

Studies on *Lernaeosis* Affecting Cultured Golden Fish (*Carassius auratus*) and Trail for its Treatment in Earthen Ponds at Kafr El-Sheikh Governorate, Egypt

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Abstract: *Lernaeosis* is crustacean parasitic disease infested cultured Golden fish (*Carassius auratus*). The present study deals with a total number of 200 infested fish were collected from earthen ponds of private fish farms at Kafr El-Sheikh and were examined for infestation. The collected fish were divided into two groups (50 for examination and 150 for experimental treatment trails. The clinical pictures were in the form of grayish to greenish colored worm-like copepods distributed along both sides of the body as well as at peduncle region especially at the base of caudal fin and the sites of attachment appeared hemorrhagic or reddening without swollen margins. Parasitological identification copepod affecting infested golden fish was isolated and identified. Histopathological examination of skin, musculature, gills, liver and spleen were investigated and recorded. Some treatment trials were conducted with different concentrations and prevention times in Golden fish (*Carassius auratus*). These results concluded that, Sodium chloride 3 mg/l dip for five minute was considered the best and effective to eliminate *Lernaea cyprinacea* which affect *Carassius auratus*.

Key words: *Lernaeosis* • *Lernaea cyprinacea* • *Carassius auratus* • Histopathological • Sodium chloride

INTRODUCTION

Crustacean parasites including *Lernaea* species cause serious economic losses and high mortality rates among ornamental fishes as a result of obtained hemorrhage and secondary infections [1]. Lernaeids are affected *Carassius carassius* and considered the most harmful ectoparasites of cultured freshwater fishes not only result from direct harm to the fish, but also from fish disfigurement which renders fish grown for food and making ornamental fish unsuitable for sale and thus impose a big loss to fishing industry [2-5]. Lernaeosis has been spreading among cyprinids in different parts of the world and present in Egyptian hatcheries since 1980 [6]. Also, it poses a major constraint to cyprinids wherever they cultured because of the damage it causes and the costs of ineffective control measures [7]. The main clinical signs of lernaeosis in *Carassius carassius* were attachment of wormlike copepods along both sides of the body, at the peduncle region and the attachment site was marked by area of inflammation around the embedded

anchors of the lernaea parasites [8]. The most common histopathological changes in *Carassius carassius* infested with lernaea were degree from acute inflammatory reactions to severe degenerative changes and necrosis in the skin and underlying musculatures.

Also, the dermis and hypodermis exhibited extensive edema, hemorrhage, leucocytic infiltration and some melano-macrophages [9]. Severe destruction, vacuolar and hyaline degeneration and necrosis were evident in the muscles with edema, congestion, hemorrhage and leucocytic infiltration replaced the necrotic muscles with numerous eosinophilic granular cells and melano-macrophages observed between muscles [10]. The efficacy of Metrophonate as a treatment of *Lernaeosis*, the ornamental fishes regularly used organophosphate pesticides Metrophonate to control lernaea infestation with the attendant problems of the development of parasite resistance, toxicity to both fish stocks and farm workers, their release to the environment causes water and soil pollution and disturbed the normal feeding behavior of the fish where the fish went off their food and

remained fasting for as long as the effects of Metrophonate persisted and not completely eliminate copepods infestation [11]. Also, the use of organophosphate insecticides, particularly trichlorphon is conducted [12]. However, the use of organophosphates creates problems due to its tissue residues [13]. On the other hand, a safer non-residual chemical that has been used effectively is 30 mg/l Sodium chlorite [14]. Sodium chloride in a concentration of 20–40 mg/l and the pH above 6, the chlorite eradicated *L. cyprinacea* from a commercial aquarium and was non-toxic to fish and killed bacteria in the surround water [1].

This study was planned to study the difference in clinical signs in infested cultured *Carassius carassius*, prevalence, histopathological alterations of *Lernaeosis*. The efficacy of Sodium chloride (NaCl) as a treatment of lernaeosis was investigated in the aquariums.

MATERIALS AND METHODS

Fish: A total number of 50 adult golden fish (*Carassius auratus*) were collected from the breeding earthen ponds at Kafr El-Sheikh Governorate, Egypt. Their body weight and length were ranged from less than 20 up to 50 g and 10 to 15 cm, respectively. All fish were kept alive in a fiberglass water tank and transported to the Lab. of hydrobiology department, NRC where they were examined immediately.

