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ORIGINAL ARTICLE

First report of *Posthodiplostomum minimum* metacercariae and resulting histopathological changes in *Bagrus bajad* from Lake Nasser, Egypt

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Abstract Parasitological studies of 100 *Bagrus bajad* fish were collected from Lake Nasser, Aswan, Egypt. Out of them 75 were infected with metacercariae in two forms; encysted in most visceral organs and free only in air bladder. These were identified morphologically as *Posthodiplostomum* minimum metacercariae (Strigeidida: Diplostomidae). The intensity of white grub in *B. bajad* was high in the intestine (30 encysted metacercariae/g). The highest prevalence season observed in summer and the lowest in winter. The histopathological examination revealed encysted metacercariae of different sizes within the intestinal musculature accompanied with granular infiltration with eosinophilia. Degenerative changes were observed in liver and kidney cells, as well as congestion of hepatic sinusoids. Spleen showed both lymphoid and melanomacrophages cells depletion.

Keywords Metacercariae · Diplostomidae · *Posthodiplostomum minimum* · White grub · *Bagrus bajad* · Histopathology

Introduction

Diplostomidae are worldwide distributed digeneans parasites of high importance, required three different hosts to complete their life cycle; snails and a wide range of fish as first and second intermediate hosts respectively as well as, piscivorous birds and mammals as final hosts (Choudhary et al. 2017). This family contains different species of *Posthodiplostomum* (Dubois 1936). The metacercariae of *Posthodiplostomum cuticola* causing “neascus” or “black spot” disease in fish (Karimian et al. 2013) and *Posthodiplostomum minimum* (MacCallum 1921) encysts in viscera and sometimes in musculature of fish given what is known “white grub” (Grizzle and Goldsby 1996; Lane and Morris 2000; Lane et al. 2015; Boone et al. 2018). The penetration of large numbers of the *P.* minimum cercariae into a fish may cause its death especially during the first few days after infection (Meade and Bedinger 1967). Fish mortality is expected when the liver is damaged by the metacercariae (Lane and Morris 2010).

P. minimum grubs have an adverse effect on the fish; reducing their growth rate, loss the condition of the infected fish and impair the immune functions leave the fish more susceptible to a variety of secondary diseases (Grizzle and Goldsby 1996) as well as, visible *P. minimum* parasites render the fish undesirable by the consumers.

Bagrus bajad (Forsskål 1775) is a siluriformes freshwater fish in the family: Bagridae, inhabits many lakes, swamps and rivers in Africa including River Nile. *B. bajad* lives and feeds on or near the bottom. Adults are piscivorous, prey on small fish, particularly *Alestes* spp. Also, *B. bajad* feeds on insects, crustaceans, mollusks and vegetable matter. It is an important food fish in addition to its flesh is of good quality and of economic importance commonly sold as food (Pauly and Froese 2007).

Despite records of *P. minimum* infections in several fish species, the identification and pathogenesis of this parasite in *B. bajad* are lacking. So the aim of this study is to characterize the *P. minimum* of *B. bajad*, recording the clinical signs, postmortem lesions, predilection sites of

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metacercariae, intensity of infection in each organ, seasonal prevalence and histopathological features of infected fish.

Material and methods

Study area and fish samples

Lake Nasser is an artificial lake created after the construction of Aswan High Dam on the River Nile in Egypt in the 1960s. It extends 300 km within the Egyptian borders, with a shoreline of about 7000 km and lies between Lat 22°00′–23°58′N and Long 31°19′–33°19′E. It represents the main source of freshwater in Egypt.

100 *B. bajad* (25 fish/season) of different sizes; total length (30–50) cm and weights (600–1000) gm were collected, alive and randomly, from different localities of Lake Nasser during the period of May 2017 to April 2018. The collected fish were kept in glass aquaria in the laboratory of Fish Diseases, Faculty of Fish and Fisheries Technology, Aswan University.

Clinical, postmortem and parasitological examinations

The investigated fish were subjected to the full clinical examination. They were euthanized rapidly by severance of the head and brain from the spinal cord followed by brain pithing according to AVMA (2013). The procedures complied with local and national animal welfare laws, guidelines and policies.

Detection of the external, internal gross lesions and parasitological examination for detection of free or encysted metacercariae (EMC) were performed according to (Lucky 1977; Woo 1995; Noga 2010).

