Songklanakarin J. Sci. Technol.
30 (2), 147-151, Mar. - Apr. 2008

Songklanakarin Journal of Science and Technology
http://www.sjst.psu.ac.th

Original Article

Gonapodasmius epinepheli observed in cage cultured orange spotted grouper (*Epinephelus coioides*) in Southern Thailand: geographical distribution of parasite and host response

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Received 30 October 2006; Accepted 15 May 2008

Abstract

Gonapodasmius epinepheli Abdul-Salam, Sreelatha & Farah, 1990, a didymozoid trematode, was found in orange spotted grouper (*Epinephelus coioides*, Hamilton, 1822) cultured in cages in Southern Thailand, both on the east coast (Gulf of Thailand) and the west coast (Indian Ocean). The parasite encysted on the primary lamellae of the gills. Histological sections revealed that larvae were primarily embedded underneath the gill epithelium and eggs of the parasite were distributed in some organs such as gill epithelium, heart and macrophage centers in the head kidney. Pathological changes and host response were shown mainly on gill lamellae, e.g. reduction and destruction of secondary lamellae. Prevalence of parasite and its life cycle are discussed in this report.

Keywords: *Gonapodasmius epinepheli*, digenetic trematode, grouper, *Epinephelus coioides*

1. Introduction

Orange spotted grouper (*Epinephelus coioides*, Hamilton, 1822) is an important economic marine fish, cultured in Thailand and nearby regions (Malaysia, Singapore, and Indonesia). This fish has been cultured intensively in the southern part of Thailand during the past decade and is expected to expand rapidly in the near future as grouper production in Thailand has increased from 6,500 tons in 1992 to 11,000 tons in 2002 (Fishery Information Technology Center, 2005).

Only a few parasitic infestations in this fish, such as kidney sphaerosporosis and some parasitic helminthes (Supamattaya *et al*., 1990; Cribb *et al*., 2002), have been reported to date. External parasitic infestations and some bacterial infections in grouper have also been mentioned (Chong & Chao, 1984; Donyadol & Direkbussarakom, 1987). In 1990 Abdul-Salam *et al*. observed a new trematode species (Didymozoidae: *Gonapodasmius epinepheli*) from a grouper species (*Epinephelus tauvina*) in the Arabian Gulf. The report described the major taxonomic characteristics of the parasite e.g. a bulbous swelling at the distal end of the ovary, prevalence of a bulge in the genital junction region and a dense mass of glandular cells around the intestinal limbs, and finally identified as a new species. In the present study host response and pathogenicity of *G. epinepheli* are

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reported in *E. coioides* and the distribution and prevalence of infection of this parasite in South China Sea (Gulf of Thailand) and Indian Ocean (Andaman Sea) are discussed.

2. Materials and Methods

Six hundred and sixty grouper of varying size, with a weight range of 38 to 1500 g, were collected from cage cultures at selected locations in southern Thailand. Samples from cage cultures of the east coast (Gulf of Thailand) and the west coast (Andaman Sea) were collected during September 2004 - September 2005. All samples were transferred to Aquatic Animal Health Research Center, Prince of Songkla University, for further studies. Fish were examined and recorded for the presence of parasites, especially in the gills. White and yellow cysts of parasites of varying size from primary gill lamellae were measured under stereomicroscope. Whole parasites were separated and examined under the light microscope. These subsequently were stained with carbolfuchsin and prepared for permanent slides. Fresh eggs and adult parasites were measured under an ocular micrometer. Gills and internal organs of infected fish were fixed in 10% buffered formalin for histological studies (Humason, 1979). Identification of parasites was performed using the key to digenetic trematodes as described by Yamaguti (1971).

3. Results

All sizes of grouper (38–1,500 g) were found to be infected with *Gonapodasmius epinepheli*. Also some small fish (40-184 g) caught from the wild were already infected by this parasite (approximately 1-2% of caught population). The incidence of infection was higher in medium sized fish than in small and large fish. With regard to geographical distribution, the prevalence of infection was higher on the west coast, where the culture activities are more intensive, than on the east coast (Table 1).

