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Fish disease

Prevalence and Histopathological Studies of *Trichodina spp.* Infecting *Oreochromis niloticus* in Behera Governorate, Egypt.

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ABSTRACT

Trichodinids are commensal ectoparasitic protozoa, having direct life cycle and may become pathogenic hindering with respiration and feeding of fishes. The study aimed to determine the seasonal dynamics of Trichodina spp. infecting Oreochromis niloticus and their Histopathological effects. Microscopic examination of gills of 747 Tilapia at different localities of Behera province, Egypt was carried out all over one year extended from January to December 2019. The Histopathological examination of infected gills was performed. The results showed that, the prevalence of Trichodina spp. was 4.36%. The locality and weights of examined fish had no significance on the occurrence of Trichodina spp. The infection rate was affected significantly by the season. The highest infection was recorded in autumn whereas the lowest infection was in summer. Moreover, the measurements and morphological description of recovered Trichodina spp. were reported. Trichodina spp. appeared as multiple ring-shaped structures attached to the supporting gill structure and surrounded by few inflammatory cells and there was congestion, fusion, thickening and distortion of gills secondary lamellae.

Keywords: Histopathology, Oreochromis niloticus, Prevalence, Trichodina

INTRODUCTION:

The growing demand for food sources, particularly protein, has made aquaculture to be one of the fastest growing food protein sources in the world. A variety of freshwater fish, carp, tilapia and catfish has been cultured in many parts of the world (FAO, 2014) to meet the demands and preferences of consumers. However, the introduction of these fish beyond their native range have caused the co-introduction of parasites along with their hosts to new localities and transmitted to native hosts, causing emergence of new diseases in the native fish (Lymbery *et al.*, 2014).

Oreochromis niloticus represents the main popular cultured fish produced in Egypt. Tilapia was initially described as more disease resistant than other species of cultured fish (Roberts and Sommerville, 1982). However, the intensification of fish culture practices creates disease problems that originate from overcrowding or deteriorating water quality such as unsuitable water temperature, pH, carbon dioxide and free ammonia concentrations (Sarig, 1968; Dujin, 1973; and Kugel *et al.*, 1990).

It is usually known that external parasites constitute the largest group of pathogenic organisms in warm water fish (Snieszko and Axelrod, 1971). Parasitic diseases of fish have a superior position and have received a significant attention in Egypt of one of subtropical country Eissa *et al.*, (2000). Away from their direct damage effect on fish tissues, parasitic agents may act as stress factors rendering the fish more susceptible to other diseases Hoffman *et al.*, (1990).

Trichodina, a genus of ciliate protists, belongs to the family Trichodinidae and is well known as the causative agent of trichodiniasis in

numerous aquatic animals, especially both cultured and wild fish (Van As and Basson, 1989; Martins, *et al.*, 2010 and Marcotegui, *et al.*, 2018). Trichodina can serve as a facultative ectoparasite proliferating and invade hosts during unsuitable conditions in environments, such as poor water quality and food deficiency (Khan, 2004 and Huh *et al.*, 2005). The life cycle of trichodina is monoxenic, reproduction occurs by means of binary division, conjugation under certain conditions can occur (Van As and Basson, 1987 and Martins *et al.*, 2015). Direct transmission makes trichodinid ciliates able to invade their hosts within a short period, especially fish that are kept under less than optimal conditions (Lom, 1995).

Pathogenesis of trichodinids related to the method they infect their hosts, since when the parasite is fixed firmly onto its host, the border of the aboral membrane creates a suction movement on the surface of the epithelial cells, which likely causes irritation to the tissues of the fish (Basson, and Van As, 2006). Thus, a high abundance of these parasites and their constant circular movements may seriously damage the epithelium of their hosts, thereby triggering physiological alterations (Van As, and Basson, 1987). Trichodina spp. causes hypertrophy of gills epithelium, fusion of gill secondary lamellae and mucus accumulation (Yemmen *et al.*, 2011; Abdelkhalek *et al.*, 2018). Furthermore, secondary lamellae damage is occurred (Schalch *et al.*, 2006). These pathological changes induce respiratory dysfunction and mortality to affected tilapia (Schalch *et al.*, 2006).