The body surface, fins, gills, liver, spleen, kidney, stomach, intestine, heart, brain, eyes, air bladder, urinary bladder, gall bladder, mesentery and musculature were examined separately in separate petri dishes containing 0.9% physiological saline with a dissecting microscope. Parts of these organs were examined microscopically by direct compression between two glass slides for any parasitic nodules. The collected parasites were washed, fixed, stained and permanently mounted according to the methods described by (Lucky 1977; Woodland 2006). The density of infection with encysted metacercariae was recorded by taking several slices (1 g) from different parts of each organ tissue. Compress it between two glass slides and count the metacercariae in each gram then calculate the mean (El-Naffar and El-Shahawi 1986). The density of infection with free metacercariae in air bladder was recorded by counting them in each dissected fish then calculate the mean. The obtained results were statistically analyzed using the mean and the standard error of the mean (SEM) by using the SPSS software (SPSS 2007).

The detected free and encysted metacercariae were identified according to (Gibson 1996).

Histopathological examination

Sections of intestines, liver, kidney and spleen of the infected fish were preserved immediately in 10% formalin and processed for histopathological evaluation, using the routine paraffin embedding method as described by Bancroft and Gamble (2007).

Results

Grossly examination of *B. bajad* revealed the presence of several wounds on the infected fish skin (Fig. 1). While internally, the presence of numerous white small cysts; *P. minimum* encysted metacercariae in different organs of the infected fish (intestine, stomach, urinary bladder, mesentery, liver, spleen and the kidney) were recorded. Parasitic cyst wall was thin, and delicate. The encysted form was not larger than 1 mm and they reside in and around the fish's organs. They were not found in the musculature, heart, brain and eyes (Fig. 2). The free metacercariae were isolated only from the air bladder.

The metacercariae were belonging to:
Superfamily Diplostomoidea (Poirier 1886)
Syn. Strigeoidea (Railliet 1919)
Family Diplostomidae (Poirier 1886)
Subfamily Crassiphialinae
Genus*:* *Posthodiplostomum* (Dubois 1936))
P. minimum (MacCallum 1921) (Dubois 1936) (metacercaria)
Syn.: *Neascus vancleavei* (Agersborg 1926).

Fig. 1 *Bagrus bajad* from Lake Nasser infected with *Posthodiplostomum minimum* showing hemorrhagic patches on the caudal peduncle (black arrows)

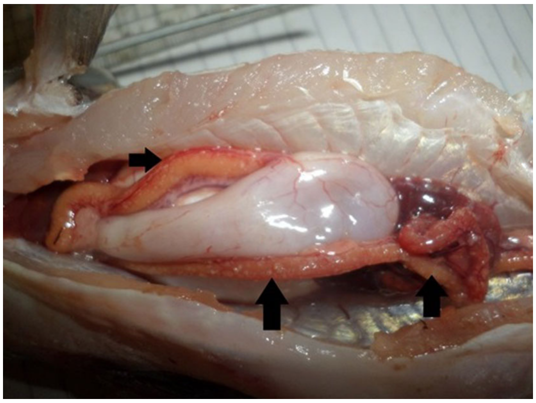

Fig. 2 *Bagrus bajad* showing white spots scattered all over the intestinal tract (arrows) and congested intestine and stomach

The body of free metacercaria was linguiform, obviously divided into two distinct regions by annular constriction; anterior part was scoop-shaped with distinct ventral concavity and posterior part (hind body) was oval to spherical. A pseudosucker was absent. The oral sucker was present normally, while the ventral one may be small. Primordiums of gonads were occasionally visible (Fig. 3). The encysted metacercariae of *P. minimum* were round or oval in shape, surrounded by two layers; the inner layer being of parasitic origin and the outer layer formed by the host. 1–3 metacercariae may be enclosed in the same cyst. The density of metacercariae per gram was high in the intestine (30/g) and in the urinary bladder (25/g) as in Table 1 and Fig. 4.

The overall prevalence of white grub; *P. minimum* metacercariae (free or encysted) in the fish was 75%. Intestine, urinary bladder, mesentery, stomach, liver, kidney and spleen were infected with encysted metacecraiae. The free *P. minimum* metacercaria was collected from the air bladder only. The intestine showed the highest (71%) prevalence of encysted metacercariae while air bladder showed the highest prevalence (70%) of free metaceracariae. spleen showed the lowest infection rate (38%) (Fig. 5) Seasonal occurrence of metacercariae was found to be the highest during summer and the lowest during winter as shown in Table 2.

