HISTOPATHOLOGICAL ALTERATIONS IN SMALL INTESTINE OF RABBIT FISH (SIGANUS RIVULATUS) INFECTED BY HELMINTH PARASITE (SCLEROCOLLUM SP.), RED SEA COAST, SUDAN

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Abstract

Wild rabbit fish (Siganus rivulatus) Forsskål (Teleostei, Siganidae), a herbivorous fish were caught from Suakin near Sudanese Red Sea during February 2010 - January 2011, which were examined for histo-pathological alterations in small intestine infected by a helminthes parasite (Sclerocollum sp.). Sclerocollum sp. was reported for the first time from Suakin near Sudanese Red Sea. Effect of a parasite included: abundance of lymphocytes cell, eosinphils, red blood cells and goblet cells in parasitized intestine; which were significantly more than intestine devoid of infection. The histopathologicaly study indebted that the worms damaged the architecture of intestinal tissues besides hemorrhage, hyperplasia in mucosa and submucosa, melanomacrophage aggregation and necrosis of mucosa and sub-mucosal layer. The 237 fishes (Siganus rivulatus) caught for investigation the helminthes parasite. The condition factor of healthy fishes was 2.94±0.03, which was highly significantly different value (p< 0.02) compared to helminthes fish (2.81±0.04). The prevalence of infection was observed in 38% of fishes. A direct damage by Sclerocollum sp in the form of destruction of villi, hemorrhage, fibrotic capsule round proboscis, erosion of submucosa along parasites capsule, and separation of submucosa from mucosa, was observed.

Key words: Sea, Siganidae, Sclerocollum, Helminth
**Introduction**

Phylum acanthocephalans comprises of endoparasites worms representing 1100 species (Golvan, 1994), nearly one-half are found as adult forms in intestine of fishes. A phylum of parasitic worms, characterized by the presence of proboscis, armed with the spines, which it uses to pierce and hold the gut wall of its host (Near et al., 1998). Adult acanthocephalans that infect fish as definitive hosts belongs to four classes namely Palaeacanthocephala, Archiacanthocephala, Polyacanthocephala and Eoacanthocephala (Weber et al., 2012). The scientific name of Helminth parasite is *Sclerocollum rubrimaris* (Schmidt and Paperna, 1978). Gorgorhynchinae was first described from the Red Sea rabbit fish *Siganus sp* in Gulf of Aqaba northern Red Sea. The parasites distribution among the wild populations was studied (Diamant, 1989). The same parasites were reported in siganid and scombrid fishes in Sharm El- Sheikh Coast, South Sinai (Hassanine, 2006). Ultrastructure and genetic diversity of two Sclerocollum infected siganid and lutianid fishes in Red sea, Egypt (Abdou and Mahfouz, 2006). Adults of different *Sclerocollum* species infecting siganid fish were recorded. *S. oramin* was found in the Arabian Gulf (Amin et al., 1984) and *S. canaliculatus* was observed in the coast of United Arab Emirates (El-Naffar et al., 1992) and *S. sutor* was reported in Kenyan coast (Martens and Moens, 1995). The acanthocephalans was observed in other fish families including lutjanidae, mullidae, and scaridae in the Arabian Gulf area (Al-Kawari, et al., 1996). Mammals often reveal symptoms of high morbidity when infected with acanthocephalans, while fish seem to tolerate high intensities of worms deeply penetrating into intestinal wall without pronounced symptoms of disease (Taraschewski, 2000). Acanthocephalans embed their spiny proboscis into mucosal epithelium. Attachment is frequently between villi. At the site of attachment, cells are destroyed and fibroblast, lymphocytes and macrophages are mobilized below the lamina propria (Dezfuli, 1990), where chronic fibrinous inflammation, resulting in an increased amount of connective tissue, causes thickening (Bullock, 1963).

