SCANNING MORPHOLOGY, PREVALENCE AND HISTOPATHOLOGY OF SOME ACANTHOCEPHALANS INFECTING SOME RIVER NILE FISH

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Summary

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Acanthocephalan morphology and their adverse pathological impact on fish are of great concern. Two species of acanthocephalans were recorded from 800 samples of live freshwater fish collected randomly during 2017–2018 from Lake Nasser, Aswan, Egypt. The recovered species were identified morphologically as *Acanthogyrus (Acanthosentis) tilapiae* from three *Tilapia* spp. (*Sarotherodon galilaeus*, *Oreochromis niloticus* and *Tilapia zillii*) and *Rhadinorhynchus niloticus* from *Lates niloticus*. The intensity of parasitic infection and the seasonal prevalence were higher in *L. niloticus* than in *Tilapia* spp. The clinical signs and post mortem lesions of infected fish were reported. Morphological description of the detected parasites using light microscope was then confirmed by electron microscopy to amplify ambiguous details. The histopathological findings of the intestine of naturally infected fish with acanthocephalan parasites were investigated and described. The main damage caused by them is destruction of the mucosal epithelium of the villi, necrosis and degeneration of intestinal epithelial cells.

Key words: Acanthocephala, histopathology, *Lates niloticus*, *Oreochromis niloticus*, *Tilapia* spp.

INTRODUCTION

Fish as food meets the basic nutritional requirements and compensates the deficiencies of vitamins, proteins and minerals to humans all over the world (Nabi *et al.*, 2015).

In Lake Nasser, *Tilapia* spp. and *L. niloticus* are the most predominant fish

species and are considered the main source of high-quality protein suitable for human consumption at a relatively low cost.

Acanthocephala is a monophyletic group and all members are parasitic. It includes at least 1,150 species of rela-

tively small vermiform endoparasites (Kennedy, 2006). Acanthocephalans are widely spread thorny headed parasites, infecting many hosts – either mammals or fish and represent a wide category of worms. They are sexually separate, worldwide distributed; the adults inhabit the fish intestine and feed on the intestinal walls giving some adverse effects (Alava & Aguirre, 2005). Acanthocephalans have no digestive tract, only their body walls have numerous pores and canals which directly absorb nutrients from the intestinal lumen of the infected fish host (Schmidt & Roberts, 2005).

In Lake Nasser, the high temperature is maintained nearly throughout the year and this favours the development of arthropods. Those arthropods are the intermediate host of acanthocephalan species where the larval acanthella and cystacanth stages of acanthocephalan developed (Schmidt, 1990; Amin, 1998).

A. tilapiae was originally described by Baylis (1948) from the intestine of the infected *Tilapia lidole* of Lake Nanyassa at East Africa. Imam (1971) and Abu El-Ezz (1988) recorded *A. tilapiae* from *O. niloticus*, *T. aurea* and *S. galilaeus* collected from the Egyptian River Nile. *O. niloticus* and *S. galilaeus* were referred as hosts for *A. tilapiae* from Lake Nasser (El-Naffar *et al.*, 1983; Al-Bassel, 1990; Ebraheam, 1992) while Bayoumy (1996) and El-Naggar (2003) obtained it from *O. niloticus*, *T. zilli* and *S. galilaeus* collected from different parts of the Nile.

Adult's identification is mainly based on the arrangement of hooks on the proboscis which is the important visible portion of the worm (Nabi *et al.*, 2015). Electron microscopy is an excellent method used additionally to light microscopy to observe and detect the neglected detailed

features of acanthocephalans (Sheema *et al.*, 2017).

There are very few data concerning acanthocephalan species that infect different fish species of Lake Nasser, Egypt. So this work aimed to use different diagnostic techniques to identify acanthocephalan parasites infecting *Tilapia* spp. and *L. niloticus* of Lake Nasser. Another goal was to record the clinical signs and post-mortem lesions of the infected fish, to determine the seasonal prevalence of natural infection as well as the histopathological changes induced by these infections.

MATERIALS AND METHODS

Study area and fish samples

Lake Nasser (southern Egypt) is one of the biggest artificial lakes in Africa that extends about 300 km from the body of the High Dam on the River Nile till the Egyptian Sudanese borders. The Great Desert is borders the lake from the west and the Eastern Desert up to the Red Sea from the east.

A total number of 800 live samples of freshwater fish of different lengths and weights were collected randomly from the natural fish populations of Lake Nasser from January 2017 to December 2018 (Table 1). The collected fish samples were *Tilapia* spp. which contains the three following species: *Sarotherodon galilaeus*, *Oreochromis niloticus* and *Tilapia zillii* as described by El-Sayed (2006) and *Lates niloticus*. They were transported alive to the Laboratory of Fish Diseases, Faculty of Fish and Fisheries Technology, Aswan University. The fish were clinically examined, and then they were euthanised by manually applied cranial concussion followed by pithing of the brain (Anonymous, 2013).

