% of total of sampled animals	vtx1/ vtx2	eaeAO157	fliCh7
8	4	+	-	+
7	3.5	+	-	-
3	1.5	-	-	+
4	2	+	+	+
Total: 22	11			

reaction contained 50 ng of extracted DNA as template, 1 U Taq DNA polymerase, 3.2 mM of each primer, 200 µM of each dNTP, 2.5 µL PCR buffer, and 2 mM $MgCl_2$. The reaction was carried out with amplification thermal cycler (Eppendorf model 22331, Germany). The PCR reactions were initiated by denaturation at 94°C for 4 min followed by 35 cycles, each cycle consisting of DNA denaturation at 94°C for 30 sec, annealing at 62°C for 30 sec, and extension at 72°C for 75 sec. The cycles were followed by a final extension at 72°C for 10 min. Amplified PCR products were separated by 1.5% agarose gel electrophoresis and finally stained with ethidium bromide and photographed.

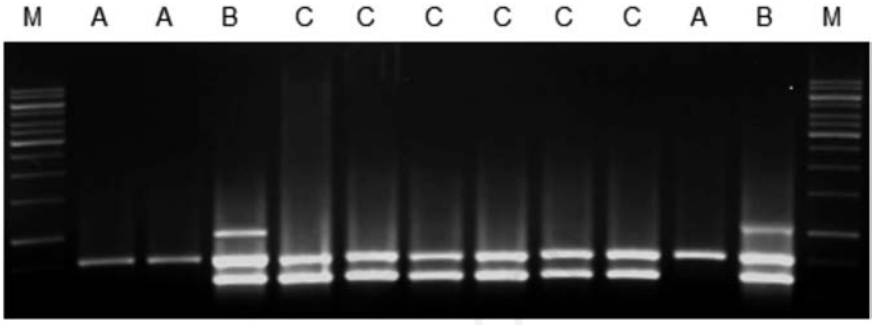

Figure 1. Lane M: Size Marker, Lane A: VTEC, Lane B: *E.coli* O157 H7, Lane C: vtx1+2 and H7 positive isolates.

Results

Of 200 samples transferred to the laboratory during the study period, 22 samples were positive by CT-SMAC culture (sorbitol-negative colonies) (Table 1). These 22 samples were analyzed by vtx1, vtx2, and *eaeA*O157-specific gene primers. Of the 22 sorbitol-negative samples investigated by PCR, 12 isolates carried any of vtx1 and vtx2 genes and also had *fliCh7* gene-specific *locus*. Among them, 4 isolates had *eaeAO157* gene and identified as *E. coli* O157:H7. Three isolates carried *fliCh7*-specific *locus* and 7 isolates had only verotoxin genes (*VTEC*) (Figure 1).

Discussion

This study was the first prevalence report of *E. coli* O157:H7 in bovine feces in Tabriz, northwest of Iran. Although the samples in this study were monitored to be low, according to our results, the prevalence of *E. coli* O157:H7 was low in the cattle. Some studies reported various incidences of this strain in cattle feces widely depending on the geographical location and season. This prevalence rate varied from 0.1% to 62 % in different countries.[5] This rate was reported between 0.2% to 27.8 % at slaughter.[4] The result of this study and other similar ones carried out in different parts of Iran showed the prevalence rate of this organism to be low, in comparison to other countries.[9,10]

These results are in agreement with other published studies about prevalence of *E. coli* O157:H7 in human population in Iran. In 2007, Nahaei *et al.* reported the low prevalence of this strain, 0.58%, isolated from patients with acute diarrhea in Tabriz hospitals.[11] Another study in Shiraz (southwest of Iran) on 719 children with diarrhea examined by polyclonal antibody test and PCR showed that *E. coli* O157: H7 was not a cause of bloody diarrhea in that area.[12] Some other studies carried out in Iran also reported similar results.[13] Some papers bring up this question that whether *E.coli* O157:H7 is a common pathogen causing diarrhea in Iran or not.[12]

A variety of factors are mentioned for low prevalence of *E. coli* O157:H7 in human diarrhea in Iran including; low consumption of fast foods, consumption of lamb and goat meat instead of beef, and few industrialized slaughterhouses in Iran.[12] The low prevalence rates of *E. coli* O157:H7 in cattle feces, as the important animal reservoir of the mentioned bacterium,[9,10] should be added to previous factors. According to a report, the prevalence rate of this bacterium in sheep is similar to cattle.[14]

Conclusions

The results of this study showed *E. coli* O157:H7 and even verotoxigenic *E. coli* does not play a critical role in human diarrhea. This finding was confirmed by some papers in human diarrhea in Iran that focused on other parasitic and bacterial agents such as *Entamoeba histolytica*, *Giardia lamblia,* and *Shigella* spp.[11-13]

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