

Isolation of *Tetrahymena pyriformis* From Several Infected Pet Fish Species And a Regime For Its Treatment.

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Summary

Tetrahymena pyriformis has been firstly isolated from brood Goldfish (*Carassius auratus*) Common, Fantail, Ryukin, Veiltail and Moor, Black molly (*Poecilia sphenops*), Gourami (*Trichogaster trichopterus*), red Swordtail (*Xiphophorus helleri*), Guppy (*Poecilia reticulata*), deep Angelfish (*Pterophyllum scalare*) and brood Algae eater (*Hypostomus plecostomus*) in Iraq. The infected fish suffered from mucus hypersecretion large hyperemic areas on the skin, and slight shedding of the scales. The incidence percentage of infection was 100 %. Rising water temperature was up to 29 – 30°C for one time and methylene blue was at a concentration of 5 ppm for 24 hours. Sodium chloride at a concentration of 2 % for one minute and two minutes has no obvious effect on *Tetrahymena pyriformis*. Treatment with NaCl at the concentration of 1% for 20 minutes for three consecutive days achieved the best results.

عزل *Tetrahymena pyriformis* من عدة أنواع من اسماك الزينة المصابة و أسلوب
لعلاجها.

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الخلاصة

تم عزل *Tetrahymena pyriformis*، لأول مرة في العراق، من أمهات السمكة الذهبية (*Carassius auratus*) والمولي الأسود (*Poecilia sphenops*)، الجورامي (*Trichogaster trichopterus*) وذنب السيف الأحمر (*Xiphophorus helleri*)، و Guppy (*Poecilia reticulata*) وسمكة الأنجل (*Pterophyllum scalare*) و أمهات الكناس (*Hypostomus plecostomus*) ، التي كانت تعاني من افراط فرز المخاط ، احتقان مساحات كبيرة على الجلد وسقوط خفيف للحراشف. كان معدل نسبة الإصابة 100% حيث كانت كل الأسماك مصابة. لم يكن لرفع درجة حرارة الماء إلى 29-30 م° لمرة واحدة وإضافة المثيل الأزرق بتركيز 5 جزء بالمليون لمدة 24 ساعة تأثير في *Tetrahymena pyriformis*. وكذلك كلوريد الصوديوم بتركيز 2% لمدة دقيقة واحدة ودقيقتين. العلاج بواسطة NaCl بتركيز 1% لمدة 20 دقيقة لثلاثة أيام على التوالي كان له أفضل النتائج.

Introduction

Tetrahymena pyriformis, synonym Guppykiller, is a free-living ciliated pear-shaped protozoan that becomes only a problem at times of overcrowding and poor water quality (1,2). Anterior end pointed, posterior rounded, 53 (30 to 70) μm long by 20 to 45 wide. uniformly covered by cilia arranged in 27 (23 to 29) meridional rows; 1 or 2 rows reaching only to posterior border of buccal apparatus, the rest reach anterior pole or suture above buccal apparatus which has an undulating membrane of coalescent cilia and with 3 obliquely set membranelles, each 3 ciliary rows wide. The buccal apparatus locate near the anterior pole of the body. The parasite has an 11- by 9- μm It has oval to round macronucleus lies near a 2 to 3 μm micronucleus (3).

It has a worldwide distribution and affects a wide range of hosts, particularly live-bearing fishes, cichlid, and various tetra species. The Dwarf cichlid appears particularly susceptible (1).

Infestations show signs of necrosis and hemorrhagic areas on the skin. Occasionally, tissues surrounding the eye are invaded, resulting in exophthalmus (1). In severe cases the parasites rupture the body walls and the fish eviscerate. Histologically there is massive invasions of the skin, musculature, and viscera by this organism; in some there was marked inflammation, but in others there was little tissue reaction (3,4).

Tetrahymena has been reported from fishes, amphibians, baby chicks, and possibly from man (5).

