Differentiation of *Yersinia enterocolitica* Serotype O:5,27 Strains by Phenotypic and Molecular Techniques

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Restriction endonuclease analyses of virulence plasmid DNA (REAP) and chromosomal DNA and other phenotypic characteristics were used to study the differentiation of *Yersinia enterocolitica* serotype O:5,27 strains. There was a close correlation between REAP patterns and the geographical distribution of serotype O:5,27. Human isolates produced only one REAP pattern, which was also found with isolates from pigs and dogs.

*Yersinia enterocolitica* serotype O:5,27 is considered to be an etiologic agent of human infection worldwide (1), with a wide distribution in domestic animals and pets (5). The first outbreaks caused by *Y. enterocolitica* serotype O:5,27 occurred among two adult groups in Mie Prefecture of Japan in 1988 and 1989 (8). However, the sources of infection in these cases were not determined.

There are reports of several techniques to differentiate *Y. enterocolitica* strains (7, 9); restriction endonuclease analyses of virulence plasmid DNA (REAP) and chromosomal DNA (REAC) were used in addition to traditional phenotypic characteristics such as biochemical properties, O and H antigens, antibiotic susceptibility, and bacteriophage lysis patterns. Examinations of serotype O:5,27 isolates have been limited to isolates from a few sources.

We examined strains of serotype O:5,27 by biotyping, serotyping, phage typing, REAP, and REAC of 138 field isolates from 24 humans, 1 hamburger, 3 raw pork samples, 97 pigs, 7 dogs, and 6 domesticated cattle in Japan, the United States, Canada, Australia, The Netherlands, and Germany (Table 1). Biotyping and serotyping were performed at the Institute of Hygiene, National Reference Center for Enteritis Pathogens, Hamburg, Germany, by methods described elsewhere (1). Phage typing was carried out at Tottori University, Tottori, Japan, by the method of Nicolle (10). Plasmid DNA was detected by the method of Kado and Liu (6). REAP was performed with the enzymes *BamHI* and *EcoRI* as described by Nesbakken et al. (9), with 0.7% agarose gel electrophoresis. In the REAC experiments, plasmid-cured bacteria were obtained by repeated cultivation on magnesium oxalate agar (4) at 37°C. Chromosomal DNA was extracted by the method of Owen and Borman (11), and REAC was done with enzyme *HaeIII* by the method of Kapperud et al. (7) and subjected to vertical polyacrylamide gel (0.75 mm thick) electrophoresis for 19 h at 100 V.

Serotype O:5,27 isolates belonged to H-antigen groups abc, abcv, bc, v, and nonmotile; to biotypes 2 and 3 (biotype 2 strains are indole positive, and biotype 3 strains are indole negative); and to phage type X (Table 1). Plasmid profiles were done for all strains. One hundred twenty-three strains contained a single 40- to 50-MDa *Yersinia* virulence plasmid, but 15 strains lacked the *Yersinia* virulence plasmid (Table 1). REAP revealed three distinct restriction patterns of plasmid DNA among the 123 strains examined (Fig. 1 and Table 1). REAP patterns I and II are the same as the REAP pattern reported by Kapperud et al. (7) and Nesbakken et al. (9). REAP pattern III was observed for the first time in the present study. Strains producing each of the three REAP patterns have a single REAC pattern (Fig. 2).

REAC, REAP, and analysis of other phenotypic characteristics revealed a close correlation between the REAP pattern and the geographical distribution of serotype O:5,27. REAP pattern I was produced by isolates from pigs, cattle, and dogs only in Japan. REAP pattern II was produced by isolates from each of the six countries represented. REAP pattern III was produced by isolates from pigs in Japan and the United States and from raw pork samples imported from the United States and Taiwan (unpublished data). Human isolates produced only REAP pattern II, which was also produced by samples from pigs.

Epidemiological investigations of *Y. enterocolitica* have focused mainly on findings with pigs as a source of infection with serotype O:3. Serotype O:5,27 has been isolated from pigs all over the world (3, 5). Asakawa et al. (2) and our group (unpublished data) isolated serotype O:5,27 from raw pork. We found little documentation on the isolation of serotype O:5,27 from other species of animals. In the present study, we obtained six isolates of serotype O:5,27 from cattle, seven from dogs in Japan, and one from hamburger in The Netherlands. Three isolates from dogs and the isolate from the hamburger had the same REAP pattern II as that seen with human isolates. Although these observations suggest the possibility that pigs and dogs are the sources of serotype O:5,27 infections in humans, the causal relationships between humans and animals such as pigs and dogs should be clarified in the future with careful epidemiological studies.

REAP pattern I isolates from pigs on one pig farm in Shimane Prefecture, Japan (3), were classified into two biotypes and three H-antigen groups (Table 1). It is not clear whether this means that different clones are involved or whether a single clone is changing in its phenotypic properties. The same may be true for serotype O:3 (7). Thus, it was
TABLE 1. Phenotypic and genotypic characterization of *Y. enterocolitica* O:5,27 strains*<sup>a</sup>

<table>
<thead>
<tr>
<th>REAP pattern</th>
<th>H-antigen type</th>
<th>Biotype</th>
<th>Source</th>
<th>Total no. of isolates</th>
<th>No. of isolates from:</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Japan</td>
</tr>
<tr>
<td>I</td>
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</tr>
<tr>
<td></td>
<td>3</td>
<td>Pigs</td>
<td>15</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>abcv</td>
<td>2</td>
<td>Pig</td>
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</tr>
<tr>
<td></td>
<td>v</td>
<td>2</td>
<td>Pig</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>_b</td>
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<td>Pig</td>
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<td>1</td>
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<tr>
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</tr>
<tr>
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<td>138</td>
<td>97</td>
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</table>

*The strains (except our own 41 isolates) were received from the following investigators: Y. Hakozaki, S. Kaneko, T. Yajima, N. Sakurai, K. Shiozawa, M. P. Doyle, A. Borczyk, D. A. Shiemann, R. M. Robins-Browne, E. de Boer, and S. Aleksic.*

*<sup>b</sup> nonmotile.

*<sup>a</sup>* shown that currently it is difficult to interpret the meaning of the differences among the different typing results, and this may be clarified in the future with careful epidemiological studies. The H antigens are monophasic and remain stable.
after repeated subcultivation of the isolates and after the storage of the isolates as stab cultures (1). H-antigen typing is not commercially available but did allow for differentiation of serotype O:5,27 strains which could not be distinguished by any other typing methods used. Therefore, the combination of REAP, biotyping, and H-antigen typing is optimal for the differentiation of serotype O:5,27 strains.

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REFERENCES


