



Research Article

Prevalence of Monogenean Trematodes of Tilapia species from River Nile in Beheira Province, Egypt

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ABSTRACT

The objective of this study was to investigate the prevalence of Monogenean Ectoparasites in Tilapia species from the Nile region in Beheira Province, Egypt. This study was carried out from January to December 2019 at two different localities of Beheira governorate to determine the prevalence of *Cichlidogyrus* spp. and *Gyrodactylus* spp. in Tilapia fish and their histopathological effects. Of the 870 examined Tilapia fishes, 83 (9.54%) were infested with *Cichlidogyrus* spp. and 13 (1.49%) were infested with *Gyrodactylus* spp. The *Cichlidogyrus* spp. had the highest infestation rate with a significant difference in all seasons. According to the Tilapia weighing, there were no significant differences between the weight of fish and infestation rates. Concerning the Tilapia species, the infestation rate with *Cichlidogyrus* spp. was recorded to be the highest in *T. zillii* (26.4%), whereas the infestation rate with *Gyrodactylus* spp. was similar in all Tilapia species. The affected gills manifested thickening and necrotic changes by histopathological examination. The parasites reported may affect the health and quality of fish leading to condemnation at inspection.

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Introduction

The infectious diseases of freshwater fish under natural conditions usually have low incidence because of the reduced risk of contact between parasites and fish. Among the variety of freshwater fish, tilapia has become one of the most important sources of animal proteins in the world (Wang and Lu 2015). Lacking health management measures lead to many disease problems in fish (Eissa 2002). Usually, about 80% of fish diseases are parasitic. Generally, the infestation with fish parasites is an economic loss not only due to mortalities, tissue damage and growth reduction but also due to the high expenses of treatment drugs (El-Asely et al. 2015). Moreover, the fish serves as an intermediate host for many parasites which reduces the food value of fish and results in high mortality. The majority of

freshwater fishes carry heavy infestation of parasites of protozoa or helminths (Reed et al. 2012). Furthermore, the Monogeneans are the most important Ectoparasites of fish belonging to the flatworms group found on gills and skin (Reed et al. 2012). Monogeneans have a direct life cycle and the larva is usually a small ciliated oncomiracidium that hatches from the egg and swims to locate and infect another host (Mono, 2015). The monogeneans include more than 100 families and most of the species are host and site-specific. The few studies have illustrated the host-parasite relationship of monogeneans using different strategies of attachment to the hosts as well as their reproductive mechanisms (Hirazawa et al. 2010). Generally, the intensity of parasitic infection of freshwater fishes is determined by the assessment of water quality and mortality of fish (Wilson et al. 2019). The present study was carried out to investigate the prevalence of monogenetic Trematode infestation

among freshwater fish (*Tilapia* species) as well as evaluation of histopathological changes induced by the detected parasites.

Materials and Methods

Study period and area

The study was carried out all over one year (four seasons) extending from January to December 2019 (Winter, Spring, September, Autumn) at two different localities of Beheira governorate (Kom Hamada and Delingat) to determine the prevalence of *Cichlidogyrus* spp & *Gyrodactylus* sp. in *Tilapia* fish and their histopathological effects.

Fish samples

A total number of 870 *Tilapia* fish were collected once weekly (18) from the examined locality and transported in live to Parasitology Lab, Faculty of Veterinary Medicine, University of Sadat City, Beheira, Egypt.

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

Clinical Examination

Alive collected fish were physically inspected for their color changes, swimming behavior, respiratory signs, and other abnormalities on gills such as petechial hemorrhage, ulcers and slimness according (Noga, 2010).

Parasitological examination

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

Histopathological examination

Gross examination was done immediately after collection of *Tilapia*, and the gills were examined for any evident changes. Then gills tissues were fixed in 10% neutral buffer formalin for 3 days. The fixed samples were processed and embedded into paraffin blocks. Sections of 3- μ m thickness were cut and stained with Hematoxylin and Eosin (HE) stain for light microscopic examination according to Bancroft and Gamble (2002).

Statistical analysis

Chi-square test and Fisher's Exact Test using SPSS software was performed the statistical analysis.

