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Histopathological Effects of Trichodiniasis in Farmed Freshwater Rainbow trout, *Oncorhynchus mykiss* in West of Iran

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***Abstract*—**The aim of present study was to monitor the presence of *Trichodina* sp. in Rainbow trout, *Oncorhynchus mykiss* collected from various fish farms in the western provinces of Iran during January, 2013- January, 2014. Out of 675 sampled fish 335, (49.16%) were infested with *Trichodina.* The highest prevalence was observed in the spring and winter followed by autumn and summer. In general, the intensity of infection was low except in cases where outbreaks of Trichodiniasis endangered the survival of fish in some ponds. In light infestation *Trichodina* is usually present on gills, fins and skin of apparently healthy fish. Clinical signs of Trichodiniasis only appear on fish with heavy infections and cases of moderate ones that are usually exposed to one or more stress factors including, rough handling during transportation from ponds, overcrowdness, malnutrition, high of free ammonia and low of oxygen concentration. Clinical signs of Trichodiniasis in sampled fish were sluggish movement, loss of appetite, black coloration, necrosis and ulcer on different parts of the body, detached scales and excessive accumulation of mucous in gill pouches. The most obvious histopathological changes in diseased fish were sloughing of the epidermal layer, aggregation of leucocytes and melanine-carrying cells (between the dermis and hypodermis) and proliferative changes including hyperplasia and hypertrophy of the epithelial lining cells of gill filaments which resulted in fusion of secondary lamellae. Control of Trichodiniasis, has been achieved by formalin bath treatment at a concentration of 250 ppm for one hour.

***Keywords*—**Gill, Histopathology, Rainbow trout, Trichodina.

I. Introduction

TODAY the concept of development of aquaculture activities as a potential source of food supply is receiving much attention in Iran due to available river, lakes and marginal seas. This has resulted in rapid growth in fish farming practice. The intensification of such fish culturing creates disease problems that originate from overcrowdness or deteriorating water quality such as unsuitable water temperature [1], pH [2], carbon dioxide and free ammonia concentrations.

The majority of fish ectoparasitic protozoa are commensals but some of them may produce serious diseases and mortality especially in fry and fingerlings. The pathological effects of these parasites have been dealt with by [3] and [4]-[8]. Parasitic protozoa have a direct life cycle that enables them to spread rapidly and widely in their hosts [9]. Such rapid growth and multiplication of these parasites greatly increase their rate of infestation among fish causing mass mortality. *Trichodina* is common ectoparasites which in most cases is pathogenic to both freshwater and marine fish [3]. It is previously reported [1] that trichodiniasis is caused by *Trichodina* spp.; the infection being stimulated by the high density of fish in ponds. Under such conditions, it may be an epizootic. In cases of stress, several species of trichodina may become pathogenic interfering with feeding and respiration of small fish [10]. The lesions mostly induced by this parasite are hyperplasia and necrosis of the epidermal cells. These cells are usually subjected to severe attacks by parasites. This results in cellular growth and excess mucus production [10]. Many authors have observed a complete destruction of gill epithelium in similar cases [11]-[13]. For the control of Trichodiniasis, formalin is recommended by many authors that have experimented with different concentrations ranging between 166 to 250 ppm applied directly in short-term bath [5], [14], [11].

The present work was aimed to achieve the following objectives: First, to investigate the incidence of *Trichodina* in freshwater Rainbow trout (*Oncoryhnchus mykiss*) in different farms in the western provinces of Iran. Secondly, to examine and evaluate the magnitude of injury that induced by this parasite in infected tissues and organs of the fish. The third purpose of this experiment was to evaluate the efficiency of formalin in removing of the parasite from affected fish.

II. Materials and Methods

In present study, 675 fish were collected regularly from various ponds of fry, fingerlings, growing and brood stock, during a fish health survey of different fish farms in the western provinces of Iran and also during emergency cases where some fish mortalities were observed during the period of January, 2013-February, 2013. After observation of fish behaviour in various ponds, collected samples were transported (alive) to the laboratory in large plastic bags which were aerated upon arrived to the laboratory. The fish were checked daily for clinical signs, according to the method described by [5].

Skin fins and gills smears of these fish were made and carefully searched for *Trichodina* sp. The severity of infection with *Trichodina* sp. was estimated on an arbitrary scale using ordinary microscope under a 10 objective lens where no parasites were observed (–), low infection with single parasite

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in several fields of vision (+), medium infection with 2 to 5 parasites per field of vision (++) and severe infection with more parasites per field of vision (+++).

After examining parasites alive, they were fixed to microscope slides with methyl alcohol then stained with Giemsa according to [15].

