Myxozoans *Myxobolus* sp. and *Henneguya* sp. co-infection in kidney of *Piaractus mesopotamicus* (Characiformes: Serrasalmidae)

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Abstract

This study evaluated the myxozoan infection and histopathology of the kidney of the freshwater fish *Piaractus mesopotamicus* from intensive fish farming in Brazil. A total of fifty-five fish were examined and the organs processed according to usual histological methods by staining with haematoxylin-eosin and Ziehl-Neelsen. In renal tissue free myxospores of *Myxobolus* sp. (85.5% prevalence) and *Henneguya* sp. (56.4% prevalence) were observed. The presence of myxospores was associated with histological alterations in both stromal and renal parenchyma. Myxospores were found mostly in the peritubular interstitial tissue and in low intensity in the glomerulus which caused nuclear hypertrophy and loss of Bowman space. An increase in the glomerular tuft and a reduction in the lumen of the collector tubules was also observed, besides high number of melanomacrophage cells in the glomerulus. This study reports for the first time detection of mixed infection by myxozoans in just one organ of pacu and discuss on the possible transport of myxospores in the circulating blood.

Keywords: Fresh water fish; histopathology; inflammation; Myxosporeia; pacu

1. Introduction

*Piaractus mesopotamicus* Holmberg, 1887, popularly known in Brazil as “pacu”, belongs to the Family Serrasalmidae, is a teleost fish native to the Paraná-Paraguay Basin. It is an emergent species in the world aquaculture, and presents great economic importance in the South America (Belo et al., 2014; Valladão et al., 2016), China (Lin et al., 2015) and United States (Witmer and Fuller, 2011). This species has proven to be a good bioindicator of water quality (Farias et al., 2016), and in accordance with Castro et al. (2014) the pacu has been used in ecotoxicity studies for registration of chemicals in Brazil.
High stocking density and inadequate handling are responsible for increased stress that affect negatively the pacu health causing increased disease susceptibility (Belo et al., 2005; 2012; Manrique et al., 2015a). On the other hand, members of the class Myxosporea use not only wild and cultured fish (Capodifoglio et al., 2016) but also amphibians, reptiles (Eiras, 2005), aquatic birds (Bartholomew et al., 2008) and terrestrial mammals (Friedrich et al., 2000) as hosts. These parasites have been recognized as a key limiting factor in the development of aquaculture because they infect a large variety of commercially important fishes, and these parasites may develop intra- and intercellularly (histozoic) or may be located in the organs and body cavity (celozoic) (Lom and Dyková, 2006).

Myxosporean parasites are known to be responsible for several forms of damage, including myoliquefaction of the host (Eiras et al., 2007), reduction of the capacity of respiration (Molnár and Székely, 1999), damage to the ovaries (Mansour et al., 2013) changes in meat quality (Manrique et al., 2015b) and changes in the renal tissue (Molnár, 2007; Manrique et al., 2012; Abdel-Baki et al., 2015).

So far, the occurrence of two Myxobolus species in pacu, M. cuneus infecting the connective tissue (Adriano et al., 2006) and the skeletal muscle (Manrique at al., 2016), and M. colossomatis in branchial arches and gill (Müller et al., 2013), and two Henneguya species H. pellucida in swim bladder (Adriano et al., 2005a) and H. piaractus in gill lamellae (Adriano et al., 2005b; Azevedo et al., 2010; Müller et al., 2013).

In this paper, we report on a mixed infection with myxospores of a Myxobolus sp. and a Henneguya sp. in the posterior kidney of P. mesopotamicus and on histopathological changes in the renal tissue caused by these parasites.

2. Materials and methods

2.1. Fish samples
Fifty-five live young fish of *P. mesopotamicus* with 124.0 ± 3.7 g mean weight and standard length 19.9 ± 2.7 cm were captured during August 2014 from a pond of intensive fish farming in Southeast Brazil, São Paulo State.

2.2. Experimental procedures

The living fish were euthanized by fish immersion in an alcoholic solution of benzocaine 1:500 v/v anesthesia/water (0.1 g benzocaine per mL of ethanol) according to the ethical procedures approved by Ethics Committee (CEUA-UNESP protocol nº 020092/09) for posterior blood collection from the caudal vein using syringes containing 10% EDTA to make the blood smears, that were stained with Giemsa to evaluation of structures examined in optical microscope. Then necropsy was performed for collection of the posterior kidney for histopathology and a small fragment for analysis in fresh mounts.