In some species, fibroplasia extends to layers of the muscularis mucosa (de Buron and Nickol, 1994). Goblet cell hyperplasia occurs widely in acanthocephalan infections. The covering created by copious secretion of mucus and the presumed presence of antibodies within probably reduces the number of parasites that succeed in establishing (Thomas, 2002). Apart from a proliferation of goblet cells increased the number of eosinophils, neutrophils and monocytes at the attachment site which characterized the histological effects of acanthocephalans. Mobilization of leucocytes occurs regardless of whether the fish species is suitable for development of the parasites. An interspecific difference in the response of leucocytes in fishes parasitized was observed in *P. Ambiguus* (Hamers et al., 1992). In eels, *A. Anguilla* is suitable definitive host, the response was much less intense than in carp, *Cyprinus carpio* or rainbow trout, *Oncorhynchus mykiss*, both unsuitable hosts that expel the acanthocephalans within a few days. The leucocytes damage acanthocephalan tegument extensively in carp; hence, the cellular defense is a factor in determining host specificity for *P. ambiguus* (Hamers et al., 1992). There is no difference in the number of goblet cell, granular cells in uninfected mosquito fish, *Gambusia affinis* and those infected with *Octospiniferoides*.
chandleri. In green sunfish infected with *Leptorhynchoides thecatus*, there are a significantly greater number of goblet cells in parasitized pyloric caeca than in unparasitized caeca in the same fish (de Buron and Nickol, 1994). This suggests a parasites – induced response that might lessen damage from the erosive nature of the worms. The mucins from goblet cells also contribute to the expulsion of acanthocephalans immunized hosts (Taraschewski, 2000). Infection by acanthocephalan causes damage to the mucosal epithelium of the gut by the main body of the worms; in addition, the attachment organ can damage the gut at attachment site (Amin and Heckmann, 1992). Cellular structure of fibrotic tunnel that forms around the neck and proboscis bulb (Dezfule et al., 2002a). *A. anguillae* is also known to perforate the intestine of its host and attach to the liver. In gold fish, *C. auratus*, large portions of this organ are replaced by proliferative tissue, often with patches of pancreas, surrounding the embedded proboscis (Taraschewski, 2000). Chronic fibrosis, destruction of intestinal villi and necrosis and degenerative changes in mucosal epithelium adversely affect motility and absorptive efficiency of the fish intestine (Dick and Choudhury, 2006). The irreversible mechanical damage caused by the attachment of the armed proboscis affect the architecture of the intestinal tissues leading to pathological changes. Loss/degeneration of the intestinal villi, formation of granular tissues and capsule formation associated with host immune responses seriously affect the digestive and absorptive efficiency of the animal. In heavy infections they can cause occlusion of the gut and invasion/migration of the parasites into uncommon locations (Nickol, 2006). Shortly after infection, the proboscis is surrounded by necrotic tissue, which becomes hemorrhagic and inflamed after few days. During second week after infection by species that penetrate deeply, inflamed tissue around the anterior portion of the worm is dominated by monocytes and macrophages maturing into epithelioid cells, and an outer belt of connective tissue appears. Acanthocephalans in moribund or dead animals are frequently assumed to indicate deleterious effects; worms have been observed extending from the rectum or protruding through the trunk (Taraschewski, 2000). Enzyme with trypsin-like activity secreted by *P.Laevis* in chub, *L. cephalus* degraded collagen (Polzer and Taraschewski, 1994) and thus is capable of degrading one or more of the major components of the host’s gastrointestinal tissue.

The objectives of this investigation are to assess infection of helminthes on small intestine of Rabbit fish pathologically in Red Sea coast Area, Sudan.

