Isolation of *Campylobacter sputorum* ssp. *mucosalis* from Proliferative Hemorrhagic Enteropathy in Pig

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(Received: November 13, 1981)

*Campylobacter sputorum* ssp. *mucosalis*, a causative agent of proliferative hemorrhagic enteropathy in pigs, was first described and named by Lawson & Rowland [5]. Although outbreaks of this disease were observed in the United States [1, 2], the United Kingdom [5, 8, 9], Australia [7], Sweden [3] and Japan [4], only a few researchers reported the isolation of *C. sputorum* ssp. *mucosalis* [3, 5, 7].

This communication concerns the isolation and identification of *C. sputorum* ssp. *mucosalis* from a pig which had proliferative lesions in the ileum.

We made an attempt to isolate *C. sputorum* ssp. *mucosalis* from the ileal mucosa with proliferative lesions of a pig slaughtered at a prefectural abattoir in Ibaraki in October, 1981. The thickened and wrinkled mucosa of the affected ileum was washed repeatedly with Trypticase soy broth (TSB, BBL) to remove the intestinal contents. One gram of the mucosa was cut off with scissors, emulsified in two volumes of TSB with a glass-mortar, and then serial 10-fold dilutions were prepared in TSB. One-tenth ml of each dilution was inoculated onto Lawson & Rowland’s medium [5]. This medium was composed of Blood agar base No. 2 (Oxoid), 5% horse blood, novobiocin (Upjohn) at 5 μg/ml, and brilliant green (Merck) at 1:60,000. The inoculated plates were introduced into an anaerobic jar together with Gas Pak (BBL) without catalyst. Then, the anaerobic jar was evacuated to a negative pressure of 650 mmHg. The jar was placed in an incubator at 37°C for 3 days. Biochemical and serological examinations of the isolates were made by the method reported by Lawson et al. [6].

As a result, *C. sputorum* ssp. *mucosalis* was isolated from the affected mucosa and the viable count was in the order of $2.3 \times 10^7$ CFU per gram. Colonies on Lawson & Rowland’s medium were about 1.5 mm in diameter, round, umbonate, light yellowish, and composed of Gram-negative, short and irregularly curved rods. Electron microscopic observation of the isolate demon-
Table 1. Biochemical and serological properties of five isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Catalase</th>
<th>Oxidase</th>
<th>Nitrate reduction</th>
<th>H₂S in TSI</th>
<th>Growth in the presence of Glycine 1.0%</th>
<th>NaCl 1.5%</th>
<th>NaCl 3.0%</th>
<th>SDC 0.05%</th>
<th>SDC 0.2%</th>
<th>Agglutination titer against anti-253/72</th>
</tr>
</thead>
<tbody>
<tr>
<td>NKS-1</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>1:2,560</td>
</tr>
<tr>
<td>NKS-2</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>1:2,560</td>
</tr>
<tr>
<td>NKS-3</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>1:2,560</td>
</tr>
<tr>
<td>NKS-4</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>1:2,560</td>
</tr>
<tr>
<td>NKS-5</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>1:2,560</td>
</tr>
</tbody>
</table>

- : Negative reaction, + : Positive reaction.
TSI: Triple Sugar Iron Agar (Eiken), SDC: Sodium deoxycholate.

Striated monotricous flagella longer than the bacterial cells. The biochemical and serological properties of five isolates corresponded well with the description by Lawson et al. [6] (Table 1). The organisms were catalase negative, produced oxidase and H₂S in TSI medium (Eiken), and reduced nitrate to nitrite. Good growth occurred on the medium containing 1.5% NaCl and that containing 0.05% sodium deoxycholate (SDC, Merck) in 48 hr of incubation, but no growth on the medium containing 1.0% glycine or 3.0% NaCl. Colonies on the medium containing 0.2% SDC appeared usually in 72 hr of incubation but not in 48 hr. In tube agglutination tests, the five isolates were agglutinated with the antiserum against strain 253/72, kindly supplied by Dr. G. H. K. Lawson, the Department of Veterinary Pathology, Royal School of Veterinary Studies, Edinburgh, Scotland. The agglutination titers were 1 : 2,560 in accord with that with the Lawson’s strain 253/72.

Histopathologically, the mucosa of the ileum was thickened by irregular hyperplastic crypts. Many crypts contained cellular debris and neutrophils. A large number of lymphocytes and plasma cells and a small number of neutrophils and eosinophils infiltrated the lamina propria. A large number of rod-shaped organisms were seen in the apical part of the cytoplasm of the hyperplastic epithelial cells stained by the Warthin-Starry method.

From the above-mentioned results, the pig was diagnosed as proliferative hemorrhagic enteropathy, and the five strains isolated from it were identified as C. *sputorum* ssp. *mucosalis*. This is the first report of isolation of *C. sputorum* ssp. *mucosalis* from proliferative hemorrhagic enteropathy in the pig in Japan.

Acknowledgments. The authors wish to thank Dr. G. H. K. Lawson, the Department of Veterinary Pathology, Royal School of Veterinary Studies, Edinburgh, Scotland, for supplying the antiserum against *C. sputorum* ssp. *mucosalis*.

References

要約
豚の増殖性出血性腸炎からの *Campylobacter sputorum* ssp. *mucosalis* の分離（短報）：中沢宗生・久保正法・杉本千尋・伊佐山康郎（家畜衛生試験場），萱池善彦（愛知家畜保健衛生所）＝愛知県下の場で得た1例の増殖性出血性変化を伴う豚の回腸粘膜から *Campylobacter sputorum* ssp. *mucosalis* を我國で初めて分離した。分離菌数は病変部粘膜1g当り2.3×10⁷ CFUであった。そのコロニーは直径1.5mm，正円で中央が隆起し薄い黄色を呈していた。分離株の主要な性状はカトラジー陰性，TSI培地で硫化水素陽性で，1.0％グリシンおよび3.0％塩化ナトリウム添加培地では発育しないが，1.5％塩化ナトリウムおよび0.05％SDC添加培地には発育し得た。ネガティブ染色法による電顕観察では極単毛の駒毛が認められた。また，分離株は本菌の抗血清に対して凝集価1:2,560を示した。以上の成績から分離株を *C. sputorum* ssp. *mucosalis* と同定した。