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PATHOGENICITY AND HISTOPATHOLOGY OF AN UNUSUALLY INTENSE INFECTION OF WHITE GRUBS (Posthodiplostomum m. minimum) IN THE FATHEAD MINNOW (Pimephales promelas)

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Abstract: A massive infection of metacercariae, particularly of the white grub (Posthodiplostomum m. minimum), was found in fathead minnows (Pimephales promelas) taken in November, 1979 from a commercial baitfish farm in Missouri. More than 2000 metacercariae were found in one fish 6 cm long. Low-grade mortality was occurring, and histologic sections showed that an intensive inflammatory response had resulted from the infection. The body cavity also contained a large volume of ascitic fluid with many blood cells, of which 92% were leucocytes. Moderate numbers of metacercariae caused a lesser inflammatory response.

INTRODUCTION

A low-grade mortality of fathead minnows (Pimephales promelas) occurred in November, 1979 and continued for several weeks at a private fish farm in Missouri. The abdomens were greatly distended (Fig. 1), exophthalmia was evident, and rupture lacerations were observed in the abdominal walls of some fish. Fish examined at the Fish Farming Experimental Station Diagnostic Laboratory at Stuttgart, Arkansas were heavily infected with the white grub, Posthodiplostomum minimum minimum (McCallum, 1921) Hoffman, 1958, and lightly infected with four other trematodes: yellow grub, Clonostomum complanatum; a black grub (unidentified species); Tetraoyle sp.; and Diplostomum sp.

There is only one previous report of a natural infection of P. minimum as a significant cause of mortality in fish. That infection involved striped bass (Morone saxatilis) at the Edenton (North Carolina) National Fish Hatchery. The white grub generally is considered to be a non-lethal parasite, often found in almost all species of freshwater fish. However, experimental studies showed that infection by large numbers of white grub cercariae could kill fathead minnows in a relatively short time. Smitherman demonstrated reduced growth rate and increased mortality of bluegills (Lepomis macrochirus) infected with this parasite. A lethal, natural infection of the white grub and concomitant
histologic changes in the fathead minnow are described.

METHODS

Initially, a thorough check of five fish, 5.7 cm long, revealed the problem to be a massive white grub infection. A pepsin digest was then used on four fish to determine numbers and sites of the metacercarial parasites. Fish were divided into four portions: the viscera, including the pericaridal region; the eyes; the remainder of the head; and the remaining portion of muscle, skin, fins, etc. Each portion, and the free parasites, from these portions were put in the pepsin solution in separate containers with the exception of the eyes, which were dissected and examined with the aid of a stereo microscope. The location, species and number of each parasite was recorded. Five fish were preserved in Bouin's solution for 24 h then transferred to 50% and then 70% ethanol. Paraffin sections were cut at 5 μm and stained with hematoxylin and eosin (H&E) and Giemsa. Blood cell counts were made on hemal-stained smears of ascitic fluid.

RESULTS

The white grub was by far the most numerous of metacercaria found, occurring in the eye, head, viscera, and in the remaining portion (Table 1). At least one yellow grub was found in each of these areas. Diplostomulum sp. was found only in the eye, and Tetracotyle sp. only in the viscera; the unidentified black grubs (found beneath the skin of the remaining portion in one fish) were not seen in the digest. A nematode, Spiroxys sp., also was found in the viscera and in the muscle (Table 1).

Only the white grub was present in sufficient numbers to cause death; 2,316 were recovered from the viscera of a single fish (not one of the 4 digested). Most fish had rupture lacerations or scars on their abdomens, likely some metacercariae had been lost through these now healed lacerations.

TABLE 1. Parasites found in four fathead minnows from an epizootic in a Missouri fish farm, November, 1979.