Fish for Experimental Treatment: A total number of infested 150 adult golden fish (*Carassius auratus*) were collected from the same breeding earthen ponds at the same body weight and length and kept alive in a fiberglass water tank and transported to the Lab. of hydrobiology department, NRC where they were examined immediately.

Aquaria: Ten aquaria (40 x 60 x 30 cm) with 100 liter of chlorine free aerated fresh water for 15 fish holding. One third of water in each aquarium was changed day after day and fish were fed on commercial fish food containing 25% protein at 3% feeding rate. Sodium chloride (NaCl) and Metriphonate (trichlorfon 97% active ingredient) were added in doses 10, 20 and 30 mg/l for 20, 10 and 5 minute respectively. While, freshly prepared Potassium permanganate (KMnO₄) was added in a doses 3.5 and 10 mg/l for 20, 10 and 5 minute respectively. The adult golden fish after exposed to different treatments were examined and transferred to four separate aerated freshwater aquaria for recovery.

Clinical Examination: The naturally infested fishes were grossly examined for determination of any clinical abnormalities and any external parasite according to Noga [15].

Parasitological Examination: Golden fish were examined externally with a hand lens. The external crustacean parasites were removed with the help of fine forceps washed several time with warm water 25 °C and left in refrigerator at 4°C until the specimens had fully relaxed. The collected parasitic copepods were fixed in glycerin 5% - alcohol 70% in test tube and permanent amounts were prepared by passage in descending grades of alcohol(70, 90% and absolute), cleared in glycerin and mounts in glycerin-gelatin according to Becky [16], then examined microscopically. Crustacean parasites were identification according to Kabata [17].

Histopathological Examination: The skin, underlying muscles, liver and kidney of naturally infested *Carassius auratus* were fixed in 10% buffer formalin. These samples were histopathologically prepared and stained with Haematoxylin and Eosin (H and E) according to Bancroft [18].

Treatment Trials: Sodium chloride (NaCl) as powder from commercial market. It was used in different concentrations 10, 20 and 30 mg/l as dip for 20, 10 and 5 minute respectively. NaCl was applied to the experimental aquaria water at the morning [19].

Metriphonate (trichlorofon 97%) from Adwia Co.S.A.E.^{10th} of Ramadan city, Egypt. It was used in different concentrations 10, 20 and 30 mg/l as immersion for 20, 10 and 5 min respectively. Metriphonate was applied to the experimental aquaria water at the morning [20].

Freshly prepared Potassium permanganate (KMnO₄) from Kima Science Co. It was used in different concentrations 3, 5 and 10 mg/l as immersion for 20, 10 and 5 min respectively. The lowest concentration in which the pink hue remains after 15 minutes was considered the end point [21]. KMnO₄ was applied to the experimental aquaria water at the morning [13]. The control group of treatment left without treatment.

RESULTS

Clinical Signs: The naturally infested fishes (*Carassius auratus*) revealed that uneasily, poor appetite, ulcers on the body surface and slow movement. The parasite appeared grossly as white colored as a short piece of



Plate (A): Showing *Lernaea* penetrate skin of Golden fish (1) and hemorrhagic circumscribed ulcers of Golden fish (2).



Fig. 1: Showing *Lernaea cyprinacea* with egg sacs. Wet mount, x40.

thread coming from beneath a single scale and protruded on the fish external body plate, A(1). The surrounding parts where *Lernaea* penetrates appeared inflamed and swollen with sever lesions including hemorrhagic circumscribed ulcers plate, A(2).

Parasitological Examination: As shown in Fig. 1, the crustacean parasite was collected from the skin of Golden fish (*Carassius auratus*). The female is long, cylindrical, rod-shaped, non-segmented and their bodies assume the shape of a worm. It has a small semispherical cephalothorax, which contains the mouth and is characterized by the presence of two pairs of well-developed symmetrical horn-shaped cephalic chitinous appendages situated at right angles to the body with elongated narrow neck. The antennae and mouthparts are rudimentary while the egg sacs are multiseriate containing very numerous eggs appearing as long slender and tube like at the posterior end. Based on the morphological characters, these crustacean parasites are related to the subclass Copepoda, family Lernaeidae, genus *Lernaea*, *Lernaea cyprinacea*.

