Prevalence of O157: H7 and Non-O157 E. coli in Iranian Domestic Sheep

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Abstract: The aim of the present study was the isolation of both E. coli O157 and non-O157 in sheep. Vero toxins (VT) 1, 2 and eae genes were tested for this propose. Sheep feces are an important source of Shiga toxin-producing Escherichia coli (STEC). Escherichia coli O157:H7 is a highly virulent food-borne pathogen and threat to public health. Rectal swab samples from sheep were collected during 2009-2010. Conventional plating and Polymerase Chain Reaction (PCR) were carried out according to virulence factors (Stx1, Stx2 and eaeA). There significant differences between prevalence of STEC and session were observed. It was at highest in spring and late summer. Six (3.92%) sheep carcasses were contaminated by E. coli O157:H7. Only six samples were positive by PCR specific for the VT2 gene and produced verocytotoxin VT2, whereas all isolates were negative for the presence of VT1 and eae virulence genes considered. Geographical variations and season may be influenced in the prevalence rate. The composition of the gastrointestinal flora may be changed by different diet and, therefore O157 STEC rate in sheep and lamb was different. Iranian sheep indicated as a natural host of E. coli O157 strains therefore, may be potentially pathogenic for humans. This is the first report of E. coli O157 detection from sheep in Iran.

Key word: E. coli O157, E. coli non-O157, sheep, Iran

INTRODUCTION

Escherichia coli O157:H7 is belonging to Enterohemorrhagic E. coli (EHEC) a highly virulent food-borne pathogen with an infective dose of near 100 cells in humans (Raya et al., 2006). Escherichia coli O157:H7 and O157:H− (non-motile) can be the main causes of disease in humans (Boyce et al., 1995). The Shiga toxin Producing E. coli (STEC) strains most frequently associated with clinical disease is serotype O157:H7 (Nataro and Kaper, 1998; Mead et al., 1999). However, in some countries, other serotypes are associated with severe disease outbreaks (Nataro and Kaper, 1998; Acheson, 2000). Since cattle and sheep, are recognized the most important reservoirs of STEC in the absence of clinical signs (Heuvelink et al., 1998; Johnson et al., 1999). STEC are widely distributed in sheep feces prior to slaughter (Fegan and Desmarshelier, 1999) and contamination of meat with both O157 and non-O157 STEC occurs during slaughter or meat processing (Armstrong et al., 1996) but consumption of contaminated foods and direct contact by infected animals or humans are the main route of transmission (Griffin and Tauxe, 1991; Boyce et al., 1995). The STEC spread, is complex and unknown (Stacey et al., 2007) but those are carried in the gastrointestinal tract of healthy animals and do not cause disease in mature ruminants even when administered at very high doses (Comick et al., 2007). The virulence factor for disease caused by STEC is not understood. However, Shiga toxin is a major factor responsible for pathogenesis (Acheson, 2000). They have cytotoxic effects on African Green Monkey kidney (Vero) cells in cultures (Konovalchuk et al., 1977). Shiga toxin 1 (Stx1) homologous with Shigella dysenteriae type 1 and Stx2 that antigenically distinct from stx1 (Paton and Paton, 1998). They caused Hemorrhagic Colitis (HC) and Hemolytic-uremic Syndrome (HUS) (Heuvelink et al., 1998). Some studies indicated that strains with only stx1 or both stx1 and stx2 less virulent and caused less frequent disease than strains harboring stx2 (Tesh et al., 1993; Nataro and Kaper, 1998; Dorn et al., 1989). Intimin encoded by eae gene is responsible for attachment of STEC to the intestinal epithelial cells (Nataro and Kaper, 1998; Acheson, 2000). The present study describes the isolation and virulence factors characterization of O157 and non-O157 STEC strains on Iranian sheep during one year period.

MATERIALS AND METHODS

Sampling: During one year period from September 2009 to August 2010, rectal swab samples from sheep were collected before slaughtering practice at several slaughterhouses located at different geographic sites in Fars province-Iran. Conventional plating, and Polymerase

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Chain Reaction (PCR) were carried out on samples for the presence of STEC (both O157 and non-O157). Three main virulence factors (Stx1, Stx2 and eae A) were tested for this proposes. A lot by means of sheep from the same source defined as a group. Fifteen percent of each lot was sampled during each month. Immediately after slaughter, the samples were collected aseptically and were placed into transfer media and kept in ice-pack container. Animals were randomly selected and no information on the farms of origin was recorded. The microbiological examination was started the day after sample collection.