Table 1. Different fish species examined during the study. Parameters are presented as mean $\pm$ SEM, n= 200 for each species

Fish species	Total length (cm)	Weight (g)
<i>Sarotherodon galilaeus</i>	22.00 $\pm$ 0.245	288.08 $\pm$ 5.69
<i>Oreochromis niloticus</i>	20.00 $\pm$ 0.1	145.20 $\pm$ 3.32
<i>Tilapia zillii</i>	12.00 $\pm$ 0.122	54.77 $\pm$ 1.13
<i>Lates niloticus</i>	27.34 $\pm$ 0.327	277.75 $\pm$ 4.86

The gross and microscopic examination of internal organs was done according to Noga (2010) to detect acanthocephalans.

Parasitological processing

The detected acanthocephalans were placed in normal saline 0.9% and processed according to Soulsby (1982). The collected acanthocephalans were identified according to Yamaguti (1963). Ten freshly collected acanthocephalans were processed according to Sheema *et al.* (2017). Samples were observed under a scanning electron microscope (JEOL 5300 JSM, Japan) at an acceleration voltage of 25 kV, and magnification power from $\times 150$ to $\times 500$.

Pieces of the intestine harbouring acanthocephalans were fixed in 10% formalin, and processed for histopathology according to Bancroft & Gamble (2007).

The obtained results were statistically analysed and presented as mean and standard error of the mean (SEM) by using the SPSS software (SPSS, 2007).

RESULTS

Gross examination of the different infected fish species with acanthocephalans revealed no pathognomonic lesions except slight abdominal distension in heavily infected fish. Acanthocephalan species were seen protruded from the anal opening of *L. niloticus* by naked eyes (Fig. 1A). Postmortem examination of the infected fish revealed presence of acanthocephalan species in the intestine of *Tilapia* spp (Fig. 1B) and in the stomach and the intestine of *L. niloticus*. Congested intestine and stomach resulted from grossly firmly attached acanthocephalan species to their mucosa (Fig. 1C,D).

The acanthocephalan species collected from *Tilapia* spp. was white in colour, cylindrical and measuring few millimeters. Acanthocephalan species collected from *L. niloticus* was white in colour, with long, cylindrical body, measuring 2–3 cm and a slender, hollow construction proboscis that formed the anterior end.

The acanthocephalan species collected from *Tilapia* spp belongs to: Species: *Acanthogyrus (Acanthosentis) tilapiae* Baylis, 1948 (Fig. 2). Live worms were white in colour. The female was longer than the male. The thorny proboscis was invaginated anteriorly with clear neck. Presence of lemnisci which is a single ganglion situated at the anterior end of the proboscis sheath was observed. The testes were of oval shape, tandem and located at the middle of the body. Posterior to them there was a single cement gland that leads to the cement gland reservoir. The bursa was located at the posterior end of the body. The ovarian balls were mixed with eggs in the parenchyma. There was a short uterine tube lying at the posterior end of the body.

Fig. 1. A. *Rhadinorhynchus niloticus* protruding from the anal opening of *Lates niloticus* (arrow); B. *Acanthogyrus (Acanthosentis) tilapiae* appearing from the intestinal wall of *Sarotherodon galilaeus* (arrow). C. Intestine of *Lates niloticus* with *Rhadinorhynchus niloticus* (arrows). D. *Rhadinorhynchus niloticus* attaching to the outer surface of *Lates niloticus* stomach (arrows).

Using electron microscope, the proboscis of *A. tilapiae* had more characteristic features as the proboscis was provided with 2 rows of spines. The longer hooks were in the front followed by the smaller ones. The neck region was constricted and separated by cuticular folds from the trunk region.

The detected acanthocephalan species collected from *L. niloticus* belongs to: Species: *Rhadinorhynchus niloticus* Meyer, 1932 (Fig. 3, 4). The body was elongated, cylindrical and had spiny proboscis. Several hooks were evenly distributed in rows. The proboscis was followed by a short neck free from spines. The pro-

boscis sheath was elongated, cylindrical and attached to the base of the proboscis. The central nervous system consisted of a single ganglion situated at the anterior end of the proboscis sheath (lemnisci). The fore third of the body was covered with spines. The male genitalia occupied the posterior half of the trunk; two ovoid testes, one behind the other followed by cement gland and finally, a clear male bursa at the posterior end. Gonopore was located terminal in both sexes. The female genital opening was presented in the end of the worm with various sizes; round or vertical slit occupying almost all the trunk diameter (note prominent lips).