The most prominent histopathological alterations were represented with the presence of different sized encysted metacercariae within the musculature of the infected intestine mostly subserosal. Sections of metacercarial cysts revealed that there were two layers, enclosed them as well as, they may contain more than one metacercariae in the same cyst. The cysts were also seen within normal tissue or associated with deep necrotic changes which mostly associated with inflammatory cells infiltration as eosinophilic granular cells, lymphocytes and macrophages. Other organs such as liver, kidney and spleen showed other secondary degenerative changes. Liver revealed marked ballooning of hepatocytes with peri-portal hepatic necrosis. The spleen showed marked congestion of the hepatic

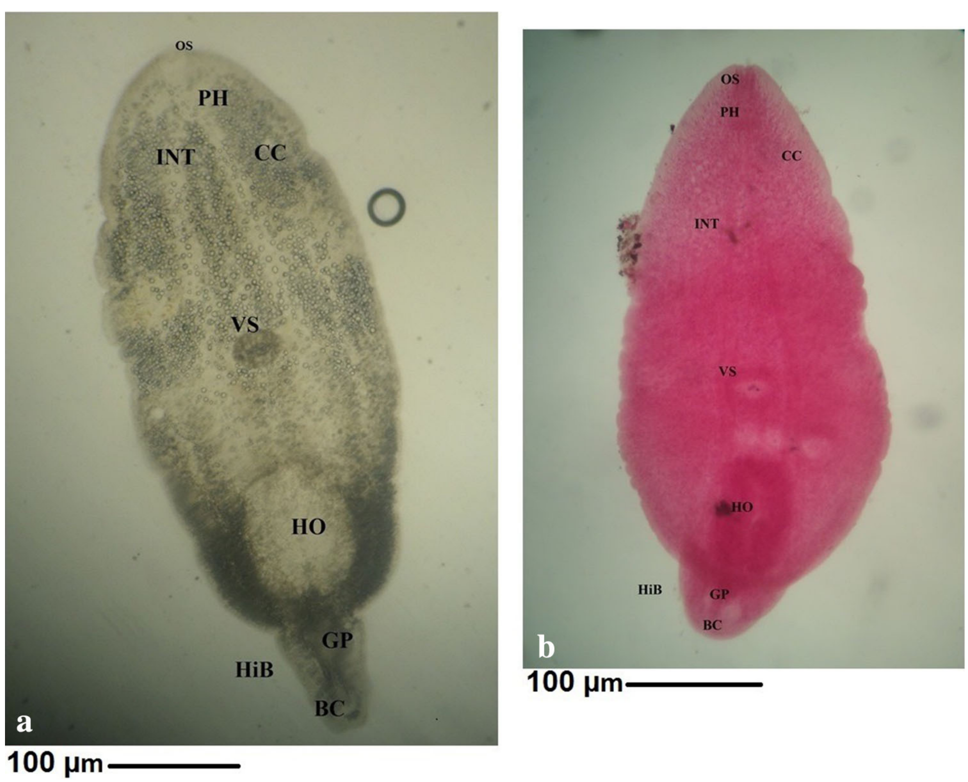

Fig. 3 *Posthodiplostomum minimum* (free metacercariae). **a** Unstained specimen × 220, **b** stained specimen (Carmine stain) × 132. Scale bar = 100 µm. *OS* oral sucker, *CC* calcareous corpuscles, *VS* ventral sucker, *PH* pharynx, *INT* intestine, *HO* hold fast organ, *HiB* hind body, *GP* genital primordia, *BC* bursa copulatrix

Table 1 Site and density of free and encysted metacercariae of *Posthodiplostomum minimum* in *Bagrus bajad* from Lake Nasser during the period of May 2017 to April 2018 (number of infected *B. bajad* = 75)

Parasite type	Site of infection	Density/g (M ± SEM)
P. minimum encysted metacercariae	Intestine	30 ± 0.4
	Urinary bladder	25 ± 0.3
	Mesentery	15 ± 0.5
	Stomach	10 ± 0.4
	Liver	9 ± 0.4
	Kidney	8 ± 0.3
	Spleen	6 ± 0.5
P. minimum free metacercariae	Air bladder	Density/dissected fish (M ± SEM)
		5 ± 0.3

Data represent the metacercariae (as the M (mean) ± SEM standard error of the mean)