Results

Oreochromis niloticus, *Sarotherodon galilaeus*, *Tilapia zillii* and *Oreochromis aureus* from two localities of Beheira Province, Egypt were examined for the occurrence of monogenean parasites and revealed the infestation with two monogenean parasites (*Cichlidogyrus* spp. and *Gyrodactylus* spp.).

From the total 870 examined fishes, 83 (9.54%) were infected with *Cichlidogyrus* spp. and 13 (1.49%) were

infected with *Gyrodactylus* spp. From 627 examined fishes from Kom Hamada: *Cichlidogyrus* spp. and *Gyrodactylus* spp. were reported in 54 (8.61%) and 9 (1.44) and reported in 29 (11.93%) and 4 (1.65%) from 243 examined fishes from Delingat city respectively. There was no significant difference ($P>0.05$) in the distribution of the parasites as shown in Table 1.

Seasonally, there was a significant difference ($P\leq 0.05$) in the spread of *Cichlidogyrus* spp. in the different seasons, while there was no significant difference ($P>0.05$) in the spread of *Gyrodactylus* spp. among the different seasons. The infection rate of *Cichlidogyrus* spp. was (4.19%, 11.92%, 11.93% and 6.42%) and *Gyrodactylus* spp. was (1.57%, 1.99%, 1.67% and 0.00%) in spring, summer, autumn and winter, respectively (Table 2).

Concerning the weight of examined fishes, no significant difference ($P>0.05$) was found in the infection rate of gill parasites among the different weight-based groups as shown in Table (3). *Cichlidogyrus* spp. and *Gyrodactylus* spp. were recorded in 59 (9.25%) and 8 (1.25%). From total 638 examined fishes in the first group (15-100 g), 47 (77.05%), 5 (8.20%), 9 (14.75%) and 0 (0%) from total 61 examined fishes in the second group (100-200 g) and 9 (50%), 1 (5.56%), 2 (11.11%) and 0 (0%) from total 18 examined fishes in the third group (>200 g), respectively.

Regarding the prevalence of monogenean parasites in the different *Tilapia* species, was found the significant difference ($P\leq 0.05$) in infection rate of *Cichlidogyrus* spp. and *Gyrodactylus* spp. among different species of *Tilapia*. The infection rate of *Cichlidogyrus* spp. was 1.05%, 16.30%, 26.36% and 0.0% in *S. galilaeus*, *O. niloticus*, *T. zillii* and *O. aureus* respectively. While *Gyrodactylus* spp. the infection rates were 0.52%, 2.90%, 2.33% and 0.0% in *S. galilaeus*, *O. niloticus*, *T. zillii* and *O. aureus* respectively as shown in Table (4). On the other hand, Table (5) showed the single infection of *Cichlidogyrus* spp. was recorded in 15 fishes (18.1%) and in 68 fishes (81.93%) as a mixed infection., Whereas, The *Gyrodactylus* spp. was recorded in only one fish (7.69%) as a single infection, while it was reported in 12 fishes (92.31%) as a mixed infection.

The morphological description and measurements of recovered monogenean parasites

Cichlidogyrus spp.

The Fig (1a, b, c) illustrated the body of the worms was elongated as the body diameters were 0.3-0.6 mm a length and 0.04-0.1mm in width. At the anterior end, three pairs of cephalic glands at two posterior ocelli (eyespots) with two small inconstant anterior ocelli. Simple intestinal branches joined posteriorly. Male copulatory complex with penis and accessory piece. Median posterior testis with Vas deferens on the right side, not encircling the intestinal branch. Median pre-testicular ovary and ventral sub-median vaginal opening. At the posterior end, two pairs of anchors, one dorsal and one ventral with two transverse bars, dorsal with two auricles while the ventral one is V-shaped with fourteen marginal hooks.

Gyrodactylus spp.

The worm is characterized by an elongated body 0.4-0.8 mm long and 0.03-0.07 mm wide at middle.

Double pointed anterior end and no eyespots. Large opisthaptor consists of two centrally positioned approximately parallel large hooks about (0.05mm) in length joined by two connecting bars, a simple dorsal bar and an approximately triangular shaped ventral bar. There are 16 marginal hooks positioned around the periphery of the opisthaptor, as illustrated in Fig (2a, b, c).

Histopathological examination

In all infected *Tilapia* sp. their gills revealed congestion, multifocal thickening and distortion.

