Vol. 17, No. 1, Spring, 2021, 106-110
DOI: 10.22055/IVJ.2020.237783.2279
Journal Homepage: http://www.ivj.ir/

Case Report

Histopathological study of gill and fish treatment in Ichthyophthiriasis of white skirt tetra (*Gymnocorymbus ternetzi*)

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Received: 02.07.2020 Accepted: 31.12.2020

Abstract

In November 2019 five samples of diseased tetra fish from a fish propagation center were referred to the faculty laboratory. Diseased fish exhibited irritation, flashing, anorexia, respiratory distress (gasping) and some fresh sore on body surface, especially head region. In this study, the classic sign of Ichthyophthiriasis (i.e., white spots) was not seen on fish skin, but pathological changes and a significant number of the parasite was seen on the gills and operculum region. The density of infestation was greater in gills and in fins and skin the intensity of the parasite was lower. The tissue scraping was examined by photomicroscope and large (50 to 1000 μm) trophonts were seen in wet and Giemsa stained dry smears. In histopathologic sections, the parasites have elicited moderate gill cellular reactions, hyperplasia and shortening of the secondary lamella. Theront or early trophont stage was seen attached to the basal layer, under the epithelium of the gill. The fish stock was successfully treated by changing the water salinity, elevating the water temperature and Lateux-Meyer mixture bath.

Key words: Histopathology, Gill, Ichthyophthiriasis, White Tetra, Aquarium

Introduction

The outbreak of Ichthyophethiriasis is very dangerous and requires effective treatment otherwise may result in heavy mortality (Matthews, 2005). However, till now there is no vaccine or prophylactic treatment available, repetitive treatments with chemical substances such as salt and formalin are needed to control the infection (GersdorffJørgensen, 2017). The common clinical signs of Ichthyophthiriasis are the characteristic disseminated white surface lesions (spots). In cases where the infection is restricted to the gills, these are not visible. Usually in the early stages of infection, large numbers of theronts can harm the fish before the parasite becomes visible on the skin. In these cases, death is caused by massive damage to the gill epithelia (Lemos, 2007).

The *I. multifiliis* distribution has probably been facilitated by the importing and translocation of cultured and ornamental fish. However, most species of freshwater fish are susceptible to Ichthyophthiriasis (Jalali et al. 2008, Kim et al, 2002, Ogut et al. 2005). It is the first report of this disease

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in white tetra. The susceptibility of different fish species to Ichthyophethiriasis is different, some fishes are more susceptible. Although this parasite has worldwide distribution and previously reported in some of local fish species such as Benny (*Mesopotamichthys sharpeyie*) in Ahvaz, but histopathology and treatment of this disease was not reported in aquarium fishes of the region so far.

Case Description

A case of white skirt tetra fish with sudden behavioral change with skin and opercula damage, with no fish mortality from a fish propagation center was referred to the faculty laboratory (veterinary school, Ahvaz, Iran). For diagnosis five samples of diseased fish (12±7 g) were examined. In the history, the owner said, the fish have had itching behavior (rubbing themselves to objects and tank walls) due to parasite irritation. Superficial ulcers on the operculum occurred possibly due to trauma and secondary bacterial infections (Fig. 1). According to the owner's report, his fish rearing tank has been well established, with partial water changes. They were testing the water and fish every week with no apparent tank problem. Distress and sudden change of fish behavior were noticed by the owner, fortunately with no fish mortality. At first, the gill and skin tissue were examined microscopically by wet mounts and dry smear, stained with Giemsa. Skin and gills scrapings were fixed in methanol and after drying, stained with Giemsa. All tissue samples were fixed in 10% buffered formalin and were processed and sectioned routinely, dehydrated, cleared, embedded in paraffin, sectioned and stained with Haematoxylin and Eosin (H&E). Then, the 5 µm sections were examined using Nikon light microscopy (Roberts, 2012).

Moderate infestation to Ich trophonts was observed and large ciliated trophonts (50 to 1000 µm) were easily seen in wet mounts (Fig. 2).

Figure 1. Tetra fish infected with *Ichthyophthirius multifiliis*. There is erosion of the epithelia, leading to ulcer formation in the operculum and jaw region of the fish.

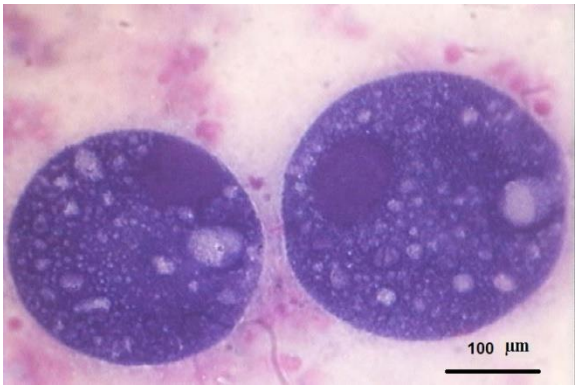

Figure 2: The mature trophont released from the affected gill tissue in Giemsa stained dry smear.