2.3. Morphological analyses of myxospores

The samples of organs were placed in a petri dish, moistened with saline solution (0.65%) and macerated with scalpel blades and placed between a glass and a coverslip for myxospore measurements in fresh (Burger and Adlard, 2010), only in the caudal kidney were observed myxospores. A total of 173 myxospores were measured from the histological sections (107 *Myxobolus* sp. and 66 *Henneguya* sp.). All analyses were performed in an Olympus BX51 light microscope with image capture in a DP73 camera and morphometry using the cellSens v.1.5 Software (Olympus).

2.3. Histopathology analyses

The posterior kidney was fixed in Bouin solution for 6 h and submitted to routine procedures in order to obtain cross sections of 5 µm thickness in paraffin and stained with hematoxylin-eosin (H&E) and Ziehl-Neelsen (ZN) for microscopical examination.

3. Results

3.1. *Myxobolus* sp. and *Henneguya* sp. myxospores
In fresh mounts of the kidney, myxospores of *Myxobolus* sp. (Fig. 1) and *Henneguya* sp. (Fig. 2) were identified. The myxospores were measured from the histological sections stained with ZN and compared with others *Myxobolus* and *Henneguya* species of Brazilian native Characiformes fish (Table 1), and showed characteristics similar to those reported in the literature. However, no myxospores were recorded in blood smears or in other organs.

3.2. Histopathology

The analysis of histological sections stained with ZN showed that neither plasmodial nor sporogonic stages of the above species were found in the kidney. Nevertheless, disseminated mature myxospores were located in the renal interstitium, in the wall and the lumen of the glomeruli, and in the tubules. The prevalence of *Myxobolus* sp. was 85.5% (47/55) and *Henneguya* sp. was 56.4% (31/55).

Most of the myxospores seemed to be intact, and their sporoplasm, polar capsules and the spore wall stained intensely (Fig. 3 and 4), some other damaged myxospores, however, were surrounded and incorporated into melanomacrophage cells. Melanomacrophage cells were regularly found inside the malpighian corpuscle, in the lumen and among epithelial cells of the convoluted channels or free in the renal interstitium. In some of the slides stained with hematoxylin and eosin, the debris of the decayed myxospores was also observed (Fig. 5 and 6). A special feature of the infection was that melanomacrophage centers were not found in the renal interstitium, but agglomerated melanomacrophage cells were located inside the Bowman capsules and tubules (Fig. 5 and 6). The cellular infiltration in the renal parenchyma (Fig. 5 and 6) was in the form of aggregation of mononuclear cells.

4. Discussion

The kidney of freshwater fishes is a complex organ with two different functions. The trunk kidney and the hind kidney have excretory function, while the head kidney has a haematopoietic function. The structure of the hind kidney is similar to those of mammals and
birds, having glomeruli in Bowman capsule, convoluted tubules and urinary ducts surrounded by the renal interstitium (Harder, 1975). The large number of myxosporean parasites located in different parts of the kidney, mainly in the trunk kidney, and they can develop in several ways (Molnár, 2007). Some species, like *M. erythrophthalmi* of *Scardinius erythrophthalmus* form large plasmodia in the renal interstitium (Molnár et al., 2009), while others develop in the epithelium and the lumen of the urinary channels or in the renal glomeruli (Molnár and Eszterbauer, 2015).

Csaba et al. (1984) described that *Sphaerospora renicola*, a sphaerosporid type myxosporean completes its presporogonic development circulating in the blood and arrives at the lumen of renal tubules for finishing its sporogonic development, where it performs spore production. The pathogenic effect of myxosporeans shows also a great variation. Capodifoglio et al. (2016) have observed that the infection by *M. hilarii* in the kidney of *Brycon hilarii* caused compression, deformation and destruction of the tubular cells and adjacent tissue. Myxospores of several species develop in organs (muscles, liver, connective tissue, abdominal cavity) from where their mature myxospores have been carried by the blood stream to the organs (gills, skin, kidney) (Molnár and Eszterbauer, 2015). Apart from these, spores are stuck, engulfed by macrophages and destroyed. We suppose that both, *Myxobolus* and *Henneguya* myxospores, found by us free in the kidney tissues or engulfed by macrophages, belong this type of species.