Histopathological Alterations

Skin: Anchor worm penetrating skin (Plate 2 e), the underlying tissue showed fibrin deposition with massive mononuclear cell infiltration and edema (Plate 2f), ulcerative lesion in the skin with complete loss of epidermal cells, the dermal connective tissues and

subcutaneous adipose tissue have been necrotized with edematous dissociation and infiltration of melano-macrophage cell as well as dissociation of underlying muscle fiber (Plate 2a).

Musculature: Muscle showed anchor worm penetrating lesions. A worm inserts the anterior holdfast and neck into the musculature. Around the perforation, the granulation tissue is markedly produced (Plate 2 a). The muscle cells are necrotized, resulting in fragmentation or coagulation of the myofibrils. Necrotized muscle cells are infiltrated by inflammatory cells, including macrophages and lymphocytes (Plate 2b), presence of hemorrhage in the interstitial tissue (Plate 2c), Congestion of interstitial blood vessel with perivascular aggregation of melano-macrophage cell (Plate 2 d).

Gills: Some gill lamellae are fused with proliferation of interlamellar epithelial cells (Plate 3b).

Liver: Liver revealed congestion of sinusoids and hepatocytes are necrotized and the laminar structure has been destroyed. Necrotized cells mostly showed nuclear degeneration as pyknosis and hyperchromatosis, resulting in cellular fragmentation and lysis of cells (Plate 3c). Hepatopancreas showed degeneration and necrosis of pancreatic acinar cells which are infiltrated with mononuclear and melano-macrophage cells (Plate 3d).

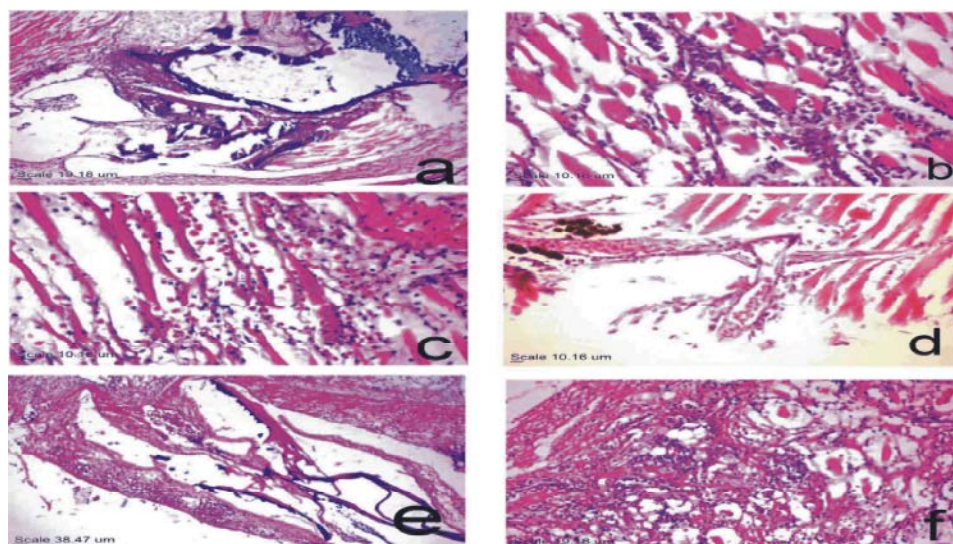


Plate 2a-d: Muscle of fish showing a- anchor worm penetrating lesions. A worm inserts the anterior holdfast and neck into the musculature around the perforation, the granulation tissue is markedly produced (scale bar, 19.18 um); b- Necrotized muscle cells infiltrated by inflammatory cells, including macrophages and lymphocytes (scale bar, 10.16 um); c- hemorrhage in the interstitial tissue (scale bar, 10.16 um); d- Congestion of interstitial blood vessel with perivascular aggregation of melano-macrophage cell (scale bar, 10.16 um). e and f, skin showing; e- anchor worm penetrating skin (scale bar, 10.16 um); f- fibrin deposition with massive mononuclear cell infiltration and edema (scale bar, 19.18 um)

