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Histological confirmation of epizootic ulcerative syndrome in two cyprinid species from Lake Liambezi, Zambezi Region, Namibia

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Epizootic ulcerative syndrome (EUS) is a fish disease caused by an infection of the oomycete, Aphanomyces invadans. During a fish biodiversity assessment of Lake Liambezi, Zambezi Region, Namibia, in August 2011, two Barbus haasianus and three Barbus unitaeniatus with circular ulcerative skin lesions were collected. Lake Liambezi receives inflow from the Zambezi, Chobe and Linyanti rivers. The presence of EUS in the two species was confirmed histologically by: a loss of epidermis at the site of the lesion; hyphae visible deep into the muscle layer with an associated granulomatous inflammatory reaction; and muscle fibre degeneration visible with associated penetrating hyphae. This paper provides further histological confirmation of EUS from Lake Liambezi and the first record of the disease in B. haasianus.

Key words: Barbus haasianus, Barbus unitaeniatus, Chobe River, fungal pathogen, histology.

Epizootic ulcerative syndrome (EUS) is a fish disease, which results in high biodiversity and economic losses (FAO 2009; Oidtmann 2012). The disease is caused by the oomycete Aphanomyces invadans David & Kirk 1997, as listed in the Index of Fungi (IMI 1997), which causes epidermal lesions that penetrate into the underlying muscle tissue, ultimately resulting in skin erosion and exposure of underlying musculature and ulceration (Lilley & Roberts 1997). Globally, the first reports of EUS outbreaks were from South East Asia, when EUS caused high mortalities of wild and cultured fish (Lilley & Roberts 1997). In sub-Saharan Africa the first report of A. invadans and an associated EUS outbreak was from Botswana in 2007, followed by Zambia in 2008 (Andrew et al. 2008). Subsequently 27 fish species from the region have been shown to be susceptible to EUS (FAO 2009; Choongo et al. 2009; Songe et al. 2012; Huchzermeyer & Van der Waal 2012). Lesions, suspected to be associated with EUS have previously been reported from Lake Liambezi by Van der Waal (2008; 2012).

The present study reports the findings of an EUS assessment of 91 fish, representing 16 species in seven families, that were sampled from Lake Liambezi, Zambezi Region, Namibia, as part of a biodiversity assessment in August 2011 (Fig. 1) (17°52.942’S, 24°23.706’E). As noted by Van der Waal (2012), this is highly relevant because the 300 km² Lake Liambezi is used extensively by artisanal fishermen and EUS-associated fish mortalities could impact on both food security and on local economies. Van der Waal (2012) further emphasized the importance for continued targeted surveillance for the prevalence of EUS in the lake. The lake also receives inflow from the Zambezi, Chobe and Linyanti Rivers which may facilitate spread of the pathogen into the currently unaffected Kwando River via the Linyanti swamps. The objective of this paper was therefore to provide insight into the current extent of EUS in Lake Liambezi. This was done by means of a histological assessment on fish that macroscopically exhibited signs of EUS.

Fish were sampled by means of gill nets, electrofishing and angling. Live fish were transferred to a field laboratory, identified using keys provided by Skelton (2001), measured, and a presumptive EUS identification, based on the presence of typical clinical signs, including skin damage and haemorrhagic ulcerative skin lesions, was conducted in accordance with FAO (2009).

Of the 91 fish inspected, only five individuals showed macroscopic alterations to the skin (Table 1). The clinically affected fish species were Barbus haasianus David, 1936 and B. unitaeniatus Günther, 1866. Individuals of both these species exhibited varying degrees of severity of skin ulcerations. The lesions were mostly focal and varied...
from a red inflamed area to an open ulcerated wound. In the more severely affected samples, scales were absent from the lesion. Affected tissues from these five fish were collected, fixed in 10% neutral buffered formalin and prepared for histology using the methods described by McHugh et al. (2011, 2013). Sections were stained using haematoxylin and eosin and examined using an Olympus BX 51 compound light microscope to determine the presence of oomycete hyphae and associated

Table 1. Fish species, number and mean lengths of fish species captured in Lake Liambezi and presence of lesions confirmed by histology as epizootic ulcerative syndrome (EUS) infections. Abbreviations: n, number; ML, mean length; S.D., standard deviation.

<table>
<thead>
<tr>
<th>Fish species</th>
<th>n</th>
<th>ML ± S.D. (range in mm)</th>
<th>Clinically confirmed</th>
<th>Histologically confirmed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cichlidae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Coptodon rendalli</em> (Boulenger, 1896)</td>
<td>4</td>
<td>148.3 ± 63.6 (84–211)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Oreochromis andersonii</em> (Castelnau, 1861)</td>
<td>5</td>
<td>182.8 ± 72.3 (100–279)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Oreochromis macrhor</em> (Boulenger, 1912)</td>
<td>4</td>
<td>217.8 ± 95.4 (80–291)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Sargochromis codringtonii</em> (Boulenger, 1908)</td>
<td>7</td>
<td>139.4 ± 10.8 (125–154)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Serranochromis macrocephalus</em> (Boulenger, 1899)</td>
<td>8</td>
<td>106.4 ± 6.1 (101–115)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Serranochromis robustus</em> (Günther, 1864)</td>
<td>3</td>
<td>239.7 ± 31.9 (211–274)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Tilapia spramani</em> Smith, 1840</td>
<td>3</td>
<td>98.3 ± 6.0 (90–104)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Clariidae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Clarias gariepinus</em> (Burchell, 1822)</td>
<td>1</td>
<td>325</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Clarias ngamensis</em> Boulenger, 1915</td>
<td>5</td>
<td>437.6 ± 11.6 (425–455)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Clarias theodora</em> Weber, 1897</td>
<td>3</td>
<td>239</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
pathology in tissue sections. The demonstration of mycotic granulomas in histological sections of affected tissues and organs is recognized as one of three diagnostic tests recommended for the confirmatory diagnosis of EUS (OIE 2012; FAO 2009).