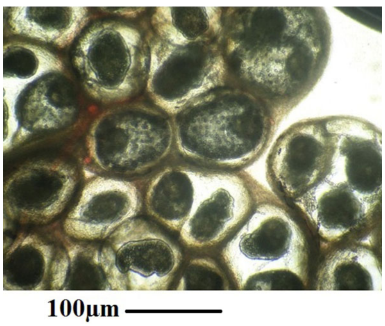

Fig. 4 *Posthodiplostomum minimum* encysted metacercariae in the urinary bladder wall. X 200. Scale bar = 100 µm. Unstained specimens

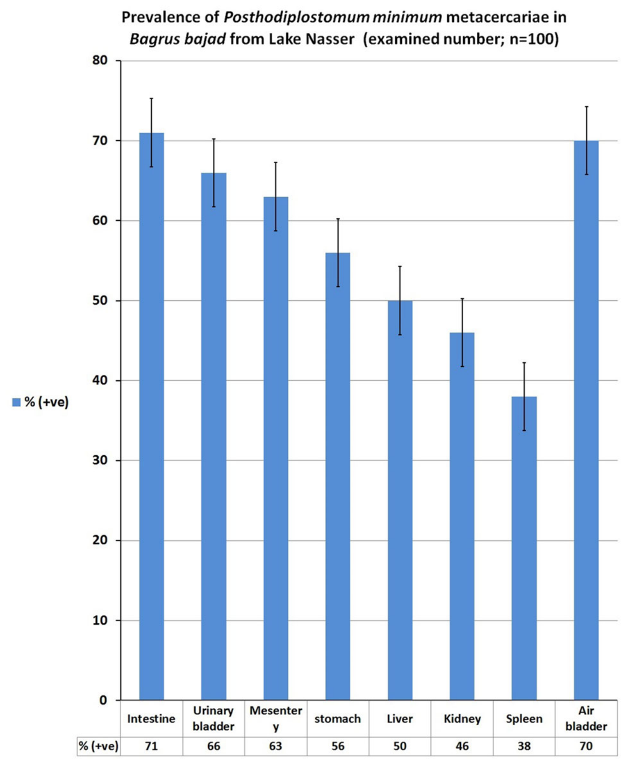

Fig. 5 Prevalence of *Posthodiplostomum minimum* metacercariae in *Bagrus bajad* from Lake Nasser during the period of May 2017 to April 2018 (examined number = 100)

sinusoids with depletion of both lymphoid elements and melanomacrophages cells. Kidney demonstrated features of interstitial nephritis associated with degeneration and necrosis of the renal tubules and infiltration of inflammatory cells including eosinophilic granular cells, lymphocytes and macrophages (Figs. 6, 7).

Discussion

Parasitic fish diseases constitute about 80% of fish diseases in Egypt (Eissa 2006). Which may have negative consequences on fish health by reducing weight gain and increasing mortality rates (Barber and Svensson 2003).

Actually, parasitological studies of *B. bajad* from Lake Nasser are very scarce and no reports on *Posthodiplostomum* metacercariae infection among this fish species in the lake. This grub does not attract as much attention as the yellow grub caused by *Clinostomum* sp. This may be due to it is smaller and encysts in the visceral organs (e.g. the stomach, the intestine, the kidneys, the liver…etc.) rather than throughout the branchial cavity or skin.

Gross examination of *B. bajad* revealed the presence of several wounds on the fish skin resulted from invasions of cercariae. *Posthodiplostomum* spp. in fish are complicated infections; need snails as an intermediate host of their propagation and transmission to fish; therefore hemorrhagic patches, wounds and ulcers on different parts of the fish's body due to blood congestion and hemorrhage at the point of cercaria penetration were always found on the

Table 2 Seasonal prevalence of free and encysted metacercariae of *Posthodiplostomum minimum* in *Bagrus bajad* from Lake Nasser (n = 25/season) during the period of May 2017 to April 2018

Infected organ	Season							
	Spring		Summer		Autumn		Winter	
	No.	%	No.	%	No.	%	No.	%
Intestine	18	72	22	88	16	64	15	60
Urinary bladder	17	68	20	80	15	60	14	56
Mesentery	16	64	20	80	14	56	13	52
Stomach	14	56	18	72	13	52	11	44
Liver	13	52	16	64	12	48	9	36
Kidney	12	48	15	60	11	44	8	32
Spleen	10	40	13	52	9	36	6	24
Air bladder	20	80	23	92	17	68	10	40

