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Histopathological Changes in the Eyes of *Aphanius vladykovi* (Teleostei: Cyprinodontidae) Infected With *Ornithodiplostomum* sp.

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Abstract: Histopathological changes in eyes of a native fish *Aphanius vladykovi*, naturally-infected with metacercariae of *Ornithodiplostomum* sp. were studied. The specimens were collected from Gandoman lagoon in west Iran. A total of 142 fish were studied during spring and summer 2010. According to the results, all studied fish were infected with the parasites. The eyeballs of infected fish were fixed and processed for routine histological investigations. Histopathological changes and tissue reactions included degeneration and necrosis of external layer of cornea, inflammatory response to the parasite, fibrosis around the cilliary bodies. Histopathological changes induced by the parasites would adversely affect the proper functioning of the eye of the host fish.

Key words: *Ornithodiplostomum* sp. · Histopathology · *Aphanius vladykovi* · Gandoman Lagoon

INTRODUCTION

Aphanius vladykovi which is also called Zagros tooth carp is found as a native fish in the Choghakhor and Gandoman Lagoons [1, 2] and Beheshtabad River [3], in the upper reaches of the Karun River basin. Presence of the fish in the upper Marun River and the upper Khersan River is also proved [4]. These localities could serve as genetic reservoir due to the limited geographic distribution. The fish is now listed as a vanishing species and needs a particular attention to be preserved. Parasites of the eyes of fresh and brackish water fishes of Iran were first studied by Barzegar *et al.* [5]. Previously, little attention was paid to the eye parasites of fishes in Iran and the records were limited mostly to infection and disease caused by *Diplostomum spathaceum* metacercariae in the lens of eyes of several freshwater fish species. However, extensive studies have recently been carried out on the parasites of eyes in riverine and lacustrine fishes inhabiting waters in the Zagros mountain area in western Iran. Therefore, our knowledge about the parasites of eyes of fish has increased enormously and several new species have been identified [3, 6-9]. Members of Diplostomatidae family have a complex life cycle with a water snail (Lymnaea) acting as the first intermediate host [10]. Larval parasites, cercariae, are released from the infected snails and actively seek out fish which act as the second intermediate host and it is the invasion of cercariae which can cause such extensive damage in the fish. The snails can release millions of cercariae over a very short period of time resulting in an overwhelming invasion of the fish. The cercariae mostly penetrate through the body surface, eyes and gills of the fish and migrate to their target organ. On their migration through the tissues of the fish to their final destination, the parasites cause severe damage and this results in the affected fish swimming abnormally (often side swimming), becoming dark, lethargic, off their food and often rapidly developing serious spinal deformities. The economic significance of the eye parasites is associated with specific effects or non-specific side effects of them, including impairment of vision that leads to exophthalmia, cataract and in some cases complete collapse of the eye, which may be the cause of growth inhibition and death of significant portions of fish. Heavy infestation of *Aphanius vladykovi* with metacercariae of the trematode *Ornithodiplostomum* sp. in Gandoman lagoon was reported before [2] as all the fish specimens were infected with the parasite during spring and summer 2010. This study, as a supplementary study, is aimed to investigate the histopathological changes induced by *Ornithodiplostomum* sp. in the eyes of a naturally-infected native fish.

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MATERIALS AND METHOD

Fishes were caught from the Gandoman lagoon and were transported the same water to the laboratory where the fish were anesthetized by eugenol and killed instantly. We reported different parasites including metacercariae of *Ornithodiplostomum* sp. in *Aphanius vladykovi* before (2) and in this paper only histopathological lesions of the parasite are described. For this purpose the eyeball was removed from the fish and microscopic examination was carried out under the light microscope.

Identification of parasites was carried out in accordance with the keys given by Bauer, 1978 and Bray, *et al*. [11, 12].

For histological study, the eyeball was immediately preserved in 10% buffered neutral formalin. The amount of fixatives was 10 times volume to the bulk of the tissue fixed. The samples were then taken in a perforated plastic holder covered with perforated steel plates. Dehydration, clearing and infiltration processes were carried out in an automatic tissue processor using a series of graded ethanol, two changes of chloroform and, finally, in three consecutive series of molten wax. The samples were then embedded in paraffin wax and sectioned at 5 µm thickness. Sections were stained with Meyer's hematoxylin and eosin and mounted with Canada balsam. The prepared sections were then examined under a light microscope (Olympus, Japan) for general histological studies [13]. In this study, only fish heavily infected were selected. For comparative purposes, the eyes from uninfected fish were also processed simultaneously.

RESULTS

A total number of 142 fish were studied during spring and summer 2010. All fish samples were infected with *Ornithodiplostomum* sp. Histopathological changes in the eyes are presented in Figures 1 and 2. Despite no gross change in fish's eyes, different histopathological changes were observed in fish including degeneration

