Histology, ultrastructure and prevalence of *Henneguya piaractus* (Myxosporea) infecting the gills of *Piaractus mesopotamicus* (Characidae) cultivated in Brazil

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ABSTRACT: The histopathological and ultrastructural characteristics of *Henneguya piaractus*, a parasite of the gill lamellae of *Piaractus mesopotamicus*, are reported here. Histological analysis showed that the plasmodia were of the intralamellar type. The development of the plasmodia resulted in marked dilatation of the infected lamellae, with the neighbouring lamellae being displaced laterally. Discreet epithelial hyperplasia was observed, but there was no inflammatory reaction. Ultrastructural analysis showed that the plasmodium had a single thin wall that was in direct contact with the host cells. Pinocytic canals and points of phagocytosis were observed in the wall. The prevalence of the parasite varied according to host size, with the lowest prevalence occurring in hosts up to 10 cm long.

KEY WORDS: Myxozoa · Pacu · Histology · Ultrastructure · Lipid droplets · Prevalence

INTRODUCTION

Aquaculture is a rapidly developing activity in Brazil. Among the species being considered for fish farming, much interest has focused on *Piaractus mesopotamicus* (Holmberg, 1887), a large riverine fish commonly known as pacu. This interest is attributable primarily to the economic importance of this species, its adaptability to diverse culture conditions, its excellent food-to-growth conversion and its resistance to disease (Hernandez 1989). In this report, which is part of an ongoing investigation into the characteristics of myxosporean parasites of freshwater fish cultivated in Brazil, we describe the ultrastructural and histological characteristics of *Henneguya piaractus* Martins & Souza, 1997, a parasite of pacu gills. In particular, the interactions between the plasmodium and adjacent cells, the spore characteristics, and the histopathological alterations were examined.

MATERIALS AND METHODS

Young specimens of 4 fish species: pacu *Piaractus mesopotamicus* (Characidae), curimbatá *Prochilodus lineatus* (Valenciennes, 1836) (Prochilodontidae), matriná *Brycon cephalus* (Gunther, 1869) (Characidae) and piaçu *Leporinus macrocephalus*, Garavello & Britski, 1988 (Anostomidae), obtained from breeding facilities, were maintained together under fish farm conditions in a pond at the Center for the Research and Management of Continental Fishing Resources (CEPTA/IBAMA) in the municipality of Pirassununga, in the state of São Paulo, Brazil. The fish were monitored for 2 yr (March 2000 to February 2002), and each month 5 specimens of each species were examined for the presence of myxozoan parasites. Immediately after collection, the fish were transported alive to the laboratory, where they were killed by transection of the...
spinal cord before being measured and autopsied. Measurements were obtained from fresh mature spores using a micrometer incorporated into a microscope eyepiece and were expressed as the mean ± standard deviation (SD). For histological analysis, parasitised gills were fixed in 10% buffered formalin for 24 h, embedded in paraffin, cut into 4 µm thick sections, and stained with haematoxylin and eosin and sirius red (Adriano et al. 2002). For ultrastructural analysis, fragments of gills containing plasmodia were fixed in 2.5% glutaraldehyde in cacodylate buffer (2 h), post-fixed in 1% OsO₄ (2 h), dehydrated in increasing concentrations of acetone, and embedded in Epon-Araldite resin. Ultrathin sections double-stained with uranyl acetate and lead citrate were examined in a LEO 906 electron microscope operated at 60 kV.

The occurrence of the parasite throughout the year was examined by grouping the monthly samples according to the season of collection. The effect of season and host (fish) size on the prevalence of the parasite was assessed using the χ² test, with the level of significance set at p < 0.05.

RESULTS

Of the fish species studied, only pacu were found to have the parasite. Of 120 pacu examined, 45 were 5 to 10 cm long, 41 were 10.1 to 20 cm long and 34 were 20.1 to 36 cm long. Fifty-four fish (45%) had plasmodia with the season (χ² = 6.66, df = 5) between the spring of 2000 (6.6 and 13.3%, respectively), with no significant variation (χ² = 6.66, df = 5) between the spring of 2000 and the summer of 2001 (Fig. 13). The prevalence also varied significantly with host size, with the lowest prevalence occurring in hosts up to 10 cm long. When these smaller fish were included from the analysis, there was no significant variation in prevalence. The greater prevalence of the parasite was assessed using the χ² test, with the level of significance set at p < 0.05.

