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

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Summary


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 ludole* of Lake Nayassa 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-Basser, 1990; Ebraheam, 1992) while Bayoumy (1996) and El-Naggar (2003) obtained it from *O. niloticus*, *T. zillii* 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 (Anonymouse, 2013).
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 ×150 to ×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.

<table>
<thead>
<tr>
<th>Fish species</th>
<th>Total length (cm)</th>
<th>Weight (g)</th>
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<tbody>
<tr>
<td><em>Sarotherodon galilaeus</em></td>
<td>22.00±0.245</td>
<td>288.08±5.69</td>
</tr>
<tr>
<td><em>Oreochromis niloticus</em></td>
<td>20.00±0.1</td>
<td>145.20±3.32</td>
</tr>
<tr>
<td><em>Tilapia zillii</em></td>
<td>12.00±0.122</td>
<td>54.77±1.13</td>
</tr>
<tr>
<td><em>Lates niloticus</em></td>
<td>27.34±0.327</td>
<td>277.75±4.86</td>
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</table>
Using electron microscope, the proboscis of *A. tilapia* 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 proboscis 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).
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

<table>
<thead>
<tr>
<th>Species</th>
<th>Season</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tilapia spp.</td>
<td>Summer</td>
<td>Highest</td>
</tr>
<tr>
<td>Tilapia spp.</td>
<td>Winter</td>
<td>Lowest</td>
</tr>
<tr>
<td><em>L. niloticus</em></td>
<td>Winter</td>
<td>Highest</td>
</tr>
<tr>
<td><em>L. niloticus</em></td>
<td>Summer</td>
<td>Lowest</td>
</tr>
</tbody>
</table>

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

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

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

<table>
<thead>
<tr>
<th>Fish species</th>
<th>Sarotherodon galilaeus</th>
<th>Oreochromis niloticus</th>
<th>Tilapia zillii</th>
<th>Lates niloticus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring* – N (%)</td>
<td>15 (30)</td>
<td>13 (26)</td>
<td>10 (20)</td>
<td>28 (56)</td>
</tr>
<tr>
<td>Summer* – N (%)</td>
<td>23 (46)</td>
<td>18 (36)</td>
<td>15 (30)</td>
<td>20 (40)</td>
</tr>
<tr>
<td>Autumn* – N (%)</td>
<td>20 (40)</td>
<td>15 (30)</td>
<td>14 (28)</td>
<td>25 (50)</td>
</tr>
<tr>
<td>Winter* – N (%)</td>
<td>13 (26)</td>
<td>15 (30)</td>
<td>14 (28)</td>
<td>30 (60)</td>
</tr>
<tr>
<td>Total prevalence** – N (%)</td>
<td>71 (35.5)</td>
<td>61 (30.5)</td>
<td>53 (26.5)</td>
<td>103 (51.5)</td>
</tr>
<tr>
<td>Number of parasites per infected fish***</td>
<td>5.4±0.102</td>
<td>6.4±0.243</td>
<td>4.0±0.142</td>
<td>12.0±0.33</td>
</tr>
</tbody>
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*N=50 fish per season; ** total number of the infected fish; ***number of acanthocephalan (mean ± SEM standard error of the mean).
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 26.3% 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 Qena governorate, Egypt and also higher.
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-Naffār 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; Bayouny 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 mucosa due to their hooked proboscis and fibrinopurulent exudate was observed.

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