Myxosporean species infecting the pacu have different site and tissue affinities. From the two *Henneguya* species, *H. piaractus* is a parasite of the gills, while *H. pellucida* infects serous membranes in the abdominal cavity (Adriano et al., 2005a). Of the two *Myxobolus* spp. found in pacu, both *M. cuneus* and *M. cf. colossomatis* are found to be parasites of the connective tissue and develop in the internal organs (Adriano et al., 2006; Müller et al., 2013). However, a third *Myxobolus* species mentioned by Manrique et al. (2015b; 2016) seems to
infect the skeletal muscle. Of the above species, *Henneguya* sp. releases its spores directly to
the outside from its gill cysts, spores of some other species among them those developing in
the muscle, however, could leave the living host via blood stream, a part of which enter the
kidney (Molnár and Székely, 2014).

We agree with authors (McGeorge et al., 1996; Belem and Pote, 2001; Molnár et al., 2009;
Bjork and Bartholomew, 2010) that myxospores of most *Myxobolus* spp. developing in
internal organs, and first of all in the skeletal muscle can reach the kidney via the circulating
blood, and myxospores found by us free in the renal tissues and captured by
melanomacrophage cells belong to these species. By the shape and measurements spores
found in the kidney we cannot exclude that myxospores of the muscle species were also
among them.

At a similar way we think that *Henneguya* sp. myxospores found in the kidney belong to
*H. pellucida*. It is well known (Molnár and Kovács-Gayer, 1985; Holzer and Schachner,
2001; Molnár, 2007) that melanomacrophage centers of the kidney and some other organs are
the major place for destroying spore stages, larvae and eggs of parasites and through innate
and non-specific immune responses, as well as by cellular host activity they eliminate
pathogens (Manrique et al., 2014; Sitja-bobadilla et al., 2015). It is rather curious that in our
case instead macrophage centers myxospores were damaged and eliminated in solitary
macrophages or groups of macrophages accumulated in the Bowman capsule or in the
convoluted tubules.

Besides macrophage activity around myxospores, cellular infiltration in the renal
parenchyma (Fig. 5 and 6) with mononuclear cells were recorded; we could not, however
relate this infiltration with cellular host answer against myxospores. In our study the
myxosporean infection in the kidney cannot be regarded as fatal, but histological changes
found show that due to these disseminated myxospores remarkable local damages can develop
in the kidney. Studies made on *Myxobolus cyprini* by Molnár and Kovács-Gayer (1985) call attention that myxospores of some myxosporean species developing in inner organs and in the muscle, leave the host body through the kidney but a part of these myxospores are captured and eliminated by macrophages.

The findings of this investigation demonstrated that further studies should focus their attention to find the exact place of plasmodial development, and how myxospores were carried to the kidney, leading as a consequence to changes in fish health, as well in order to eliminate the pathogen.

**Conflicts of interest**

The authors have no conflicts of interest to declare.

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**Figure legends**

**Fig. 1.** Photomicrography of the isolated fresh myxospores of the myxosporean *Myxobolus* sp. infecting the kidney of *Piaractus mesopotamicus*. Scale bar = 5 µm. (B).

**Fig. 2.** Photomicrography of the isolated fresh myxospores of the myxosporean *Henneguya* sp. infecting the kidney of *P. mesopotamicus*. Scale bar = 5 µm.

**Fig. 3.** Photomicrography of the posterior kidney of *Piaractus mesopotamicus*. In one of the renal tubules (star) relatively intact myxospores of *Myxobolus* sp. (arrowhead) and *Henneguya* sp. (arrow) are seen. Some free myxospores in the renal parenchyma around tubules are also seen. ZN staining. Scale bar = 20 µm.

**Fig. 4.** Enlarged picture of the posterior kidney of *Piaractus mesopotamicus*. Note the mature myxospores of *Myxobolus* sp. (arrowhead), mature spore of *Henneguya* sp. (arrow) free, melanomacrophages (MM) and macrophages (MØ). ZN staining. Scale bar = 10 µm.

**Fig. 5.** Inflammatory infiltrate (I), predominantly with mononuclear cells, in the renal parenchyma around a damaged tubule and glomerulus. In the lumen and the damaged
epithelium of the tubule melanomacrophage (arrowheads) cells are seen. Glomeruli (G) and the Bowman capsule are also damaged (dashed line, arrow). Inside the blood vessel (star) red blood cells and a mononucleate cell is seen. H & E staining. Scale bar = 20 µm.

**Fig. 6.** A part of the kidney with renal tubules (star) and glomerulus. Renal interstitium surrounding an intact glomerulus is infiltrated by inflammatory, predominantly mononuclear (I) cells. An infected, damaged glomerulus (G) is filled by melanomacrophage centers (MMC). The wall of the Bowman capsule (dashed line, arrow) is also damaged. Some free melanomacrophages (arrowhead) are located. H & E staining. Scale bar = 20 µm.