

Hole-in-head disease: New method of treatment in flower horn ornamental fish

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Abstract. In this study we generally describe the hole-in-head disease and introduce a new method of treatment that called cupping. Cupping is important and old treatment for specific diseases in human, used by Iranian and Chinese medicinists in the past. This disease, often called hexamitiasis, is caused by a member of the genus *Hexamita* or *Spironucleus*. This parasite has two forms of infection one of them is external and cause hole-in-head disease and the other form is internal that cause intestinal problems. Hole-in-head disease cause death in flower horn fishes which are expensive and very popular members of Cichlidae family. Flower horn fish is a genetically improved hybrid between *Amphilophus labiatus* and *Amphilophus trimaculatus*. This simple method of cupping combined with stress factors removal was applied by us for the treatment of external hexamitiasis resulting in more than 50% cases of fish health recovery. The first signs of health in treated specimens become visible after 4-6 days of treatment.

Key words: *Hexamita*, slimy material, cupping, old therapy, flower horn fish.

Introduction. During the history of medicine and health traditional methods and diets demonstrated effective for human or animal health (Vesa et al 2009; Jafaryan et al 2010; Luangpirom & Taweebot 2010; Luangpirom & Komnont 2011). Notably many human traditional therapies were adopted by veterinarians or aquaculturists and viceversa (Csep et al 2010; Farahi et al 2010; Sakkaravarthi et al 2010).

Diseases play an important role in cichlids cultivation and are frequently the most important profit-limiting factor in intensive aquaculture. *Hexamita* is a flagellated protozoan found in the gastrointestinal tracts of a variety of cold and warm water fish (Aldrige & Shireman 1987; Uldal & Buchmann 1996; Hajimoradloo & Sahandi 2010), including several species of cichlids which are popular aquarium pets. Hexamitiasis can be a serious health problem in angel fish and discus. This parasite lives in digestive canal and special place is first part of intestine that located after stomach (Mokhier 1941). External form of this disease cause hole-in-head. *Hexamita* is probably transmitted through the water from contaminated fecal material. The flagellated stage makes its way to the lumen of the upper intestine. In digestive canal parasites swim freely in the intestinal and fecal fluids. The parasite may be present in small numbers under normal circumstances. Generation time for the flagellated form is thought to be 24 hours. *Hexamita* can be transferred to the other parts of body by blood after increasing of their population number. Occasionally hexamita is found in healthy fish. Stress from malnutrition, shipping, over-crowding, or poor water quality may lead to rapid reproduction of the protozoan, resulting in disease. This flagellated protozoa can infect fish externally and internally. They are characterized by one or more flagella that cause the parasite to move in a whip-like or jerky motion. Because of their small size and their movement, the parasites can be observed only at 200 or 400x magnification under the microscope (Aldrige & Shireman 1987).

Hexamita is a member of Protozoa and was firstly found in Europe by Schmidt in 1920 (Mokhier 1941). Hexamitiasis is a parasitic disease that can affect both freshwater

and marine fishes. In some cases, a lesion appears on the head and flanks of the fish, hence its alternative name: hole-in-head disease. *Hexamita* infections may also be responsible for head and lateral line erosion (HLE) disease in fish. The genus *Hexamita* was formerly called *Octomitus* because of eight hair-like flagella which project from the organism (Mokhier 1941).

Among freshwater fish, hexamitiasis and hole-in-head disease most commonly affect perciform fish, particularly cichlids (Sano 1971). Carps, including koi and goldfish, are also vulnerable. A wide variety of marine fish can suffer from head and lateral line erosion, but angelfish and surgeonfish seem to be particularly susceptible. This disease has multiple names and one of them is octomitiase (Mokhier 1941) and was firstly found on trout. This parasite is known as digestive canal parasite (Axelrod 1989) and has 4 pair flagella and move with them. It reproduces by dichotomy way and can be spread into environment from anus of sick fish. It can be the cause of catarrhal enteritis (Sano 1971). Since Moore (1922) first reported a flagellate, *Hexamita salmonis*, in the intestine of trout more than ten species of commensal and pathogenic diplomonad flagellate belonging to *Hexamita* and *Spironucleus* have been described from fishes (Xiao Wu-han 1993). Base only on light microscopy flagellate parasites of fish have often been incompletely described. Various theories about this parasite's biology and pathogenicity have been proposed (Davis 1956; Uzmann & Hayduk 1963; Uzmann et al 1965). However, only a few studies have actually provided us with clear evidence of host infection, site selection and association with pathological conditions.

