Characterization of Campylobacter lanienae from Pig Feces

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ABSTRACT. To isolate Campylobacter spp., the feces of healthy cattle, pigs, and broilers were examined between June 1999 and January 2000. Campylobacter lanienae strains were isolated from the feces of healthy pigs, but not from the feces of cattle or broilers. In six C. lanienae isolates, there was only 21–38% DNA-DNA homology to Campylobacter hyointestinalis subsp. lawsonii strain NCTC12901. Thus, the primary host of C. lanienae is likely to be the pig and C. lanienae appears to be a species distinct from C. hyointestinalis subsp. lawsonii. In addition, an intervening sequence of 226 bp in the 16S rRNA gene was found in four isolates.

NOTE. Campylobacter lanienae is a new bacterial species that was first reported in 2000 [8]. In that study, C. lanienae was isolated from the feces of healthy abattoir workers in Switzerland. These abattoir workers were mainly exposed to cattle and pig carcasses. Therefore, the primary host of C. lanienae was thought to be cattle and/or pigs. However, an investigation by the Swiss National Reference Laboratory for Foodborne Diseases failed to produce an isolate of C. lanienae from animal sources (cattle, pigs, dogs, cats, sheep, poultry and mice). Therefore, the primary host of this organism was not clarified. In addition, On [10] questioned whether C. lanienae is a distinct species from Campylobacter hyointestinalis subsp. lawsonii or a C. hyointestinalis subspecies, because C. lanienae shares 16S RNA gene sequence similarities of 97.2–97.7% with C. hyointestinalis subsp. lawsonii. Thus, further analysis is clearly necessary to characterize C. lanienae in detail.

We attempted to isolate campylobacter from the feces of healthy cattle, pigs and broilers between June 1999 and January 2000. Fecal samples were provided by Livestock Hygiene Service Centers across Japan and were transported to our laboratory in Seedswab No. 2 (EIKEN KIZAI, Japan) under cool conditions. The feces were inoculated onto Campylobacter blood-free selective agar (Oxoid, U.K.) and incubated for 48 hr at 37°C in a micro-aerobic atmosphere of 85% N₂, 10% CO₂ and 5% O₂. Colonies were picked from each plate and then cultured several times on Mueller-Hinton agar (Oxoid, U.K.) with 5% defibrinated horse blood. Identification was based on colony morphology, Gram staining, oxidase, catalase, indoxyl-acetate hydrolysis, and hippurate hydrolysis tests, susceptibility to cephalothin (CET) (30 μg) and nalidixic acid (NA)(30 μg), growth at 25 and 43°C and H2S production in triple sugar iron (TSI) medium (EIKEN CHEMICAL, Japan) [1, 4, 9]. In addition, isolates were examined by three polymerase chain reaction (PCR) identification methods to differentiate Campylobacter jejuni, Campylobacter coli and Campylobacter lari [2, 7]. Using the serial approach, we obtained 166 isolates from the feces of 183 healthy cattle, 180 pigs and 156 broilers. One hundred nine, fifty and one isolates were identified as C. jejuni, C. coli and C. lari, respectively. However, the remaining six isolates (FK171, FK172, FK173, FK174, FK175 and FK176) from 4 pigs could not be identified because the six isolates had unknown restriction banding patterns after digestion with AluI which was used to differentiate C. lari from Campylobacter upsaliensis in a PCR identification method based on the 23S rRNA gene [2]. Therefore, the six isolates were analyzed by several additional standard phenotypic tests [nitrate reduction and growth in 0.04% triphenyltetrazolium chloride (TTC), 1% glycine, 1.5% NaCl and 3.5% NaCl] [4, 9] and 16S rRNA gene sequence analysis. The phenotypic characteristics of the six isolates are shown in Table 1. The phenotypic characteristics of each isolate and C. lanienae NCTC13004T were completely identical. The 16S rRNA genes of the six isolates were amplified by PCR and their DNA sequences were determined. For PCR, primers 16SUNI-L and UNI16S-R [6] were used to amplify an expected 1500 bp region of the 16S rRNA gene sequences. The amplified products were separated by electrophoresis in 1.0% (w/v) agarose gel and stained with ethidium bromide. Although two strains, FK171 and FK176, yielded an expected band of 1,500 bp, four strains FK172, FK173, FK174 and FK175 produced a band of 1,700 bp (data not shown). These strains were thought to have an intervening sequence (IVS) of 200 bp in the 16S rRNA genes. The amplified products were recovered from agarose gels and were sequenced directly using an ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystems, U.S.A.). Automated DNA sequencing of sample DNA was performed on a DNA sequencer (model 373A, PE Biosystems, U.S.A.). All sequences were confirmed by sequencing both strands. FK171 differed from FK176 in 9 out of 1467 nucleotides (0.6%) and had the most similarity to C. lanienae NCTC13004T. The 16S rRNA gene sequences of FK172, FK173, FK174 and FK175 had high similarity to C. lanienae...
nae NCTC13004T (99.4%) and FK171 (99.6%), but they also harbored an IVS of 226 bp. This IVS had greatest similarity with an IVS in the 16S rRNA gene of C. sputorum BU112B (72.7%) (accession no. AF022768). In order to analyze the phylogenicity of the isolates in this study, their 16S rRNA sequences (with IVS removed) were compared with GenBank data from representative Campylobacter species using the CLUSTAL W program [12]. The evolutionary distance matrix of the aligned sequences was calculated using the two-parameter model for multiple substitutions [5]. The tree was constructed from the distance matrix by the neighbor-joining method [11]. The reliability of the tree topology was estimated by the boot-strap method [3]. FK171, FK176 and FK172 clustered with C. lanienae strains (Fig. 1). The six isolates were identified as C. lanienae by the phenotypic characteristics and the 16S rRNA gene sequence analysis. Isolates FK172 and FK173 and similarly FK175 and FK176 were isolated from two pigs, respectively. The four pigs harboring the isolates were bred and housed on separate farms.