Of the fish species studied, only pacu were found to have the parasite. Of 120 pacu examined, 45 were 5 to 10 cm long, 41 were 10.1 to 20 cm long and 34 were 20.1 to 36 cm long. Fifty-four fish (45%) had plasmodia of Henneguya piaractus in their gill lamellae. The plasmodia were polysporic, white, round or ellipsoidal, and measured 25 µm (immature plasmodia) to 2.5 mm (mature plasmodia) in length. The measurements for fresh spores (Fig. 1) (n = 30) are shown in Table 1.

Histological analysis of infected gills of Piaractus mesopotamicus showed that the plasmodia were of the intralamellar type and occurred between the gill lamellar epithelium and the capillary (Figs. 2 to 4). The parasite caused stretching of the epithelium with accentuated deformation, as well as compression of the capillary and adjacent tissues (Fig. 3). The initial developmental stages of the parasite occurred in all regions of the gill lamellae (basal, medial and distal regions) (Figs. 2 & 3). In advanced stages, the plasmodia occupied the entire extent of the gill lamellae and produced marked dilatation and discreet epithelial hyperplasia. The extensive dilatation of infected lamellae caused displacement, deformation and eventually fusion of the neighbouring lamellae (Fig. 4). No inflammatory reaction was observed in the infected gills.

Ultrastructural analysis showed direct contact between the plasmodial wall and the host cells (Figs. 5 to 9). The plasmodial wall consisted of a single layer of the plasmodial wall and the host cells (Figs. 5 to 9). The plasmodial wall consisted of a single layer

### Table 1. Henneguya piaractus. Measurements of fresh spores

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Measurement</th>
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<tbody>
<tr>
<td>Total length</td>
<td>59.6 ± 2.3 µm</td>
</tr>
<tr>
<td>Body length</td>
<td>12.8 ± 0.7 µm</td>
</tr>
<tr>
<td>Width</td>
<td>4.1 ± 0.2 µm</td>
</tr>
<tr>
<td>Length of caudal process</td>
<td>46.4 ± 2.1 µm</td>
</tr>
<tr>
<td>Polar capsule: Length</td>
<td>6.5 ± 0.4 µm</td>
</tr>
<tr>
<td>Width</td>
<td>1.2 ± 0.2 µm</td>
</tr>
<tr>
<td>No. of polar filament turns</td>
<td>8–9</td>
</tr>
</tbody>
</table>

DISCUSSION

Although there was a significant difference in the prevalence between the seasons, this finding most likely reflects the time required for infection of the fish and the appearance of plasmodia after the initial contact with the host rather than true seasonal variation. In agreement with this conclusion, the lowest prevalence of the parasite occurred in the autumn and winter of 2000 (beginning of the study), with no significant variation (χ² = 6.66, df = 5) between the spring of 2000 and the summer of 2001 (Fig. 13). The prevalence also varied significantly with host size (χ² = 25.24, df = 2). The lowest prevalence occurred in hosts up to 10 cm long, with no significant difference (χ² = 0.02, df = 1) between fish 10.1 to 20 cm long and those >20 cm long (Fig. 14).
Adriano et al.: *Henneguya piaractus* infecting pacu cultivated in Brazil

parasite in larger fish was similar to that of other myxosporean species, such as *Henneguya crepílini* infecting the gills of *Stizostedion lucioperca* (Molnár 1998) and *Myxobolus muelleri* and *Myxobolus dujardini*, parasites of *Ptychochelus oregonensis*, *P. caurinus* and *Richardsonius blateatus* (Mitchell 1988).

The specimens of *Piaractus mesopotamicus* examined were confined to a pond with 3 other fish species: *Brycon cephalus* (Characidae), *Prochilodus lineatus* (Prochilodontidae), and *Leporinus macrocephalus* (Anostomidae), but *Henneguya piaractus* was found only in pacu, indicating host specificity. Molnár (1998) suggested that *Henneguya* species may have a relatively strict host specificity. However, *H. piaractus* has been reported to infect pacu, tambaqui *Colossoma macropomum*, a large characid native to the Amazon river basin, and tambacu, a hybrid of these 2 species (*P. mesopotamicus* male × *C. macropomum* female), in a fish farm (Martins et al. 1999). This may indicate that *H. piaractus* has a host specificity restricted to closely related species—pacu and tambaqui are characids that can crossbreed with each other.

