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Pathological Studies on the Small Intestine of Wild Rabbit Fish (*Siganus Rivulatus*) Infected by Helminthes Parasite (*Procamallanus Sp*) in Red Sea Coast Area, Sudan

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Pathological Studies on the Small Intestine of Wild Rabbit Fish (*Siganus Rivulatus*) Infected by Helminthes Parasite (*Procamallanus Sp*) in Red Sea Coast Area, Sudan

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Abstract

Wild rabbit fish (*Siganus rivulatus*) Forsskål (Teleostei, Siganidae) herbivores fish were collected from two sites (Dongnab and Swakin) on Sudanese Red Sea Coast during February 2010 - January 2011 and then examine for helminthes parasites. Microscopic anatomy of intestine with worms and without worms compared to each other. Helminth parasites belong to *Procamallanus sp*. The abundance of lymphocytes cell, eosinphils, red blood cells and goblet cells in parasitized intestine was significantly greater than in intestine without worms. Histopathological of intestine showed hyperplasia of mucosa, necrosis and hemorrhage in villi, hyperplasia of submucosa, cloudy swelling of columnar cells, melanomacrophage aggregation and separation of submucosa from muscular layer.

Key words: Fish, Rabbit, Worm and Parasite

Introduction

Parasites nematodes are considered as one of the earliest known groups of helminthes fishes. It is infected the freshwater, marine and brackish water fish species and sometimes cause substantial damage to the host. Although parasitic nematodes can infect almost all organs in a fish, the majority of currently known species have been described from the intestine. Most nematodes infected fish as adults, but a large proportion of them occur as larval stages. These are usually parasites of piscivorous birds, mammals or reptile, or less frequently of predatory fishes (Dick and Choudhury, 2006). The pathological affects of helminthes on their hosts and the immune response of the fish to infection are two aspects of the host – parasites interaction. Although some information on pathology has accumulated, little is known so far of the immune response of fish to nematode infection. (Williams and Jones 1994). Nematode infections in marine fishes cause range of problems. Some of these are associated with pathogenicity of these parasites to the fish host, while others are health hazards connected to human ingestion of live nematodes in fresh or undercooked fish (Dick and Choudhury, 2006). The present study was done to characterize the histopathologcial changes in intestine of rabbit fish **(***Siganus rivulatus*) due to effect of *Procamallanus sp* (helminthes parasites). Camallanids, relatively large reddish orange colored nematodes, infected the alimentary tract of some commercially important marine fishes in the Red Sea include two siganids, good prospective for cultivation (Ben-Tuvia *et al*., 1973). Dzikowski et al (2003) found significant increase prevalence of gut helminthes nematodes *Procamalanus elatensis* and *Cucullanus sigani* infected wild rabbit fish in the Gulf of Aqaba, Red Sea and also *Procamalanus elatensis* infected wild rabbit fish collected from northern Red Sea