Gills and skin specimens from clinically infested fish were immediately immersed into Bouin's fixative for 24 hours, washed and dehydrated in an ascending series of ethanol for embedding in Paraffin (Merck). Following embedment in Parafin, transversal and longitudinal sections of 6 µm were cut on a Leica RM2255 microtome and collected on glass slides and stained with Haematoxylin and Eosin [16] and [17].

During outbreaks of Trichodiniasis among fish in some ponds, dissolved oxygen concentrations were measured with Orion model 850 oxygen meter and ammonia (as ammonia-nitrogen) was determined by the Nessler method (Hach model DR/2000).

Clinically infested fish in ponds were treated by addition of formalin at a concentration of 250 ppm for one hour [14]. Treated fish were checked after 24 hours.

III. Results

A. Identification of the Parasite

Trichodina were recorded from the body surface, fins and gills of Rainbow trout (Fig. 1). In fresh unstained preparation, the parasite is very motile and appears as a circular or bell-shaped ciliated organism. The adhesive disc (saucer-shaped), several circular rows of cilia and a circle of more centrally lying hooklets could be easily seen in preparations stained with Giem's stain, Fig. 2.

B. Clinical Picture of the Infection

Out of 675 fish, 335 were found infested with Trichodina (49.6% infestation). Data indicate that the highest incidence of infestation was found in growing fish (61.11%) followed by fingerling (54.28%), Brood stock (46.87%) and lastly fry (34.31%).

The number of fish examined was 180, 175, 160 and 160 for each age group, respectively. The highest infestation with Trichodina sp. was found in spring (62.9%) followed by winter (56.25%) then autumn (41.37%) while the lowest infestation rate was in summer (35%). A picture of infected skin is shown in Fig. 2. As indicated in the present study, the intensity of infection with Trichodina sp. was generally low except few cases were fish mortalities have been observed in some ponds. In light infestation, Trichodina sp. is usually present in small numbers of skin, fins and gills of the infected fish and no clinical signs can be described. On the other hand most clinical signs and mortalities in some ponds were noticed in fish with heavy infections and some cases of moderate ones due to exposure to one or several stress factors. These factors may include, rough handling during transportation, overcrowdness, malnutrition, high free ammonia (ranged between 0.8-2.16 mg/l) as well as low dissolved oxygen (with average 1.4 mg/l in clinically infected ponds). Clinical signs and postmortem examination revealed that diseased fish showed sluggish movement, loss of appetite, emaciation, loss of condition with larger head and darker skin than normal. Some infected fish showed detached scales with pale skin patches and more slimy skin. Erosion and ulcer on different parts of the body, blood spots at the base of fins as well as laceration of membranous parts. On the other hand excessive accumulation of mucous in the gill pouches and around gill filaments were frequently noticed in these infected fish. Hyperaemic and also pale gill filaments were observed in some studied fish. Hypertrophy of gill filament cells was another observed symptom in studied fish. Signs of asphyxia including surfacing of fish, gasping of air bubbles on the surface of water were also observed in heavily infested fish. Finally they lost their normal swimming behaviour and died.

C. Histopathology

The skin of fish infected with *Trichodina* species showed, sloughing of the epidermal layer and the remained dermis was oedematous and infiltrated with leucocytes and melanine-carrying cells which aggregate between the muscle layers (Fig. 3).

On the other hand, the epithelial lining cells of the gill filaments showed, proliferative changes, including, hyperplasia and hypertrophy. In most cases, the epithelial proliferation of the lamellae started from their apices and extended towards the basal portations. Sometimes, those proliferative changes extend to other lamellae where one, two, or three lamellae appeared adherent to each other. In some fish, epithelial proliferation was extensive leading to fusion of secondary lamellae (Fig. 4).

D. Therapeutic Control

After 24 hour from the treatment with formalin (250 ppm), parasitological examination revealed that the *Trichodina* sp. were completely eradicated and most fish regained their normal feeding response, mortality ceased, and fish began to appear healthy.

IV. Discussion and Conclusion

Parasitic infestation of cultured fish in tropical and subtropical countries represents a serious problem for aquaculture due to severe economical losses either as directly or indirectly [18]. The data obtained from present study indicate that the Rainbow trout have a variety of clinical and post mortem changes from infection with *Trichodina* sp. *Trichodina* sp. Are commensals parasites on fish and have been reported in *Oncorhynchus mykiss* [19]. It is also apparent in the present study that *Trichodina* spp. prevails on skin and gills of *Oncorhynchus mykiss*. On the other hand, the present findings confirm those of Snieszko and Axelrod [20] in which they mentioned that *Trichodina* spp. occur on the gills and skin in numbers that could obscure the normal structure of the epithelium.