Flower horn fish is a genetically selected/improved hybrid between *Amphilophus labiatus* and *Amphilophus trimaculatus*. Because of its man-made origin this variety of fish does not have scientific/latin name. Some of experts think that this fish was created by physical-chemical shock induced mutations (Habibi 2009). It is an ornamental fish that cultured in south east of Asia for export to western countries (Sandford 2007). During this travel due to stress caused by weakness of transferring standards these fishes lose their ability of facing with diseases.

Purpose of Study. In this study we generally describe the hole-in-head disease and introduce a new and simple method of treatment that called cupping. Cupping is important and old treatment for specific diseases in human, used by Iranian and Chinese medicinists in the past.

Material and Method. In this study sick fishes were obtained from Sahand Aquarium Ornamental Fish Store (Tehran, Iran). There are three main aspects we should discuss here: signs of disease, autopsy and the treatment we propose for this disease in flower horn fish.

1. Signs of disease. This disease starts with physical signs such as losing weight and appetite for food. Weak or stressed fishes seem to be most susceptible to heavy infestation. With this disease, activity of flower horn fishes decrease and fishes lay on closed places and do not have normal movement. Color of fish will change into dark and crest of fish become brighter than the other parts of the body. White slimy material will often be released from gills, mouth, holes of head and anus of sick fish. After 48 hours bright layer will cover the eyes. Fish will face with cataract and after a few days sick fish will lose the eyes. Crest of fish will swell and become brighter that the other part of the head. At the end of disease process fish will face with lack of oxygen and gills replete with blood so that the transfer of oxygen to other parts of the body will be difficult. Fish swim to the upward of the water and after arrived to surface of water fall to the depth. There is no control and balance on fish swimming. Majority of sick fish without treatment will die in 14–16 days. Sick fish in all time of their life can transfer the disease to other fish; that is why the aquaculturist have to wash the hand after handling the fish. Confirmation of *Hexamita* infestation is easily done by making a squash preparation of the intestine and examining it with a light microscope at 200 and 400x. The flagellates move rapidly and erratically. They are most easily seen in areas where the mucosa is broken. If the infestation is severe they are numerous and easily found.

2. Autopsy. In autopsy, part of intestines that located after stomach in sick fishes have bright-yellow slimy material (Mokhier 1941). Preparing samples from this bright-

yellow material and biopsy with microscopy *Hexamita* parasites can be watched. Fins of the fish are dark and there is bloody tissue injury on base of fins. After infestation of host body, *Hexamita* passed to the blood and promoted to all parts of the body. Fish scales are covered by slimy material that colored to whitish or yellow. There was internal bleeding prior to death.

3. Treatment. After first sign of hexamitiasis we started treatment process. For this case we prepared a tank of 70 L capacity. Water of tank had 28°C and an automatic heater was used to control the temperature, and an air pump for oxygen supply was used all the times. Environment of the tank must be calm and lack of potential stressors of fish. After diagnosis we started to cup the crest of the host animal. Traditional small sized cups (of 20-30 mm diameter) for medical use were disinfected with H₂O₂ (not alcohol), washed with distilled water and used for cupping. We draw easily 3 superficial lines with a blade on the surface of the crest of fish. Most often this operation is not necessary due to already existing holes. About 2/3 of the crest should be absorbed by cupping with much attention, especially the affected regions of the head. After cupping, all around the injury parts become dark and white slimy material starts to be released out of this area.