Fig. 2. Carmine stained adult *Acanthogyryus (Acanthosentis) tilapiae* from *Tilapia* spp. P= proboscis, L= lemnisci.

A. male; AT=anterior testis, PT=posterior testis, CG=cement gland, CGR=cement gland reservoir, B=Bursa.

B. OB=ovarian balls, Eg=eggs, UT=uterine tube. Scale bar=100 µm.

C–E. Electron microscopy of *A. tilapiae* showing two rows of hooks on the proboscis.

The seasonal prevalence of acanthocephalan species among the different fish types was recorded in Table 2. From the table, the highest infection rates for *Tilapia* spp. were observed during summer and the lowest – in winter, while the highest infection rates for *L. niloticus* were

observed during winter and the lowest were in the summer. *L. niloticus* had the highest prevalence among the examined fish species in all seasons (51.5%). The overall prevalence of acanthocephalan infection in *Sarotherodon galilaeus*, *Oreochromis niloticus*, *Tilapia zillii* and

Fig. 3. Carmine stained adult *Rhadinorhynchus niloticus* from *Lates niloticus*. **A.** P=proboscis, N=neck, PS=proboscis sheath, L=lemnisci. **B.** TS=trunk spines. Scale bar=100 µm. **C, D.** Electron microscopy of *Rhadinorhynchus niloticus* showing spiny proboscis, neck and trunk spines.

L. niloticus were 35.5, 30.5, 26.5 and 51.5%, respectively.

The histopathological sections in the intestine of *O. niloticus* and *L. niloticus* infected with acanthocephalans revealed several changes. Severe destruction of the mucosa, loss of intestinal crypts with presence of fibrinopurulent exudate in the intestinal lumen was the main histopathological changes induced by acanthocephalan species infections. Furthermore, eosinophils and fibroblasts were the most commonly detected cells within the inflammatory tissue especially at the site of parasite attachment (Fig. 5, 6).

DISCUSSION

The morphological description of *A. tilapia* by both light and electron microscopies is similar to that already described (Bayoumy *et al.*, 2006; Abo Msalam, 2007; Amin & Heckmann, 2012). The morphological description of *R. niloticus* by light microscope was firstly reported by Meyer (1932) similar to that described by El-Shahawy *et al.* (2017).

A. tilapia infecting *Tilapia* spp. is of nearly uniform frequency during the different seasons while *R. niloticus* infecting *L. niloticus* shows clear seasonal variation

Fig. 4. Carmine stained adult *Rhadinorhynchus niloticus* from *Lates niloticus*. **A, B.** male posterior end; AT=anterior testis, PT=posterior testis, CG=cement gland, B=Bursa. Scale bar=100 μ m. **C, D.** female gonopore (round or vertical slit).

Table 2. Seasonal prevalence of of *Acanthocephala* spp. in the different fish species from Lake Nasser during the period from January 2017 to December 2018

	Fish species			
	<i>Sarotherodon galilaeus</i>	<i>Oreochromis niloticus</i>	<i>Tilapia zillii</i>	<i>Lates niloticus</i>
Spring* – N (%)	15 (30)	13 (26)	10 (20)	28 (56)
Summer* – N (%)	23 (46)	18 (36)	15 (30)	20 (40)
Autumn* – N (%)	20 (40)	15 (30)	14 (28)	25 (50)
Winter* – N (%)	13 (26)	15 (30)	14 (28)	30 (60)
Total prevalence** – N (%)	71 (35.5)	61 (30.5)	53 (26.5)	103 (51.5)
Number of parasites per infected fish***	5.4 $\pm$ 0.102	6.4 $\pm$ 0.243	4.0 $\pm$ 0.142	12.0 $\pm$ 0.33

*N=50 fish per season; ** total number of the infected fish; ***number of acanthocephalan (mean $\pm$ SEM standard error of the mean).

Fig. 5. **A.** Cross section in *Acanthogyrus (Acanthosentis) tilapiae* in intestinal lumen; **B.** Intestinal section showing the anterior end of *Rhadinorhynchus niloticus* (arrowhead) associated with necrotic enteritis (arrow) H&E; **C.** neutrophils are noted in the fibrinopurulent exudate in the gut lumen of *Oreochromis niloticus* infected with *Acanthosentis tilapiae*, H&E; **D.** Intestinal section showing the posterior end of *Rhadinorhynchus niloticus* (arrowhead) associated with necrotic enteritis in *Lates niloticus* (arrow), H&E.

and its distribution and abundance depends mainly on its intermediate hosts' population dynamics (Ohtaka *et al.*, 2002).

