Infestation of green tiger barb (*Puntius tetrazona*) with *Capillaria* sp. and *Hexamita* sp. parasites in an ornamental fish farm

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Received: January 2022 Accepted: February 2022

Abstract

The green tiger barb (*Puntius tetrazona*) is a tropical ornamental fish that is very popular in research and the fish aquarium trade. In an ornamental fish farm, losses of 6 to 12 per day were reported in green tiger barb juvenile aquariums. After examining the farm environment and aquarium water quality, fish samples were taken. Euthanasia was performed and after the autopsy, wet smears from the skin, gills, and intestine were taken. In the microscopic examination of samples, internal parasites including *Capillaria sp.* and *Hexamita sp.* were detected from two different aquariums. Based on references, treatment with levamisole for *Capillaria sp.* and metronidazole for *Hexamita sp.* was started, which was associated with a reduction in mortality. After two weeks, a re-sampling of the treated aquariums was performed and the samples were negative for *Capillaria sp.* and *Hexamita sp.*. As a result, levamisole and metronidazole can be reported as suitable prescriptions for the treatment of these diseases in green tiger barb.

Keywords: Green Tiger Barb, *Capillaria* sp., *Hexamita* sp., Levamisole, Metronidazole.

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Introduction

The barb fish is one of the ornamental fish of the *Cyprinidae* family, which is small in size and originates from Malaysia, Indonesia, and its surrounding areas. In addition, many people are interested in breeding it due to its variety of colors, beauty, and easy maintenance (Li *et al.,* 2007). Tiger barb is one of the most popular species among ornamental fish. It's two green and albino strains were created due to two natural genetic mutations (Froese and Pauly, 2011). Green tiger barb (*Puntius tetrazona*) is a popular tropical ornamental fish in the fish aquarium trade and research (Liu *et al.*, 2020).

The infection with ciliate parasites like *Hexamita sp.* and with worms like *Capillaria* sp. is important findings in ornamental fishes (Peyghan, 2015).

Diplomonad flagella can involve the intestinal tract and cause skin and systemic infections to a lesser extent in shellfish, amphibians, saltwater and freshwater fish, salmonids, cichlids, gadids, and cyprinids (Woo and Poynton, 1995). Contamination with these parasites can cause significant mortality in the aquaculture industry (Poynton *et al*., 2004). These parasites have been reported from cold, temperate, and warm waters in Asia, North America, and Europe (Moore, 1922; Awakura, 1992; Sterud *et al*., 1997). The first reports of diplomonads in Europe were in 1903 of *Urophagus intestinalis* along with *Hexamitus intestinalis* from rainbow trout *Oncorhynchus mykiss* (Moroff, 1903). *Hexamita sp.* is a flagellated protozoan that is an intestinal and gallbladder parasite of a variety of cold and warm freshwater fish that commonly affects salmonid fish and is also found in common carp and ornamental fish (Aldridge and Shireman, 1987; Uldal and Buchmann, 1996; Jalali, 1997; Hajimoradloo and Sahandi, 2010). Due to their small size and rapid whipping movements, these parasites can only be seen under a microscope with a magnification of 200 or 400 times (Aldridge and Shireman, 1987). *Hexamita sp.* is probably transmitted through water contaminated with feces of sick fish (Sahandi and Hajimoradloo, 2011). The flagellated stage, which occurs after 24 hours, settles at the beginning of the intestine and after reproduction, it is transferred to other parts of the body through the blood (Mokhier, 1941; Sahandi and Hajimoradloo, 2011). The external form of this parasite causes lesions on the head and sides of the fish, hence it is known as a hole-in-head disease. Head and lateral line erosion disease (HLLE) in fish can also occur as a result of *Hexamita* sp. infection. In normal conditions, *Hexamita* sp. can be found in small numbers, even in healthy fish, and as a result of any stress, such as malnutrition, transportation, excessive density, or low water quality, it may multiply and cause disease (Sahandi and Hajimoradloo, 2011). Hexamitosis is a common subsequent infection in fish that are immune suppressed (Jalali, 1997).

Nematodes infect different species of farmed and wild aquatic animals and

are present in small numbers, often in healthy fish, but if their number increases, they can cause illness or even death (Yanong, 2002). In addition, there is a possibility of symptoms in juvenile fish infected with a small number of nematodes and it can cause a decrease in their growth rate (Yanong, 2002). *Capillaria* sp. is a zoonotic helminth parasite that infects many mammals, birds, fish, and invertebrates (Francis-Floyd and Reed, 1994). They have a direct life cycle and do not require an intermediate host (Wildgoose *et al*., 2001) and can spread from fish to fish by ingesting infective larvae (Yanong, 2002). When *Capillaria sp.* are present at high parasitic levels, they can act very aggressively, penetrate the intestinal mucosa, and induce significant inflammatory responses, causing intestinal obstruction, food restriction, and finally chronic losses (Heckmann *et al*., 1987; Kent *et al.*, 2002) that lead to intestinal obstruction and nutritional limitations that lead to chronic mortality (Wildgoose *et al*., 2001; Thatcher, 2006; Rahmati-Holasoo *et al*., 2022).

The purpose of this study is to diagnose and treat two common internal parasites in aquarium fish, namely *Hexamita* sp. and *Capillaria* sp., which cause economic losses for ornamental fish breeders.

