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Typing of Stx2 Genes of Escherichia coli O157 Isolates from Cattle

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Enterohemorrhagic Escherichia coli (EHEC) infection in Japan is classified as a category III notifiable infectious disease under the Infectious Diseases Control Law. Shiga toxin-producing E. coli of serogroup O157 (O157) is the major causative agent of EHEC infection in Japan (1). The number of large outbreaks caused by O157 has diminished since numerous nationwide occurrences in 1996. However, 3,922 new symptomatic and asymptomatic cases of EHEC infection were reported in 2006 (1). The number of sporadic cases, including diffuse outbreaks caused by a single clone in different areas, has increased (2). Cattle are an important source of EHEC infection in Japan (3). Shiga toxin (Stx) is a cytotoxin; it is the major virulence factor produced by EHEC. All Stx's have been classified into two major classes, Stx1 and Stx2, based on toxin-neutralization and DNA hybridization tests. The Stx1 class is largely homogeneous, although several variants of Stx2 exist, as judged by neutralization tests, comparison of activity in different cell lines and, more recently, DNA sequencing (4). However, few variants of Stx2 genes exist among O157 isolates compared with other serotypes (5). We previously reported that strains carrying only the stx2vha gene (sometimes expressed as stx2c) were probably less virulent and caused bloody diarrhea less frequently (6).

70 of 72 strains isolated from feces or carcasses of cattle or calf possessed only stx2vha isolated from cattle and to compare the prevalence of these genes with those among strains isolated from humans.

In all, 330 O157 isolates collected during 2000-2003 were studied: as presented in Table 1, of the 253 isolates of human origin, 182 isolates were from patients, and 71 were from asymptomatic human carriers. At the Osaka Municipal Meat Inspection Center, 77 bovine isolates were obtained. They had the following origins: feces (n = 36), ruminal contents (n = 27), carcass, liver or other (n = 14). The presence of stx1 in the O157 isolates was investigated using PCR with commercial primers-TaKaRa EVT-1/2 (for stx1) and TaKaRa EVS-1/2 (for stx2) according to the manufacturer’s instructions (Takara Bio Inc., Otsu, Japan). The Stx2 genotypes were analyzed using the predicted restriction enzyme fragment length polymorphisms of PCR products (PCR-RFLP) (6,7). Because no O157 strains were positive for stx2e or stx2ev (6), these variant genes were not examined in this study. For bovine isolates (and a part of human isolates), pulsed-field gel electrophoresis (PFGE) was carried out according to Izumiya et al. (8) using a CHEF DR III (Bio-Rad Laboratories Inc., Hercules, Calif., USA). Bovine isolates showing identical XbaI-PFGE patterns were compared further using additional restriction endonucleases SfiI (New England Biolabs Inc., Beverly, Mass., USA) and NotI (New England Biolabs). Restriction endonuclease digestion was performed using 30 U of each enzyme. The pulse time was increased from 5 to 50 s over 22 h for SfiI-digested DNAs and from 4 to 8 over 11 h, then 8 to 50 s over 9 h for NotI-digested DNAs. For human isolates, PFGE was carried out at the National Institute of Infectious Diseases, Tokyo between 2001 and 2003.

Various PFGE patterns were observed in both human and bovine isolates. The 163 human O157 isolates between 2001 and 2003 were classified into 124 different types (data not shown); no EHEC O157 outbreak was recognized in Osaka City between 2000 and 2003. The 77 bovine O157 isolates were classified into 49 different types (data not shown).

In all, 330 strains were assigned to seven groups according to their stx type (Table 1). After the strains were assigned to three groups based on Stx2 genotypes, 45 (58.4%) of 77 bovine isolates possessed only stx2vha, 20 (26.0%) strains possessed stx2, and 8 (10.4%) strains possessed both stx2 and stx2vha. In terms of isolates from human patients, 125 (68.7%) of 182 strains possessed only stx2, whereas 34 (47.9%) of 71 strains from asymptomatic human carriers possessed only stx2vha. There were only 5 isolates possessing only stx1: 4 from cattle and one from a patient. Two isolates from patients were untypable using PCR-RFLP (Table 1). No O157 strains in this study were positive for stx2vhb.

We found that O157 strains possessing only stx2vha are prevalent among bovine isolates or strains from asymptomatic carriers, whereas major patient isolates possessed only stx2. Most O157 strains isolated from bovine sources (possessing only stx2vha) might not readily cause disease to humans. In Australia, human disease is only rarely associated with EHEC O157. Fegan and Desmarchelier (9) examined Australian O157 strains for the Stx2 genes and reported that 70 of 72 strains isolated from feces or carcasses of cattle or calf possessed only stx2vha (stx2c). LeJeune et al. (10) used the antiterminator Q gene of bacteriophage 933W (Q933) as a genetic marker. The stx2 and stx2-gene variants are under similar regulatory control as other phage late genes; it is governed by the interaction of the transcription antiterminator Q with the late promoter P6 (11). The Q933 gene target was more commonly identified in human disease-associated strains of O157 than from strains of bovine origin (10). In addition, they suggested that distribution of the Q933 gene

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target among O157 strains from bovine origin in Japan was minor (10). These results are consistent with those of our study: major O157 strains isolated from bovine sources are less virulent.

Actually, Stx2vha is a major variant form of Stx2 present in E. coli. We previously reported that strains carrying only stx2vha were probably less virulent and caused diarrhea less frequently (6). In this study, we recognized that strains carrying only stx2vha are prevalent among both cattle and healthy human carriers. Cattle seem to be a source not only of stx2 possessing O157 organisms but for those possessing the possibly less-virulent stx2vha; however, caution is necessary because E. coli strains carrying stx2vha were also isolated from patients with hemolytic uremic syndrome (12).

**REFERENCES**


**Table 1. Genotype of Stx2 genes of Escherichia coli O157 strains from cattle or human**

<table>
<thead>
<tr>
<th>Genotype of stx2 genes</th>
<th>Cattle (n = 77)</th>
<th>Patient (n = 182)</th>
<th>Asymptomatic human carrier (n = 71)</th>
<th>Total (n = 330)</th>
</tr>
</thead>
<tbody>
<tr>
<td>stx1 stx2 stx2vha stx2 and stx2vha</td>
<td>4 (5.2)</td>
<td>1 (0.5)</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>stx1 and stx2</td>
<td>15 (19.5)</td>
<td>108 (59.3)</td>
<td>27 (38.0)</td>
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</tr>
<tr>
<td>stx2</td>
<td>5 (6.5)</td>
<td>17 (9.3)</td>
<td>3 (4.2)</td>
<td>25</td>
</tr>
<tr>
<td>stx2vha</td>
<td>17 (22.1)</td>
<td>12 (6.6)</td>
<td>5 (7.0)</td>
<td>34</td>
</tr>
<tr>
<td>stx1, stx2 and stx2vha</td>
<td>28 (36.4)</td>
<td>19 (10.4)</td>
<td>29 (40.8)</td>
<td>76</td>
</tr>
<tr>
<td>stx2vha</td>
<td>2 (2.6)</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>stx2 and stx2vha</td>
<td>6 (7.8)</td>
<td>23 (12.6)</td>
<td>7 (9.9)</td>
<td>36</td>
</tr>
</tbody>
</table>

1): Two strains were untypable by PCR-RFLP according to stx2 gene.