So the current study was aimed to determine the prevalence and histopathological effects of Trichodiniasis. in Oreochromis niloticus in Behera governorate, Egypt.

MATERIALS AND METHODS

Study period and area

The study was carried out all over one year extended from January to December 2019 at different two localities of Behera governorate (Kom Hamada and Delingat) to determine the prevalence of Trichodina spp. in Tilapia fish and their Histopathological effect.

Fish samples

A total number of 747 Tilapia fish were collected once weekly from the examined locality and transported a live to Parasitology Lab, Faculty of Veterinary Medicine, .University of Sadat City, Menoufia, Egypt

Tilapia fish of different body weights were acquired by nets and hook of fishermen from freshwater canals in the study area. The examined fishes were divided into 3 groups according their weights: Group 1 (15-100 g, Group 2 (100-200 g) and Group 3 (> 200 g).

Clinical Examination

Alive fish were physically inspected for their behaviors, colors changes, respiratory signs, and any abnormalities on gills, petechial hemorrhage, ulcers and slimness according to (Noga, 2010).

Parasitological examination

Wet smears of gills were prepared and examined in order to detect the presence of Trichodina spp. and spread with a drop of normal saline, covered with a clean cover slip and examined microscopically (Wet mount preparation), specimens from gills were mixed with drop of iodine solution on clean slide and covered with a clean slip (iodine stained preparation) (Lucky, 1977).

Preparation of permanent mount for Identification of recovered protozoa: -

The smears from scrapings were made and left to air dry, then smears were fixed in absolute methanol, stained by Giemsa stain that freshly prepared and diluted with distilled water before staining with (10 ml of the stain were added to 90 ml of distilled water). The fixed films were dipped into the prepared diluted stain for twenty minutes. After staining, the films were rinsed with running tap water and air dried. The stained preparations were examined with an oil immersion objective lens (Lucky, 1977). All measurements are in micrometers and based on 30 trichodinid specimens and follow the uniform specific characteristics given by (Lom, 1958) and the description of denticule elements by (Van As and Basson, 1989).

Identification of the recovered Trichodina spp.:

The identification of the recovered *Trichodina spp*. was carried out according to (Lom and Dykova 1992), (Paperna, 1996) and (Yamaguti, 1963).

Histopathological examination:

After collection of fish, necropsy was done immediately, and the gills were examined for

any gross change. The gills tissues were fixed in 10% neutral buffer formalin (NBF) for microscopic investigation. Three days after fixation in NBF, fixed gills tissues were processed and embedded into paraffin blocks. Paraffin blocks were cut into 3-µm thickness and stained with haematoxylin and eosin (HE) stain according to (Bancroft and Gamble, 2002).

Statistical analysis

Statistical analysis was performed by Chi square test and Fisher's Exact Test using SPSS software.

RESULTS

Prevalence of Trichodina spp.

Oreochromis niloticus from two localities of Behera province, Egypt were examined for the occurrence of Trichodina spp. Table (1) revealed the prevalence of Trichodina spp. Infecting Tilapia in Behera governorate. The total infected Oreochromis niloticus was 36 (4.82%). This protozoan was reported in 22 (4.36%) in Kom Hamada and in 14 (5.76%) in Delingat city. Concerning the weight of examined fishes, Trichodina spp. was recorded in 30 (6.45%) in the first group (15-100 gm), 5 (8.93%) in the second group (100-200 gm) and 1 (5.88%) in the third group. Concerning to single or mixed infections, only Trichodina spp was reported in 5 (0.67%), mixed Trichodina with digenean encysted metaceracria in 24 (3.21%), mixed with monogenea in 2 (0.27%) and mixed with monogenea and digenean encysted metacercaria in 5 (0.67%). The locality and the weight of examined Oreochromis niloticus had no significant effect on the occurrence of Trichodina spp. (Table 1).

Regarding the monthly incidence of Trichodina spp. in the examined fish, the highest infection rate was in December (21.8%), whereas the lowest infection rate was recorded in August (1.2%).

