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NOTE

An unusual site of infection by a microsporean in the turbot *Scophthalmus maximus*

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ABSTRACT: We report a microsporean infection which affected a population of juvenile turbot *Scophthalmus maximus* (Rafinesque, 1810) in a fish farm in the Ria de Vigo (NW Spain), causing high mortality. The uninuclear spores (4.25 × 2.00 μm) contained a characteristic posterior vacuole with a large inclusion body; the xenomas, some displaying microvilli-type projections on their surface, were situated in the connective tissue between muscle fibres, leading to severe myodegeneration. The infection was also observed (though with less spectacular pathological effects) in the liver and the intestine, this being the first time that microsporidiosis has been recorded in these organs in the turbot. On the basis of morphological and pathological characteristics we consider that the aetiological agent of the infection might be *Tetramicra brevifilum*. In fish which survived the infection, all signs of the disease disappeared.

A great number of viral, bacterial and parasitic diseases affecting flatfish have been described (Roberts 1978). In the case of farmed turbot, such diseases can be divided into 2 categories (Person-Le Ruyet 1990): (1) major diseases, common on farms; (2) other diseases occurring occasionally and dangerous only if allowed to persist. According to these criteria, microsporean infections are generally considered to belong to the second category, although they may on occasion lead to serious losses (Lom 1970, Nepszy & Dechtiar 1972). The effects of microsporean infections are particularly severe where there is high host density (McVicar 1975). Microsporea-induced epizooties affecting skeletal muscle and causing important structural changes in host cells have been described for turbot (Matthews & Matthews 1980).

This report deals with an investigation of a disease associated with heavy mortalities among young turbot on a fish farm in NW Spain.

Materials and methods. The disease problem appeared in an extensive production unit near the Ria de Vigo (NW Spain). We examined specimens (average total length 15 cm) collected in May 1991. In preparation for light microscopy, specimens were fixed in 10% buffered formalin, embedded in paraffin and processed following standard histological procedure. Tissue sections (5 μm) were stained with haematoxylin-eosin.

Spores were obtained by maceration of infected host tissues in distilled water; the resulting suspension was then filtered through increasingly fine meshes to remove tissue fragments (Canning & Lom 1986). The filtrate was centrifuged at 2000 × *g* for 30 min; this filtration/centrifugation process was repeated various times until a pure sample of spores was obtained.

Xenoma measurements were taken from micrographs.

Results and discussion. At the macroscopic level, the infection was characterized by the presence of clearly visible whitish nodules, of ca 1 mm diameter, distributed irregularly over the entire body wall (Fig. 1A). In some cases, particularly in host specimens with high infection intensity, the parasite also occurred in the liver (Fig. 1B) and the intestine (Fig. 1I).

In muscle, the xenomas were located in connective tissue between the muscle fibres. They were rounded, ovaloid or fusiform in shape, in some cases displaying dendritic projections. Various stages in the development of the parasite could be distinguished in the interior of the xenomas, from the merogonic and sporogonic stages to stages with completely formed

