The Study on the Pathogenicity of *Nocardia asteroides* to Largemouth Bass *Micropterus salmoides* Lacepede

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The largemouth bass *Micropterus salmoides* Lacepede was given an intramuscular (IM) or intraperitoneal (IP) injection of *Nocardia asteroides* isolated from a diseased cultured largemouth bass, and typical granulomatous lesions similar to those of the natural infection were induced. The experimental fish showed 100% mortality 14 or 21 days after IP or IM injection of 8 mg of bacteria, and 28 days after IM injection of 0.8 mg of bacteria. The bacterium could be easily reisolated from the liver and kidney of dead fish. Many typical focal granulomata developed at the site of injection and in organs such as liver, spleen, kidney, eye, brain, pancreas, gill, stomach, intestine, and ovary. The result shows that the bacterium is clearly pathogenic to the largemouth bass.

Nocardiosis of fish is a systemic infection, and the lesions are localized in both the skin and internal organs with the nodular structure of typical granuloma. The first case of a fish infection caused by *Nocardia asteroides* was described and identified by Valdez and Conroy in 1963. Several species of fish such as rainbow trout, *Salmon gairdneri* (Snieszko et al., 1964), Formosa snakehead fish *Channa maculata* Lacepede (Hsu et al., 1977; Chen et al., 1989) have also been found to be affected by *N. asteroides* or *N. sp*. Snieszko et al. (1964) indicated that the fingerling or yearling trouts could be infected by injection of *N. asteroides*. The lesions occurred between 1 and 3 months after injection. Nocardiosis of largemouth bass was first described by Chen and Tung (1991) in southern Taiwan. It caused a great commercial loss in this species. Attempts were made to transmit the disease by the inoculations of *N. asteroides* isolated from diseased fish and to study the pathogenicity of the bacterium.

**Materials and Methods**

A total of 140 healthy, 22–25 g in body weight, or 10–13 cm in body length, cultured largemouth bass *Micropterus salmoides* Lacepede were purchased for the experimental study. The fish were kept in fresh water at 24–28°C and sanitized in 3 ppm furatadone for 24 h then 1 ppm benzalkonium chloride for another 24 h. They were fed commercial diet at a one day interval and fasted one day before and during the experiment. They were equally divided into 4 treatments and kept in separated tanks. *Nocardia asteroides* used in this study was originally isolated from the largemouth bass during an outbreak of nocardiosis in Pingtung, Taiwan in 1989. The bacterium grown on brain heart infusion (BHI) agar was used to make the bacterial suspensions of 1.6 mg/ml (7×10^8 CFU/ml) and 16 mg/ml (7×10^9 CFU/ml) in 0.85% NaCl solution. A 0.5 ml of each bacterial suspension was inoculated intramuscularly (IM) on the left side near the dorsal fin to the fish of two groups, respectively. Another 0.5 ml of a high concentration of bacterial suspension (16 mg/ml) was injected intraperitoneally (IP) to the 3rd group of fish. Saline solution without bacterial cells was administered intramuscularly to the fourth group as the control. Two fish from each group were sacrificed on 3, 7, 14, 21, 28 days postinoculation for bacterial isolation and
histopathological examination. Reisolation of the bacterium was attempted from the lesions of the kidney and liver by streaking on BHI agar with a loop. The inoculated media were incubated at 25°C and observed for bacterial growth 10–14 days thereafter.

The muscle at the site of injection, gills, kidney, heart, liver, spleen and other internal organs with lesions were fixed in buffered neutral formalin solution, and processed for paraffin sections. The sections were stained with haematoxylin and eosin, Giemsa and Ziehl-Neelsen’s method before being examined microscopically.

**Results and Discussion**

The fish showed 100% mortality 14 or 21 days after intraperitoneal or intramuscular inoculation with 8 mg of bacteria. Even the fish given a low concentration of bacterial suspension (1.6 mg/ml) also showed 100% mortality 28 days postinoculation (Table 1). All the infected fish showed the signs of random swimming, circling or floating upside down some hours before the death.

Almost all the fish inoculated with *N. asteroides* exhibited the distension of abdomen with numerous yellowish-white granulomata growing on the mesentery and visceral surfaces, and diffusely distributing in several internal organs especially in the kidney, spleen, heart, liver, and swim-bladder (Figs. 1 & 2). The lesions found in the IM group received a dose of 8 mg of bacteria were more intense and extensive. The gills and the muscles both

<table>
<thead>
<tr>
<th>Bacteria inoculated (mg)</th>
<th>Route of inoculation</th>
<th>No. of fish used</th>
<th>Cumulative mortality (%) Days after injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>IP</td>
<td>35</td>
<td>16  89  100</td>
</tr>
<tr>
<td>8</td>
<td>IM</td>
<td>35</td>
<td>8   71  86  100</td>
</tr>
<tr>
<td>0.8</td>
<td>IM</td>
<td>35</td>
<td>0   27  65  83  100</td>
</tr>
<tr>
<td>0</td>
<td>Control (IM)</td>
<td>35</td>
<td>0   0   0   0</td>
</tr>
</tbody>
</table>

* Healthy largemouth bass 10–13 cm in length were used.

