Vet. Med. J., Giza. Vol. 44, No. 3. (1996):545-550.

PARASITOLOGICAL AND HISTOPATHOLOGICAL STUDIES ON THE GRASS CARP, *CTENOPHARYNGODON IDELLA* VAL. INFECTED WITH *TRICHOPHRYA PISCIUM*

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SUMMARY

The grass carp, *Ctenopharyngodon idella* Val., Collected from Delta Breeding Station Cairo, Egypt was found to be infected with the ciliate parasite *Trichophrya piscium*. In Fish ≤ 4 grams the parasite caused proliferation of the epithelial lining of the secondary gill lamelae resulting in fusion of the lamellae. Blood vessels feeding lamellae became congested resulting in a degeneration of the lamellae epithelium. There was a high incident of mortality among small fish harbording the pareasite. Large fish (≥ 10 grams) were also adversely affected by *T. piscium* although mortality was reduced. Branchial epithelial cells increased in size, blood vessels in the primary and secondary gill lamellae became dilated, and there was extensive hyperplasia in the epithelial lining of the primary gill filaments. Short term (30 minutes) treatments with formalin at three concentrations (300,400 and 500 ppm) and copper sulfate at one concentration (20 ppm were unsuccessful in eradicating the parasites from the gills. However, treatment with 5 ppm of formalin over a 4 day period and 1,2 and 5 ppm copper sulfate cleared the parasites form the fish completely within 24 hours period.

INTRODUCTION

Trichophrya piscium, a member of the phylum Ciliophora, class Kinetoflagminophorea, subclass Suctoria differs from other ciliates in that it possesses no cilia as an adult. Feeding is accomplished via endocytotic absorption by feeding tentacles (Rudzinska, 1973). It is a common parasite of the gills of many species of freshwater fishes (Schulman and Shtein, 1962; Hoffman, 1967; Rogers and Gaines, 1975). Few members of this subclass are pareasitic. Those species that are parasitic, however, are usually parasites in the colon, large intestine or caecum of horses (*Allantosoma intestinalis, A. brevicoringer, A. bicorniger)* or parasitic in other ciliates *(Syphaerophyra sol in Stylonychia mytilus , Episylis plicatilis* and *Paramecium aurelia)* (Colman, 1980; Hungate , 1978).

The grass carp, *Ctenopharyngodon idella* val., was introduced into Egypt as a biological agent to control aquatic weeds (macrophages) in the canals and drainage systems. Since the grass carp

was not native breed to Egypt, an extensive breeding program was instituted by the Delta Breeding Station (D. B. S.) to produce this fish. Because of the economic importance of this species as a control agent it was essential that these fish remained relatively free of parasitic diseases. However, the suctorian parasite, *Trichophrya piscium,* was found to parasitize the gills of grass carp. Thus, an intensive program of parasite control (Davis, 1953, Hoffman and Meyer, 1974; Hoffman, 1978) was also instituted along with the fish breeding program.

The objective of the present study was to describe the morphology of *T. piscium,* and to describe the histopathology caused by this parasite on the grass carp. Efforts were also made to develop a method of control of these parasites on carp.

MATERIAL AND METHODS

In order to determine the morphological structure of *T. piscium*, 50 adult parasites were carefully collected from infected fish, fixed in methylalcohol and stained with haematoxylin & eosin. To examine the extent of tissue damage caused by the parasite, infected fish were collected from the fish hatchery at D. B. S. Gills containing *T. piscium* were removed and fixed in Bouin's fluid for a minimum of 24 hours. The tissues were embedded in paraffin, sectioned at 4-5 microns, and then stained using haematoxylin and eosin.

Parasite control experiments consisted of exposing infected fish to various concentrations of formalin and copper sulfate. Treatments were characterized as short term bath or long term bath, depending on the length of time experimental animals were exposed to each chemical. In the short bath treatment, 10 infected fish were exposed to three concentrations (300, 400 and 500 ppm) of formalin and one concentration (20 ppm) of copper sulfate for 30 minutes. The infection status was evaluated following the 24 hours period. For the long term bath treatment, 10 infected fish were exposed to 5 ppm formalin and to 1,2 and 5 ppm copper sulfate over a 4 day period. Infection status was evaluated every 24 hours.