It is worthy to mention that infestation with *Trichodina* sp. varies between ages and sizes of *Oncorhynchus mykiss* and can be attributed to overcrowdness and consequently changes

to water quality in ponds of growing and fingerling fish, compared with those of broods stock or fry. Regarding the seasonal incidence, Trichodina infection was prevalent throughout the year with maximum rate of infestation during spring season (62.9%), followed by winter (56.25%), then autumn (41.37%) and was lowest in summer (35%). The results agree with the findings of [12], [21] and [22] who reported that, Trichodiniasis was prevalent all over the year with maximum rate of infestation during spring and winter.

It is also apparent in the present study that the intensity of infestation with *Trichodina* sp. was generally low, excepting few cases where mass mortalities have been reported in some ponds. In light infestation, fish do not show any signs of disease. However, most clinical signs and mortalities in some ponds were noticed in fish with heavy infection. Mortalities in moderated infected fish is usually associated with one or more of stress factors including, rough handling, overcrowdness, malnutrition, high free ammonia (0.8-2.16 mg/l) as well as low oxygen (1.4 mg/l).

Trichodina is an opportunistic parasite which become pathogenic under stress full conditions [10]-[13]. On the other hand, [23] reported that *Trichodina* sp. is a true and permanent parasite which was noticed to bring about marked mortalities among newly-hatched grass carp.

The common lesions observed in the present study were in the form of black colouration, sloughing of scales with pale skin patches, laceration of membranous parts of fins and sometimes sloughing of free portion of them, erosion and ulcer in different parts of the body were also observed and reported. These lesions are common in trichodina infection [12], [22]-[25]. In addition to these lesions, excessive accumulation of mucus on the skin and gills was seen on infected fish. The massive production of mucus in case of Trichodiniasis infection is regarded as a defence mechanism to eliminate the parasite or dilute its irritating effects [26]. The results of histopathological changes in skin and gills of *Oncorhynchus mykiss* infested with *Trichodina* sp. revealed sloughing of the epidermal layer and the remaining dermis was oedmatous and infiltrated with leucocytes and melanine carrying cells which aggregate between the muscle layer and hypodermis forming a thick blackish band. This last finding may explain why the heavily infected fish had acquired characteristic dark colouration. In addition to the above listed lesions, the epithelial lining cells of the secondary lamellae showed, proliferative changes, including, hyperplasia and hypertrophy resulting in their fusion. This finding may interpret the behaviour of fish, heavily infested with *Trichodina* sp., exhibiting distinct signs of respiratory disfunction. With regard to parasitic control in the present study, inspection of the parasitised fish (exposed to 250 ppm of commercial formalin, for one hour and examined after 24 hours) indicated that fish were free of the parasite. Mortality ceased and formalin treated fish appeared very healthy. Formalin is known to control ectoparasites in fish [10].

In conclusion, present study emphasized the importance of keeping good husbandry practice and favorable water quality parameters for successful rearing of fish. In case of outbreak of trichodiniasis, formalin is an effective therapeutic agent.

Fig. 1 *Trichodina* sp. attached to the skin of *Oncorhynchus mykiss*

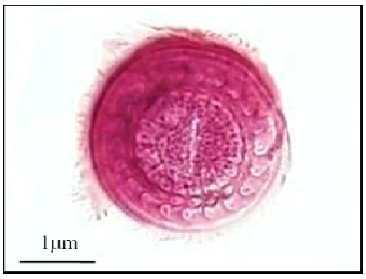

Fig. 2 *Trichodina* sp. with peripheral adhesive disc followed by several circles of cilia and a central circle of hooklets

Fig. 3 Skin section revealing loss of epidermis, disarrangement of collagen bundles and leucocytic infiltration of dermis with aggregation of melanine-carring cells

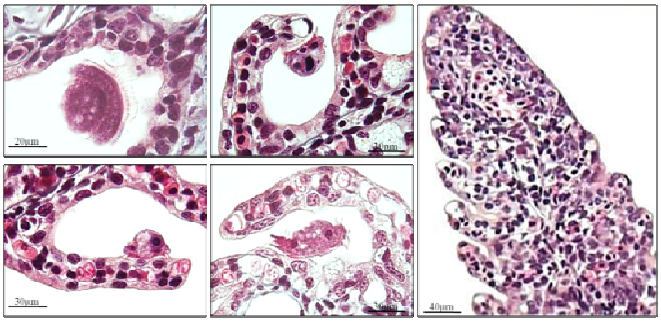

Fig. 4 Rainbow trout infected gill with *Trichodina* sp.

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