Mortalities resulting from this parasite are difficult to be controlled due to its ability to burrow deeply into the skin of host which protects the parasites from chemotherapeutics, (2). Baths with external parasiticides such as formalin may reduce external parasites but have no effect on internal parasites (1).

Materials and Methods

Fish used : 12 brood Goldfish (*Carassius auratus*) Common, Fantail, Ryukin, Veiltail, and Moor with a length of 9.5-14 cm. , 4 red Swordtail (*Xiphophorus helleri*) with a length of 7-7.5 cm., 1 Gourami (*Trichogaster trichopterus*) with a length of 6.5 cm. , 3 Black molly (*Poecilia sphenops*) with a length of 4-5 cm., 7 Guppy (*Poecilia reticulata*) with a length of 2.75-4 cm., 9 deep Angel fish (*Pterophyllum scalare*) with a length of 2-2.5 cm. , 8 brood Algae eater (*Hypostomus plecostomus*) with a length of 6-11cm. The fish held at first in 120 liters glass aquarium supplemented with dechlorinated artificially oxygenated tap water (coarse air bubble), and fed powder fish feed. Water temperature was $17\pm 2^{\circ}\text{C}$ After the clinical signs began and the mortalities start the fish were transferred to 260 liters aquarium supplemented with dechlorinated artificially oxygenated tap water (fine air bubble), fed pellets and crushed

shrimp. Water temperature was $20 \pm 2^{\circ}\text{C}$ The fish were brought from an earth pond which was supplemented with water coming from carp ponds.

Fish examination: clinical examination was done on the living fish in the aquarium; they were grossly examined for the detection of any external clinical signs. Microscopical examination was done by taking mucus scrapes from both sides of the fish and from fins and transferred into glass slides and then examined under compound microscope with a magnification power of X100 and X400, according to (6) and (7). Incidence percentage of infection was calculated according to (8). Physical treatment: Half of the aquarium water was changed and the water temperature was then risen up to $29 - 30^{\circ}\text{C}$ for one time.

Chemical treatment: The infected fish were treated with methylene blue (medical quality) at a concentration of 5 ppm. Commercial sodium chloride (NaCl) were used at a concentration of 2% for one minute, 2% for two minutes, 1% for 15 minutes and 1% for 20 minutes (as the fish tolerate the concentration of 1% for 20 minutes, this regime was used). All the chemical treatments were applied at a water temperature of 20°C .

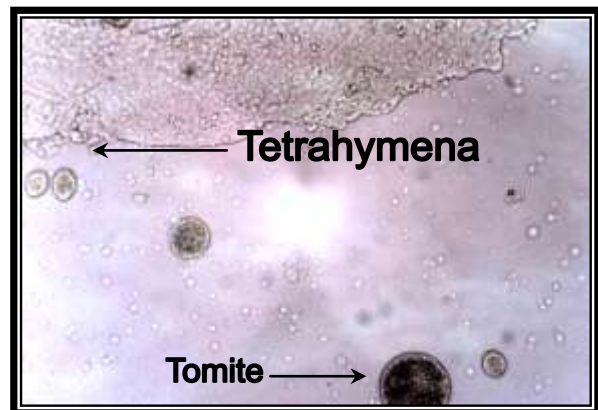
Results

Tetrahymena pyriformis has been firstly recorded in Iraq in brood Goldfish (*Carassius auratus*) Common , Fantail, Ryukin, Veiltail, and Moor, Gourami (*Trichogaster trichopterus*), red Swordtail (*Xiphophorus helleri*), Guppy (*Poecilia reticulata*), deep Angel fish (*Pterophyllum scalare*), and brood Algae eater (*Hypostomus plecostomus*) Prof. Furhan T. Mhaisen (Personal Communication).

All the examined fish were infected with *T. pyriformis*, incidence percentage of infection was (%100). Large hyperemic areas of the skin, light shedding of the scales and mucus hypersecretion of the skin were noticed on the infected fish.