(Hassanine and Al-Jahdali, 2007). The fishes presumed acquire infections from infective larvae in crustaceans. Siganids, primary herbivores, also ingest with their plant diet small invertebrates including harpacticoid copepods (Westernhagen, 1973) which may act as hosts. The pathological effect of nematode infection in fish is little studied, and most information is based on field observations. There are only few reported cases of mortality due to nematode infections. Most authors (Bauer *et al.*, 1977; Moravec, 1994; Dick and Choudhury, 1995) agree that fish nematodes damage the hosts by depriving the fish of digested food; by feeding on host tissues, sera or blood; and by direct mechanical damage through fixing to host tissues and developing or migrating in them. Nematodes generally possess a range of enzymes such as proteases, which may have tissue degrading functions (Newton and Munn, 1999). Large sized parasites cause's hemorrhages (Jilek and Crites, 1982; Dunn *et al.*, 1983), inflammation (Measures, 1988; Molnar et al, 1993), granulomas (Hauck and May, 1977; Sindermann, 1990) and mesenteric and visceral adhesions (Sindermann, 1990). C*amallanus*, *procamallanus* and procamallanus species are grab the intestinal wall with their buccal capsules while feeding on blood. Usually there is a local inflammatory reaction at attachment site. Thatcher (1991) and Sinha and Sinha (1988) suggested that nematodes could cause primary anemia by feeding on blood. In intensive infections, especially in small fishes, these camallanids can reduce growth rates and also cause intestinal blockage. More severe changes were recorded in ornamental fishes. Several authors (Stumpp, 1975; Campana - Rouget *et al.*, 1976 and Schaperclaus, 1992) reported on complete destruction of the intestinal mucosa and death of the fish in the presence of large number of *Camallanus fotedari.* Heavy infection with *Camallanus cotti* caused a reduced sexual display rate in *Poecilia reticulata* (McMinn, 1990). According to Dunn et al. (1983), there is loss of epithelium and mucosal hyperplasia, as well as hemorrhage and fibrosis in the laminar propria at the point of attachment. Growth rate, food consumption and swim activity are reduced in infected fish. The spiruroid nematodes *Camallanus oxycephalus* of the green sunfish penetrate to the mucosal layer of gut and causes damage to the columnar epithelium. At the site of penetration, ulcer developed in the mucosa and submucosal layers and there was growth of granulomatous tissue with extensive fibrosis (Meguid and Eure, 1996). Local changes in the intestine can also be provoked by seemingly less pathogenic nematodes. In the case of *Echinocephalus daileyi*, there is a special cephalic inflation and rows of hooks for attachment of the worm to the intestinal mucosa, Thatcher (1991) observed inflammation and formation of a fibrous capsule around the head bulb. Formation of capsules filled by tissue debris, oedematous fluid, fibrous exudates and leucocytes at attachment point of the nematodes. Molnar (1994) found hundreds of *A.crassus* larvae in nodules in the intestinal wall; some of the larvae were alive while others were dead and calcified. Jilek and Crites (1982) studied the pathogenicity of the habronematoid *S. carolini* in centrarchid fishes, described the third stage larvae penetrating the intestinal wall, causing traumatic enteritis, the growth of epithelioid fibroblasts around worms and accumulation of granule cells, leucocytes and macrophages. An expanding fibrocystic layer formed a capsule around the larvae; the innermost layer became necrotic but encapsulated worms were able to develop into adult. *A.simplex* larvae have been seen to induce severe inflammatory reaction in the wall of stomach of cod. Thus clusters of larvae gathered in local inflammatory foci in the stomach wall of the fish host (Berland, 1981).

Material and methods

Wild rabbit fish Forsskål (Teleostei, Siganidae) were collected from two sites on Sudanese Red Sea Coast, Dongnab (21º 03´, N 37º 10´E) and Swakin (19º 06´ N, 37º 20´ E) during February 2010 - January 2011 Fig.1. Fishes were caught by barrier net placed at depths between 1 and 2m, and identified according to Randall (1983) and Froese and Pauly (2010). Living fishes were killed immediately using an overdose of lidocaine (Lignosol) anaesthetic. Digestive tract was removed and injected with Bouin's solution in alcohol. After 24h of fixation, from 20 fish infected and uninfected fish were prepared for serial sectioning. The specimen was stained by Haematoxylin & eosin Harris, (1900) for

normal histological structure and combined Alcian blue (Mowry, 1956) for detection of neutral and mucopoly– saccharides. All fish were 15.68 (±0.39) cm standard length and 113.71 (±11.13) g body weight. To examine the incidence of nematodes, the digestive tract of each fish was cut along the longitudinal plane and adult nematodes species were collected from infected intestines. The number of the parasites and their distribution in the hosts were recorded, and then transferred to a clean 7% saline solution. For taxonomy, parasites were rinsed with tap water, fixed in a mixture of alcohol, formalin and acidic acid (AFA), stained in aceto- Carmine, differentiated, cleared and mounted with DPX. The worm was examined and photographs were taken with a camera connected to a microscope (Leica, DM750, and Switzerland). Specimen used for scanning electronmicroscope (SEM) were post fixed in 1% osmium teteroxide, dehydrated through a graded ethanol series, critical point dried and sputter- coated with gold; there were examine using a JEOL JSM-5510 scanning electron microscope at an accelerating voltage 6 kv. Parasites identification according to Fuscoo, and Overstreet (1979). Parasites and un-parasite intestine 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 descriptions by Harder (1975), Ellis (1977) and Bastide-Guillaume (1986). In parasitized 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. Comparisons between means were made by two-tailed t-tests and significant was accepted at $P \leq 0.05$.