Materials and methods

In juvenile green tiger barb aquariums at an ornamental fish farm, deaths of 6 to 12 per day were reported. After checking the health condition of the farm and the water quality of the aquariums, some of the sick fish from each aquarium, in aerated plastic bags containing the water of the aquariums, were taken to the laboratory for examination. After euthanizing, wet mounts were taken from the skin and gills immediately. Then the fish were dissected under sterile conditions and wet mounts were prepared from the internal organs. *Hexamita* sp. contamination was observed in the intestinal samples of fish from one aquarium and *Capillaria* sp. infection was observed in the intestines of fish from another aquarium. The symptoms of fish affected by *Hexamita sp.* were weight loss, anorexia, lethargy, cataracts, hyperemia of gills and impaired oxygen supply to tissues, imbalance in swimming, and then death within 14 to 16 days after infection. The wet mount prepared from the intestine was examined under a light microscope with 200x and 400x magnification. *Hexamita* sp. was observed as flagellate with fast and irregular movements (Sahandi and Hajimoradloo, 2011).

Treatment

Metronidazole with a dose of 500 mg per 100 liters of water was used to treat fish with hexamitosis, and levamisole with a dose of 200 mg per 100 liters of water was used to treat fish infected with *Capillaria* sp.. Aquariums containing infected fish had aeration, sand filter and water temperature of 25 degrees Celsius. After calculating the dosage of drugs based on the amount of water in each aquarium, the treatment

process began. After adding the drug to the water, feeding was stopped. The aquarium water contaminated with *Hexamita sp.* was changed after 24 hours and the aquarium water contaminated with *Capillaria sp.* was changed after 48 hours. The reduction of losses was evident after the start of the treatment, and after 2 weeks of the re-sampling of the fish, contamination was not seen. Since levamisole can partially destroy parasite eggs, to complete the treatment process and according to the protocol, the treatment was repeated 2 weeks later (Bassleer, 2004).

Results

In the microscopic examination of samples, parasites were not observed in the skin and gill, but internal parasites including *Capillaria sp.* and *Hexamita sp*. were detected from two different aquariums. Based on reference, treatment with levamisole (dosage=200 mg/100 lit, 24 h (Bassleer, 2004)) for *Capillaria* sp. and metronidazole (dosage=500 mg/100 lit, 48 h (Bassleer, 2004)) for *Hexamita* sp. was started, which was associated with a reduction in mortality. After two weeks, a re-sampling of the treated aquariums was performed and the samples were negative for *Capillaria* sp. and *Hexamita* sp. Chemical drugs work effectively in the treatment and control of fish parasites (Pahor-Filho *et al*., 2017). Levamisole is an anthelmintic belonging to the imidotiazol group, which helps control parasites in animals (Cruz *et al*., 2010) and it has had a positive effect on fish parasites (Findlay *et al*., 2000). Metronidazole, as an antiparasitic and antibacterial compound, is one of the most widely used drugs worldwide (Khalil *et al*., 2007) and is used to treat a wide variety of parasitic and anaerobic bacterial infections (Peyghan *et al*., 2010).

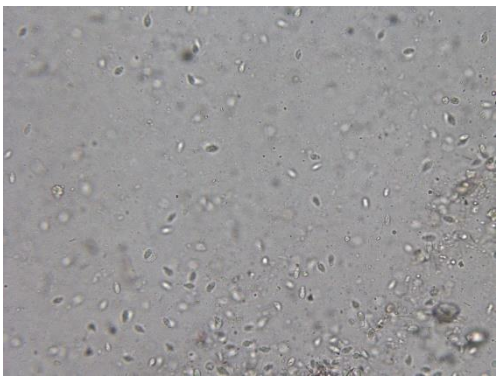

Figure 1: *Hexamita* sp. X20.

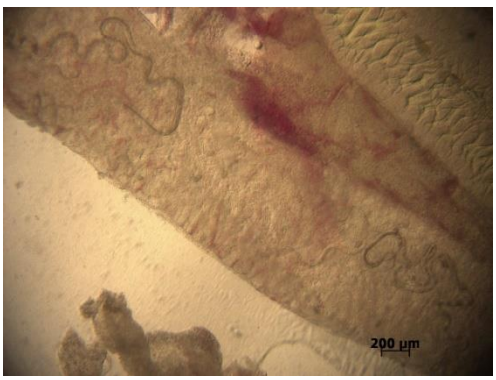

Figure 2: *Capillaria* sp. X40

Conclusions and discussion

One of the common mistakes of fish breeders is the wrong diagnosis and treatment of their fish's disease with inappropriate drugs or chemicals (Sahandi and Hajimoradloo, 2011). Based on this study, Levamisole and metronidazole can be reported as suitable prescriptions for the treatment of *Capillaria* sp. and *Hexamita* sp., respectively in green tiger barb.

Aquarium fish, like natural fish, have a limited physiological ability to deal with stress (Petrescu-Mag *et al*., 2007; Petrescu-Mag *et al*., 2008). Parasitic diseases often occur after stress caused by factors such as low water quality, water level reduction, transportation, inappropriate nutritional factors, overcrowding, inappropriate treatments or drugs, or the presence of other pathogens (Sahandi and Hajimoradloo, 2011). Proper hygiene helps prevent the spread of nematodes and reduce the burden of infection. The aquarium fish should be evaluated periodically during the breeding period for the presence of nematodes. Since live foods, such as oligochaete worms, may act as disease vectors, it is recommended that they be avoided if possible. In addition, proper hygiene of aquariums and ponds helps to reduce the frequency of infectious stages (Yanong, 2002). Biosecurity principles must be followed, including having a quarantine section and complying with its principles, segregation of the net and accessories of each aquarium, and prevention of rodents, birds, insects, and other animals from entering the farm and food storage. Every year a large number of different species of ornamental fish are imported from Southeast Asian countries to Iran. Uncontrolled import of infected live fish can lead to the transfer of *Capillaria* sp, *Hexamita* sp, and other parasites to valuable fish and severe economic losses. Therefore, it is necessary to evaluate the health of fish before exporting from these countries (Peyghan, 2015).

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