Cysts containing trematodes were varied in size, depending on host size. The appearance of cysts depended on their size. Small cysts were whitish and became more yellow in a bigger ones. In small fish (38-200 g), cysts were found with a size range 0.28 x 1.67 mm - 3.25 x 4.83 mm ($\bar{x}$ = 1.32 $\pm$ 0.62 x 4.18 $\pm$ 0.76, n = 72). In medium fish (200-700 g) and large fish (700-1,500 g), cysts of parasites were much bigger, with a size range of 0.46 x 3.34 - 4.60 x 7.90 mm (= 2.43 $\pm$ 1.14 x 6.25 $\pm$ 1.50, n = 32).

The worms separated from the cysts showed a variation in color which depended on the sex. Male worms were whitish while female worms were more yellowish due to the color of eggs inside their bodies. One to three males were observed together with one female in each cyst. Males were normally smaller and shorter than females. Capsule eggs were observed in the female uterus with a size range of 18.85$\pm$ 1.09 x 10.51$\pm$0.73 mm. An egg operculum was not observed by light microscope. The morphology of the parasites and developing eggs examined under the light microscope and the site of infection, as well as the type of host, suggests that this parasite is identical to *Gonapodasmius epinepheli* described by Abdul-Salam *et al.* (1990).

Gills were the major site of infection predominantly on the curve of lower gill arch (Figure 1). Histological sections showed that parasites were tightly packed underneath the mucosal layer of the primary gill lamellae (Figure 2). In this region all secondary lamellae have disappeared. The cyst wall was composed of epithelial cells of primary gill lamellae, overlying several layers of connective tissue. No parts of the cyst wall consisted of parasitic tissue (Figure 3). In the infected area, parasitic cysts expanded and destroyed most of the secondary lamellae (Figure 4).

Some host reactions were also observed, such as a proliferation of capillaries along the cyst wall with lymphocytic infiltrations in the surrounding area (Figure 5). In some cases an increasing number of eosinophilic granulocytes in

Figure 1. *Epinephelus coioides*. Separated gill arch showing parasitic cysts on the gill lamellae.

Table 1. Prevalence of *Gonapodasmius epinepheli*, in grouper (*Epinephelus coioides*) from the Gulf of Thailand and the Andaman Sea during September 2004 - September 2005.

Size of fish/Location	No. of infected fish / No. of fish examined			Total
	Small (38-200 g)	Medium (200-700 g)	Large (700-1,500 g)	
East coast (Gulf of Thailand)	2/61 (3.28%)	1/26 (3.85%)	6/76 (7.89%)	9/163 (5.52%)
West coast (Andaman Sea)	35/252 (13.89%)	32/129 (24.80%)	24/122 (19.67%)	91/503 (18.09%)
Total	37/313 (11.82%)	33/155 (21.29%)	30/198 (15.15%)	100/666 (15.02%)

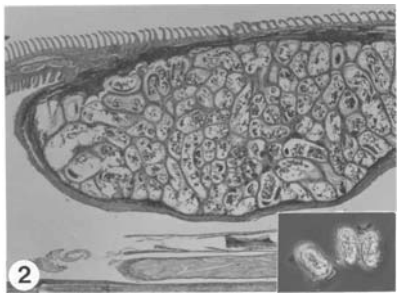

Figure 2. *Epinephelus coioides*. Cyst of trematode found attached to the primary lamellae, H & E, x40, higher magnification of eggs. (inset, x 560).

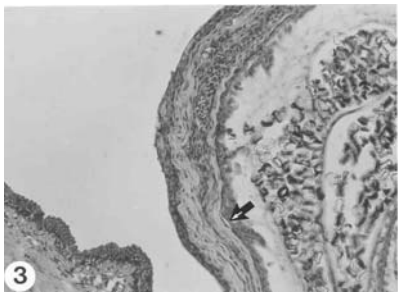

Figure 3. *Epinephelus coioides*. Cyst walls are composed of host tissue; the detached area (arrow) indicates contact between parasites and cyst wall. H & E, x 230.

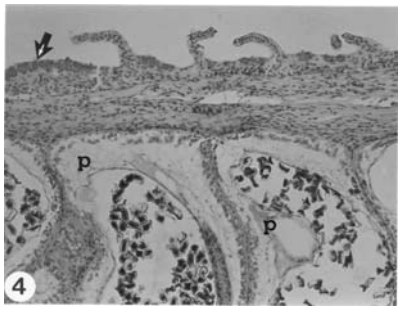

Figure 4. *Epinephelus coioides*. Area of infection showing the reduction of secondary lamellae. (P = parasite), arrow indicate lining of goblet cell in primary lamella epithelium. H & E, x 230.