Picture (1): *Tetrahymena pyriformis*



Picture (2): *Tetrahymena pyriformis* and *Ichthyophthirius multifiliis* tomites

Rising water temperature up to 29-30°C has no effect on *Tetrahymena pyriformis*, neither methylene blue in a concentration of 5ppm for 24 hours. Deep Angelfish (*Pterophyllum scalare*) and red Swordtail (*Xiphophorus helleri*) did not tolerate methylene blue treatment.

Examination of the infected fish after two days from the treatment with NaCl at a concentration of 2% for 1 and 2 minutes show no obvious effect on *Tetrahymena* numbers, and the fish were highly stressed, deep Angel fish (*Pterophyllum scalare*) could not tolerate this treatment.

Treatment with NaCl at a concentration of 1% for 20 minutes reduced *Tetrahymena* numbers. After a second treatment the parasite were controlled, and after the third treatment the parasite were eradicated as shown in Table (1).

Table (1): Efficacy of treatment on Tetrahymena in infected pet fish

Type of treatment	Day of treatment			
	0	1	2	3
NaCl (%1) for 20 min.	(+++)	(+) - (++)	(+)	(-)
NaCl (%2) for 2 min.	(+++)	(++)-(+++)		
NaCl (%2) for 1 min.	(+++)	(++)-(+++)		
Methylene blue	(+++)	(+++)		
Rising water temperature	(+++)	(+++)		

(+): Weak response

(++) : Moderate response

(+++): Good response (there is no detected parasites)

(-) : Negative response

Discussion

Tetrahymena pyriformis has been recorded for, the first time in Iraq, in Goldfish (*Carassius auratus*) Common, Fantail, Ryukin, Veiltail, and Moor, Black molly (*Poecilia sphenops*), Gourami (*Trichogaster trichopterus*), red Swordtail (*Xiphophorus helleri*), Guppy (*Poecilia reticulata*), deep Angel fish (*Pterophyllum scalare*), and Algae eater (*Hypostomus plecostomus*).

Epizootics have been reported among overwintering carp in Poland (9). *Tetrahymena pyriformis* also infects grass carp *Ctenopharyngodon idella* (10). One case of *Tetrahymena* species infected was reported in catfish (3). The organism has been known to affect the fry of various cultured fishes and northern pike (4).

The first report of *Tetrahymena* species from fish is that of (11) who found it in the peduncle musculature and spinal canal of rainbow trout.

Tetrahymena pyriformis is a free-living protozoan that becomes only a problem at times of overcrowding, poor water quality, water with a high organic matter content and containing excessive organic debris (4,2). It is possible that various strains of *Tetrahymena* have the potential for being more virulent, especially when the water is high in organic matter and fish are immunologically depressed (1). In this study, at the beginning, fishes were kept at a high density and there was a high density of organic matter content which could explain the infestation of fish by *Tetrahymena*. The parasite probably reach the pet fish pond through the water coming from the carp pond in the fish were carrier or infected.

In addition to its importance in fish diseases, *Tetrahymena* displays a degree of cellular structural and functional complexity comparable to that of human and other metazoan cells. The richness of *Tetrahymena* biology makes it a genetic unicellular animal model organism (12, 13).

It seem to be that *Tetrahymena pyriformis* could tolerate water temperature up to 29 – 30 c° for 24 hours and it did not effect by methylene blue at a concentration of 5 ppm for 24 hours (water temperature was 20 c°).treatment with NaCl revealed that *Tetrahymena pyriformis* was more affected by 1% NaCl concentration for 20 minutes for three times than 2% NaCl for one minute and two minutes for one time. The effect of NaCl solution on *Tetrahymena* could be due to its hypertonic activity, in which water flows from the unicellular parasites leading to its collapse and shrinking, and then its death (14, 15).

This treatment was effective in external *Tetrahymena* infestation, but it is not known whether it is active against internal *Tetrahymena* infection.

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