The results in (Table 2) recorded the seasonal prevalence of *Trichodina spp.* in examined 747 *Oreochromis niloticus*. The infection rate with *Trichodina spp.* was 6.67% in winter, 1.57% in spring, 0.93% in summer and 7.16% in autumn. The highest *Trichodina spp*. infection was 7.16 % in autumn season followed by 6.67% in winter season. While the lowest *Trichodina spp.* infection was 0.93 % in summer. The season had significant effect on the prevalence of *Trichodina spp.* in *Oreochromis niloticus* as shown in table (2).

The Morphological description and measurements of Trichodina spp .

Morphological descriptions of Trichodina spp. were recorded. Measurements of their body diameter, diameter of central adhesive disc and number of denticles were reported. Trichodina spp. are disc shaped and their body diameters were 76 µm (64- 88um). The diameter of their adhesive disc was 67.5 µm (55-80 µm), while the denticle ring diameter was 40 µm. The number of denticles was 28 (27-29). (Fig. 1a, b, c).

Histopathological examination

Grossly, fish gills were thickened and congested, and sometimes distorted as shown in (Plate 2).

Microscopic examination of gills revealed that gill filaments were slightly thickened with inflammatory cells infiltration and gills epithelial cells were necrosed and sloughed. Blood vessels in the primary lamella were congested and gills secondary lamellae were distorted fused and congested as shown in (Fig. 3a). Mild inflammation and congestion were observed in between the bony cartilage and supporting muscle in the gill base. Mast cell/ eosinophilic granule cells containing multiple eosinophilic granules were observed (Fig. 3b). Trichodina spp. appeared as multiple ring-shaped (disc shape) structure attached to the supporting gill structure and surrounded by few inflammatory cells (Fig. 3 c, d).

Table (1) prevalence of *Trichodina spp.* Infecting Oreochromis niloticus in different localities of Behera governorate and different weights of examined fishes

Parameter	Classification	N= 747	*Trichodina spp.* Infecting		Chi-square	P-value	Sig.
			Freq.	%			
Locality	Kom Hamada	504	22	4.36%	0.70	0.40	NS
	Delingat	243	14	5.76%			
Weight of examined fishes	15-100 gm	549	30	5.46%	1.12	0.57	NS
	100-200 gm	56	5	8.93%			
	> 200 gm	17	1	5.88%			

Table (2) Seasonal prevalence of *Trichodina spp*. In Oreochromis niloticus in Behera governorate, Egypt**:**

Season	N=747	*Trichodina spp.* infection		Chi-square	P-value	Sig.
		Freq.	%			
Winter	30	2	6.67%	13.14	0.004	Yes
Spring	191	3	1.57%			
Summer	107	1	0.93%			
Autumn	419	30	7.16%			

The Morphological description and measurements of Trichodina spp.

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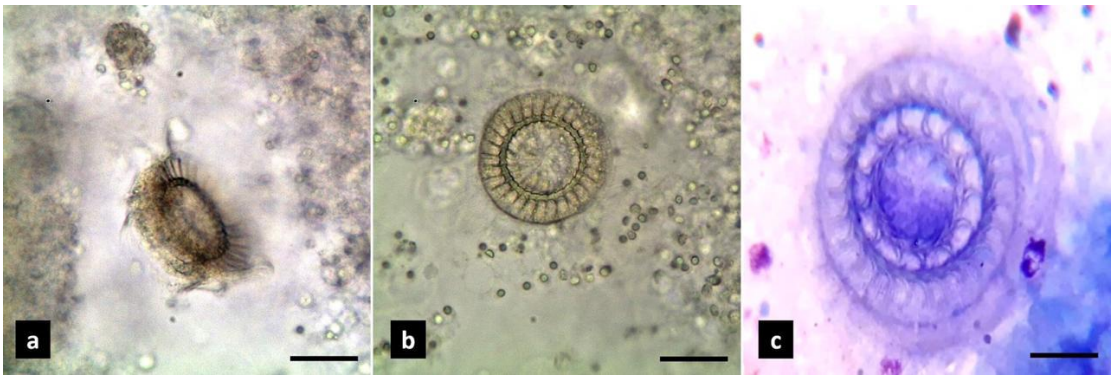

Fig. 1a, Fig. 1b: wet mount *Trichodina spp*. (X40)
Fig. 1c: Giemsa stained smear of *Trichodina spp*. (X100)

Plate (2): The fish gills were congested, and sometimes distorted (arrow).

