High Prevalence of Enterohemorrhagic *Escherichia coli* (EHEC) O157 from Cattle in Selected Regions of Japan

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NOTE  Bacteriology

Human hemorrhagic colitis and hemolytic uremic syndrome caused by enterohemorrhagic *Escherichia coli* (EHEC) is found in many countries of the world including Japan [10, 13, 17, 20, 21], and it is one of the most problematic infectious diseases at present. The major virulence factor of EHEC is the production of Shiga toxin (Stx), which can be subdivided into Stx1 and Stx2 [11]. Although various serotypes of EHEC have been associated with human disease, O157 is the most important pathogen.

EHEC O157 can be isolated from various animals such as cattle [2, 4, 5, 8, 9, 12, 16, 23], sheep [6], deer [1], and so on. In particular, it is often isolated from faeces of cattle showing no clinical signs of disease. It was reported that the prevalence of EHEC O157 in cattle was 0.2–28% in the United States [7, 8, 23], 1.9% in Australia [5], and 4.2–20% in Europe [2, 4, 9, 16]. Therefore, cattle can harbour the organisms in their faeces and are regarded as a natural reservoir and source of infection [3]. In Japan, Kanda et al. [12] isolated EHEC from the faeces of 0.3% of cattle delivered to a slaughterhouse in 1987. In a nationwide survey of EHEC O157, the organism was isolated from 1.4% of faeces and 0.3% of dressed carcasses [22]. However, there have been few reports on cattle reared on farms. The aim of the present study was to investigate the prevalence of EHEC O157 in the faeces of cattle on dairy and beef farms.

Faecal samples were collected from a beef farm in central Honshu, and from dairy farms and a veterinary hospital in Hokkaido from 1997 to 1998. Except for the hospitalized cattle in the veterinary hospital, few cattle had any clinical signs.

Faeces were collected from the rectum. About 10 g of faecal sample was added to 100 ml of ME broth containing 20 µg/ml novobiocin [18]. After 18 hr of incubation at 42°C, 10 µl was inoculated on CT-SMAC agar [24] and incubated at 37°C for 18 to 20 hr. Sorbitol-nonfermenting colonies were selected and regarded as EHEC. Serotyping of isolates was carried out by slide agglutination test with anti-O157 serum (Denka Seiken, Tokyo, Japan).

O157 strains were then further characterized. Isolates were tested for stx genes by PCR with consensus primer pairs amplifying stx1 and stx2. The PCR was performed as described previously [19]. The bacteria were grown overnight at 37°C in 3 ml of tryptone soy broth. A 100-µl sample from each culture was centrifuged and washed with distilled water. The bacterial sediment was resuspended in 100 µl distilled water and heated at 100°C for 5 min. Ten microliters was used for PCR. The PCR primers and programs used in the present study are shown in Table 1.

Production of Stx was confirmed by reversed passive latex agglutination (RPLA) test with a verotoxin detection kit (VTEC-RPLA SEiken, Denka Seiken, Tokyo, Japan).

The results of the isolation of EHEC O157 from faeces are shown in Table 2. EHEC O157 was isolated from 42 (13.0%) of 324 bovine faeces. Of the 4 farms and the facilities tested, 3 farms and the facilities were positive for EHEC O157. The isolation rate varied among the farms and facility. T Farm in Hokkaido showed the highest isolation rate (33.7%), followed by Y Farm in Honshu and R Farm in Hokkaido. Two calves and a cow in the veterinary hospital were positive for EHEC O157. EHEC prevalence in heifers was higher than that in calves and other cattle. Among the cattle positive for EHEC O157, none showed any clinical signs, except 2 calves in the veterinary hospital with diarrhea.