Microscopically, the gills were severely infested with monogenean spp. causing thickening of gills (Fig. 3a). Gill epithelium showed epithelial hyperplasia (Fig. 3b). The parasite was observed in gill filaments' secondary lamellae. The gills' secondary lamellae were fused to encyst the parasite (Fig. 3c). Another area of gills showed necrosis and sloughing of gill epithelium with congestion of blood vessels (Fig. 3d).

Discussion

The present study was carried out on 870 *Tilapia* fishes to determine the seasonal dynamics of different gill parasites and the effect of some biological factors as body weight, which is referring to the age and the different species of *Tilapia* on parasite occurrence in *Tilapia* fishes from two localities: Kom Hamada and Delingat in Beheira Province, Egypt.

The present study aimed to throw more light on the prevalence of different monogenean parasites infecting *Tilapia* spp. and their histopathological effects.

The fishes showed signs of respiratory distress manifested by rapid breathing and aggregation of fish near the water surface "Piping" which also mentioned by (Younis et al. 2009; Reda et al. 2010; Noor El- Deen et al. 2010, 2015; Aly et al. 2020). Grossly, the gills were congested, pale or marbled appearance, covered with mucoid secretions destructed or fried appearance of gills some fishes showed white spots in the gills which were also described by (Younis et al. 2009; Noor El- Deen et al. 2010, 2015; Reda et al. 2010; Aly et al. 2020). In the current study, the infection rate of *Cichlidogyrus* spp. was (9.54%). The highest *Cichlidogyrus* spp. infections were recorded during autumn and summer (11.93% & 11.92%), while their lowest infection was reported in spring season (4.19%). This result disagrees with Mahmoud et al. (2009) who recorded the highest infection rate of *O. niloticus* by monogeneans (*Macrogyrodactylus congolensis* and *Cichlidogyrus tubicirrus magnus*) in spring. Moreover, the lowest infection in autumn and disagrees with Marzouk et al. (2013). The highest prevalence of *C. halli* in *O. niloticus* from fish farms in Sharkia, Kalubia and Giza governorates was in spring and the lowest infection rate was in summer. Moreover, agrees with El-Seify et al. (2011) as the highest prevalence of Monogenean parasites (*Cichlidogyrus tilapiae*, *Cichlidogyrus aegypticus*, *Cichlidogyrus cirratus*, *Macrogyrodactylus clarii*) in *O. niloticus* in Kafr Elsheikh Province was in autumn

and the lowest prevalence was in spring. The prevalence of *Cichlidogyrus* spp. in this study was fluctuated due to some ecological Conditions.

The infection rate of *Gyrodactylus* spp. in the present study was (9.54%). The highest *Gyrodactylus* spp. infections were recorded during summer (1.99%) while their lowest infection rate (no infection) was reported in the winter season agreeing with Gado et al. (2017a) of the infection rate of monogenean trematodes (*Dactylogyrus* spp. and *Gyrodactylus* spp.) in *O. niloticus* in Kafr El-Sheikh governorate. Furthermore, the highest infection rate in summer and the lowest rate of infection in winter and agrees with Shehata et al. (2018) who recorded that the prevalence of parasites among examined fish was the highest in summer and the lowest in winter. Moreover, disagrees with Mahmoud et al. (2009) who recorded the highest rate of infection of *O. niloticus* by monogeneans (*Macrogyrodactylus congolensis* and *Cichlidogyrus tubicirrus magnus*) in spring and the lowest in autumn.

The prevalence of monogenean parasites in this study was fluctuated due to some ecological conditions such as oxygen, temperature and water quality. This observation was discussed with Hassan (1999) and Yemmen et al. (2010).

In this study, the *Cichlidogyrus* spp. appeared with characteristic four cephalic lobes, three pairs of cephalic glands and two posterior ocelli (eyespot) with two small inconstant anterior ocelli. In addition, simple intestinal branches joined posteriorly, male copulatory complex with penis and accessory piece, two pairs of posterior anchors, one dorsal and one ventral with two transverse bars, dorsal with two auricles while the ventral one is V-shaped with fourteen marginal hooks. The Morphological description of *Cichlidogyrus* spp. and their body diameters agreed with (Pariselle and Euzet 1996, 1998; Florio et al. 2009; Morsy et al. 2012; Noor El-Deen et al. 2015; Agos et al. 2016; Lim et al. 2016; Hussein et al. 2019; Zhang et al. 2019; Aly et al. 2020).

