

***Hepatoxylon trichiuri* (Cestoda: Trypanorhyncha) plerocercoids in cage-reared northern bluefin tuna, *Thunnus thynnus* (Osteichthyes: Scombridae)**

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Northern bluefin tuna (Thunnus thynnus) were caught by purse seine boats and brought to a farm for a rearing cycle of 1.5 years. Mortalities occurred in the first weeks of acclimatization at the facility. Parasitological examination revealed plerocercoids of the cestode Hepatoxylon trichiuri embedded in the stomach mucosa. The larvae were found in 28.4% of the fish and the mean abundance was 3.12 per fish. Histopathology revealed disseminated erosion at the site of attachment, atrophy of fundic glands, lymphocytic migration, and hyperplasia of connective tissue in the lamina propria. These symptoms, however, together with the parasite abundance, could not have triggered the mortalities. This is the first record of this parasite in northern bluefin tuna.

Key words: histopathology, bluefin tuna, *Hepatoxylon trichiuri*, Cestoda

INTRODUCTION

Cage rearing of northern bluefin tuna (*Thunnus thynnus*) in the Adriatic Sea is a relatively recent and growing type of aquaculture. Tuna are caught from the wild and raised intensively in floating semi-offshore cages for six months to 1.5 years. Fish are fed fresh anchovies and other mixed small pelagic fish or frozen imported herrings. Mortality is usually observed during the acclimatization period and is presumed to be due to the lengthy transportation and handling period during transfer to the cages. Sudden or severe changes in abiotic parameters such as temperature, weather conditions, and oxygen also can induce mortality and, so far, only a small proportion of mortalities in farmed tunas have been attributed to pathogens (SAWADA *et al.*, 2002). The majority of studies concerning tuna

pathogens are parasitological reports from wild tuna populations or recent findings in farmed fish (MUNDAY *et al.*, 2003; MLADINEO & TUDOR, 2004; DEVENEY *et al.*, 2005; MLADINEO, 2006).

Hepatoxylon trichiuri (Cestoda: Trypanorhyncha) has been isolated from diverse geographic areas and environmental conditions: from deep-water fish (KLIMPEL *et al.*, 2001) to northern sea fishes such as redfish (*Sebastes mentella*) in Irminger Sea (BAKAY & MELNIKOV, 2002), Atlantic salmon (*Salmo salar*; BAKKE & HARRIS, 1998), and swordfish (ROSSO *et al.*, 1997). It is mainly regarded as a biological tag for identification of fish stock arriving from southern parts (SEWELL & LESTER, 1995; OLIVA & BALLÓN, 2002), while no histopathology associated with infection has been reported.

Merluccids (*Macruronus magellanicus*, *Merluccius hubbis*, *M. gayi*, *M. capensis*,

Macromesistius poutassou) harbor plerocercoids of *H. trichiuri* in the visceral cavity in a variety of prevalence and abundance ranges (KRZEPTOWSKI, 1980; KUSZ & TREDER, 1980; SARDELLA & TIMI, 1996; OLIVA, 2001). The parasite was found encapsulated in the mesenteries of orange roughy (*Hoplostethus atlanticus*) from Australian waters (LESTER *et al.*, 1988). Among tuna species, *H. trichiuri* has been recorded only from albacore (*T. alalunga*; JONES, 1991) and yellowfin tuna (*T. albacares*; BUSSIERAS & BAUDIN-LAURENCINE, 1973), species not found in the Mediterranean.

MATERIAL AND METHODS

Bluefin tuna (12-15 kg) were caught near Jabuka Island, Adriatic Sea, at a depth of 20 m by commercial fishers and brought to the farm on the northwestern part of Brač Island. Mortalities occurred during the capture and towing process, the transfer to cages, and the first week at the facility. Dead fish were collected for parasitological examination. Plerocercoids of the cestode *H. trichiuri* were isolated from stomach mucosa and identified according to KHALIL *et al.* (1994). It was suggested that the plerocercoids

caused the mortality since these parasites had not previously been observed in bluefin tuna.