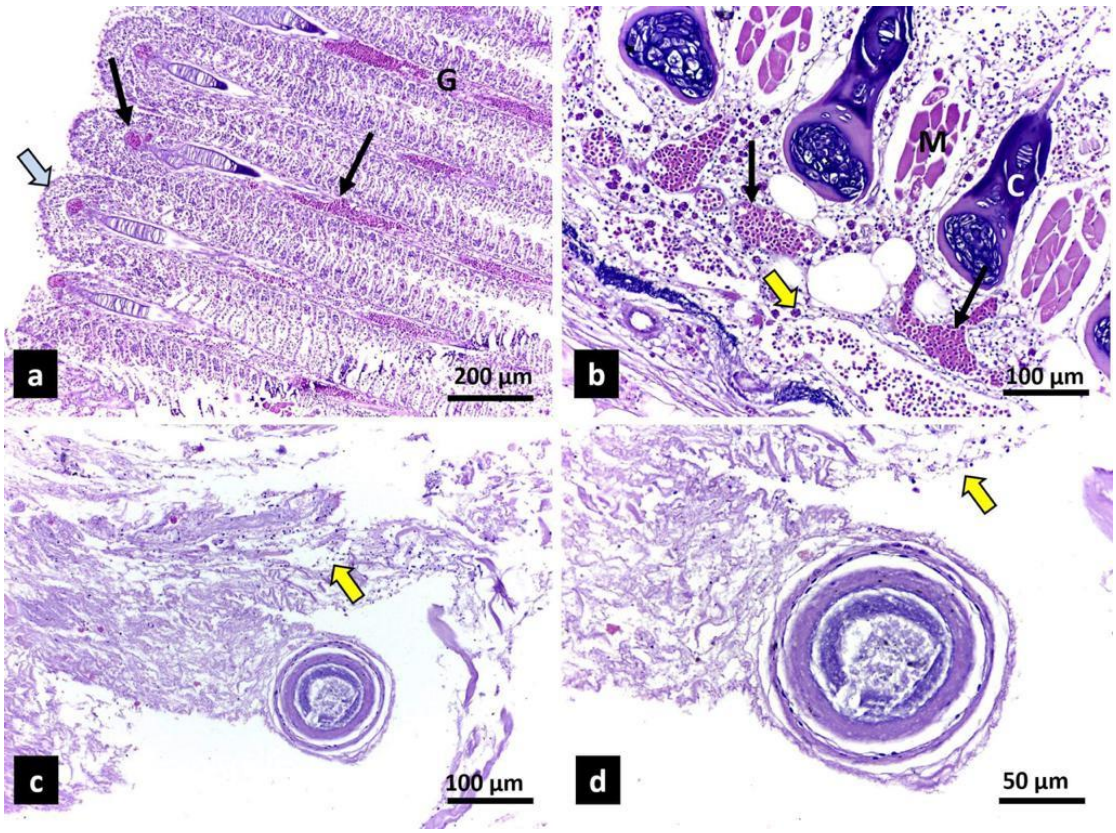

Fig. 2a: Gill filaments (G) were slightly thickened with inflammatory cells, blood vessels in the primary lamella were congested (thin arrow) and gill epithelium was necrosed and sloughing (thick arrow). H&E X10.
Fig. 2b: Mast cell/ eosinophilic granule cells (thick arrow) and congestion of blood vessels (thin arrow) were observed in between the bony cartilage (C) and supporting muscle (M) in the gill base. H&E X20.
Fig. 2c: *Trichodina spp*. appeared as multiple ring-shaped structure and surrounded by mild inflammatory reaction. Fig. 2d: high magnification of previous image. H&E X20 and 40, respectively.