Results

1. Parasite: The digestive tract of *Siganus rivulatus* consist of long esophagus, muscular stomach and an intestine. From the examined samples of *Siganus rivulatus*, an intestinal helminth belong to *Procamallanus sp* Fusco, and Overstreet, 1970 (cucullanidae Cobbold, 1864) Fig.2.

2. Intestine: The number of lymphocytes, eosinophils, red blood cells and goblet cells in parasitized intestine along 100µm length of the supranuclear portion of mucosal epithelium was significantly great than in unparasitized portion as shown in Table 1.

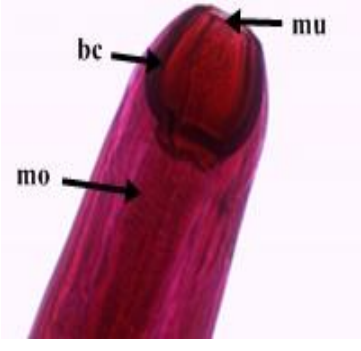
3. Histology: Normal histological feature of unparasitized intestine of *Siganus rivulatus* revealed in Fig. 3, 4, 5, 6 and 7. Histological investigation showed great variability among parasitized and unparasitized *Siganus rivulatus* including caseous necrosis of mucosal epithelial layer of some villi Fig.10. Hyperplasia of sub mucosa lead to separation it from muscular layer Fig. 13. Lymphocytic aggregation, cloudy swelling of columnar epithelial layer Fig. 9. Parasites infection accompanied by damage of the intestinal villi, hemorrhage was observed in the villi lumen and in the arteries in submucsa layer of many fished examined Fig. 8, 9, 11 and 12. Melanomacrophage aggregation and hemosiderin pigment this may be represent important link in the intestinal immune system which catch antigen and pass it into macrophage Fig.11. Eosinophilic infilteration was clearly accumulated in parasitic infected intestines Fig.10. Goblet cells in parasitized intestine were enlarged and appeared to produce excess mucus around parasites attachment sites as immune response Fig. 14, 15, and 16.

Discussion

The pathogenic effect depends on the species, size, and number of parasites. While survival of the fish also depend on the site of infection. Significant differences of lymphocytes cells number may due to present of link in the intestinal immune system which catch antigen and pass it into macrophage and lymphocyte underlying it to activate immune responses against antigen (Junqueira, et al 1998). Esinophilic inflilteration is stimulated by

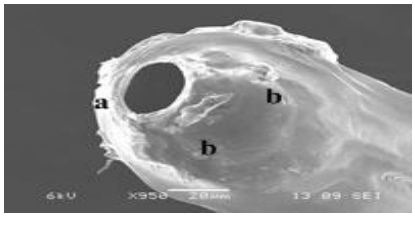
helminthes parasites and increase in number in inflammatory sites, it is same results those presented by Patt and Patt, (1969) and Robbins, (1995). All section cross villi revealed hemorrhages because *procamallanus* species are grab the intestinal wall with their buccal capsules while feeding on blood, which may lead to hemorrhages, inflammation and villi necrosis (Jilek and Crites, 1982; Dunn et al., 1983 Measures, 1988 and Molnar et al, 1993). Increase in number of goblet cells and hyperplasia result of mucosal hyperplasia. Hyperplasia of mucosa layer might due to parasites attachment, which leads to make the space between muscular layer and submucosa layer (Dunn et al. (1983). Fish infected with *Catalans fotedari* are characterized by complete destroy of intestinal mucosa(Stumpp, 1975; Campana- Rouget et al., 1976 and Schaperclaus, 1992). The same results were observed in this study. Where, there was cloudy swelling of the mucosal epithelial cell.

Fig.1 Two sites of samples collection along Sudanese Red Sea Coast. Modified from www.Googleearth.com, (2010). (The photo is not drawn to scale).