Materials and methods

Map of Study area

Sample Collection

Rabbit fishes were caught from Suakin Area (19° 06 N, 37° 20 E) by barrier net (3x3 cm mesh size) which put at depth 1 – 2 m. Experiment was done in 2010-2011. The naming and identification of collected fishes were done according to method described by Randall (1983), and Froese and Pauly (2010). Map of study area was shown in plate 1
Histopathological evaluation
Living fish were killed immediately using an overdose of lidocaine (Lignosol) anaesthetic (mention concentration). To examine the incidence of acanthocephalans, the digestive tract of each fish was cut along the longitudinal plane and adult acanthocephalans species was collected from infected intestines. The number of the parasites and their distribution in the hosts were recorded, and then transferred to a clean physiological 0.6% saline solution. Parasites were identified by using the certain key for identification of acanthocephalans (Schmidt and Paperna, 1978). After 24h of fixation, from 20 fishes (infected and uninfected) the intestines of fishes were prepared for serial sectioning. The specimen was stained (Haematoxylin & eosin (Harris,n1900), for normal histological structure and combined Alcian blue (Mowery, 1956), for detection of neutral and mucopoly saccharides. Parasitized and un-parasitized intestines were selected randomly for examination, because histological features don't vary along a single intestine or among intestine of uninfected fish (Williams and Nickol, 1989). For each intestine examined, the numbers of lymphocytes, eosinophils, red blood cells and goblet cells were determined. Blood cells were identified according to the method described (Bastide, 1986). In intestine, a count the lymphocytes, eosinophils, red blood cells and goblet cells were made along 100µm length of the supranuclear portion of mucosal epithelium in section affect and not affect by the parasites.

Statistical analysis
Statistical Package for Social Science (SPSS- Version 11) program was used to run the data analysis with each test being conducted at 0.05% level of probability by t-test.
Results and discussions

Parasites Identification and Morphology

From the examined samples of *Siganus rivulatus*, the acanthocephalan genus *Sclerocollum sp* were found in Suakin fishes recorded in interior and posterior of intestine (Fig. 1). These results are agreed with those values obtained by Schmidt and Paperna (1978). The results of *Sclerocollum Sp* helminthes parasite present in the intestine of *Siganus rivulatus* are similar to those finding (Schmidt and Paperna, 1978; Diamant, 1989; Hassanine, 2006) and Abdou and Mahfouz, 2006). The most acanthocephalans have a life cycle involving a single arthropod intermediate host and a vertebrate definitive host (Amin *et al.*, 2004), but certain acanthocephalans utilize one or more species of a second class of vertebrate as a paratenic, or rarely post-cyclic, host (a predator vertebrate feeding on a prey fish harbouring the worm, and in which the worm can re-establish).

General Pathology of Fish Intestine Infected with *Sclerocollum Sp*.

The fish which examined seem to tolerate high intensities of parasites infection because there are no pronounced symptoms of disease. *Sclerocollum sp* predominated in posterior region of intestine appeared swollen with anterior ends of some worms projecting into the peritoneal cavity enclosed in a connective tissue capsule at site of proboscis attachment, the surface of intestine appeared thickened and with high mucus. Count of lymphocytes, eosinophils, red blood cells and goblet cells in parasitized intestine along 100µm length of the supranuclear portion of mucosal epithelium was significantly high at $P \leq 0.05$ (Table 1). My observations suggest that intestinal helminthes of *S. rivulatus* have a strong tendency to over dispersed (aggregated) distribution, characterized by irregular fluctuations of the percentage of infected fishes in relation to the intensity of infection occurs (Kennedy, 1975). Such an aggregated pattern of distribution may increase the chances of mating or may reduce the effects of interspecific competition (Dobson and Roberts, 1994). Penetration of the intestinal wall of fishes by acanthocephalan proboscis has been reported by several authors (Mc donough and Gleason, 1981). Thus, such interactive site segregation of helminthes may result in harm to the fish host. Helminthes infected rabbit fish revealed older ages when compared with healthy fishes. Most helminthes parasites cause injure with no high significant on host’s general health. However, the intensity of infection usually increases with age (Moravec and Rehulka, 1987). A negative correlation exists between disease and condition factor (CF) in fishes (Moller, 1985). A value of one (1) for CF is indicative of a very good health status (Adams *et al*., 1993). The values of condition factor of helminthes fishes were lower when compare with healthy fishes but these values still in range (Adams et *al*., 1993).