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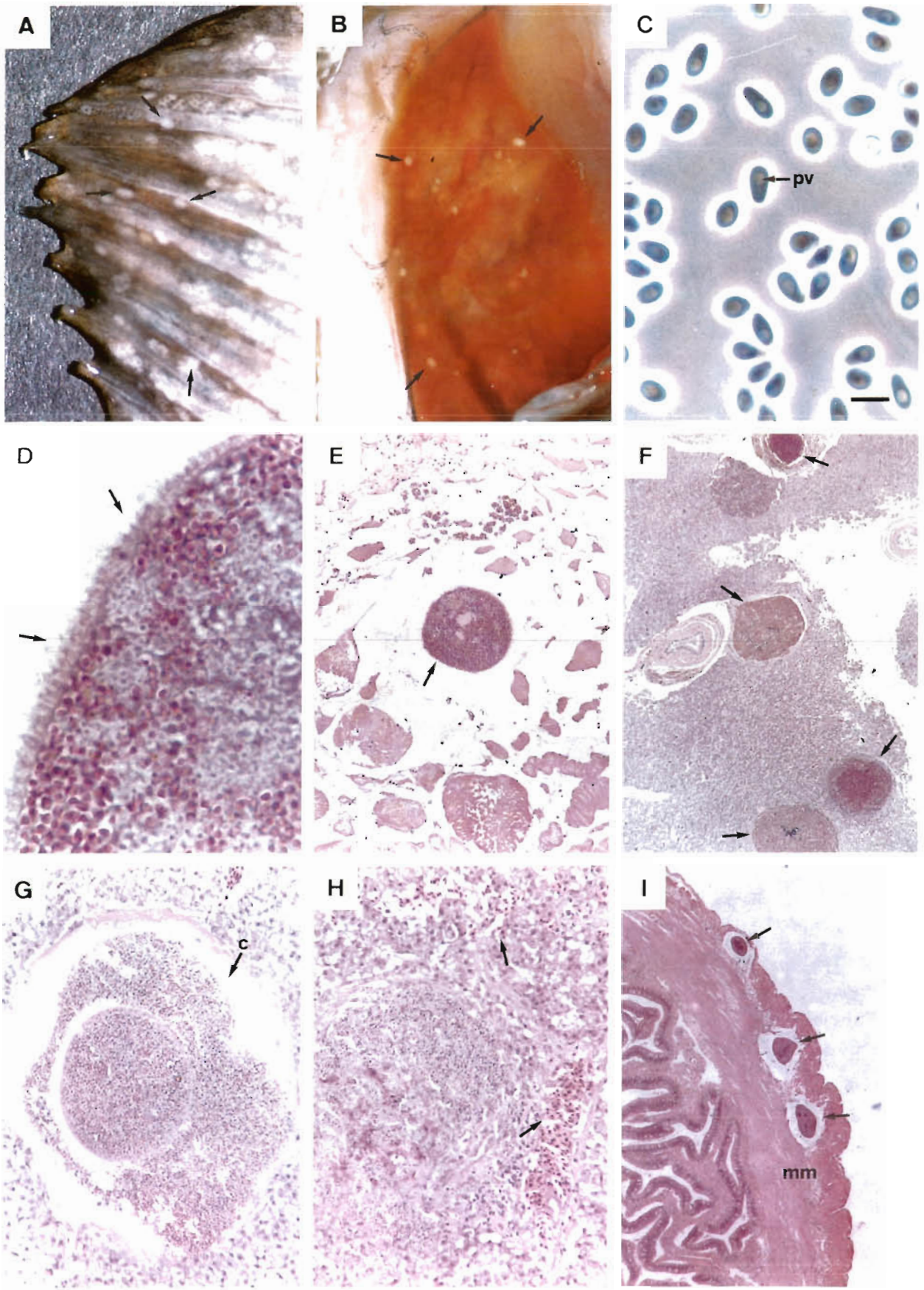
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spores and the typical organization of the Microspora (Canning 1977). Uninuclear spores were oval in shape (average length 4.25 µm, maximum width 2 µm) and, unlike the majority of fish-infecting microsporeans, showed a large vacuole in the posterior region, occupying about 1/3 of the spore and containing a large spherical inclusion body (Fig. 1C). In all cases the xenomas were larger than 150 µm and bore microvilli-type structures on their surface (Fig. 1D). From a pathological viewpoint, the observed muscular lesion was associated with myodegeneration involving vacuolization of the sarcoplasm and separation of myofibrils which led, at macroscopic level, to a visible liquefaction of muscle tissue (Fig. 1E).

Around the xenomas we also detected a large number of monocytic cells ingesting spores which had probably been released as a result of xenoma rupture. The role played by these cells in the phagocytosis and active digestion of microsporean spores, and their consequent importance in the control of infection, have been described by Dyková & Lom (1980). Microsporean infections producing liquefaction of skeletal muscle have generally been attributed to species of the genus *Pleistophora* (Canning & Lom 1986, Pulsford & Matthews 1991); descriptions of this genus, however, do not coincide with the characteristics of the organism observed in this case, both in terms of the size of the spore and its nodules and in terms of the size and structure of the xenomas. On the basis of these characteristics and various others (location of the infection, presence of microvilli-type projections on the xenoma surface, and host species), we consider that the aetiological agent of the infection reported in this study might be *Tetramicra brevifilum* (Matthews & Matthews 1980).

In the hepatic parenchyma the majority of xenomas were rounded in shape with diameter greater than 200 µm (Fig. 1F). In some cases there were microvilli-type projections on the surface, but most xenomas lacked these structures and were characterized by a marked proliferation of the surrounding connective tissue (Fig. 1G), sometimes showing haemorrhage and necrosis (Fig. 1H). As noted previously, however, the pathology in the liver was not as spectacular as that in muscle.

In the intestine (Fig. 1I) the infection was limited to the *muscularis mucosa*, the xenomas displaying microvilli-type and dendritic surface projections. Free spores were also found in the *lamina propria*. In pathological terms the intestinal infection was characterized by destruction of myofibrils and considerable muscle degeneration, but to a much lesser extent than observed in skeletal muscle.

This microsporean infection of the liver and intestine in turbot, described here for the first time, might well be a result of the massive infection intensities occurring in the specimens examined. Survivors, on the other hand, regained their normal appearance within about a month, all signs of the disease appearing.

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Fig. 1. *Scophthalmus maximus.* Macroscopic effects of microsporean infection in turbot. (A) Xenomas in the dorsal fin. (B) Xenomas in the liver The small whitish nodules (arrows) are the points of infection. (C) Groups of microsporean spores showing the large posterior vacuole (pv) (scale bar = 5 µm). (D) Section of a xenoma in connective tissue among muscle cell, showing microvilli-type structures (arrows) on its surface (H&E; 800 ×). (E) Severe muscle degeneration around a xenoma (arrow) (H&E; 80 ×). (F) Section of hepatic tissue showing rounded microsporean xenomas (arrows) (H&E; 32 ×). (G) Detail of a secondary xenoma surrounded by thick capsule of connective tissue (c) in liver (H&E; 160 ×). (H) Histological section of hepatic tissue showing haemorrhagic zones (arrows) around xenoma (H&E; 160 ×). (I) Xenomas (arrows) located within the *muscularis mucosa* (mm) of the intestine (H&E; 32 ×)

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