Moreover, *Gyrodactylus* spp. observed in this study characterized by elongated body double-pointed anterior end and no eyespots. A large opisthaptor consists of two centrally positioned large hooks about joined by two connecting bars, a simple dorsal bar and an approximately triangular-shaped ventral bar. There are 16 marginal hooks positioned around the periphery of the opisthaptor. The Morphological description of *Gyrodactylus* spp. and their measurements agreed with (Cone et al. 1995; Florio et al. 2009; Younis et al. 2009; Morsy et al. 2012; Gado et al. 2017b; Ashmawy et al. 2018; Zhang et al. 2019). Histopathological lesions in gill tissue due to monogenean infestation can be summarized as gill epithelium having hyperplasia, congestion of blood vessels, the gills' secondary lamellae were fused together and sometimes encyst the parasite. Also, gills showed necrosis and sloughing of gill epithelium.

Table 1: Prevalence of monogenean parasites in examined fish samples in Beheira province

Locality	No. examined	<i>Cichlidogyrus spp.</i>		<i>Gyrodactylus spp.</i>	
		No. infected	% of infection	No. infected	% of infection
Kom Hamada	627	54	8.61	9	1.44
Delingat	243	29	11.93	4	1.65
Chi square		2.239		0.053	
P-value		0.157 ^{NS}		0.763 ^{NS}	

NS Non-Significant difference at P>0.05

Table 2: Seasonal Prevalence of monogenean parasites in examined Tilapia.

** High significant difference at P≤0.01

Season	Examined fish	<i>Cichlidogyrus spp.</i>		<i>Gyrodactylus spp.</i>	
		No. infected	% of infection	No. infected	% of infection
Spring (write months)	191	8	4.19	3	1.57
Summer	151	18	11.92	3	1.99
Autumn	419	50	11.93	7	1.67
Winter	109	7	6.42	0	0.00
Chi square		11.338**		1.998	
P-value		0.010		0.573	

Table 3: Prevalence rates of monogenean parasites in examined fishes with different weight groups

Weight (g)	No. Examined	<i>Cichlidogyrus spp.</i>		<i>Gyrodactylus spp.</i>	
		No. infected	% of infection	No. infected	% of infection
15 - 100	638	59	9.25	8	1.25
100 - 200	61	9	14.75	0	0.00
>200	18	2	11.11	0	0.00
Chi square		1.954		1.002	
P-value		0.376 ^{NS}		0.606 ^{NS}	

NS Non-Significant difference at P>0.05

Table 4: Prevalence of monogenean parasites in different *Tilapia sp.*

Tilapia species	No. Examined	<i>Cichlidogyrus spp.</i>		<i>Gyrodactylus spp.</i>	
		No. infected	% of infection	No. infected	% of infection
<i>S. galilaeus</i>	381	4	1.05	2	0.52
<i>O. niloticus</i>	276	45	16.30	8	2.90
<i>T. zillii</i>	129	34	26.36	3	2.33
<i>O. aureus</i>	84	0	0.00	0	0.00
Chi square		97.587***		8.010*	
P-value		0.000		0.046	

*Significant difference at P≤0.05; *** Very high significant difference at P≤0.00

Table 5: Single and mixed infection of monogenean parasites in examined Tilapia

Parasite	Number of infected fish	Single infection		Mixed infection	
		No. infected	% of infection	No. infected	% of infection
Encysted metacercaria of Digenean parasites	627	526	83.89	101	16.11
<i>Cichlidogyrus spp.</i>	83	15	18.07	68	81.93
<i>Gyrodactylus spp.</i>	13	1	7.69	12	92.31