RESULTS

Trichophrya piscium had an elongated ovoid body form with 8 to 10 transparent tentacles on the anterior end (Figs. I & IB).There was a prominent macronucleus (haematoxylin stained). However, the micronucleus was small and barely visible using standard microscope techniques. Measurements of *T. piscium* were listed in Table (1).

Trichophrya piscium was observed on the gills of grass carp throughout the year. Although larger fish (≥ 10 grams) showed detrimental effects of parasitic infestation. Small fish (≤ 4 grams) were more susceptible. The most pronounced effects on large fish were hypertrophy of epithelial branchial cells, hyperplastic proliferation of lamella epithelial cells and dilation of the branchial blood vessels within both the primary and secondary lamellae (Fig. 2). Small fish (≤ 4 grams) were more susceptible to their death, small fish suffered

Table (I) Biometric dimensions of *T. piscium* (measurements in microns).

	Hoffman (1978)	Present study
Number of parasites measured	-	50
Number of tentacles	3 - 27	8 - 10
The length of tentacles	-	3.8 - 10.9
(average ±S.E.)	-	(6.4 ±3.7)
The length of the body	30 - 40	30 - 118
(average ±S.E.)	-	(74.5 ±4.5)
The width of the body	10 - 12	7 - 52
(average ±S.E.)	-	(47.3 ±4.2)
The length of macronucleus	-	25 - 45
(average ±S.E.)	-	(29.6 ±3.9)
The width of macronucleus	-	8 - 15
(average ±S.E.)	-	(13.2 ±2.4)

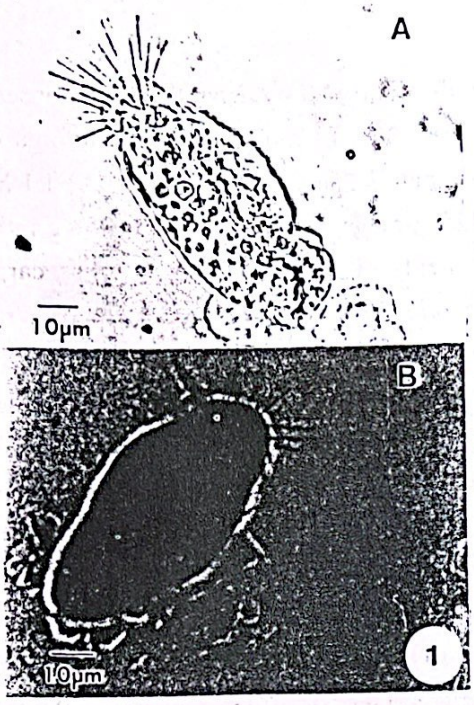

Fig. 1: (A) *Trichophrya piscium* (fresh preparation). (B) *T. piscium* (haematoxylin and eosin stain).

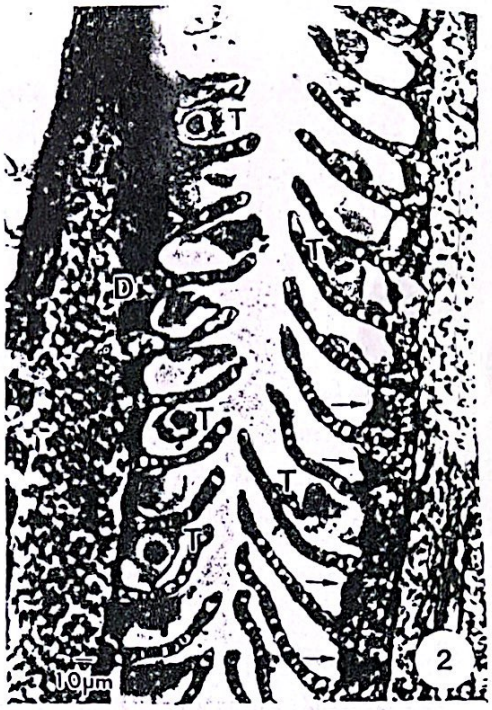

Fig. 2: Section through the gill filaments showing *T. piscium* (T) between the secondary gill lamellae. Mild hyperplastic proliferation of the secondary lamellae (arrows) and congestion of main lamellar blood vessel(D).