Bacterial Isolation by selective media: The transfer media which described above contain modified tryptase-soy broth (Merck VM61475930) supplemented with 0.05 mg L^{-1} cefixime (Darri Pakshir-Ir-32) and 2.5 mg L^{-1} potassium tellurite (Scharlai PO0380) (mTSE). The samples were homogenized and incubated overnight at 37°C on a rotary shaker (100 rpm). One loop full of enrichment culture was spread plated onto sorbitol MacConkey agar (Hi Media M298), supplemented with cefixime and potassium tellurite (SMAC-CT) as well. All plates were incubated over night at 37°C. Ten to eleven sorbitol and non-sorbitol fermenting colonies were selected for further experiments. The sorbitol negative and positive colonies were picked and further identification was performed using biochemical test such as indol, rhamnose, urease activity and motility.

Antiserum test: Every colony was assayed for expression of the O157 antigen by latex agglutination with the Wellcolox™E. coli O157:H7 Latex Test (Mast, UK). O157 positive and negative colonies were selected for PCR assay.

DNA extraction: All samples that were previously positive and negative were screened for presence of stx1, stx2 and eaeA using multiplex PCR. One milliliter overnight culture was used for DNA extraction by DNTP kit (Cina-gene) and 2 μL of elution results was used as template DNA in a 25 μL reaction PCR.

PCR and reaction: Twenty five micro liter reactions mixture containing 0.2 μM of each primers (Table 1) (Holland et al., 2000; Yilmaz et al., 2006; Urdahl et al., 2003) 1-F/IIR. The mixture continued by 0.2 μM of each deoxynucleotide triphosphates (Roche, Germany Company), 1X PCR buffer (10 mM Tris-HCl [pH 8.3], 50 mM KCl, 0.001% gelatin), 1.5 mM MgCl₂. Finally, 2.5 U of Taq DNA polymerase (Roche Molecular system) was used and then 2 μL DNA templates were added. The reactions were carried out with a Thermocycler (Eppendorf, Germany) and cycling condition with some modification was as follows: 94°C for 2 min, 30 cycle of 94°C for 30 sec, 57.5°C for 45 sec and 72°C for 30 sec and a final extension at 72°C for 10 min. Ten μL of the amplification PCR product were analyzed on 1% agarose (Merck) gels containing 0.4 μg mL^{-1} ethidium bromide, visualized under UV illumination, saved and photographed. Each agarose gel electrophoresis run included DNA molecular size standards (100 bp Ladder).

RESULTS

Thirteen samples were shown at least one sorbitol negative colonies on CT-SMAC identified susceptible as an O157. Forty nine (32.02%) colonies with sorbitol positive were considered as a non-O157 STEC. Out of 62 (40.52%) STEC from 153 animals, 7 (4.57%) and 45 (29.41%) recognized O157 and non-O157 by PCR respectively (Table 2). For all animals, the location of slaughter was not linked to the location of origin of the animals. Any sorbitol positive E. coli isolate harboring at least one shiga toxin was considered as a non-O157 STEC.

The monthly prevalence of ETEC in sheep was obtained 2-40% and was at its highest in spring and late summer (Fig. 1). As is noted, the prevalence rate of non-O157 is more than O157. There was observed significant differences between prevalence rate in spring and summer with O157 and non-O157.

The PCR assay revealed that some isolates possessive stx1, stx2 and both of them and also eae genes. PCR results indicated that of 153 isolate, 98 (64.05%) had stx2 and 53 (34.64%) possessive stx1. Both

<table>
<thead>
<tr>
<th>Product size (bp)</th>
<th>Oligonucleotide sequence (5'-3')</th>
<th>Primers</th>
</tr>
</thead>
<tbody>
<tr>
<td>614</td>
<td>ACA CTG GAT GAT CTC AGT GG</td>
<td>stx1-f</td>
</tr>
<tr>
<td></td>
<td>CGT AAT CCC CCT CTC TTA TG</td>
<td>stx1-r</td>
</tr>
<tr>
<td>179</td>
<td>CGA TGA CAA CGG AGA GCA GCT</td>
<td>stx2-f</td>
</tr>
<tr>
<td></td>
<td>CCT GCC AAC TCA GCA CCT TG</td>
<td>stx2-r</td>
</tr>
<tr>
<td>450</td>
<td>AAG CGA CTG AGG TCA CT</td>
<td>eae-f</td>
</tr>
<tr>
<td></td>
<td>ACG CTG CTC ACT AGA TGT</td>
<td>eae-r</td>
</tr>
</tbody>
</table>

