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

Jalal Shayegh
Department of Veterinary Medicine College, Shabestar Branch, Islamic Azad University, Shabestar, Iran

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 (eaeAO157), 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.

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^-3 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 C test using Indole test in SIM medium (Merk, Germany), citrate utilization test in Simmons citrate agar (Merk, Germany), and the Methyl red and Voges-proskauere 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 CHCl3-isooamyl 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 (eaeAO157), and H7 flagella antigen (fliCh7) with special primers according to the literature.

The oligonucleotide primers (MWG, Germany) used in this study were designed according to Gannon, 1997. Each 25 µL
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 MgCl₂. 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.

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.

<table>
<thead>
<tr>
<th>Number of sorbitol-negative E.coli isolates</th>
<th>% of total of sampled animals</th>
<th>vtx1/ vtx2</th>
<th>eaeAO157</th>
<th>fliCh7</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>4</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>35</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Total: 22</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**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 eaeAO157-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. This rate was reported between 0.2% to 27.8% at slaughter. 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.

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. 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. Some other studies carried out in Iran also reported similar results. Some papers bring up this question that whether E.coli O157:H7 is a common pathogen causing diarrhea in Iran or not.

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. The low prevalence rates of E. coli O157:H7 in cattle feces, as the important animal reservoir of the mentioned bacterium, should be added to previous factors. According to a report, the prevalence rate of this bacterium in sheep is similar to cattle.

**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.

**References**

4. Hussein HS. Prevalence and pathogenicity of Shiga toxin-producing