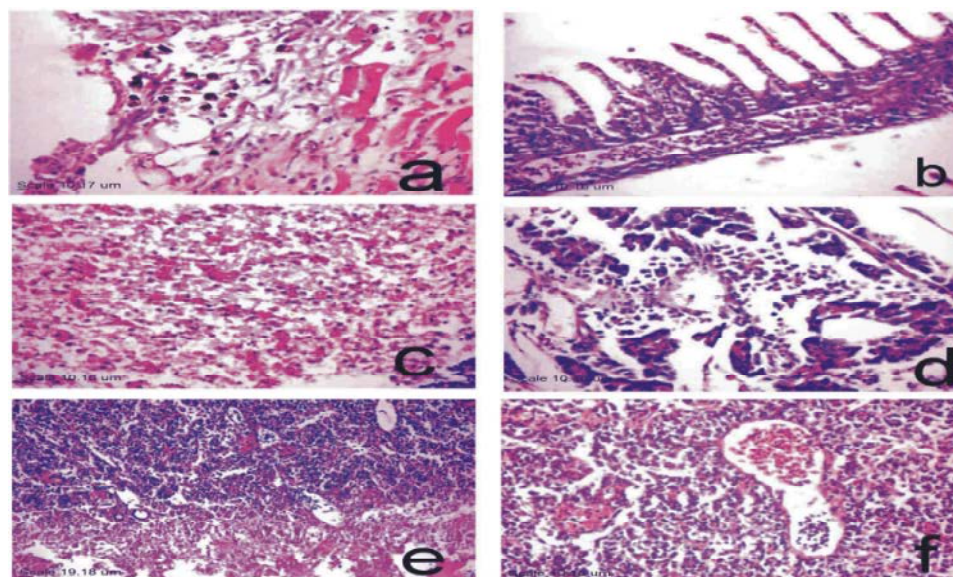


Plate 3: a- ulcerative lesion in the skin with complete loss of epidermal cells, the dermal connective tissues and subcutaneous adipose tissue have been necrotized with edematous dissociation and infiltration of melano-macrophage cell as well as dissociation of underlying muscle fiber (scale bar, 10.17 um); b- proliferation of interlamellar epithelial cells with fusion of gill lamellae (scale bar, 10.16 um); c- nuclear degeneration as pyknosis and hyperchromatosis, resulting in cellular fragmentation and lysis of hepatocytes (scale bar, 10.16 um); d- degeneration and necrosis of pancreatic acinar cells which are infiltrated with mononuclear cells (scale bar, 10.16 um); e- Splenic capsular necrosis (scale bar, 19.18 um); f- necrosis of splenocytes with nuclear pyknosis (scale bar, 10.16 um).

Table 1: Showing condition of the fish and copepods after different treatments and time of exposure to remove *Lernaea cyprinacea* from Golden examined fish

Treatment	No. of examined fish	Time	Treatment Concentration	Condition <i>Mugil cephalus</i>	Condition Copepods	No. of free copepods in aquarium	No. of attachment copepods
Control	15		12 h	Good	Active	0	35
Sodium chloride	15	20min	10 mg/L	Good	Active	0	28
Sodium chloride	15	10min	20 mg/L	Good	Dead	21	0
Sodium chloride	15	5min	30 mg/L	Stressed	Dead	16	0
Freshly prepared Pot. permanganate	15	20min	3 mg/L	Good	Active	5	18
Freshly prepared Pot. permanganate	15	10min	5 mg/L	Good	Dead	21	5
Freshly prepared Pot. permanganate	15	5min	10 mg/L	Stressed	Dead	16	2
Metriphonate	15	30min	10 mg/L	Good	Active	12	10
Metriphonate	15	20min	20 mg/L	Good	Dead	30	5
Metriphonate	15	10min	30 mg/L	Stressed	Dead	30	0

Spleen: Spleen showed capsular necrosis (Plate 3e), congestion of splenic sinusoids and splenocytes were necrotized and showed nuclear pyknosis (Plate 3f).

Treatment Trials: AS shown in table (1); treatment trails of naturally infested Golden fish (*Carassius auratus*) with *Lernaea cyprinacea*, were applied using Sodium chloride (NaCl), Potassium permanganate (KMnO₄) and Metriphonate (Trichlorofon). It was shown that, the best suitable and effective concentration of Sodium chloride (NaCl) used for treatment without harmful effect on fish meanwhile causing eradication of *L. cyprinacea* from infested aquarium and was non-toxic to fish was 20 g/l as dip for one minute. While, the effective concentration of Metriphonate (Trichlorofon) used for treatment without harmful effect on fish meanwhile causing a great damage to the parasite were 20 mg/l for 20 min. Also, Potassium permanganate in concentration 5 ppm for 10 min was used in experimental fish aquaria without harmful effect on fish meanwhile, causing a greet damage to the parasite.