Ultrastructural analysis showed that sporogenesis in *Henneguya piaractus* followed the general pattern of other *Henneguya* species (Current 1979, Azevedo & Matos 2002, 2003, El-Mansy & Bashtar 2002, Vita et al. 2003). However, numerous spherical lipid droplets were immersed in the sporoplasm cells of *H. piaractus*. Similar lipid inclusions have also been reported in the
inner zone of the plasmodial wall of *Myxobolus* sp. (Desser & Peterson 1978), in association with capsulogenic cells in *Sphaerospora dicentrarchi* (Sitjà-Bobadilla & Alvarez-Pellitero 1992), in generative and sporoplasmic cells of *Sphaerospora testicularis* (Sitjà-Bobadilla & Alvarez-Pellitero 1993), in sporoplasmic
but have not been previously reported for the genus *Henneguya*. Although these inclusions may serve as energy reserves (Sitjà-Bobadilla & Alvarez-Pellitero 1993, Redondo et al. 2003), their true functional and metabolic significance remains to be established (Redondo et al. 2003).

The plasmodial wall of *Henneguya piaractus* consisted of a single membrane, as in other myxosporean species (Current & Janovy 1978, Current 1979, Hallett & Diamant 2001, Dohole et al. 2002), and contained pinocytic canals that extended into the plasmodial ectoplasm, as also seen in several other *Henneguya* species (Current & Janovy 1976, 1978, Current 1979, Rocha et al. 1992, Hallett & Diamant 2001, Azevedo & Matos 2002, 2003, El-Mansy & Bashtar 2002). However, there was no coat, nor was the wall surrounded by a capsule of collagen fibres.

A similar organization of the plasmodial surface has been described in *Henneguya listerine* (Hallett & Diamant 2001) and for the interlamellar plasmodia of *Henneguya exilis* (Current & Janovy 1976). In *H. exilis*, regions of the plasmodial surface are in direct contact with the host cells, with the cytoplasm of the host cell appearing to pass into pinocytic canals of the plasmodial wall (Current & Janovy 1976). In *H. listerine*, the pinocytic canals of the plasmodial wall function as a nutrient transport system (Hallett & Diamant 2001), and in *Henneguya suprabranchiae* these canals supply various developing stages with the nutrients necessary for growth (El-Mansy & Bashtar 2002).

In addition to pinocytic canals, the plasmodial wall of *Henneguya piaractus* also contained several points of phagocytosis that engulfed parts of the host cells. Thus, this species can obtain nutrients by pinocytosis and phagocytosis. Phagocytosis followed by intracellular digestion within a food vacuole was observed in *Kudoa quadratum* (Uspenskaya 1982). The phagocytosis of chondrocytes was observed in *Myxobolus cerebralis*, and extrasporo-
tomicus. Prevalence of H. piaractus in relation to host size

Fig. 14. Henneguya piaractus infecting Piaractus mesopotamicus. Prevalence of H. piaractus in relation to host size

Henneguya piaractus causes important pathological alterations in the gills of cultivated pacu (Martins et al. 1997), including haemorrhage and severe inflammatory foci in the gill epithelium. Two layers of elongated, fibroblast-like cells and an inflammatory mononuclear infiltrate surround the parasite. Infected fish generally remain near the pond banks or congregate near inflowing water. Feeding activity decreases over time and the fish become lethargic and swim erratically, with an apparent loss of equilibrium before eventually dying (Martins et al. 1997). As shown here, in advanced stages of infection, the plasmodium occupied the entire gill lamella and caused marked dilatation and discreet epithelial hyperplasia. The extensive dilatation of the infected lamellae pushed the neighbouring lamellae sideways and caused deformation and, eventually, fusion. In a massive infection, these alterations may partially compromise the gill functions by reducing the epithelial area and by compressing the blood capillaries. These effects corroborate the potential pathogenicity of H. piaractus reported by Martins et al. (1997).

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