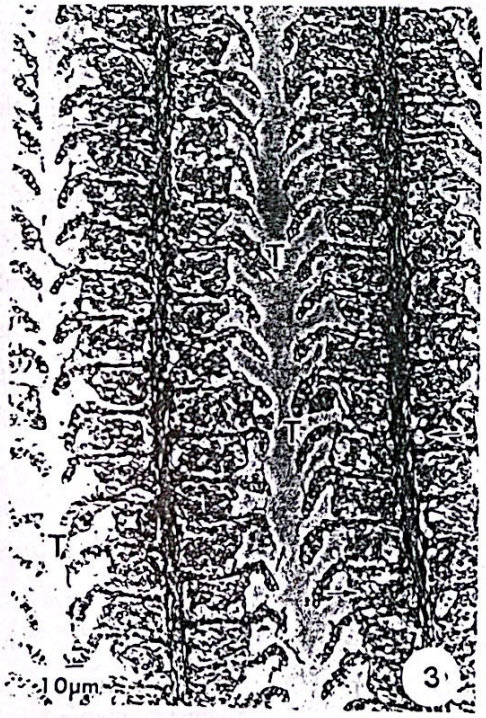

Fig. 3: Section through the gill filaments showing epithelial proliferation among the secondary gill lamellae and vacuolar degeneration (arrows). *Trichophrya piscium* (T) between the secondary gill lamellae.

a fusion of their gill lamellae, proliferation and vacuolation of branchial epithelial lining and ultimately a degeneration and necrosis of the secondary gill lamellae (Fig. 3).

Short term exposure of *Trichophrya piscium* to 300, 400 and 500 ppm formalin and to 20 ppm copper sulfate for 30 minutes were unsuccessful in eradicating the parasite from the gills. However, when lower concentrations (5 ppm formalin and 1, 2 and 5 ppm copper sulfate) were used, the parasites were completely eliminated from the gills after four day period at the concentration of 5 ppm formalin and after one day at the concentrations of 1, 2 and 5 ppm copper sulfate.

DISCUSSION

Morphological analysis confirmed that the parasite found on the grass carp was *Trichophrya piscium* (Hoffman, 1978). A Comparison of these data with previous accounts clearly indicated that *T. piscium* had not previously been described from grass carp (Shirman and Smith, 1983). By most published accounts, *T. piscium* has been regarded as harmless endocommensal that fed on plankton (Lom, 1971; Corliss, 1979) or on fish cells (Kozicka, 1966; Hoffman, 1967), but was incapable of producing lesions. Clearly in this study, that was not the case. The damage caused by this parasite was extensive, even causing mortality in fish less than 4 grams in weight. It is reasonable to speculate that the presence of numerous parasites on the gill filaments of grass carp might cause fish moralities. Also, with high parasite numbers, intensive tissue responses might be seen. The histopathological findings of this study parallel those described by Abdel Meguid (1995) and Ramadan et al. (1995) on the gill fillaments of heavily infected grass carp with *Trichodinella epizootica* and *Trichodina nobilis*. Gill lamellae were fused, branchial cells destroyed and effective gas exchange was probably terminated. Although larger fish survived the infection, damage to the gills was also severe. Branchial cells became larger, and epithelial lining cells became hyperplastic. One could speculate that gas exchange was adversely affected since larger and fewer cells and damaged cells would be less effective in gas exchange. The transfer factor, the relationship between the branchial surface and its ability to exchange gases would probably decrease, given the extent of the damage to the lamellae. Dilation of various blood vessels would cause fluid leakage, possible affecting total blood volume and electrolyte concentration. All of these factors would certainly impact on the ability of the fish to forage. A lessening in the foraging ability would ultimately lead to a proliferation in the aquatic macrophytes / weeds in canals and drainage ditches. This proliferation could lead to total blockage in some areas and would be the initial stage in the eutrophication process.

Control of the parasite, therefore, is essential to the overall well being of this aquatic system. It appears that small, sustained doses of either formalin or copper sulfate are sufficient to render these fish parasite *(T. piscium)* free.

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