Infected tissue was fixed in modified DAVIDSON's fixative and processed for routine histology. Tissue was dehydrated in increasing concentrations of ethanol, embedded in paraffin (Histowax, Leica), cut into 5-8 μm sections, and stained with hematoxylin and eosin. Each section was examined under a compound microscope at 100 and 400x magnification. Photographs were made using an Olympus C-4040 digital camera and processed with Olympus DP-Soft software.

RESULTS

Hepatoxylon trichiuri larvae were found in 28.4% of the examined tuna. Mean abundance was 3.12 per fish. Parasites were easily visible, pale yellow, 20.2 ± 3.1 by 0.4 ± 0.2 mm, and difficult to detach from the tissues in which they were found (Fig 1). They were found mostly in the fundic region of the stomach.

Histological sections had erosions and deterioration of the mucosal columnar epithelium at the attachment site of the plerocercoids (Fig. 2). At sites where bothridial hooks penetrated the gastric glands,



Fig. 1. *Hepatoxylon trichiuri* from the stomach wall of northern bluefin tuna (*Thunnus thynnus*)

the glands were atrophied and had sloughed off. Neighboring glands had compensatory hypertrophy and evidence of extensive mucus production. Surface erosion was apparent, with an overlying mass of detritus and secondary bacterial infection. Focal hemorrhages were observed in younger processes but connective tissue layers were not thickened. The submucosa under these erosions were thickened and numerous lymphocytes were present, however, the erosions did not penetrate the submucosal stomach layer. The reaction in the *lamina propria* consisted of hyperplastic thickening of its layers. The parasite did not induce formation of a connective tissue pseudocyst or capsule and there was no observable macrophage accumulation.

DISCUSSION

Based on the histological changes associated with the *H. trichiuri* infection and the level of

abundance detected in this study, it is extremely unlikely that the infection led to the mortalities. The stomach erosions did not involve deep stomach layers, so no ulcerations could be expected to have formed. Haemorrhages were focal and present in the early stage of infection (during attachment of the plerocercoids) and appeared to cease in later stages. Later, only a minor connective tissue reaction and lymphocyte infiltration were observed. The atrophy of gastric glands was limited to the attachment site and did not impede ongoing secretion processes in non-infected stomachs.

The plerocercoids induced local changes in the stomachs with a mild inflammatory response. In stressful rearing conditions, such shallow erosions can become the entry site for secondary pathogens (particularly bacteria) and thereby threaten the health of the host. Pathological effects, combined with secondary infections that may occur during the 1.5 year rearing cycle, require assessment, though in this