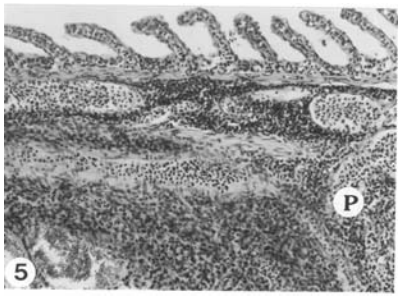

Figure 5. *Epinephelus coioides*. Lymphocytic infiltration into the area of parasitic infestation. (P = parasite), H & E, x 230.

the head kidney were associated with the infection of this parasite. The parasites themselves were often partially or wholly destroyed. In some cases, only the residual body of parasitic cuticles and egg shells were detected in the cysts whereas most organs had degenerated (Figure 6). However, cysts with intact parasites were also observed between those with totally degenerated ones.

The earliest stage of trematode infection which could be detected by light microscope showed an individual parasite with clear cuticle and small suckers embedded underneath the gill epithelium, and most often found in lymphatic vessels (Figure 7). Additionally, early stages of parasites, characterized by small black eye spots were detected in small gill vessels. In the heart tissues, many eggs of the parasite were phagocytized by endocardial cells (Figures 8, 9). These eggs were found intact in some cases but usually destroyed. A large number of eggs engulfed by macrophage centers in the head kidney were also observed (Figure 10).

4. Discussion

Invasions by didymozoid trematodes (*Gonapodasmius* sp.) have been recorded in many species of grouper mostly in gill, buccal cavity and skin (Yamaguti, 1971), but few studies have reported trematode and helminth infection from southeast Asia, especially from mariculture activities (Chong and

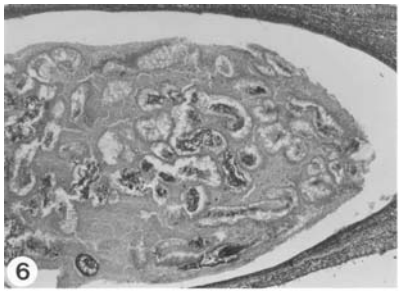

Figure 6. *Gonapodasmius epinepheli*. Organs and eggs of the parasite are destroyed and parasite showing destruction of organs and eggs, the whole structure is shrunken and detached from the cyst wall. H & E, x 40.

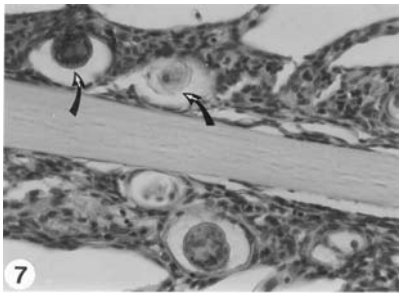

Figure 7. *Epinephelus coioides*. Early encystment of early developmental stages of parasite under mucosal layer of gill epithelium. Arrows indicate the parasite in lymphatic vessel, H & E, x 560.

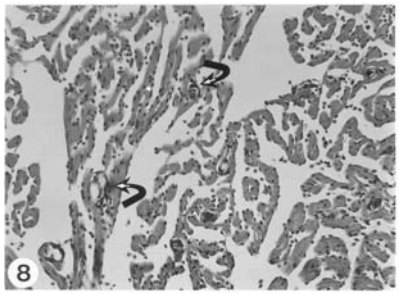

Figure 8. *Gonapodasmius epinepheli*. Eggs of parasite are engulfed by endocardium of the ventricle (arrows), H & E, x 230.

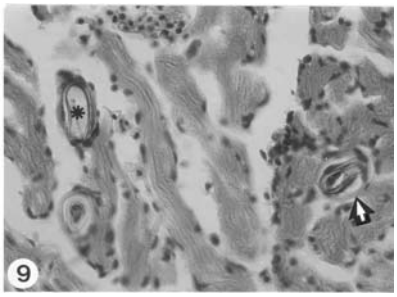

Figure 9. *Gonapodasmius epinepheli*. Some parasite eggs are encapsulated (arrow indicating layer of fibrocysts) and some are engulfed by endocardial cells (*), H & E, x 560.