These numbers means one or more organs were infected in a single *B. bajad*. Musculature, Heart, brain and eye were free totally from infection

No. number of infected fish

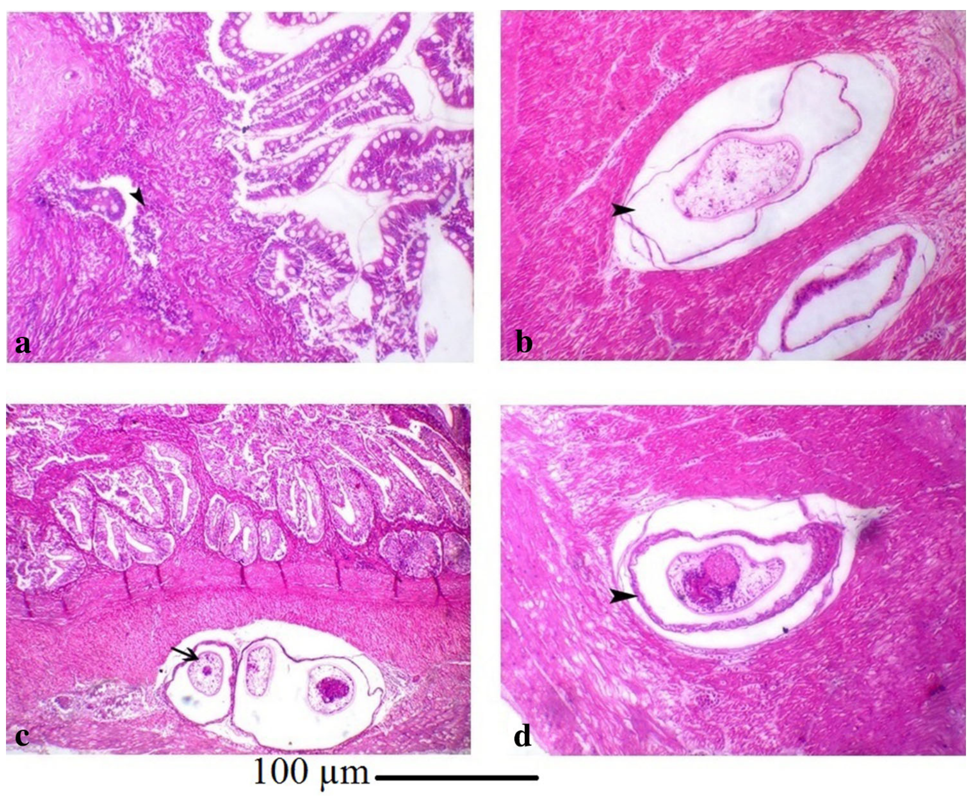

Fig. 6 Intestine of *Bagrus bajad* showing **a** necrotic changes within the muscle layer accompanied with granular eosinophilic cells infiltration (arrowhead), **b** parasitic encysted metacercaria burdened within the muscle layer (arrowhead) **c** parasitic encysted metacercaria burdened within the muscle layer mostly towards the serosal layer (arrow), **d** live encysted metacercaria within the muscle layer (arrowhead), H&E, X100. Scale bar = 100 µm

infected fish (Spall and Summerfelt 1970; Ritossa et al. 2013 and Hamouda 2014).

Lane et al. (2015) recorded “white grub” *P. minimum* metacercariae in many fish species. Boone et al. (2018) detected white grub (*P. minimum*) metacercariae in the visceral organs of many centrarchids freshwater fishes. Kvach et al. (2017) detected *Posthodiplostomum cf. minimum centrarchi (Strigeiformes: Diplostomidae)*, from seven localities in Europe, recording the cysts in the mesentery and internal organs of two fishes (*Lepomis gibbosus* and *Micropterus salmoides*) from sites in Bulgaria, the Czech Republic and Portugal. Zhang et al. (2018) found internal *P. minimum* cysts in the livers tissues in many North Americans freshwater fishes.