After the cupping operation, we added 2% methylen blue to the water tank to avoid the infection with other pathogenic agents; this substance can be effective also for the treatment of hexamitiasis. The methylen blue treatment should be repeated during the next four days and each day 30% of water should be changed by siphoning the sediments (uneaten food and faeces) from the bottom. Maturated clean water and not directly fresh water should be used for water change operation. The maturated water added to the tank each day should be without chloramines that would be harmful for fish. Feeding plays an important role in health recovery in the first days. Portions of rich nutritional food should be of good quality (cow heart, spinach and multi vitamins) but small and repeated to not alter the water quality. The first signs of health recovery appear after 4-6 days.

Results and Discussion. Confirmation of *Hexamita* infestation is easily done by making a squash preparation of the intestine and examining it with a light microscope at 200 and 400x. The living flagellates move fast and erratically. They are most easily seen in areas where the mucosa is broken. If the infestation is severe they are numerous and easily found. The species of *Hexamita* we identified in the flower horn fish by microscopy has 8 flagella and ovate shape.

The external form of hexamitiasis is referred to hole-in-head disease and cupping recommendation will be good way for treatment. *Hexamita* parasites can migrate in any part of the body (Poppe et al 1992) and this matter is occurring by blood circulation, but crest of this fish is a sensitive organ and can be good place for parasites. For fish treatment, cupping of head can decrease amount of slimy material that contain *Hexamita* parasite and this decreasing of parasite density help fish compete with disease. Of course, for cupping we need much attention to not put the fish life in danger; due to its small size, fish can be easily killed by us. Methylen blue plays role of guard in front of other pathogens that can cause disease by this injury of cupping and it has effect on *Hexamita* parasite too.

This method of cupping combined with stress factors removal was many times applied by us for the treatment of external hexamitiasis resulting in more than 50% cases of fish health recovery. The first signs of health in treated specimens appear after 4-6 days of treatment.

Some common mistakes of fish culturists are misdiagnosing disease problems and treating their sick fish with the wrong medication or chemicals. This disease is most often started by stress. Environmental effects such as poor quality of water, decreasing of water level, shipping, poor nutritional factors, overcrowding, improper treatments or drugs, or the presence of other pathogenes can grind this disease. Drugs such as antibiotics do not have enough effect on eucaryotic parasites and moreover because of continue using of antibiotics *Hexamita* demonstrated resistant in front of antibiotics. This study shows among others that *Hexamita* is a secondary pathogen that causes disease

by physical weakness of fish. As this parasite is primarily intestinal, one that starts its activity from intestine, maybe the use of antibiotics, probiotics or other traditional methods of treatment in fish diet can assist this fish to reduce the risk of disease. Among the chemicals frequently used for hole-in-head disease therapy are: dimetridazole, metronidazole, pyrimethamine, albendazole, fenbendazole and magnesium sulfate or different combinations of these chemicals. Dimetridazole seems to be the best of them; administration of dimetridazole per L of water should be 100mg L⁻¹; treatment should be repeated to each 12 hours.

An important stressor is shipping. After any transferring of flower horn fish we must take care of fish feed quality; rich nutritional food is required (cow heart, spinach, multi vitamins or specific high quality feeds from the market).

Water quality should be maintained at optimal parameters. For this safe filtration that can kept water quality in normal range is important. Range of pH should be 6.5 to 7.0 and temperature of water 26 to 30°C. This features can be achieved by standard measurements, filtration and heating methods/systems for aquarium fish keeping.

Measures of prevention. In aquarium as in the wild, fish has limited physiological capability to fight against stress (Petrescu-Mag et al 2008, 2007; Petrescu-Mag & Petrescu-Mag 2010). Beside the stress factors removal and good maintenance practices, hole-in-head disease can be controlled by attention to new fish that enter into aquarium. The use of probiotics significantly contribute to fish health, indirectly affecting their ability to fight against different kind of diseases.

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