In this study, six C. lanienae strains shared 16S rRNA gene sequence similarities of 96.9–97.3% with C. hyointestinalis subsp. lawsonii. It is inadequate to determine if taxa represent the same or distinct species. Therefore, DNA-DNA hybridization experiments were used to clarify the taxonomic position of C. lanienae. Assay of DNA similarity by colorimetric hybridization in microdilution wells was performed using the procedure of Yaeshima et al. [13]. Hybridization was carried out at 29°C. C. lanienae NCTC13004T had 95–110% DNA-DNA homology to FK171, FK172 and FK176. On the other hand, C. lanienae strains had 21–38% DNA-DNA homology to C. hyointestinalis subsp. lawsonii NCTC12901T (Table 2). Thus, C. lanienae appears to be a species distinct from C. hyointestinalis subsp. lawsonii.

C. lanienae was isolated from the feces of healthy abattoir workers, who were mainly exposed to cattle and pig carcasses in Switzerland [8]. In this study, we have shown that C. lanienae can be isolated from the feces of healthy pigs in Japan. The primary host of this strain may be the pig.

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Table 2. DNA-DNA hybridization results for \textit{C. lanienae} isolates

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Strain</th>
<th>NCTC13004&lt;sup&gt;T&lt;/sup&gt;</th>
<th>FK171</th>
<th>FK172</th>
<th>FK176</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{C. lari}</td>
<td>ATCC35221&lt;sup&gt;T&lt;/sup&gt;</td>
<td>24</td>
<td>15</td>
<td>14</td>
<td>4</td>
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<tr>
<td>\textit{C. hyointestinalis subsp. lawsonii}</td>
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<td>35</td>
<td>21</td>
<td>38</td>
</tr>
<tr>
<td>\textit{C. lanienae}</td>
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<td>95</td>
<td>110</td>
<td>104</td>
</tr>
<tr>
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<td></td>
</tr>
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</tr>
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<td>105</td>
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REFERENCES