NOTE

Haematology and histopathology of Nile tilapia parasitised by *Epistylis* sp., an emerging pathogen in South America

G. M. R. Valladão, N. Levy-Pereira, P. H. O. Viadanna, S. U. Gallani, T. H. V. Farias and F. Pilarski*

Pathology Laboratory of Aquatic Organisms (LAPOA), Universidade Estadual Paulista-UNESP, Faculdade de Ciências Agrárias e Veterinárias, Centro de Aquicultura da UNESP (CAUNESP), Jaboticabal, São Paulo, Brazil.

Abstract
Twenty Nile tilapia (*Oreochromis niloticus*) parasitised by *Epistylis* sp. were collected for haematological and histopathological analysis. Fish with >400 zooids showed increase in lymphocyte and decrease in neutrophil population (p<0.05), compared to the less parasitised group (<400). Histopathologic analysis showed that *Epistylis* sp. induces a chronic inflammatory response in the host, possibly provoked by constant contact with the fish skin.

*Epistylis* is a sessile peritrich found fixed on inert substrates and in live organisms by their structure called scopula. Many sessile peritrichs are considered epibionts, however, the genus *Epistylis* was associated with severe mortalities of fish in North America in the seventies (Esch et al., 1976; Miller and Chapman, 1976), being recognised as an important fish ectoparasite. The outbreaks occurred in water bodies with high levels of organic load, thermal pollution and in combination with the presence of bacterial pathogen *Aeromonas hydrophila* (Lom, 2006).

Haematological and histological studies are important for assessing the general health status of a fish population. Both approaches are complementary, and can be used to determine how certain disease can develop and how parasites can survive in the host. This approach was especially followed by some authors to understand the effect of ciliated protozoans in fish host (Witeska et al., 2010; Valladão et al., 2014), but specifically for *Epistylis* sp., there is a lack of such knowledge.

Nile tilapia can be affected by various parasites; the most common parasites are trichodinids (Valladão et al., 2013) and monogeneans (Pantoja et al., 2012). Because high degrees of *Epistylis* sp. parasitism in tilapia are unusual, the aim of our preliminary study was to assess potential differences in haematological and pathological parameters in fish with low and high infection of *Epistylis* sp.

Twenty juvenile males of the Nile tilapia (*Ore-
Ochromis niloticus) reared in cages (18 m³, at density of 50 kg/ m³) of a fish farm at Tietê River (22°01'43"S; 48°54'40"W), São Paulo, Brazil, were presenting variable infection rates of Epistyliis sp. and were randomly sampled for haematological and pathological analysis. Fish were weighed and measured (62.58 ± 7.75 g and 15.38 ± 0.66 cm, respectively), and whole left side of body surface was scraped using a slide and cover slip (including head and fins region). The right side was fixed for collection of tissue for histological analysis. Slides were examined using a Nikon E200® microscope. To determine the degree of parasitism, the number of colonies and zooids from the samples containing the peritrich were counted. Results were expressed as number of colonies and zooids per fish, and based on the result, fish were divided into two groups: group 1 (lower infection rate), with less than 400 zooids and group 2 (higher infection rate), with more than 400 zooids per fish. The experimental procedures were authorised by the Animal Ethics and Welfare Committee (CEBEA) of São Paulo State University (FCAV/UNESP), under the protocol 22.517/10.

Twelve fish were anesthetised in a tank containing benzocaine solution (100 mg/ L) until the loss of equilibrium was noticed. Approximately 2 mL of blood were collected by puncture of the caudal vessel, using 3 mL syringes coated with heparin (100 IU), and added to polystyrene tubes. Six blood samples from each group were analysed for haematological parameters, while a drop of blood sample was used to prepare smears in duplicate. Smears were stained with May-Grünwald Giemsa-Wright and used for total and differential leukocytes as well as the total thrombocytes count (Hrube & Smith, 1998). The erythrocyte count was performed in a Neubauer chamber, after diluting the blood in citrate-buffered formaldehyde in a proportion of 1:200. The number of cells in the blood smear, either leukocytes or thrombocytes, was counted in relation to 2000 red blood cells in the microscope field.