Almost all isolates possessed the stx gene, and 75% of the positive strains carried both stx1 and stx2 genes. Stx production in the isolates was confirmed by RPLA test although there were a few inconsistencies (Table 3). Of 30 isolates possessing both stx1 and stx2 genes, 7 tested negative for...
It was revealed that the isolation rate of EHEC O157 was 13.0% and the organisms were widely distributed in bovine faeces. High prevalences of EHEC O157 in bovine faeces, 27.8% in the United States [7], 19.7% in the United Kingdom [2] and 16.1% in the Czech Republic [4], have been shown previously. In Japan, it was previously reported that the isolation rate of EHEC O157 was 0.3% in faecal samples from slaughterhouse cattle in 1987 [11], 1.4% in faeces and 0.3% of dressed carcasses in 1996 [22], and 6.5% in faeces in 1999 [15].

Kobayashi et al. [14] reported that the EHEC infection rate in fecal samples from calves, heifers, and cows on farms were 46%, 66% and 69%, respectively. Among the isolates, however, only one EHEC O157 strain was found. In the present study, the isolation rate of EHEC O157 from faeces was remarkably high in comparison with previously reported results. The findings in the present study suggest that the prevalence of the organisms is dependent on the area or farm, and certain farms were heavily contaminated by the organisms, but the number of farms examined in this study was limited. Therefore, it is necessary to carry out a more widespread bacterial survey of farms in different areas.

The results of bacterial isolation are influenced by the isolation method. In the present study, about 10 g of faeces was inoculated into the enrichment broth for the isolation of EHEC. Perhaps the culture method using this somewhat large amount of faeces in the culture broth was one of the factors in the high isolation rate achieved in the organisms. Inconsistencies were observed in detection results for stx gene by PCR and Stx by RPLA test. Although strains possess both stx1 and stx2 genes, some strains were negative for either Stx1 or Stx2 in the RPLA test.

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A remarkably high isolation rate was shown on T Farm, which raised heifers only. There were many opportunities for the heifers to come into contact with each other in padd...

<table>
<thead>
<tr>
<th>Region</th>
<th>Farm</th>
<th>Kind of farm</th>
<th>No. of samples</th>
<th>No. of positives</th>
<th>Rate (%)</th>
<th>No. of positive faeces from</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>calf heifer cow</td>
</tr>
<tr>
<td>Honshu</td>
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<td>Beef</td>
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<td>6</td>
<td>27.2</td>
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<tr>
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<td>R</td>
<td>Dairy</td>
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<td>5</td>
<td>18.5</td>
<td>5/27</td>
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<tr>
<td></td>
<td>T</td>
<td>Dairy</td>
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<td>28</td>
<td>33.7</td>
<td>28/83</td>
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<tr>
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<td>Dairy VM</td>
<td>131</td>
<td>0</td>
<td>0/4</td>
<td>0/8 0/119</td>
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<tr>
<td></td>
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<td>44</td>
<td>1</td>
<td>2.3</td>
<td>1/44</td>
</tr>
<tr>
<td></td>
<td>In hospital</td>
<td></td>
<td>17</td>
<td>2</td>
<td>11.8</td>
<td>2/7</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>324</td>
<td>42</td>
<td>13.0</td>
<td>2/11 34/113 6/200</td>
</tr>
</tbody>
</table>

a) Veterinary hospital.

b) No. of positive/No. of examined.

| Table 3. Detection of stx gene by PCR and Stx by RPLA test from EHEC O157 isolated from bovine faeces |
|---------------------|---------------------|---------------------|
| **PCR**             | **RPLA**            |
| stx gene + or –     | Stx + or –          |
| stx1 stx2 stx1+stx2 | Stx1 Stx2 Stx1+Stx2 |
| 1 (2.5) 9 (22.5) 30 (75.0) | 3 (7.5) 14 (35.0) 23 (57.5) |
The high prevalence of EHEC O157 from cattle docks and pastures. Heifers harbouring the EHEC O157 directly contaminated other heifers. They also contaminated the environment, and non-infected heifers might easily be infected via the environment. Thus, the infectious cycle of the organisms resulted in the high isolation rate on the T Farm.

Excretion of EHEC O157 from the faeces of cattle was intermittent and cattle excreting the organisms long term were rare. Therefore, it is necessary to carry out long-term observation. To clarify whether only T Farm was heavily contaminated or not, it is necessary to carry out a bacterial survey of farms of a similar type to T Farm.

REFERENCES