Interestingly, metacecariae of *P. minimum* were found free unencysted in the air bladder of *B. bajad* and this was somewhat similar to that reported by Kalogianni et al. (2017) who isolated *Posthodiplostomum* species from the

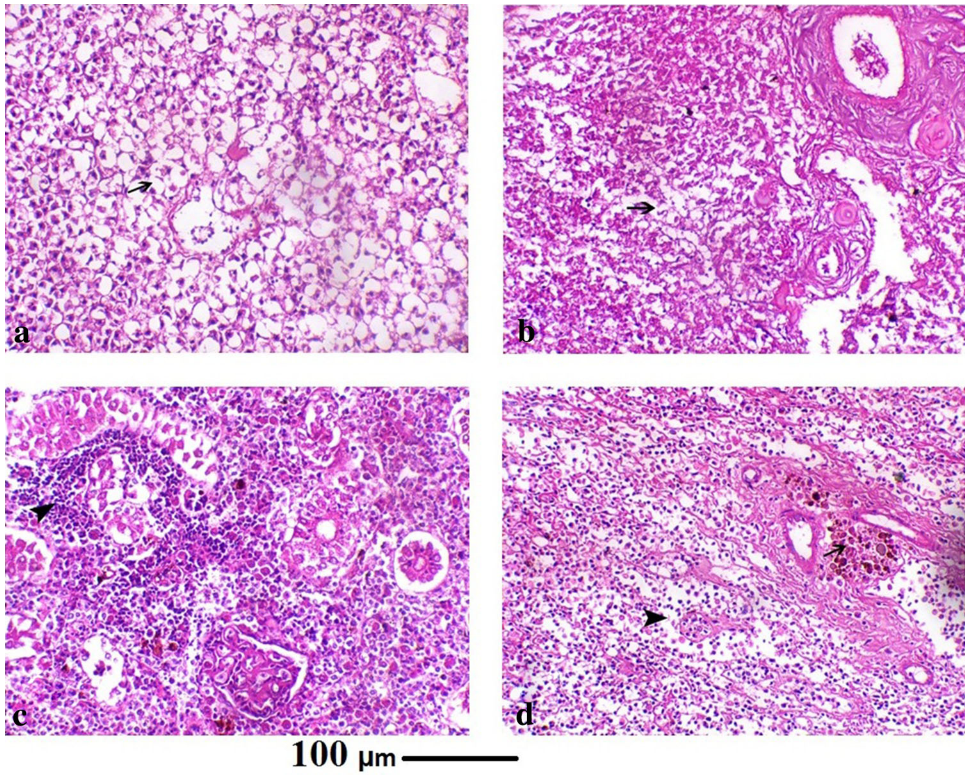

Fig. 7 **a** Liver of *Bagrus bajad* showing marked hepatic vacuolation associated with marked fatty degeneration (arrow), **b** liver showing marked hepatic degeneration mostly within the periportal area (arrow), **c** kidney showing interstitial nephritis (arrowhead), **d** Spleen showing marked both lymphoid (arrowhead) and melanomacrophages cells (arrow) depletion, H&E, X200. Scale bar = 100 µm

freshwater fishes in Greece which occurred in two forms; excysted and encysted in the abdominal cavity.

The morphological description of the recovered unencysted *P. minimum* metacercariae was typically similar to that recorded in Japan by (Nguyen et al. 2012). Also, (Athokpam and Tandon 2014) described the typical morphological features of the genus *Posthodiplostomum* (e.g. distinctly bipartite body and tribocytic organ was disc-shaped with a median slit) and showed well-developed gonads and anlagen of some genital organs, but no melanin. (Boone et al. 2018) mentioned that *P. minimum* metacercariae has limited morphological variation among a wide range of hosts.

The recorded results revealed that 75% of the examined *B. bajad* were infected with *P. minimum* and this may be due to the abundance of the snails and the presence of piscivorous birds around Lake Nasser all over the year, explains the high prevalence of *P. minimum* in *B. bajad* in this area. “White grub” is the metacercarial stage of a fluke (*P.* minimum) that occurs in many fish species, in 2 centrarchid hosts, bluegill (*Lepomis macrochirus*) with 100% prevalence and white crappie (*Pomoxis annularis*) 57%, from Spring Lake in McDonough County, Illinois (Lane et al. 2015). While, in Lower Egypt, at Dakahlyia Governorate (Mansour et al. 2003) did not record any *Posthodiplostomum* sp. infection in the examined *B. bayad* which collected from River Nile. McAllister et al. (2016) detected *P. minimum* in a Redspot Chub (*Nocomis asper*) from Oklahoma with prevalence of 25%. García-López et al. (2016) recorded *P. minimum* from different organs of infected fish with prevalence ranged from (2.7–31.60) %. Calhoun et al. (2018) recorded *P. minimum* in the liver, heart, kidney and body cavity of some freshwater fishes from California with prevalence of 36.09%. The differences between the obtained results with many studies may be due to, the different localities from which the examined fish were collected and the different water hydrochemistry in each area. Rossiter and Davidson (2018) found that the strongest predictors of *P. minimum* infection intensity were fish collection site, host body mass and species identity.

