Plasmid Profiles of *Salmonella typhimurium* var. *copenhagen* Strains Isolated from Horses

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Twelve strains of *Salmonella typhimurium* var. *copenhagen* were isolated from diseased or dead 1 to 2-year-old Thoroughbred foals on 10 farms in the Hidaka district of Hokkaido, Japan, in 1983. They showed 5 resistance patterns against 7 antibiotics and were classified into biovars of 26e (11 strains) and 26eh (1 strain) in the scheme of Duguid et al. (1975). They revealed, however, a uniform plasmid profile (3.8, 5, or 58 Mdaltons in molecular weight) in agarose gel electrophoresis.

Key words. *Salmonella*, plasmid profile, equine salmonellosis

Plasmid profiles identified by agarose gel electrophoresis have been recently used as an epidemiological marker in human and animal salmonellosis.1-5) Plasmid profile analysis is a valuable tool in epidemiological investigations of the disease1,2) and estimated to be at least as specific as phage typing in the classification of *Salmonella typhimurium*.6)

Since an outbreak of *S. typhimurium* infection among horses on a farm in eastern Hokkaido of Japan in 1976,7) enzootic outbreak of the infection occurred among horses in different districts of Hokkaido.8-11) Especially in the Hidaka district where many Thoroughbred farms are located, salmonellosis prevailed mainly among foals in 1981,9,11) and successively in 1982,10,11) Moreover, during a period from March to October in 1983, the disease due to *S. typhimurium* occurred newly on 9 farms and again on 1 farm, which suffered from it in the previous year, as shown in Table 1.

Biotyping, phage typing9,11) and antibiotic sensitivity testing7-11) have been used for epizootiological studies on *S. typhimurium* infections among horses in Japan, but plasmid profile analysis has not. The prevalence of *S. typhimurium* var. *copenhagen* of biovar 26bi and phagovar 1/19/23/29/31/36/48 was indicated among horses in the Hidaka district in 1981,9,11) To obtain more information on the epizootiology of *S. typhimurium* infection among horses in the district, plasmid profile analysis was applied to *S. typhimurium* strains isolated in 1983. The results obtained were compared with antibiotic resistance patterns and biovars.

Twelve strains of *S. typhimurium* were used for this study, as shown in Table 1.
Plasmid DNA was isolated from them by the method of Kado and Liu with some modifications. Five ml of overnight Penassay broth (Difco) culture of bacteria was centrifuged at 7000 rpm for 7 min. The resulting cell pellet was thoroughly suspended in 0.2ml of E buffer (0.04mol/l Tris-acetate and 0.002mol/l sodium EDTA, pH 7.9) and added to 0.4ml of a lysing solution (3% sodium dodecyl sulfate and 0.05mol/l Tris-HCl, pH 13.0). The resulting suspension was heated at 55°C for 30 min. Then the lysate was extracted with 2 volumes (1.2 ml) of phenol-chloroform solution (1:1 vol/vol).

The upper aqueous phase containing plasmid DNA was precipitated with 2.5 volumes of 99% ethanol and 1/10 volume of 3 mol/l sodium acetate at −80°C for 30 min. After centrifugation at 15000 rpm for 5 min, DNA precipitate was resuspended in 30 µl of TE buffer (0.01 mol/l Tris-HCl and 0.001 mol/l sodium EDTA, pH 8.0) and used for agarose gel electrophoresis. To obtain more purified plasmid DNA, another preparation method essentially the same as described by Ishiguro et al. was used. Bacterial cells collected from 400 ml of overnight L-broth culture were suspended in 10 ml of 25% sucrose in 0.05 mol/l Tris-HCl, pH 8.0). The resulting suspension was heated at 55°C for 30 min. Then the lysate was extracted with 2 volumes (1.2 ml) of phenol-chloroform solution (1:1 vol/vol).

The upper aqueous phase containing plasmid DNA was precipitated with 2.5 volumes of 99% ethanol and 1/10 volume of 3 mol/l sodium acetate at −80°C for 30 min. After centrifugation at 15000 rpm for 5 min, DNA precipitate was resuspended in 30 µl of TE buffer (0.01 mol/l Tris-HCl and 0.001 mol/l sodium EDTA, pH 8.0) and used for agarose gel electrophoresis. To obtain more purified plasmid DNA, another preparation method essentially the same as described by Ishiguro et al. was used. Bacterial cells collected from 400 ml of overnight L-broth culture were suspended in 10 ml of 25% sucrose in 0.05 mol/l Tris-HCl, pH 8.0). The resulting suspension was heated at 55°C for 30 min. Then the lysate was extracted with 2 volumes (1.2 ml) of phenol-chloroform solution (1:1 vol/vol).

