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Intestinal amoebiasis in Heckel discus *Symphysodon discus* - a case report

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Abstract

This case is reported with the intention of highlighting the presentation of primary intestinal amoebic disease in Heckel discus *Symphysodon discus*. The morphology of the parasites and the pathological changes seen using light microscopy were essential. In fresh samples of intestinal content we discovered numerous amoeba-like cells. The trophozoite measures 18.6 x 10.2 mm while the cyst is 11-25 x 10-20 (average 22.2 x 18.4 mm). To the best of our knowledge, this is a new manifestation of amoebiasis in aquarium fish.

Introduction

In spite of several reports of fish amoebiasis in cultured *Salmo salar, Scophthalmus maximus, Sparus aurata, Oncorhynchus kisutch* and *Dicentrarchus labrax* (Douglas-Helders et al., 2001; Dyková et al., 1995; Kent et al., 1988; Leiro et al., 1998; Munday et al., 2001; Nowak et al., 2002; Steinum et al., 2008; Tan et al., 2002; Woo & Poynton, 1995; Zilberg et al., 1999), there are much less reports of systemic infection caused by amoebae (Athanassopoulou et al., 2002; Bullock & Giantris, 1964; Bullock, 1966; Laoprasert et al., 2004; Lom & Dyková, 1992; Nash et al., 1988; Steinhagen et al., 1993; Taylor, 1977; Voelker et al., 1977; Woo & Poynton, 1995). There are also several reports on amoeba-like cells, and they were described in more than 20 species of fishes from different families (Antychowicz, 2007; Dyková et al., 1993; Dyková & Lom, 2004).

To the best of our knowledge, there is no information about intestinal amoebiasis in tropical freshwater aquarium fishes. This report describes the first intestinal amoebiasis in Heckel discus *Symphysodon discus*.

Materials and methods

A single adult *Symphysodon discus* was delivered to Department of Fish Diseases, University of Life Sciences in Lublin in order to diagnose the disease and propose some preventive treatment. The fish was killed and submitted for parasitology (microscopical examination of fresh samples). Parasitological examination was performed according to the methods described by Noga (1996). The fins, gills, skin, body cavity, heart, liver, spleen, kidney and intestine were examined using at first a dissecting microscope (10 to 40x magnification) and subsequently a compound microscope (100 to 1 000x magnification). Fresh sample of intestinal content was also examined using differential interference contrast (Nomarski) microscope.

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Results and discussion

The present report is based on case report of the Protozoa of the discus intestine and records the presence of the cysts of these amoebae. Medical history of a fish revealed chronic character of this illness. The affected fish lost appetite and behaved sluggishly. Progressing anorexia, extended faeces (stool), apathy and colourless were primary symptoms observed in the lab. During routine examinations external parasites were not observed. At necropsy, increased peritoneal fluid was found, and microscopic examination of gastrointestinal tract revealed swollen mucous membrane and focal cells mass created nodules. In fresh samples of intestinal contents we discovered numerous amoeba-like cells (Figure 1). These cells, probably resting forms of trophozoites, were oval to round in shape. The prominent, centrally located endoplasm was reach in granules and surrounded by a flat zone of hyaloplasm at the periphery (Figure 2). When measured under the coverslip in PBS, the size was 11.0–25.0 x 10.0–20.0 mm (average size 22.2 x 18.4 mm). The diameter of the nucleus averaged 2.5 mm (Figure 2). Resting cells isolated from the intestinal contents under coverslip in a wet chamber for a few hours at 20°C were observed to be active (Figures 3, 4). Average dimensions of trophozoites were between 18.6 to 10.2 um. Unfortunately agar plate culture of intestinal contents and selection of homogeneous amoebae cultures to morphological classification was failed, because of abundant growth of bacteria. According to some authors microbial flora is often dominant in the last phase of infection and it may be difficult or impossible to isolate amoebae as primary agent (Scholz, 1999). Moreover, bacteria from the kidney sample

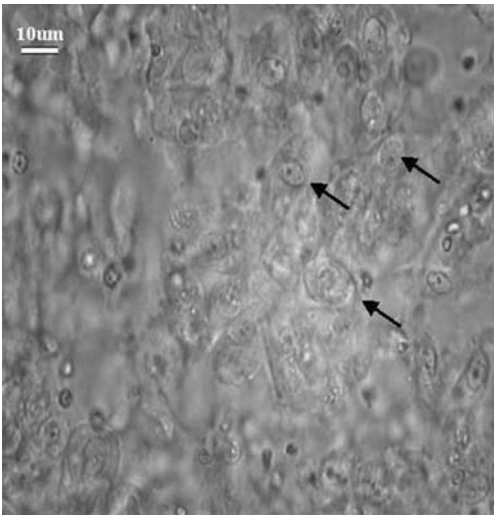

Figure 1. Numerous amoeba-like cells in intestinal contents (scale bar 10μm).