<table>
<thead>
<tr>
<th>Location in host, and parasite</th>
<th>Fish no. and (in parentheses) total length (cm).</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Viscera</td>
<td></td>
</tr>
<tr>
<td>P. minimum</td>
<td>1,528</td>
</tr>
<tr>
<td>C. complanatum</td>
<td></td>
</tr>
<tr>
<td>Spiroxys sp.</td>
<td>2</td>
</tr>
<tr>
<td>Tetracotyle</td>
<td>2</td>
</tr>
<tr>
<td>Eyes</td>
<td></td>
</tr>
<tr>
<td>P. minimum</td>
<td></td>
</tr>
<tr>
<td>C. complanatum</td>
<td></td>
</tr>
<tr>
<td>Diplostomulum</td>
<td>9</td>
</tr>
<tr>
<td>Head other than eyes</td>
<td></td>
</tr>
<tr>
<td>P. minimum</td>
<td>2</td>
</tr>
<tr>
<td>C. complanatum</td>
<td>2</td>
</tr>
<tr>
<td>Remainder</td>
<td></td>
</tr>
<tr>
<td>P. minimum</td>
<td></td>
</tr>
<tr>
<td>C. complanatum</td>
<td></td>
</tr>
<tr>
<td>Spiroxys sp.</td>
<td>5</td>
</tr>
<tr>
<td>Blackspot*</td>
<td>3</td>
</tr>
</tbody>
</table>

*aSeen only before digestion.
Associated with the numerous grubs in the visceras was 2-3 ml of a non-viscous, milky colored ascitic fluid (Fig. 2), containing many blood cells, of which 92% were white blood cells and 8% were red blood cells. Of the white blood cells, 88% were determined to be neutrophiles and monocytes, and 12% lymphocytes. Some of the cells including a few enlarged mononuclear cells had phagocytized many pigment granules.

Histologic examination of two fish severely infected with *P. minimum* revealed numerous parasites in various stages of development within the visceral cavity. They usually were surrounded by a highly cellular, fibrinous exudate, the primary cell being the macrophage (Fig. 3). Some eosinophiles and polymorphonuclear leucocytes (PMN's) also were present in the tissue. Eosinophiles appeared to be rather common in tissues of some infected minnows.

Parasites also were found adjacent to the intestine, kidney, liver, and spleen, where they stimulated a cellular inflammatory response; some were encysted in liver tissue but appeared to be causing little damage to hepatocytes. Focal accumulations of macrophages were common in liver, spleen (Figs. 4, 5), and kidney tissues. Focal lesions in the process of repair, probably in areas where migration of the parasites occurred, were occasionally seen in liver tissue. Such lesions contained fibroblasts which apparently produced collagenous connective tissue. Melanin-macrophage centers were diffusely scattered throughout the fibrinous exudate but were usually closely associated with encysted parasites (Fig. 4).

In one severely infected fish, parasites often were surrounded by what appeared to be macrophages forming continuous sheets of cells. Eosinophiles, PMN's, and individual macrophages were present (Fig. 6). Granulomas containing degenerating parasitic debris at their centers were common in this fish (Fig. 7).

Fish with mild infections of white grubs did not show the extensive

![FIGURE 2. Ascitic fluid and *P. minimum* emerge from an abdominal incision of the fathead minnow shown in Figure 1.](image-url)
FIGURE 3. Encysted metacercaria (P) of *P. minimum* is shown in highly cellular fibrinous exudate adjacent to the intestine. Note melanin-macrophage centers (m) and accumulation of macrophages adjacent to capsule of parasite (arrow) (H & E × 145).

FIGURE 4. Immature *P. minimum* metacercaria (P, lower central portion of photograph) in liver. Note influx of macrophages (arrows) into cystic space (H & E × 375).
FIGURE 5. Encysted *P. minimum* in liver. Note accumulation of macrophages (arrows) adjacent to parasite and highly cellular exudate (E) surrounding liver. Focal accumulations of macrophages (M) are diffusely scattered throughout liver tissue (H & E × 145).

FIGURE 6. Two encysted metacercariae surrounded by macrophages (arrow), forming an almost continuous sheet of cells. Eosinophils and polymorphonuclear cells are present in tissue at the periphery (H & E × 145).
DISCUSSION

This is the second report of a significant mortality caused, apparently, by the white grub. Usually, light to moderate infections occur in fish, and the parasites are soon walled-off by an inner cyst of parasite origin.\(^1\) Thus, damage to the fish is minor. An extremely abundant population of the first intermediate host, the snail (Physa), and the final host, the heron, must be present for a significant mortality to occur from the parasite. Any appropriate fish host present in the environment could presumably contract a lethal infection. Massive invasion by cercariae can kill fish,\(^5,6\) but usually only a few invade at any one time, although a rather large number of metacercariae may accumulate. As we have shown, host reaction to an extremely heavy infection is much more complex, and may include gross displacement of tissue, inflammatory response, rupture of abdomen, and death.

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