The highest prevalence of infection with metacercariae of *P. minimum* was recorded in summer. This result was of great agreement with Saleh et al. (2009) who recorded encysted metacercariae other than *P. minimum* from muscle, liver, gonads and gills of *B. bayad* at Port Said province, Egypt at 77.2% reporting high prevalence in summer, spring, autumn then winter. Also, (Schaaf et al. 2017) suggested that the warmer temperatures are associated with the increase black spot disease prevalence in threatened Steelhead trout in California due to some environmental and biotic conditions.

The intensity of infection in organs was variable ranged from 30/g in intestine and about 5/dissected fish in air bladder of *B. bajad*. (Mitchell et al. 1982) recorded massive infection of (*Pimephales promelas*) fish with more than 2000 *P. m. minimum* metacercariae. On the other hand, (Rabaldo et al. 1999) recorded 6 parasites per tissue section as the high intensity of cysts infection.

The histopathological studies associated with digenean larval stages of *P. minimum* infection in fish are very scarce. our results showing several changes in the infected organs (intestine, liver, kidney) as inflammatory cells infiltration (eosinophilic granular cells, lymphocytes and macrophages). The infected liver with marked congestion of hepatic sinusoids. This agreed to the results recorded by (Mitchell et al. 1982) who recorded an intensive inflammatory response had resulted from the infection with *Posthodiplostomum m. minimum* in fathead minnow; *Pimephales promelas* fish in USA. Mladineo (2006) recorded lymphocytic infiltrations around the parasitic cysts of the digenea *Didymocystis* spp. declaring that the intensity of observed reaction was depended mainly on the size of the cyst and on the infected tissue. Ramos et al. (2012) recorded that visceral infection of *Lepomis gibbosus* fish with *P. minimum centrarchi* revealed numerous cysts present in spleen, liver and kidney which surrounded by a cellular inflammatory response (macrophages, eosinophils and polymorphonuclear neutrophils).

(Reddy and Benarjee 2014) reported that the intestine of *Channa punctatus* infected with the trematode *Genarchopsis goppo* showed damage of the villi, inflammation, fibrosis associated with hyperplasia and metaplasia epithelial necrosis.

Liver of infected *B. bajad* with *P. minimum* showing marked ballooning of hepatocytes with periportal hepatic necrosis. The degeneration and necrosis of the hepatocytes was resulted from the pressure atrophy of the encysted metacercariae in liver cells and the toxic waste products excreted by these cysts (Ramadan et al. 2002; Shareef and Abidi 2015; Maghrabi 2017).

In the present results, kidney demonstrated features of interstitial nephritis with degeneration and necrosis of the renal tubules and infiltration of inflammatory cells. This agreed with (Shareef and Abidi 2015) who recorded that the infected kidney with digenetic trematode; *Euclinostomum heterostomum* from *Channa punctata* showed dilation of renal tubules, vacuolar degeneration, tubular epithelial cells with hypertrophy and hyperplasia, occlusion of tubules, fibrosis, hemorrhage, and congestion of glomeruli.

Conclusion

It is of great interest that, *B. bajad* from Lake Nasser possess *P. minimum* metacercariae either encysted in vital organs as liver, spleen, kidney, intestine, stomach, urinary bladder and mesentery or free in air bladder. These metacercariae have adverse effects on these organs which confirmed histopathologically for the first time. *P. minimum* predispose the infected fish to other diseases (bacterial, fungal, viral) by creating portals of entry when cercariae penetrate the skin to infect fish. The edible part of the fish (musculature) was free from parasitic infection and safe completely for human consumption. This study will be a helpful tool for further investigations about the effects of *P. minimum* infection on gross rate, fecundity, immunity and survival of this fish species.

Compliance with ethical standards

Ethics approval This work was approved by the ethics approval form (3/2017).

Conflict of interest Authors declared that there is no any conflict of interest.

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