Microscopic examination revealed uniformly ciliated organisms with crescent-shaped macronucleus, up to 1 mm in diameter that is characteristic of the monotypic ciliate, *Ichthyophthirius multifiliis.* Also, in stained smears and histopathology sections of gills revealed large number of parasites. The infected secondary gill lamellae appeared shortened, hyperplastic with severely damaged epithelial cells. In the sections, the trophont stage of the parasite was observed that covered by two or three layers of epithelial cells.

The results showed that the parasites have elicited minor gill cellular reactions. The extensive hyperplasia and shortening of the secondary lamella were the most important visible histopathological changes. Theront or early trophont stage

was seen attached to the basal layer, under the epithelium of the gill. In the secondary gill lamellae, moderate aggregation of mononuclear inflammatory cells, hyperplasia of epithelial cells and cell atrophy and necrosis were seen around the parasite (Figs 3, 4 and 5).

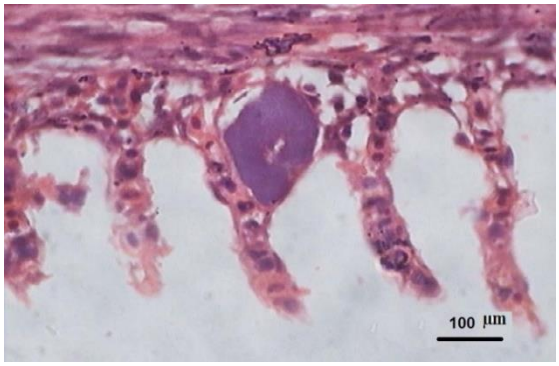

Figure 3. Theront or early trophont stage attached to the basal layer, under the epithelium of the gill (arrow). Mononuclear inflammatory and hyperplastic cells aggregation can be seen around the parasite. Atrophy and necrosis of the secondary lamellae can also be seen (H&E staining).

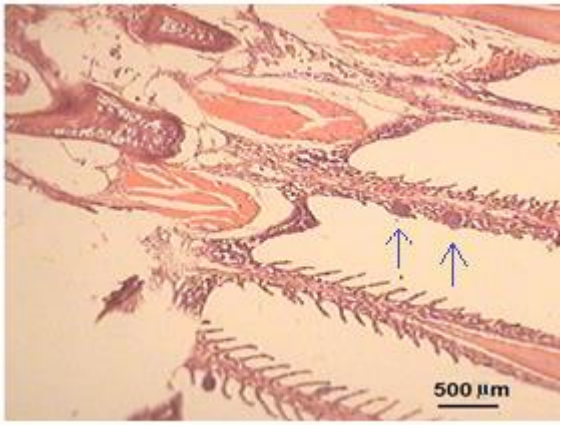

Figure 4. Primary and secondary lamellae of gill in tetra with moderate inflammatory reaction can be seen. Note the presence of the parasite (arrows) under the epithelial cell (H&E staining).

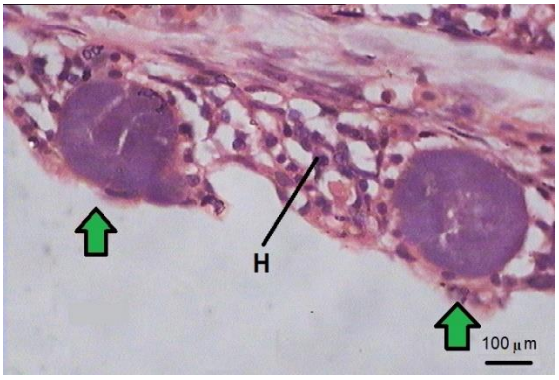

Figure 5: Gill primary lamella with 2 trophont (arrows). The parasites have elicited gill cellular reactions. The gill hyperplasia (H) and shortening of the secondary lamella can be seen (H&E staining).

The fish stock was successfully treated by changing the water salinity to 5 ppt, elevating the water temperature to 29-30 ºC gradually (2-hour interval). Fishes were treated with Lateux-Meyer mixture long term bath (25 ppm formalin and 0.1 ppm malachite green) for 24 hours.

Discussion

The operculum damage was very obvious in the affected cases that may due to irritation of the parasite and hypoxia caused by the parasite since the fishes move their gill opercula more rapidly in an attempt to obtain more oxygen. Since in comparison with skin, usually, there is very little mucus that covers the secondary lamella. In many species, the gills are more susceptible to infection than other organs. Mucus composition and quantity depend on fish species and environmental factors. Genetic factors in each species can affect the amount and composition of fish mucus (Swennes et al, 2006). Surface mucus acts as an important physical barrier. It also contains many chemicals, mucus antibodies and anti-parasitic factors.