Table 1. Antibiotic resistance patterns and biovars of S. typhimurium var. copenhagen strains isolated from horses in the Hidaka district in 1983

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Farm</th>
<th>Source</th>
<th>Antibiotic resistance to*</th>
<th>Biovar**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ap</td>
<td>Sm</td>
</tr>
<tr>
<td>1</td>
<td>A</td>
<td>Intestinal contents (Dead, 2 years old)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>Intestinal contents (Dead, 2 years old)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>B</td>
<td>Feces (Normal, foal)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>C</td>
<td>Intestinal contents (Dead, 2 years old)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>D</td>
<td>Feces (Diseased, foal)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>E</td>
<td>Lungs (Dead, foal)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>Feces (Diseased, foal)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>G</td>
<td>Feces (Diseased, foal)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>H</td>
<td>Feces (Diseased, foal)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>I</td>
<td>Feces (Diseased, foal)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>J***</td>
<td>Intestines (Dead, foal)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>J***</td>
<td>Feces (Stall floor)</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Remarks. * Ap-ampicillin, Sm-streptomycin, Su-sulfadimethoxine, Tc-tetracycline, Km-kanamycin, Na-nalidixic acid, and Cl-colistin. All the strains were sensitive to chloramphenicol, gentamicin, furatrizine, rifampin, and trimethoprim. ** Biotyped by the method of Duguid et al. Equine salmonellosis occurred also in 1982.
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Remarks. 1: strain No. 5 in Table 1, 2: strain No. 7, 3: strain No. 12, Chr: chromosomal band, OC: open circular DNA band, linear: linear DNA band, Md: Mdaltons.

Biotyping and antibiotic sensitivity testing of S. typhimurium were made by the method of Duguid et al.\textsuperscript{15} and that of Makino et al.,\textsuperscript{16} respectively. Antibiotics and their concentrations used are as follows: ampicillin (Ap), 25 μg/ml; streptomycin (Sm), 12.5; sulfadimethoxine (Su), 800; tetracycline (Tc), 25; kanamycin (Km), 25; nalidixic acid (Na), 25; chloramphenicol, 25; furatridine, 6.3; rifampin, 25; gentamicin, 12.5; trimethoprim, 25; and colistin (Cl), 12.5 U/ml.

Table 1 shows 12 S. typhimurium strains from 10 farms revealed 5 patterns of antibiotic resistance (ApSmSuTcNa, 5 strains; ApSmSuTc, 3; ApSmSuTcKm, 2; ApSmSuTcKmCl, 1; and ApSuNa, 1) and a uniform primary biovar (26) in Duguid’s scheme with 2 subbiovars (e, 11 strains; and eh, 1 strain). Gel electrophoresis of partially purified plasmid DNAs from the 12 strains indicated similar plasmid profiles, irrespective of the differences in resistance pattern and biovar. Three plasmid bands were observed in all the strains tested (No figures are presented). Gel electrophoresis of purified plasmid DNAs showed 3 plasmids 3.8, 5 and 58 Mdaltons in molecular weight, respectively, in 3 strains (Fig. 1).

Yataya et al.\textsuperscript{5} applied plasmid profile analysis to an epidemiological study on S. typhimurium infection in calves and a human infant on a farm. They indicated that S. typhimurium strains from the infant and calves carried 2 kind of plasmid DNAs (98 and 5 Mdaltons) in the same cell, and that conjugative R plasmids (98 Mdaltons) detected were indistinguishable in the restriction enzyme cleavage pattern, suggesting that the R plasmids might be of common ancestry. Therefore, they concluded that S. typhimurium had been transmitted from the calves to the infant.

The results obtained from the present study indicate that the enzootic occurrence of equine salmonellosis in the Hidaka district in 1983 must be caused by a par-
ticular strains of *S. typhimurium*. For further
characterization of the plasmids detected
here it is needed to determine whether the
uniform plasmid profiles identified are
specific to the equine strains isolated in the
Hidaka district in 1983.

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要約

馬由来 Salmonella typhimurium var. copenhagen 株のプラスミド保有像 (plasmid profile)
（短報）：佐藤義平*・中岡祐司*・石黒直隆*・大石秀夫**・仙波裕之**・加藤秀樹***・本間哲二***
長瀬 井***（*帯広畜産大学獣医公衆衛生学講座 **日高地区農業共済組合 ***北海道日高家畜保健
衛生所）——1983年に北海道日高地方の10牧場において、サルモネラ症に罹患して回復または観死し
た1〜2歳のサラブレッド仔馬から分離された12株の S. typhimurium はすべてコペンハーゲン型で,
7 種の抗菌剤に対して 5 種の耐性パターンを示し、Duguid ら (1975) の方法で11株は 26e, 1株は
26 eh の生物型に別れた。しかし、これら12株は1様な3種のプラスミド（58, 5, 3.8メガダルトン）
を保有していた。