All the parasitic characterisation and haematological parameters were tested for homogeneity and homoscedasticity with Levene and Camer-Von Mises tests, respectively, and submitted to one-way analysis of variance (one-way ANOVA) using the R software.

Fish were euthanised by disruption of the spinal cord for pathological analysis and organs collection. All gross lesions observed during necropsy were described. To assess the state of health of infected fish, internal organs (gills, stomach, spleen, liver, intestine and heart) and skin were collected. The organs were fixed in 10 % formaldehyde solution for 48 hours and latter, routinely processed for paraffin embedding. Sections of 5–6 µm were prepared and stained with haematoxylin–eosin (H&E). The slides were observed under Nikon E200® microscope.

Nile tilapia showed darkening skin, multifocal and coalescing erosions with whitish appearance (1-3 mm), diffused throughout the lateral line, lateral and head region (Figure 1a). Macroscopic structures, resembling cotton tufts were observed in the caudal part, peduncle and at bases of the fins region (Figure 1b). Aetiological agent was identified (by colonial habit; noncontractile, regularly dichotomously branched stalk; well-defined peristomial lip, and less than 3 turns of peristomial cilia), according to Li et al. (2012) as Epistyliis sp. (Figure 1c), while no other parasitic taxa were observed.
in examined fish. The degree of *Epistylis* sp. infection in sampled fish (n=20) ranged, but was not associated with the size of the animals. The average of zooids per colony did not differ between the two groups (Table 1). Gross lesions elicited in the fish group with high infection were similar to lesions observed in the fish group with lower infection.

The most heavily parasitised group had a lower number of neutrophils (p <0.05) and twice higher the number of lymphocytes (p <0.05) when compared to the less infected group. The degree of infection had no significant effect on the counts of total leukocytes, erythrocytes, thrombocytes, monocytes and basophils (p > 0.05) (Table 2). Eosinophiles were observed only in heavily infected fish.

The presence of *Epistylis* sp. was also confirmed in histological sections (Figure 2a). Fish from this study showed several microscopic changes, mainly associated with *Epistylis* sp. colonising the tegument. In all sampled fish we observed a rare subepithelial lymphocytic infiltrate (Figure 2b), moderate infiltrate of mast cells, with epithelial hyperplasia and hypertrophy of the tissue (Figure 2c). Giant cells formation was observed only in one fish from the heavily infected group. Some fish showed mast cell infiltrate and subepithelial hyperplasia, lamellar edema and mild congestion in gills. In the stomach and intestine, mild mast cell infiltrate of submucosa was observed, and hydropic and/or fatty degeneration (mild-moderate) of liver related to intensive farming feeding regime.

### Table 1. Data of the Nile tilapia parasitised by *Epistylis* sp.

<table>
<thead>
<tr>
<th>Zoo</th>
<th>W</th>
<th>L</th>
<th>MZ</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;400</td>
<td>62.91 ± 7.86</td>
<td>15.33 ± 0.66</td>
<td>26.9 ± 6.0</td>
<td>10</td>
</tr>
<tr>
<td>&gt;400</td>
<td>62.25 ± 7.54</td>
<td>15.43 ± 0.60</td>
<td>33.9 ± 2.7</td>
<td>10</td>
</tr>
</tbody>
</table>

Zoo: zooids; W: weight (grams); L: length (cm); MZ: mean of zooids in each colony.

### Table 2. Blood parameters of the Nile tilapia according to the number of zooids.

<table>
<thead>
<tr>
<th>Zoo</th>
<th>RBC</th>
<th>Thromb</th>
<th>Leuk</th>
<th>Mon</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;400</td>
<td>1.957 ± 0.063</td>
<td>24.976 ± 6.522</td>
<td>32.036 ± 2.577</td>
<td>3.770 ± 0.726</td>
<td>6</td>
</tr>
<tr>
<td>&gt;400</td>
<td>1.991 ± 0.030</td>
<td>29.898 ± 5.504</td>
<td>50.885 ± 9.050</td>
<td>3.342 ± 0.598</td>
<td>6</td>
</tr>
<tr>
<td>p</td>
<td>0.639</td>
<td>0.577</td>
<td>0.073</td>
<td>0.659</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Zoo</th>
<th>Lymp</th>
<th>Neutr</th>
<th>Baso</th>
<th>Eos</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;400</td>
<td>20.288 ± 4.052b</td>
<td>7.879 ± 0.363a</td>
<td>0.389 ± 0.118</td>
<td>0.000 ± 0.000</td>
<td>6</td>
</tr>
<tr>
<td>&gt;400</td>
<td>41.038 ± 7.815a</td>
<td>4.865 ± 0.218b</td>
<td>0.202 ± 0.139</td>
<td>0.211 ± 0.146</td>
<td>6</td>
</tr>
<tr>
<td>p</td>
<td>0.0402</td>
<td>0.0001</td>
<td>0.332</td>
<td>0.181</td>
<td></td>
</tr>
</tbody>
</table>