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Table 1. Cumulative mortality (%) of largemouth bass* inoculated with *Nocardia asteroides*.

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**Fig. 1.** Marked yellowish-white granulomata, varying in size, seen in the liver, swim-bladder, mesentery, intestine and spleen, 7 days after an IM injection of 8 mg of bacteria.

**Fig. 2.** Marked yellowish-white granulomata (arrows) seen in the anterior and posterior kidney, 21 days after an IM injection of 0.8 mg of bacteria.
Pathogenicity of *Nocardia asteroides*

**Table 2.** The presence and severity of granulomata in the organs of largemouth bass inoculated with *N. asteroides*.

<table>
<thead>
<tr>
<th>Days after injection</th>
<th>Bacteria inoculated (mg)</th>
<th>Organs</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Muscle</td>
</tr>
<tr>
<td>IM</td>
<td>8</td>
<td>++</td>
</tr>
<tr>
<td>7</td>
<td>0.8</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>8</td>
<td>+++</td>
</tr>
<tr>
<td>21</td>
<td>0.8</td>
<td>++</td>
</tr>
<tr>
<td>28</td>
<td>0.8</td>
<td>+</td>
</tr>
<tr>
<td>IP</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>–</td>
</tr>
<tr>
<td>14</td>
<td>8</td>
<td>–</td>
</tr>
</tbody>
</table>


–: No granulomata were observed.

Fig. 3. Necrotizing focus with bacterial clumps and cellular debris seen in the heart, 5 days after an IM injection of 8 mg of bacteria. Giemsa. Scale bar=100 μm.

Fig. 4. Marked granuloma in the mesentery. Bacterial clumps in the center were encapsulated by epitheloid cells or the fibrous tissue 14 days after an IM injection of 8 mg of bacteria. Giemsa. Scale bar=100 μm.
in diameter containing reddish purulent materials. The development of granulomatous lesions in various organs of the experimental groups is shown in Table 2. The bacteria were reisolated from the liver and kidney of all dead and sacrificed fish. No bacteria were isolated from the liver and kidney of fish taken randomly prior to the experiment or from the control fish.

Histopathologically, in the early stage of infection, an exudative inflammatory reaction, consisting of an acute, serous, inflammatory exudative containing cellular and bacterial debris were found (Fig. 3). In the later stage the granulomatous lesions were seen extensively in the organs described above and such other organ as brain without predilection in distribution. The nodules consisted of necrotizing foci surrounded by some epitheloid cells and fibroblasts or fibrous encapsulation (Figs. 4 & 5). Long filamentous bacteria were usually observed by Giemsa stain and acid-fast stain in the lesions (Fig. 6). The remaining areas of the affected organs were usually normal in structure.

The experimental study indicated that *N. asteroides* isolated from a naturally infected fish was pathogenic to largemouth bass. The signs and lesions produced by experimental inoculation were similar to those found in the disease outbreaks of Formosa snakehead fish and largemouth bass (Chen et al., 1989; Chen and Tung, 1991). The result proves that *N. asteroides* is an pathogen and is responsible for a great loss in largemouth bass culture in Taiwan. Affected fish either swam in a rapid tail chasing motion or on their sides in a circular movement. Many trout had a distended abdomen, containing a compact mass of microorganisms (Snieszko et al., 1964). The organism was also seen in the brain capsule.
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of infected fish held some months in the laboratory. Our experimental result showed granulomatous lesions with bacterial clumps in the brain of largemouth bass. Hence affected fish showed nervous disorder with circular movement which were probably the result of brain damage.

Affected fish showed anorexia, emaciation, and distension of the mouth or abdomen with a compact granuloma growing from visceral surface and often diffuse granuloma of the mesentery (Richards and Roberts, 1978). The clinical signs observed in largemouth bass in the present experiment were similar to some of these symptoms. The hematopoietic organs such as spleen and anterior kidney were the first organs to be affected by IM injection, but marked diffuse lesions were also seen in the heart and mesentery. The ovary, pancreatic islets, liver, stomach, intestine and eye were also affected. *N. asteroides* therefore is a systemic infectious agent in largemouth bass (Chen and Tung, 1991).

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**References**