DISCUSSION

The present study was carried out on 747 Oreochromis niloticus to determine the seasonal dynamic of Trichodina spp. and their pathological effect on the gills of infected fish. The infection rate of Trichodina spp. in the present study was 4.82%. The highest Trichodina spp. infections were recorded during autumn (7.16%) while their lowest infection was reported in summer season (0.93%). These seasonal occurrence of Trichodina spp. disagreed with (Noor El- Deen *et al.*, 2015) who found that the highest infection in summer season in Kafr El sheihk, Alabasa and Alfayom in O. niloticus. Also, the recorded seasonal prevalence of trichodinids disagreed with (Borji *et al.*, 2012) who recorded the highest occurrence of this ciliate in carp in summer season .

Trichodina spp. in the current study was recorded all over the year with highest infection rate in autumn followed by winter season. It appears that the high-water temperature affects the survival and multiplication of Trichodinids. These findings agree with (Abu El-Wafa, 1988; El-Khatib, 1989 and Hassan, 1999) who reported that trichodinids were prevalent throughout the year with highest infection rate during cold season.

The prevalence of Trichodina spp. in this study was fluctuated due to some ecological conditions as oxygen, temperature and water quality. This observation was discussed by (Hassan, 1999; Kristmundsson *et al.*, 2006 and Yemmen *et al.*, 2010)

The Morphological description of Trichodina spp. and their measurements was agreed with Kabata (1992); Yemmen *et al.*, (2010) and Noor El- Deen *et al.*, (2015).

Trichodina spp. in the present study are discoidal, their body diameters and their adhesive discs diameters agreed with (Nilsen 1995) and Yemmen *et al.*, (2010). In addition, the denticle ring size and the number of denticles of Trichodina spp. agreed with previous studies as (Nilsen 1995) and Yemmen *et al.*, (2010). The identification of different species of Trichodina depend on the number, shape and arrangement of teeth on the denticle (Kruger *et al.*, 1991)

In the present study, there was thickening of gill filaments, congestion and fusions of gills secondary lamellae which agreed with previous studies that Trichodina spp. infection causes haemorrhage, oedema, congestion, leucocytic infiltration and fusion of gills (Abd EL- Hady, 1998; Yemmen *et al.*, 2011 and Noor El- Deen, 2015).

Trichodinids are pathogenic external parasites to fish in most cases (Thomas and Wellborn, 1967), interfere with respiration and nutrition of fish (Ahmed 1976), and cause whole damage of epithelium of gills (Paperna 1980; Eisa *et al*. 1985).

Trichodinads are ciliated ectoparasites that reside in gills and skin of fish and amphibians but may inhabit U.B. of frogs (Collymore *et al*., 2013). The pathogenicity of Trichodina spp. depends on their presence in high or low numbers. When fish infected with low number of trichodinids, these organisms do not cause any lesions. But become pathogenic when they increase in number (Mitchell, 2007; Pessier, 2009; Collymore *et al*., 2013), which agree with our results in this study.

The large population of these ciliates indicates overcrowding, bad nutrition, bad quality of water (Kent and Fournie 2007; Mitchell, 2007). Trichodinads feed on organic materials in water and their increasing lead to increase in these organisms which cause tissue damage and ulcerative lesions in the affected gills (Roberts *et al*., 2009).

The pathogenic effect of Trichodina spp. on gills may be due to their attachment, locomotion, feeding and fixation that cause severe damage of the gills. Moreover, high mucous production was observed on all parts of infected fish as a mechanism of host defense to overcome the ectoparasitic infection as previously mentioned by (Yemmen *et al.*, 2011 and Abdelkhalek *et al.*, 2018).

CONCLUSION

The presence of Trichodina spp. in gills of Tilapia was confirmed by parasitological and Histopathological examinations. Its prevalence was affected significantly by season not by locality or weight of examined fish. Their pathogenic effects may be due to poor quality of the water and the presence of sewage, algae, aquatic plants and chemicals in these canals.

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