A

B



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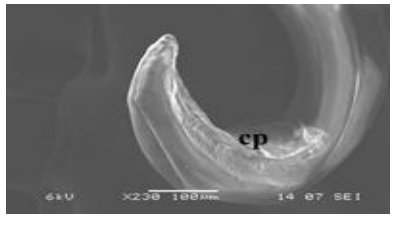

D

Fig (2): *Procamallanus sp*. A- Cephalic end of female, mu → mouth; bc → buccal capsule; mo → muscular esophagus (X40). B- Tail tip of female, a→ anus (X40). C- Cephalic end of male, a → amphid; b → cephalic papilla. D- Tail tip of male, cp → caudal papillae. SEM micrographs.

Table-1 Number of lymphocytes, eosinophils, red blood cells and goblet cells per 200-µm length of intestine parasitized and unparasitized from *Siganus rivulatus*.

Number (No) of Cells	Unparasite intestine	Parasites Intestine
No of lymphocytes	7.88 ± 0.11 [a]	5.73 ± 0.08 [b]
No of eosinophils	3.04 ± 0.09 [a]	4.19 ± 0.17 [b]
No of red blood cells	5.10 ± 0.44 [a]	8.00 ± 0.58 [b]
No of goblet cells	2.42 ± 0.14 [a]	3.06 ± 0.07 [b]

Means ± (SE) within same column followed by different superscript small letters are significantly different at ($P \leq 0.05$) based on t-test.

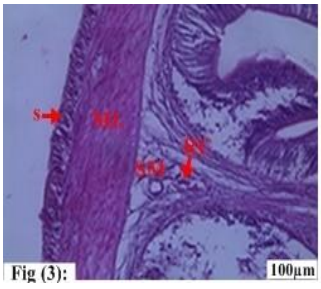

Fig. 3: Normal muscular layer (ML) and submucosa. Serosa (S); submucosa (SM) & red blood cells (RC). H&E.X40.

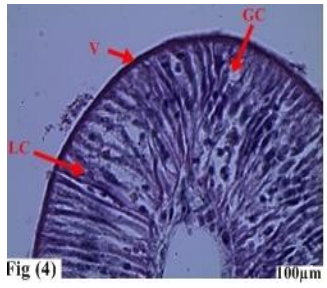

Fig. 4: Normal distribution of cells in villi (V); Goblet cells (GC) & Lymphocyte cells (LC). H&E.X100.

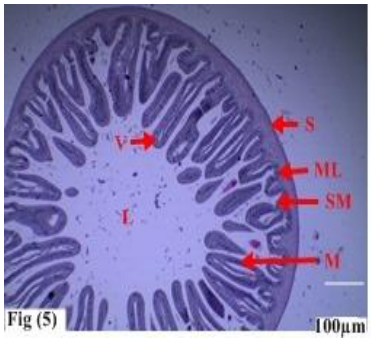

Fig. 5: Whole section of unparasitized intestine. Serosa (S); musclar layer (ML); submucosa (SM); Lumen (L) & Villi (V). H&E.X100.

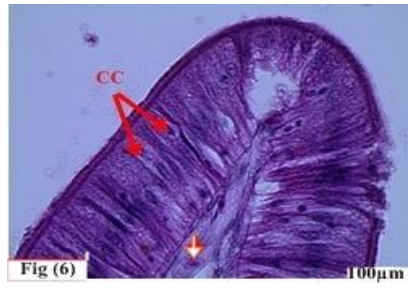

Fig. 6: Cross section villi with normal columnar cells (CC); normal distribution of red blood cells (arrow). Alcian blue X100.

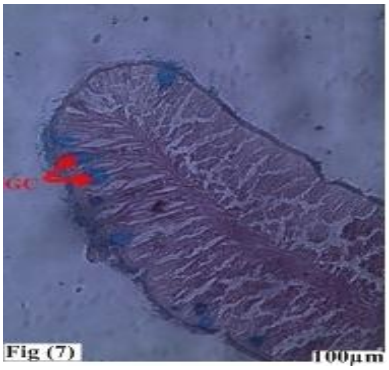

Fig. 7: Goblet cells (GC) stained with Alcian blue X40.