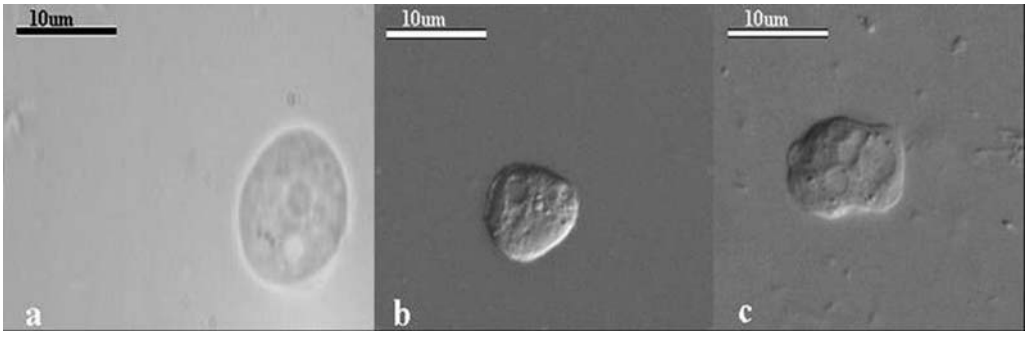

Figure 2. Light microscope image (a) and differential interference contrast images (Nomarski) (b, c) of isolated amoeba-like cells from intestinal contents (scale bar 10μm).

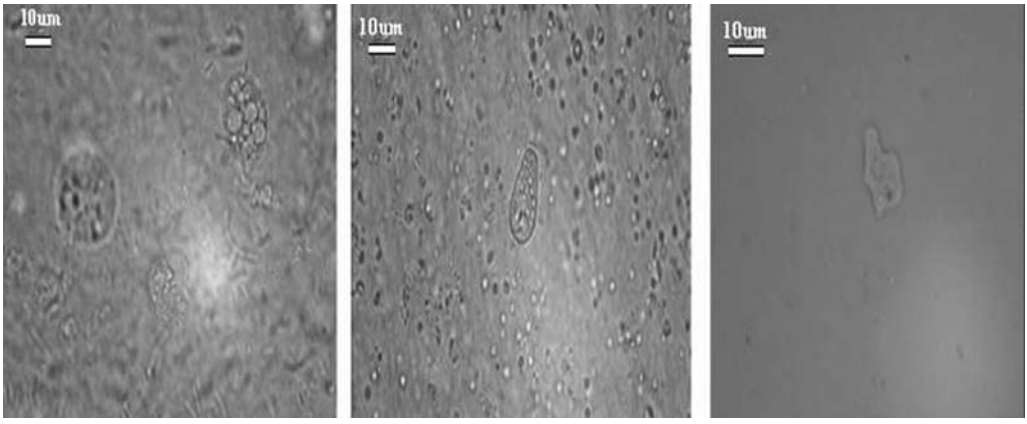

Figure 3. Rounded resting stages transformed in living trophic amoeba (scale bar 10µm).