Zoo: zooids; RBC: red blood cells; Thromb: thrombocytes; Leuk: leukocytes; Mon: monocyte; Lymp: lymphocytes; Neutr: neutrophils; Baso: basophils; Eos: eosinophils.
Parasitism caused by peritrichs has already been reported as a cause of mortality in North American (Rogers, 1971) and European fish (Zrnčić et al., 2009), especially when associated with opportunistic infection (Esch et al., 1976; Miller and Chapman, 1976). *Epistylis* sp. was never considered as one of the major health problems in South America, and has been found with low infection rates in fish (Centeno et al., 2004; Martins et al., 2008). However, recent studies showed high infection rates in tilapia in Peru (Fernández, 2012) and catfish (*Pseudoplattystoma* sp.) in Brazil (Padua et al., 2013), suggesting, along with the present study that this protozoan could be an emerging pathogen in this continent. Rogers (1971) established that aquatic environment saturated in organic matter favors epizootic outbreaks by *Epistylis* sp., which explains the high infestation observed in fish from this study, since Tiete river, where tilapia were collected, is considered strongly eutrophic (Galli and Abe, 2010).
Erosive white spots were observed in areas of *Epistylis* sp. scopula fixation, however, these parasites do not produce lytic enzymes nor have structures capable of piercing or penetrating the tissue and injuring mechanically their host (Lom, 1973; Hazen et al., 1978). We cannot discard that the observed tissue damage may have occurred due to the action of immune cells at the site of parasitism.

Haematological response of fish parasitised by *Epistylis* sp. is unknown. In this study haematological analysis showed a lower number of neutrophils in the highly infected fish and Clauss et al. (2008) reported that lower number of neutrophils may reflect an extravasation of cells to the site of action. Neutrophils are important defense cells of the non-specific system of fish and low rate in blood may indicate that fish heavily infested are more susceptible to secondary infections. Furthermore, the number of lymphocytes was two times higher than in less parasitised animals and, according to Weiss and Wardrop (2010), persistent antigenic stimulation can lead to high lymphocytic count, indicative of a chronic parasitism.

The colonisation by *Epistylis* sp. caused mild to moderate histopathological changes in tilapia that did not differ between heavily and low infected fish groups. Hazen et al. (1978) proposed that severe microscopic changes are observed only when *Epistylis* sp. is associated with bacterial diseases, especially *A. hydrophila* that is able to produce toxins. However, a significant infiltration of mast cells was observed in the infected fish of this study. Mast cell plays an important role in the immune system of teleost fish and their migration is related to the action of bacteria and other parasites (Marshall, 2004; Sfacteria et al., 2015), including *Epistylis* sp. as observed in this study. The presence of giant cells, which is usually related to chronic parasitic and bacterial diseases (Pulsford and Matthews, 1991; Aro et al., 2014), was observed in one fish in the heavily parasitised group. This might suggest that, along with the high blood lymphocyte counts, a chronic process has been initiated in tilapia, and not necessarily exclusive to *Epistylis* sp.

In conclusion, we observed that fish heavily parasitised by *Epistylis* sp. developed pathological alterations, especially the migration of mast cells to the fixation site and significant change in the number of peripheral lymphocytes and neutrophils, which associated with clinical signs described in this study, proves that this sessile protozoan has the ability to affect the fish health.

Acknowledgment to FAPESP for the scholarship (Process number 2010/14679-7).

References


Esch GW, Hazen TC, Dimock RV and Gibbons JW (1976). Thermal effluent and the