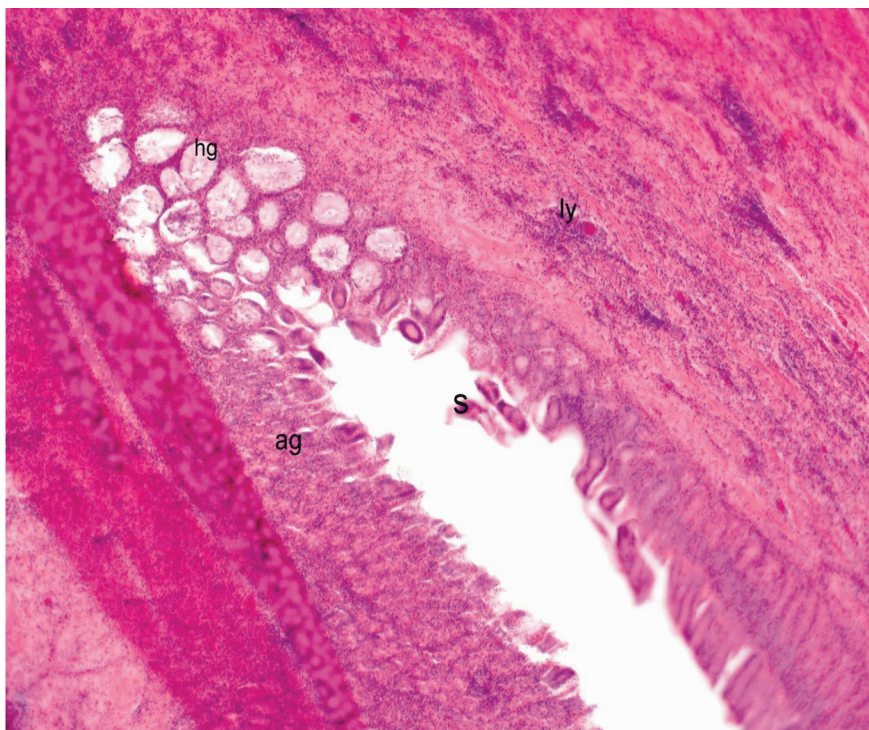


Fig. 2. Changes in stomach layers at the *Hepatoxylon trichiuri* attachment site: disruption of the epithelial layer with sloughed columnar epithelium (s), atrophied fundic glands (ag), compensatory hypertrophied fundic glands in a deeper layer (hg), and lymphocyte infiltration (ly)

case mortalities were probably related to post-capture trauma and stress.

It is worth noting the very nonspecific infection site of the plerocercoids. Pelagic fish usually harbor plerocercoids in the visceral cavity after the parasite penetrates the stomach wall. In the studied case, they were found mostly in the fundic region of the stomach. One possible explanation is that the plerocercoids were isolated during the penetration process, however no parasites were recovered from the visceral cavity, suggesting that the parasite actually lives and inhabits the stomach without migrating in the viscera. However, the histological changes are not strong evidence for a prolonged process since no connective

capsule was formed at the attachment site as usual in chronic parasitic infections.

ACKNOWLEDGEMENTS

The preliminary study and collected data could not have been achieved without the enormous technical support of the staff at the tuna facility, especially its former employees Mr. Ivor JEFTIMIADES and Ms. Ivana MILETIĆ. The study was part of the project "Biological and Ecological Characteristics of New Species in Aquaculture" founded by the MINISTRY OF SCIENCE, EDUCATION AND SPORT of the REPUBLIC OF CROATIA.

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Received: 26 January 2006

Accepted: 27 March 2006

Nalaz plerocerkoidne trakavice *Hepatoxylon trichiuri* (Cestoda: Trypanorhyncha) u kavezno uzgojenoj sjevernoatlantskoj plavoperajnoj tuni *Thunnus thynnus*

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SAŽETAK

Plavoperajne tune (*Thunnus thynnus*) ulovljene su plivaricama i tegljene do uzgajališta za uzgojni ciklus u trajanju od godine i pol dana. Parazitološka pretraga je provedena na uginućima nastalim tijekom prvih tjedana aklimatizacijskog razdoblja na uzgajalištu, otkrivajući plerocerkoidne trakavice *Hepatoxylon trichiuri* uklopljene u sluznicu želuca. Ličinke su izolirane u 28.4 % riba, srednje abundancije od 3.12 po ribi. Cilj istraživanja je bio utvrđivanje poveznosti uginuća i količine nametnika, odnosno histopatološki učinak plerocerkoidna na domaćina. Histopatološki nalaz otkrio je diseminirane erozije na mjestu prihvatanja nametnika, atrofiju fundusnih žlijezda, migraciju limfocita i hiperplaziju vezivnog tkiva *laminae propriae* želuca. Međutim, ove promjene zajedno s nađenom abundancijom nametnika nisu mogle potaknuti uginuća. Ovo je također i prvi nalaz ovog nametnika u plavoperajnoj tuni.

Ključne riječi: histopatologija, plavoperajna tuna, *Hepatoxylon trichiuri*, Cestoda