Epizootic ulcerative syndrome infection could only be histologically confirmed in one *B. haasiannus* and two *B. unitaeniatus* (Table 2). One sample of the *B. haasiannus* had damage to the skin that appeared to have been caused by the gill net from which it was collected. Pathology in the fish tissue was consistent between both *Barbus* species and comprised deeply penetrating, oomycete infection through the epidermis into the sub-dermal muscle layers eliciting a pronounced host response (Fig. 2). Numerous refractile hyphal cell walls, were visible in cross-section within the muscle tissue (Fig. 2b,c,d). Muscular degeneration or necrosis, in association with oomycete infiltration, was characterized by loss of muscular striation and in some instances the muscle fibres were almost entirely replaced by fibrous tissue and oomycete hyphae surrounded by a granulomatous inflammatory tissue reaction (Fig. 2b,c).

Table 2. Clinical and histological observations on *Barbus unitaeniatus* and *Barbus haasiannus* collected from Lake Liambezi, Zambezi District, Namibia.

<table>
<thead>
<tr>
<th>Host species</th>
<th>Gross pathology</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Barbus unitaeniatus</em></td>
<td>Skin lesion with small red spots</td>
<td>Loss of epidermis at site of lesion. Hyphae visible deep into muscle layer with associated granulomatous inflammatory reaction. Muscle fibre degeneration visible with associated penetrating hyphae. Areas of erythrocytic extravasation visible.</td>
</tr>
<tr>
<td><em>Barbus unitaeniatus</em></td>
<td>Skin lesions with inflamed muscle tissue</td>
<td>No EUS-associated pathology observed.</td>
</tr>
<tr>
<td><em>Barbus unitaeniatus</em></td>
<td>Skin ulceration with inflamed muscle tissue</td>
<td>Lesions were extensive. Total loss of epidermis at site of lesion. Oomycete hyphae visible throughout lesion penetrating deep into muscle layer. Muscle fibres mostly replaced by inflammatory tissue at centre of lesion. Muscle degeneration visible immediately adjacent to the lesion. Multiple areas of erythrocytic extravasation visible throughout the lesion. Numerous multinucleated giant cells visible throughout lesion often associated with encapsulated hyphae.</td>
</tr>
</tbody>
</table>

Continued on p. 314
The inflammatory cellular infiltration of the dermal and underlying muscle layers consisted mostly of cells with relatively large nuclei and very little or no cytoplasm (typical of lymphocytes) and cells with large nuclei and eosinophilic cytoplasm (typical of macrophages). Numerous multinucleated giant cells infiltrating the muscle layer were also observed (Fig. 2d). According to Ferguson (2006), multinucleated giant cells are frequently associated with fungal and metazoan parasitic infections. Multiple oomycete granulomas were formed from the aggregation of inflammatory cells, including numerous multinucleated giant cells often associated with encap-

<table>
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<tr>
<th>Host species</th>
<th>Gross pathology</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Barbus haasianus</em></td>
<td>Skin damage and tearing appeared to be gill net damage</td>
<td>Focal lesion, loss of epidermis at site of lesion. Oomycete hyphae visible throughout lesion and penetrating deep into the muscle. Refractile hyphae in cross section visible throughout the lesion. Extensive, granulomatous inflammatory reaction replacing muscle fibres in centre of the lesion. Muscle degeneration visible in surrounding tissue.</td>
</tr>
<tr>
<td><em>Barbus haasianus</em></td>
<td>Small skin lesion with raised scales</td>
<td>No EUS-associated pathology observed.</td>
</tr>
</tbody>
</table>

**Fig. 2.** Micrographs of muscle tissue sections from the two *Barbus* species stained with H&E. **a,** Deeply penetrating, oomycete infection of the epidermis and sub-dermal muscle layers eliciting a pronounced host inflammatory response. **b,** **c,** **d,** Numerous refractile hyphal cell walls (arrows), were visible in cross-section within the muscle tissue; **b,** **c,** necrosis of muscle tissue in association with oomycete infiltration surrounded by granulomatous inflammatory tissue reaction; **d,** numerous multinucleated giant cells infiltrating the muscle layer (mgc as indicated by arrows). Scale bars = 100 µm.
sulated oomycete hyphae. Multiple areas of erythrocytes forced from the blood vessels into the surrounding tissue were also visible within the lesions.

These findings are consistent with the pathology described for EUS infections (Chinabut et al. 1995; Vishwanath et al. 1998; Werner et al. 2002; Kiryu et al. 2003; Johnson et al. 2004; Oidtmann et al. 2008) and satisfy the OIE’s requirements for a confirmed case of EUS (OIE 2012). The current study therefore provides histological evidence for EUS in Lake Liambezi, the infection of B. unitaeniatuus and the first histological-evidence supported record of EUS in B. haasiaituus. Further investigations are needed to assess the impact of EUS on other fish species in Lake Liambezi and to determine its spread and population level impact.

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