Table 2: STEC isolation rate from sheep slaughterhouse, Shiraz-Iran 2009-2010

<table>
<thead>
<tr>
<th>Animals</th>
<th>No.</th>
<th>+ (Non-O157)</th>
<th>- (O157)</th>
<th>STEC (%)</th>
<th>O157 antisera (%)</th>
<th>PCR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ewes</td>
<td>115</td>
<td>38 (33.04)</td>
<td>10 (8.69)</td>
<td>48 (41.73)</td>
<td>8 (6.95)</td>
<td>35 (30.43)</td>
</tr>
<tr>
<td>Lambs</td>
<td>38</td>
<td>11 (28.97)</td>
<td>3 (7.89)</td>
<td>14 (36.84)</td>
<td>2 (5.26)</td>
<td>9 (23.63)</td>
</tr>
<tr>
<td>Total</td>
<td>153</td>
<td>49 (32.02)</td>
<td>13 (8.49)</td>
<td>62 (40.52)</td>
<td>10 (6.53)</td>
<td>45 (29.41)</td>
</tr>
</tbody>
</table>
Table 3: Distribution of virulence factor associated with E. coli O157

<table>
<thead>
<tr>
<th>STEC virulence</th>
<th>Stx1</th>
<th>Stx2</th>
<th>eae</th>
<th>Stx1, Stx2</th>
<th>Stx1, eae</th>
<th>Stx2, eae</th>
<th>Stx1, Stx2, eae</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of isolates (%)</td>
<td>53 (34.64)</td>
<td>98 (64.05)</td>
<td>34 (22.23)</td>
<td>44 (28.75)</td>
<td>36 (23.53)</td>
<td>59 (38.56)</td>
<td>21 (13.72)</td>
<td>153</td>
</tr>
</tbody>
</table>

Fig. 1: Monthly prevalence of E. coli O157 and Non-O157 from sheep in Fars-Iran

stx1 and stx2 were harboring at 44 (28.75%) of STEC isolates. Except five percent, all isolates fermented sorbitol on SMAC agar. Thirty four (22.23%) isolates produced intimin encoded by eae. Nineteen isolates (12.58%) possessed the combination of virulence factors (eae and at least one shiga toxin gene) that has been associated with severe disease in humans (Table 3).

DISCUSSION

In the previous study similar variations in incidence rates of disease was found. The seasonal prevalence of disease in sheep parallel the disease in cattle and sheep did not differ a great deal from previous studies. Although at significantly lower incidence of sheep according to data was observed (Talamtan et al., 2010).

Sheep, the second most commonly reared species of ruminant food animals, appears to have a role similar to that of cattle as a natural reservoir of STEC (Heuvelink et al., 1998). STEC shedding by sheep peaks during the summer (Hancock et al., 1994; Kudva et al., 1997), parallel to the human infections (Griffin and Tauxe, 1991; Boyce et al., 1995). Different diet change the composition of the gastrointestinal flora and differences in STEC isolate from sheep and lambs. In the other hand, young lambs are directly sold and transported from fattening herds to slaughterhouse but adult sheep are at several risk of coming into contact with infected animals. These data are difficult to compare with those of other studies in the world. Because they may be greatly influenced by factors such as the target population, the sampling strategy, the methodology of screening and isolation and also by possible seasonal variation in prevalence (Callaway et al., 2009; Hancock et al., 2001). In the present study, the PCR technique for identification of STEC, a method which is regarded as one of the most sensitive isolation methods for STEC was used (Johnsen et al., 2001) and then compare with conventional methods. In general, lambs are more susceptible to disease than sheep shown more sever clinical signs. The data indicated that Iranian sheep is natural hosts of E. coli O157 strains and Non-O157 as well, therefore there is a potential risk for contamination of carcasses and entry of STEC into the food chain and considered for humans public health threat. Contamination of the carcasses and environment with E. coli O157:H7 from intestinal contents of sheep during slaughtering practice is one of the most important risk factor in transmission to human (Mead and Griffin, 1998; Philips, 1999). Carcasses become contaminate at the time of slaughter and processing may introduce the organism when performed in no hygienic conditions (Chapman et al., 1993). It is difficult to directly compare results from different studies due to varying sampling and testing regimes. Therefore, critical points must be determined in the abattoir to prevent the contamination by E. coli O157:H7 (Gun et al., 2003).

CONCLUSION

This investigation was indicated the epidemiological survey on the occurrence of STEC in healthy sheep in Iran. This confirms that in Iran, as well as in other countries, sheep represent and also important reservoir of EHEC. The prevalence of the bacterium appear to be similar to those to the other parts of the world (Cormick et al., 2007; Johnsen et al., 2001). Despite the increasing significance of EHEC strains in the etiology of disease in Iran, the epidemiology of infections caused by these strains is poorly understood (Orth et al., 2006).

ACKNOWLEDGMENTS

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