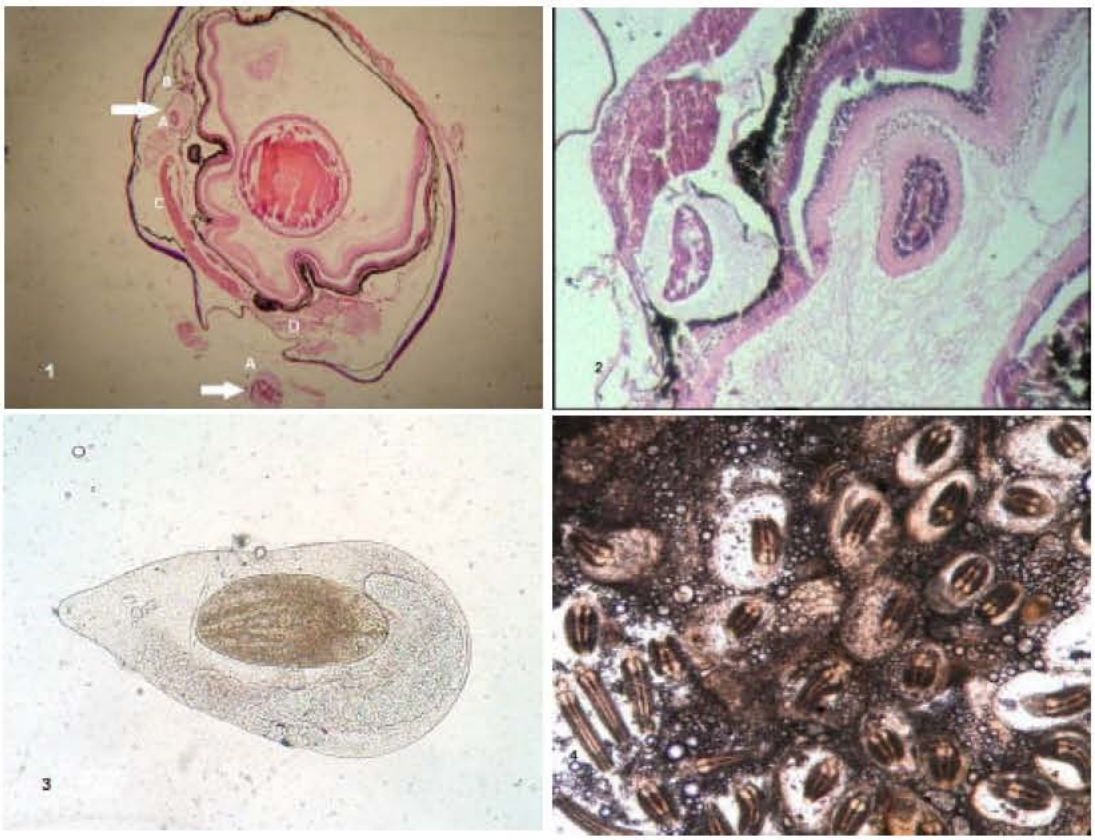

Fig. 1: A, Cross section of the parasite in anterior chamber. B, mild inflammatory response around the parasite. C, Degeneration and necrosis of external layer of cornea. D, fibrosis around the cilliary bodies (44X)

Fig. 2: Cross section of the parasite in anterior chamber and vitreous humor (70X)

Fig. 3: Encysted metacercariae (175X)

Fig. 4: Aggregation of parasites in abdominal cavity of infected fish (64X)

and necrosis of external layer of cornea, inflammatory response to the parasite, fibrosis around the cilliary bodies. Metacercariae were found in posterior chamber in vitreous humor, attached to retina. No histopathological change was found in lens of eyes of infected fishes. Parasites were also found in different organs of fish (Figures 3 and 4). The metacercariae of the parasite was found in gills, brain, esophagus and intestinal wall, gonads and muscles.

DISCUSSION

Various species of parasite at different stages of life may be found in the eye and associated structures of fish. Roberts, 2001 stated that few *Myxobolus* spp. infected the sclera (*M. hoffmani*, *M.scleroperca*), anterior chamber and iris (*M. couseii*) of both fresh- and seawater fishes of Canada [13]. Vitreous humor, lens and retina are the predilection site of several metacercariae of Strigeidida order, including *Diplostomum*, *Ornithodiplostomum* and *Tylodelphys*, where pressing against the cornea and other orbital locations cause collapse of the eye. Probably the most significant damage, called worm cataract and subsequent growth retardation, caused by *Diplostomum* spp. *D. spathaceum* is the most well known fish digenean parasite in Iran and has been frequently reported from different freshwater fish species [3, 6-9].

In this study *Ornithodiplostomum* sp. was found in many organs of *Aphanius vladykovi* including vitreous humor of eyes. Despite, from epidemiological point of view, members of the family Diplostomatidae are not host specific the parasite was not found in other fish species in Gandoman lagoon. Migration of *Ornithodiplostomum ptychocheilus* to brain of fathead minnows was studied by Matisz *et al.* [14]. They used a serial necropsy approach to characterize the migration of *Ornithodiplostomum ptychocheilus* from the point of cercarial penetration, to encystment within the outermost tissues of the brain of fathead minnows. They indicated that Diplostomules utilized peripheral nerves to access the central nerve cord, or they used specific cranial nerves to directly access the brain [14]. They reported no metacercariae in the eyes of infected fish, although some researchers believe that direct penetration of cercariae or presence of the parasite in eyes is relatively common and some cercariae may remain in the eye, eventually encysting as Hendrickson, 1978 have reported [15].

Different histopathological changes were observed in fish including degeneration and necrosis of cornea, inflammatory response to the parasite, fibrosis around the cilliary bodies. The parasites were found in posterior chamber in vitreous humor, attached to retina. No histopathological change was found in lens of eyes of infected fishes.

Exposure to parasites in nature is gradual and thus fish carry metacercariae of different ages. Nevertheless, since fish develop immunity against the parasite it is likely that a majority of parasites establish in a fish before the development of immunity [16-19]. Thus, the parasites within one fish may reach infectivity to birds at approximately the same time and an abrupt change in the susceptibility to predation may be seen in field conditions.

In conclusion, the major features of parasitic infections in the eyes of the studied fish in this study or similar studies mostly include destruction of structures in eyes by feeding and movement of the metacercariae. Such lesions can severely stress the fish, or can even lead to death. This could also lead to reduced growth and secondary infection affecting the survival of fish. According to the results the histopathological changes induced by the infections of parasites would adversely endanger the fish population in Gandoman lagoon.

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