The highest prevalence and intensity of acanthocephalans were recorded in *L. niloticus*; this is probably due to the fact that *L. niloticus* is a carnivorous fish that assists in the transmission of acanthocephalan species through feeding on arthropods that harbour the infective stages (Hamouda *et al.*, 2018) while *Tilapia* spp. are generally herbivorous/omnivorous fish (El-Sayed, 2006).

A. tilapiae in *S. galilaeus*, *O. niloticus* and *T. zillii* were recorded with prevalence of 35.5%, 30.5% and 26.5% respectively.

These results are different compared to those of El-Naffar *et al.* (1983) who isolated acanthocephalans from *O. niloticus*, *S. galilaeus* and *T. zillii* of Lake Nasser with prevalence of 45.66%, 4.3% and 0% respectively. Saoud & Wannas (1984) recorded acanthocephalans from *O. niloticus* and *S. galilaeus* of Lake Nasser with prevalence of 96.7% and 100% respectively.

L. niloticus has an overall infection rate of 51.5% which is higher than that recorded by El-Shahawy *et al.* (2017) – 12.5% overall infection rate of *R. niloticus* in *L. niloticus* from River Nile at Quena governorate, Egypt and also higher

Fig. 6. Histopathological section in the intestine of *Lates niloticus* infected with *Rhadinorhynchus niloticus*; **A.** Central fibrinopurulent exudate in the gut lumen, H&E; **B.** Necrotic enteritis characterised by severe ulceration and necrosis, accompanied by an intense inflammatory cells infiltration and a patchy loss of intestinal crypts, H&E; **C.** Spines print of the proboscis when anchored firmly in the intestinal mucosa, H&E; **D.** Damaged intestinal epithelium showing desquamation; severe inflammatory reaction in lamina propria close to the site of parasite attachment and increased mucus production, H&E.

than that recorded by Hamouda *et al.* (2018) – 24.5% overall infection rate of *R. niloticus* in *L. niloticus* from Lake Nasser. Our rates were however lower than those reported by El-Naffar *et al.* (1983) and Saoud & Wannas (1984) who isolated acanthocephalan species from *L. niloticus* from Lake Nasser with prevalence of 80% and 95% respectively. This could be attributed to abiotic and biotic conditions of the environments where the studies were carried out as well as the date when fish samples were collected.

Extent of infection was higher during dry month periods for *Tilapia* spp. while the extent of infection for *L. niloticus* was higher during rainy month periods and this may be attributed to the difference between fish species, behaviour of fish and the type of diet and feeding habits.

Fish infected with acanthocephalans showed severe pathological lesions as parasites attach to the host intestinal wall by their hooked proboscis. In heavy infection they cause occlusion of the gut and invasion/migration of the parasites into

uncommon locations as peritoneum resulting in adhesion and bleeding of different internal organs (McDonough & Gleason, 1981; Schmidt & Roberts, 2005; Nickol, 2006). Infected fish demonstrated severe destruction of the intestinal mucosa and loss of crypts as acanthocephalans embedded their hooked proboscises in the lining mucosa of the stomach and intestine (Dezfuli *et al.*, 2002; Bayoumy *et al.*, 2006; Khurshid & Ahmed, 2012). Also, toxic metabolic by-products produced by acanthocephalans caused occlusion of intestine, blood vessels and other ducts resulting in congestion in the infected organs (Schmidt & Roberts, 2005).

Eosinophils and fibroblasts were the commonest detected cells within the inflammatory tissue. They may be attributed to the acanthocephalans' hooks secreting some enzymes, a trypsin-like collagenolytic serine proteinase, that aids the penetration of the proboscis within the host intestinal wall. So, it is supposed that the proboscis' enzymes might enhance host inflammatory responses (Dezfuli, 1991; Polzer & Taraschewski, 1994; Taraschewski, 2000).

CONCLUSION

There are very few reports on the acanthocephalan parasites especially those of fish of Lake Nasser. Their presence in fish is of a great interest. Several fish species from Lake Nasser possessed acanthocephalan parasites in their intestine that may infect other internal organs. Morphologic descriptions of two acanthocephalans; *Acanthogyrus (Acanthosentis) tilapiae* from three *Tilapia* spp. (*Sarotherodon galilaeus*, *Oreochromis niloticus* and *Tilapia zillii*) and *Rhadinorhynchus niloticus* from *Lates niloticus* were recorded. Destruction of the intestinal mu-

cosa due to their hooked proboscis and fibrinopurulent exudate was observed.

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