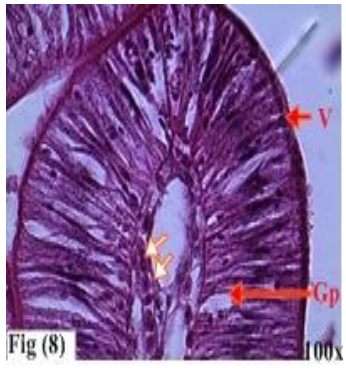

Fig.8: Hyperplsia of the goblet cells (Gp) and hemorrhage inside villi lumen (arrow). H&E.X100.

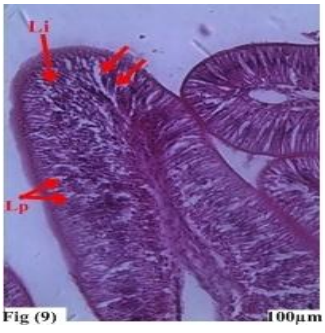

Fig 9: Cloudy swelling of columnar epithelial layer (arrow) and Cellular infilteration (Lp). H&E.X40..

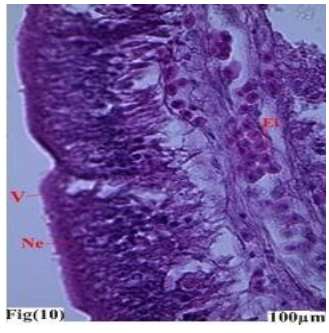

Fig. 10: Necrosis of mucosal epithelial layer & eosinophilic infilteration,(Ei) (arrow). H&E.X100.

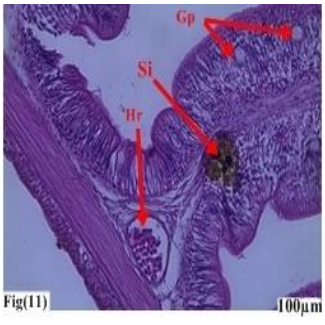

Fig.11: Melanomacrophage aggregation and hemosiderin pigment (Si); Hemorrhage in arteries (Hr) & Goblet cells hyperplasia (Gp). H&E.X40.

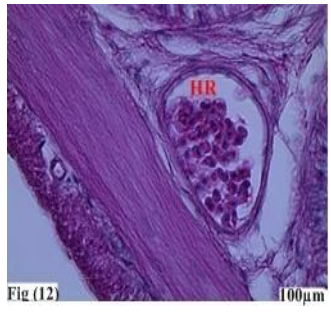

Fig. 12: Ahigh power from fig. 11. Showed Heavy hemorrhage in arteries H&E. X100.

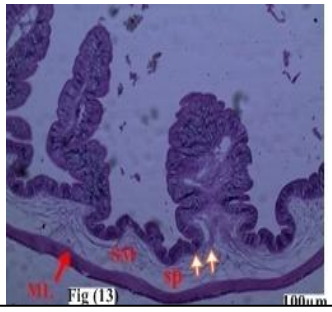

Fig. 13: Separation of submuucosa from muscular layer, (Sp) (arrow) & hyperplasia of mucosa. H&E.X4.

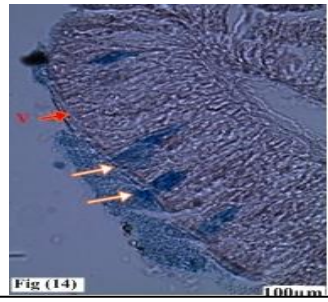

Fig. 14: Excess of mucous formation. Alcian blue X100.

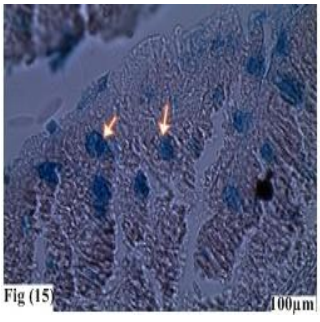

Fig 15 Hyperplasia of Goblet cells, (Gp) (arrow). Alcian blue X100.

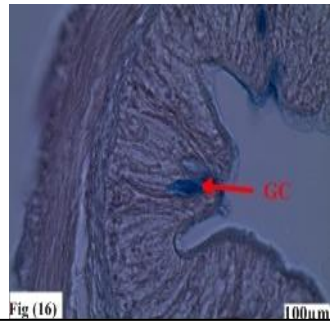

Fig :16: Hyperplasia of Goblet cells, (Gp) (arrow). Alcian blue X100.

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