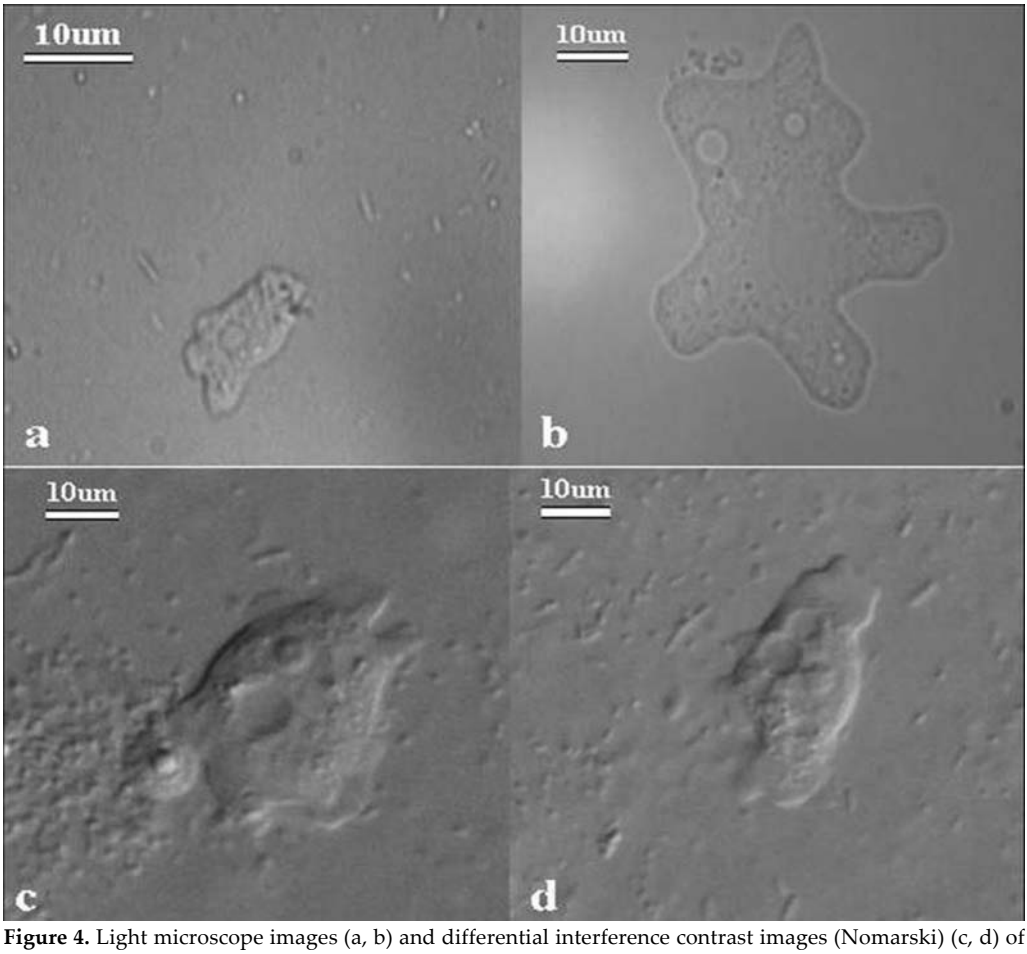

Figure 4. Light microscope images (a, b) and differential interference contrast images (Nomarski) (c, d) of active trophozoite (scale bar 10µm).

of fish submitted to the lab were not isolated. To the best of our knowledge, this is a new manifestation of amoebiasis in aquarium discus. This may represent a new problem to the aquarium fish.

Based on necropsy and parasitology results, surviving fish were given 25 mg/kg metronidazole orally once daily for 5 days. Oral metronidazole was an effective medical treatment for amoebiasis (Noga, 1996).

The presence of *Entamoeba* species from the rectum of the marine gadoid fish, *Pollachius virens*, was first reported by Bullock and Giantris (1964). These trophozoites had a much vacuolated cytoplasm and *Entamoeba* type nucleus with a small endosome and peripheral chromatin granules. Parasitic cells resembling amoeba trophozoites were also found histologically and ultrastructurally to be associated with systemic infection in European catfish (*Silurus glanis*) (Nash et al., 1988), perch (*Perca fluviatilis*) (Dyková et al., 1998), goldfish (Voelker et al., 1977; Steinhagen et al., 1993), pompano (*Trachinotus falcatus*) (Athanassopoulou et al., 2002), and oscar (*Astronotus ocellatus*) (Laoprasert et al., 2004). Study on isolation and identification of amoebae, isolated from gills and kidneys of diseased oscar (*Astronotus ocellatus*), which collected from ornamental fish farm in Thailand, was performed by Laoprasert et al. (2004). It was the first report on isolation and identification of various groups of amoebae from that ornamental fish *Amoebae* isolated from diseased oscar fish were classified into three different groups. The first group isolated from kidney was classified in the genus *Acanthamoeba*, the second group isolated from the gills was classified in the genus *Vannella*, and the third group isolated from the gills and water formed three stages: trophozoite, flagellum and cyst (Laoprasert et al., 2004). The case of *Amoeba*-like infection in cultured warmwater marine fish was described by Athanassopoulou et al. (2002), an unusual systemic infection in pompano *Trachinotus falcatus* L. from Singapore. All pompano showed marked systemic infection of *Amoeba*-like parasites in gills, kidney, intestine, pancreas and spleen.

Some free-living amoebae may change their mode of life and become harmful. Free-living amoebae that may become pathogenic for fish include members of the genera *Acanthamoeba*, *Cochliopodium*, *Negleria*, *Thecamoeba*, *Vahlkampfia*, and *Paramoeba*, the members of the latter genus undoubtedly being of the greatest veterinary importance (Scholz, 1999). In the literature on pathogenic free-living amoebae, special attention has been paid to human pathogens, among others to strains of the genus *Acanthamoeba*. Dyková et al. (1999) described 14 *Acanthamoeba* strains isolated from organs of asymptomatic freshwater fish. Franke and Mackiewicz (1982) described that amoebae of the genera *Acanthamoeba* and *Negleria* have been implicated as the etiological agents of amoebic meningoencephalitis in man and animals. These amoebae have been isolated from a few fishes, reptiles and amphibians associated with the water habitat. Harriff et al. (2007) suggests that the passage through amoebae leads the bacteria to enter the cells of the intestinal lining. Free-living amoebae are found in the water, and further studies on the role of these organisms as reservoirs for bacteria (mycobacteria) pathogenic for fish should undertake (Harriff et al., 2007). The

amphizoic amoebae as a newly emerged veterinary problem require much attention by fish parasitologists.

In conclusion, an occasional occurrence of amoebiasis in discus is described and discussed. The significance of these findings is unknown, but in previous report of AGD and systemic infections in other fish environmental stresses were considered to be responsible for stimulating the disease (Woo & Poynton, 1995). The major areas for studies should include interactions between water quality, environmental contaminations and dietary factors.

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