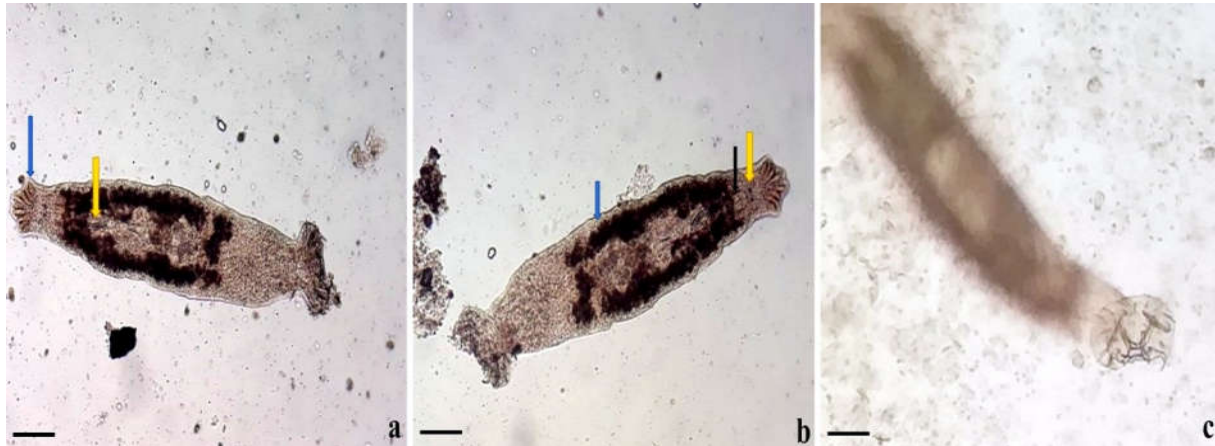


Fig. 1: a. Wet mount of *Cichlidogyrus* spp. showing cephalic glands (blue arrow) and male copulatory complex (yellow arrow). b. *Cichlidogyrus* spp. with two posterior eyespots (yellow arrow), pharynx (black arrow) and intestinal branches (blue arrow). c. Posterior end of *Cichlidogyrus* spp. showing dorsal and ventral anchors with transverse bars and marginal hooks.

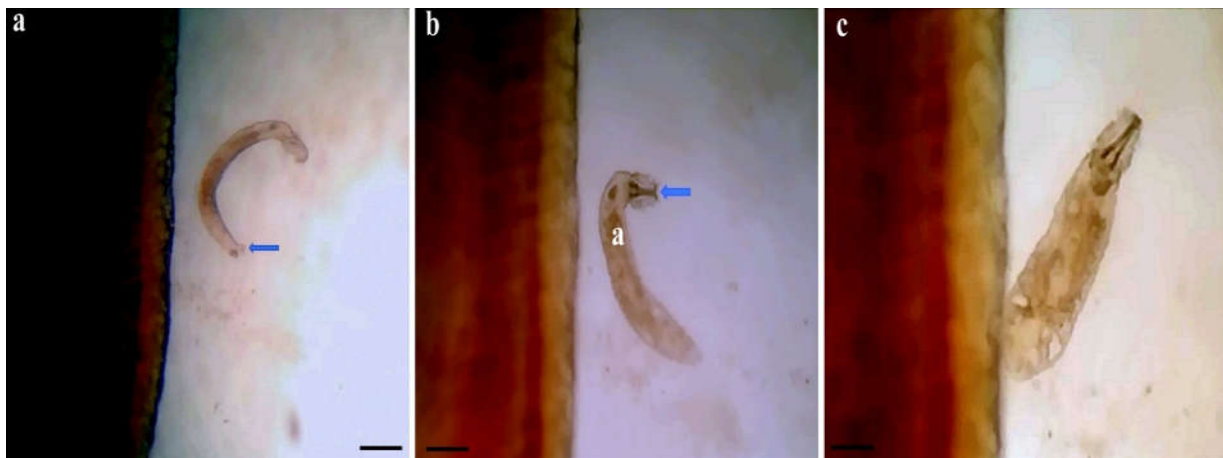


Fig. 2: a. Wet mount of *Gyrodactylus* spp. b. Double pointed anterior end of *Gyrodactylus* spp. (arrow). c. Large opisthaptor with two centrally positioned approximately parallel large hooks and marginal hooks (arrow).

All of these histopathological lesions were previously mentioned by (Jalali and Barzegar 2005; Abdel Latif et al. 2009; Arafa et al. 2009; Mahmoud et al. 2009, 2014; El-Mansy et al. 2011; Mahmoud and Gindy 2011).

Conclusions

In conclusion, the current study was attention to monogenean parasites of Tilapia underlining the need for epidemiological studies and appropriate preventive and control programs, in order to monitor their occurrence and prevent their negative consequences for the economy, biodiversity, scientific research, animal and public health. Some aspects of biology and epidemiology, regarding both animal and human hosts, should be further explored.