Histopathology of Fish Intestine Infected with *Sclerocollum Sp*.

Normal histological feature of unparasitized intestine of *Siganus rivulatus* are shown in (Fig. 2, 3, 4, 5 and 6). Histopathological studies showed sever damage to the wall of intestine. In parasitized intestine, mucosal folds at the sites of parasites attachment had their tips eroded and
appeared flattened with increase mucus production (Fig. 7). Always, proboscises were anchored in the lamina propria, but sometimes they penetrated the muscle layers (Fig. 8 and 9). Lymphocytes and neutrophils were abundant around proboscis; some erythrocytes were present (Fig. 8, 9 and 10). Necrosis of mucosal epithelium and villi were seen in many areas they were totally destroyed, reducing the absorptive function of fish (Fig. 11, 12). In intestinal villi near the site of parasites attachment, epithelial cell were found hyperplasia and separate sub mucosa from muscular layer (Fig. 13 and 14), capillaries were ruptured and clump of RBCs seen free in the lumen (Fig. 10). In the some of the capillaries the number of RBCs visible was very low. The presoma of the parasites were found to pierce the mucosal epithelium, lamina propria, musclaris and serosa of the wall of intestine forming a tunnel surrounded collagenous fibers (Fig. 8 and 9). Connective tissue proliferation, inflammation and aggregation of lymphocytes and eosinophils indicated of host immune responses. Histopathological studies revealed severe pathological changes and the mechanical damage caused by worms have totally destroyed the architecture of the intestinal tissues; however the worms did not produce any visible disease sign on general health of the fish (Fig. 11).

Generally in parasitic infections, the host immune system reacts in different ways to the parasite stages and the cellular responses invariably attempts to isolate and destroy the parasites. The most severe pathology is often a combination of efficient parasite replication and excessive host response (Feist and Longshaw, 2008). Histopathological changes in fish intestines due to acanthocephalan infections depend on various factors such as species of parasite and host nature of the infected tissues and host-parasite interactions. Length of the neck and proboscis, presence or absence of a proboscis bulb affects the pathological outcome. The mechanical destruction to the host's intestinal tissue is usually followed by host immune responses like proliferation of fibroblasts and granular cell infiltration around the invader, resulting in the formation of a collagenic capsule around it (Schelhaas, 1980). In the present study case, the fish showed to tolerate high intensity of parasites without appearing of any sign of illness, same results were presented by Taraschewski, 2000). In heavy infections, the intestine appeared to be packed with parasites, almost blocking the intestinal lumen, which will adversely affect the movement of digested materials within the intestine and the absorption of nutrients. de Buron and Nickol (1994) reported occlusion and significant distention of caeca in Lepomis cyanellus infected with the acanthocephalan Leptorhynchoïdes thecatus (de Buron and Nickol, 1994). The Sclerocollum sp hooks at attachment sites have considerably damaged the tissue architecture of the host intestine leading to villi necrosis when compared to normal (Fig. 5), same as observed by (Amin and Heckmann, 1992), fibroplasia extent to muscular mucosa similar lesion described by (de Buron and Nickol, 1994) and destroyed villi (Fig. 11), this agree with (Sanil et al., 2010). Increase number of goblet cells, eosinophils aggregation and lymphocytes infiltration were stimulated by parasites infection has been well documented (Fig. 7) as documented by (de Buron and Nickol, 1994; Thomas, 2002; Hamers et al., 1992 and Dick and Choudhury, 2006). The nature of the pathological changes observed in the intestinal tissues, via compression of mucosal epithelium at the point of attachment and the tissue damage to the various layers of the intestine
is in perfect agreement with the changes described by (Wanstall et al., 1982) as shown in (Fig.7). For *P. laevis* infection in *Salmo gairdneri*, the degeneration and necrosis of the mucosal epithelium and excessive mucus secretion in *Rachycentron canadum* infected with the acanthocephalan *Serrasentis nadakali* are shown as described by (George and Nadakal, 1981). Fig. 8, 11 and 13 shows clearly the extent of tissue damage to the intestinal mucosa and only small portion of villi remained intact. Parasite induced fibrosis in the intestinal wall along with the associated biochemical reactions will induce loss of gut motility (Nickol, 2006). Further, severe damage to the intestinal folds will drastically reduce the absorptive area available for the digestive and absorptive functions of the animal.