DISCUSSION

The present study deals with *Lernaeosis* among cultured golden fish, *Carassius auratus*, at earthen ponds in private ornamental fish farms in Kafr-El-Sheikh Governorate, Egypt.

The main clinical signs observed in infested fish with *Lernaeosis* showed acute and chronic inflammatory changes in the skin characterized by macroscopic reddening and swollen margins (hemorrhagic nodules) with ulceration and loss of scales at the site of

penetration. These results may be attributed to bacteria and ciliated protozoans or fungus infections. These results were similar to that recorded by Eissa *et al.* [22].

The infested golden fish showed attachment of worm-like grey to greenish colored copepods which was distributed along both sides of the body and peduncle region especially at the base of caudal fin in *Carassius auratus*. Similar findings were recorded by Abd El-Rahman [23] and these findings mean that the lernaea parasite preferred the scaleless areas of the fish body.

Regarding the identification of *Lernaea* sp. affecting golden fish, the present investigation recorded only *Lernaea cyprinacea* from *Carassius auratus*. This result according to the description was recorded by Paperna [24].

Lernaea cyprinacea is common pest in freshwater aquaculture of cyprinids and often associated with high mortality in earthen ponds, particularly in small golden fish which is clearly visible to naked eye. These results nearly agree with that recorded previously by Woo [1] and Tasawar *et al.* [25].

The results of the present study revealed severe histopathological alterations especially in the skin and muscles of infested fish. These alterations characterized by ulcerative lesion in the skin with complete loss of epidermal cells and necrosis of subcutaneous adipose tissue which showed edematous dissociation and infiltration of melano-macrophage cell. Also a much more dramatic response to *Lernaea* species was seen in the muscle, in which the parasite inserts part of its crustacean body and evokes severe acute inflammation with necrosis and fragmentation of the myofibrils as well as infiltration by inflammatory cells mostly macrophages, lymphocytes

and melano-macrophage cell. These results are similar to that recorded by Abd El-Galil *et al.* [10] and Ferguson [26]. Liver revealed congestion of sinusoids and hepatocytes are necrotized and the laminar structure has been destroyed. Necrotized cells mostly showed nuclear degeneration as pyknosis and hyperchromatosis, resulting to cellular fragmentation and lysis of cells [27]. Severe congestion with blood vessels dilatation was observed necrosis in splenic capsular and congestion of splenic sinusoids and splenocytes were necrotized and showed nuclear pyknosis. This may be attributed to absorption of the *Lernaea* metabolic end products from the site of infestation to the fish body and reached to the internal organs especially kidney as it is an excretory organ [7].

Concerning the trail of treatment although the relatively high dose used of Metrophonate, the non-promising effect of its use as a treatment may be attributed to the development of parasite resistance as a result of its recurrent and disordered application in the investigated hatchery for many previous years in addition to the efficacy of Metrophonate decreased at high temperature and it kills the copepodid stages but not the nauplii or female adults [28] and Abd El-Galil *et al.* [10] who recorded that Dipterex eradicated *lerna* when used at a concentration of 30 mg/l bath for 5 minute. Potassium permanganate (KMnO₄) should be used freshly prepared and in absence of organic matters in pond which is reacted with organic matter far away the parasite. The treatment trails of *Lernaea cyprinacea* in Golden fish (*Carassius auratus*) using different concentrations in different duration of Sodium chloride (NaCl), Potassium permanganate (KMnO₄) and Metriphonate. The present study was revealed that the best suitable and effective concentrations of Sodium chloride was 30 mg/l as bath for 5 minute for killed all parasite as it leads to dehydration of parasites and killed bacteria in the surrounding water. Such findings are in agreement with that recorded by Dempster *et al.* [29] and Woo [1] who recorded that sodium chloride completely eradicated *lerna* when used at a concentration of 40 mg/l bath for one minute, while, Metriphonate had harmful on fish. Meanwhile causing a great damage to the parasite were 20 mg/l for 20 min. Such findings agree with Eissa [28] who recorded that Metriphonate eradicated *lerna* when used at a concentration of 30 mg/l bath for 5 minute. From the present investigation, it could be concluded that the sodium chloride (NaCl) 10% is effective and safe for treatment of *Lernaeosis* in Golden fish (*Carassius auratus*).

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