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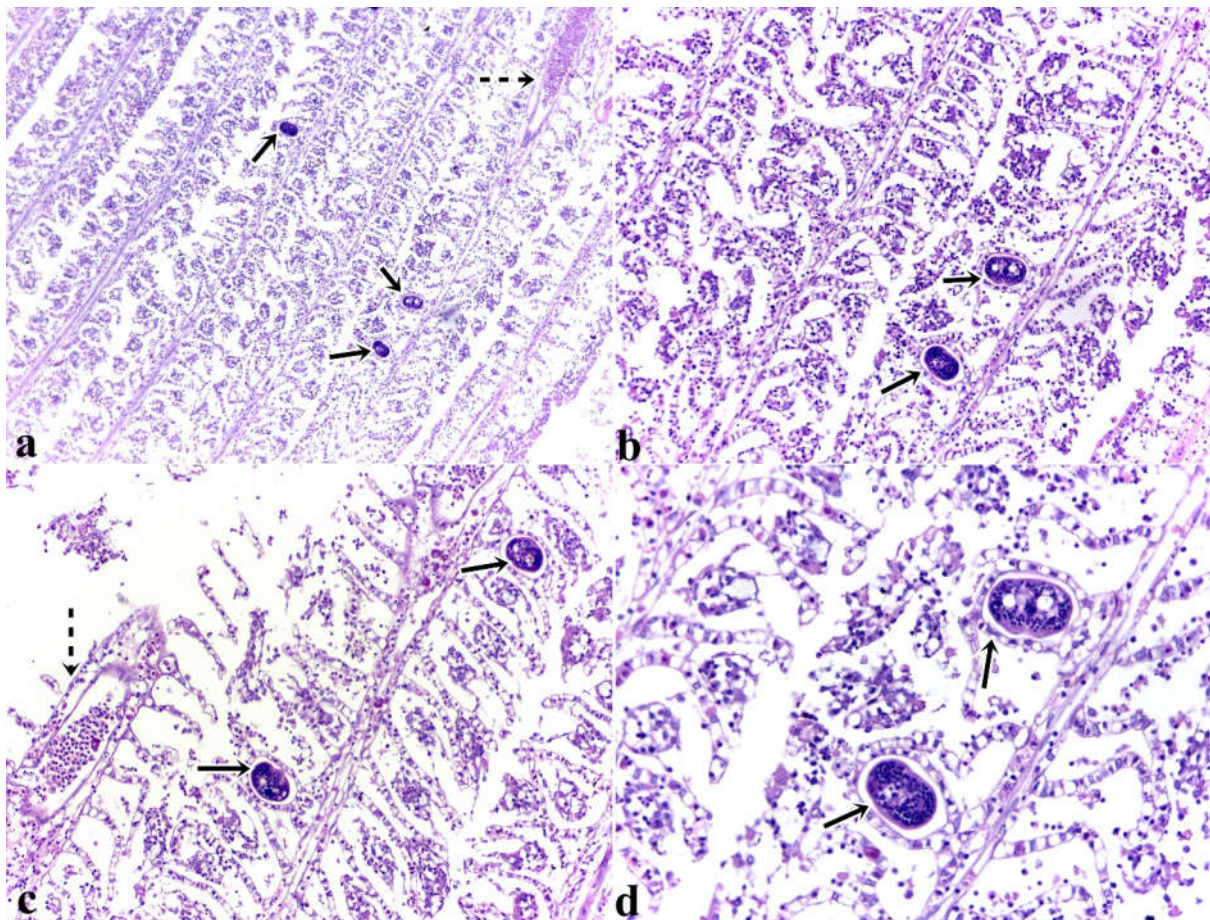


Fig 3: a. The gills were severely infested with *monogenean* spp. causing thickening of gills (arrows) with congested blood vessels (dashed arrow). H&E X10. b. Gill epithelium had hyperplasia (arrows). H&E X20. c. Gills showed necrosis and sloughing of their epithelium with presence of parasite (thin arrow) and congestion of blood vessels (dashed arrow). H&E X20. d. The gills`secondary lamellae were fused to encyst the parasite (arrows). H&E X40.

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