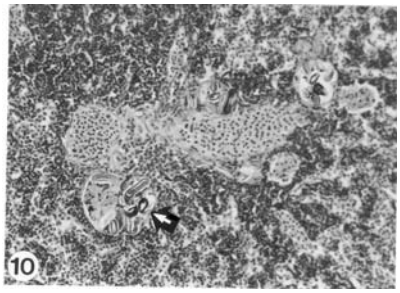

Figure 10. *Gonapodasmius epinepheli*. Eggs are trapped by macrophage centers in the head kidney (arrows), H & E, x 230.

Chao, 1984; Kabata, 1985; Paperna, 1991, Moravec and Scholz, 1991).

Characteristics of this parasite observed in the present study were the same as those of *Gonapodasmius epinepheli* reported from grouper (*Epinephelus tauvina*) from the Arabian Gulf (Abdul-Salam *et al*.,1990). Moreover, the site of infection and geographical distribution lead to the conclusion that this parasite is identical to the trematode found in *E. coioides*. The distribution of this parasite covers the South China Sea (Gulf of Thailand) and the Indian Ocean (Andaman Sea). There is no evidence to suggest where the parasite originated from, as cage culture of grouper has developed on both coastlines simultaneously. In southern Thailand, fish larvae from the east coast are transferred to the west coast for culture and vice versa, therefore, the distribution of this parasite in both areas is possible. However, the percentage of infection was higher on the west coast during September 2004 - September 2005 (Table 1). This was probably due to the fact that grouper culture is more intensive on the west coast of Thailand compared with the east coast (Pomeroy *et al*., 2002). The infection rate also depended on the size of fish. In the west coast the prevalence was higher in medium sized and large fish than in small fish. Infected small grouper may have introduced the parasite from the wild to cultured fish, since some small fish caught from the wild were already found to be infected with this parasite. Culture systems can change the equilibrium of the host parasite relationship and are also often associated with poor environmental conditions, which may lead to increased incidence in outbreaks of this parasite. Similar observations have been made in pond-cultured trout infected by *Sanguinicola fontinalis* (Hoffman *et al*., 1985).

Histopathological changes associated with *G. epinepheli* in grouper (*E. coioides*) were observed mainly in the gills. The area in which the parasite encysted showed lymphocytic infiltrations and a reduction of secondary lamellae. Extended parasites which encysted underneath the gill epithelia caused a proliferation of connective tissue and fibrocytes around the cyst wall. In some cases, a large number of early developmental stages of parasite was observed under the mucosal layer of primary lamellae. This stage showed a similar structure to the early stages of other trematode worms. Similar patterns of infection were found in the blood fluke (*Sanguinicola* sp.) and in carp (Degener, 1980). Early infective stages in host tissue are usually observed under gill epithelium. Therefore, it can be concluded that the gills are the main target of invasion. This is also because the soft tissue of the gill is easy to penetrate and the parasite can easily reach the blood vessels for nutrient supply.

In addition to the early stages of parasites, eggs of *G. epinepheli* could be observed not only in gill lymphatic vessels but also in the heart and kidney. In the gills tissue, eggs were found in small blood lacunae or lymphatic vessels, whereas in heart tissue they were usually observed inside endocardial cells. These degenerated eggs showed empty or deformed egg shells. There were only mild host responses and lymphocytic reactions were observed in the heart and gill. In the head kidney, eggs were sometimes seen in sinuses but were mainly recorded inside macrophages, where destruction of the parasite was similar to that seen in the cardiac endocardium. The fact that nearly all of these eggs were destroyed led us to the conclusion that the distribution of eggs directly in the final host cannot establish a re-infection. According to the typical life cycle of most digenetic trematode the eggs can be released into the surrounding water to find an intermediate host. This can occur through the perforation of cysts by mature worms or by an active penetration of eggs through the capillaries. Some of the eggs probably shedding from mature worm to the blood circulation system are apparently distributed by the blood stream to

various tissues and by the immune system of the host.

Although the life cycle of *Gonapodasmius* is not known to date, it may follow the same cycle as other trematodes (Odening, 1974). Some possible intermediate hosts near the cage cultures, e.g. green mussels or marine gastropods, may play an important role in the distribution of this parasite. Further studies should be undertaken to clarify the biology and life cycle and to establish efficient control measures.

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