The presence of encapsulated proboscis, Fig. 8 and 9 penetrating the muscularis and submucosa layers indicates of host immune response. In *Lepomis cyanellus*, the proboscis occasionally reached into the muscularis and mucosa (de Buron and Nickol, 1994). In green sun fish infected with *L. thecatusthe erythrocytes indicating hemorrhage, at the sites of attachment in villi* (de Buron and Nickol, 1994). The same condition was present when *Sclerocollum sp* occur in *Siganus rivulatus*.

![Fig.1: Sclerocollum sp. A- Anterior Part of Male, pb → Proboscis; le → Lemniscus; pc→ Proboscis receptacle. B- Mid Part of Male, ts→ Testis, cg→ Cement gland. C- Posterior Part of Male, br→ Bursa. (X40 light micrograph). D- Anterior Part of Female, Pb→ Proboscis Hooks. E- View of Proboscis Showing 14 Rows of Hooks. F- Posterior Part of Female Showing Genital Pore (arrow). SEM micrographs.](image-url)
**Fig. 2:** Normal Muscular Layer (ML) and submucosa. Serosa (S); submucosa (SM) H&E.X40

**Fig. 3:** Normal Distribution of Cells in Villi (V); Goblet cells (GC) H&E.X40. & Lymphocyte cells (LC). H&E.X100

**Fig. 4:** Whole Section of Unparasitized Intestine. Serosa (S); Muscular layer (ML); Submucosa (SM); Lumen (L) & Villi (V). H&E.X4

**Fig. 5:** Cross Section Villi with Normal Columnar Cells (CC); Normal Distribution of Red Blood Cells (arrow). Alcian blue X100

**Fig. 6:** Normal Goblet Cells (GC) in V Stained with Alcian blue X40

**Fig. 7:** Eroded Villi and Flatten Intestinal Fold (Arrow), Eosinophilic Infiltration (Ei). H&E.X40
**Fig. 8:** Parasites Penetrating the Wall of Intestine, Presoma of the Parasites (Pr). H&E. X40

**Fig. 9:** Enlarged View of (Fig.9) Showing Lymphocytes Infiltration (Li). Muscular Layer (ML), Presoma of The Parasites (Pr).

**Fig. 10:** Erythrocytes Aggregation as Hemorrhage (Hr) in Submucosa (SM).

**Fig. 11:** Destroy of Intestinal Villi (Arrow) and Necrosis of Mucosal Epithelium. H&E.X40

**Fig. 12:** Loss the Architecture of the Intestinal Tissues. Separation of Submucosa from Muscular Layer. Lumen (L) (Arrow). H&E.X4

**Fig. 13:** Enlargement View of Fig. 12 Showing Hyperplasia of Villi (Arrow) and Necrosis (Ne). H&E.X10
Table 1 shows health intestine and intestine infected by Helminth parasitize

<table>
<thead>
<tr>
<th>Number (No) of Cells</th>
<th>Health Intestine</th>
<th>Parasitized Intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of lymphocytes</td>
<td>4 ± 0.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.3 ± 0.13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>No. of eosinophils</td>
<td>4 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.3 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>No. of red blood cells</td>
<td>4.4 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.9 ± 0.14&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>No. of goblet cells</td>
<td>2.43 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.08 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
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Mean values with same letters within column are significant difference at P ≤ 0.05

Conclusion

In present study, the direct damage by *Sclerocollum sp* included destruction of villi, hemorrhage, fibrotic capsule around proboscis, erosion of submucosa along parasites capsule and separation of submucosa from mucosa.

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