Our result showed that, the fishes were in the early stage of the disease. Mild infestations usually cause little pathologic effect but in heavy infestations, can cause serious gill damage (Lemos et al, 2007). Under stressful condition, young non-immune fishes appear to be more

susceptible so the disease can quickly be overwhelming a susceptible fish population (Xu et al, 2002, Zhao et al, 2008).

In practice, formalin and malachite green treatment is the most reliable methods of treatment of Ichthyophthiriasis (Roberts, 2012, Schachte, 2011). Our experience showed that a saltwater bath can dislodge superficial or unattached Ichthyophtherius and is especially useful for small fish. In practice, depends to aquaculture system and facilities, a variety of chemicals such as hydrogen peroxide, peracetic, acetic and peroctanoic acid have been used for treatment of this disease (Gersdorff Jørgensen, 2017, Picón-Camacho, et al. 2012).

In many studies, natural herbal compounds such as polyphenol curcumin, that obtains from the rhizome of *Curcuma* and extract of *longa Polygonum cuspidatum*, identified as emodin have had some therapeutic effects. The efficacy of curcumin was evaluated good *in vitro* and *in vivo* which resulted in 100% mortality of I. *multifiliis* theronts at a concentration of 1 mg/L (Yan-Meng, et al., 2017, Sheng-Yu, et al., 2018). Also *in vitro* study on the antiparasitic effect of active compounds isolated from *Zingiber officinale* against *I. multifiliis* showed that the compounds identified as 10-gingerol, 6-dehydroshogaol, and 6-dehydro-10-gingerol. 10-gingerol had antiparasitic efficacy on theronts with 100% mortality (Yao-Wu, et al, 2019).

However, using only salt treatment (5 ppt) cannot kill or completely remove the parasite from the fish. However, the use of salt may help in osmoregulation and may help fishes maintain osmotic balance, thus reducing osmotic stress (Miron et al, 2004). In our suggested treatment the fishes were treated with combination of formalin and malachite green and the water was changed 24 hours after each occasion. Malachite green is a potential carcinogen and it is not approved for use in edible fishes. Therefore, it must be handled with care. In our case treatment, the malachite green contaminated water discarded safely (did not discharged to municipal drain).

Acknowledgement

We wish to acknowledge the Shahid Chamran University of Ahvaz for financing this work. We thank the pathology technician for assistance in preparing the tissue sections

Conflict of interest

The authors declare that there is no conflict of interest.

Funding

This work was financially supported by the Veterinary Faculty, Shahid Chamran University of Ahvaz (grant no: SCU.VC98.413).

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Received: 02.07.2020
Accepted: 31.12.2020

DOI: 10.22055/IVJ.2020.237783.2279

بررسی هیستوپاتولوژیک آبشش و درمان ایکتیوفتریوزیس در ماهی تترای سفید (*Gymnocorymbus ternetzi*)

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تاریخ دریافت: ۱۳۹۹/۴/۱۵ تاریخ پذیرش: ۱۳۹۹/۱۰/۱۱

چکیده

در آبان ماه سال ۱۳۹۸، پنج قطعه ماهی تترا سفید از یک مرکز تکثیر ماهیان آکواریومی به بخش آبزیان ارجاع داده شد. ماهی‌ها دچار خارش و تحریک جلدی، حرکات عصبی، زخم در نواحی اطراف آبشش، بی اشتهایی و اختلالات تنفسی (آمدن به سطح آب و بلع هوا) بودند. در بررسی میکروسکوپی ماهیان مبتلا علایم مشخصه بیماری ایکتیوفتریوز در ماهیان مبتلا مشاهده گردید. با این حال علایم ظاهری متداول در بیماری یعنی لکه‌های سفید در ماهیان مبتلا مشاهده نگردید. در بررسی میکروسکوپیک از نظر شدت بیماری، به ترتیب آبشش‌ها، باله‌ها و پوست ماهیان مبتلا، آلودگی به انگل ایکتیوفتریوس را نشان دادند. در بررسی گسترش مرطوب انگل‌ها در اندازه مختلف (۵۰-۱۰۰۰ میکرومتر) مشاهده گردیدند. علاوه بر آن ماهیان مبتلا بیش‌ترین ضایعات در آبشش‌ها مشاهده گردید که شامل کوتاه شدن لاملاهای ثانویه، هیپرپلازی، تجمع سلول‌های آماسی و حضور انگل در لایه‌های زیر سلول‌های پوششی بوده است. ماهیان گله مبتلا با افزایش شوری آب، افزایش دما و حمام با محلول لوتکس-مایر با موفقیت درمان گردیدند.

کلمات کلیدی: هیستوپاتولوژی، آبشش، ایکتیوفتریوزیس، تترای سفید، آکواریوم

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