

The prevalence and intensity of gastrointestinal endoparasite worms of cantang grouper (*Epinephelus fuscoguttatus* - *lanceolatus*) on floating net cages at Lamong Bay Surabaya, Indonesia

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Abstract. Cantang groupers (*Epinephelus fuscoguttatus-lanceolatus*) is a hybridized grouper fish of Brackishculture Center, Situbondo. In Indonesia, currently information about the parasite infection in cantang groupers is still few. This study aims to determine the prevalence and intensity of endoparasite worms that infect the gastrointestinal of cantang groupers (*E. fuscoguttatus-lanceolatus*) on the floating net cages at Lamong Bay, Surabaya. The method used in this study is survey method and analyzed descriptively. The endoparasite worms found in the gastrointestinal of cantang groupers were *Anisakis physeteris* and *Neoechinorhynchus longnucleanus*. The highest prevalence is single infection of *Neoechinorhynchus longnucleanus* was 3 % (occasionally) with intensity of 1 individual/fish and the lowest prevalence was single infection of *Anisakis physeteris* is 1 % (occasionally) with intensity of 1 individual/fish.

1. Introduction

Groupers are one of leading Indonesian export commodities widely cultivated. The production levels of groupers from 2009 to 2013 in Indonesia were 8.791 tons, 10.398 tons, 10.580 tons, 11.950 tons and 18.864 tons respectively [1]. Cultivation of groupers in Surabaya is located in Lamong Bay uses a floating net cage system. Cantang grouper (*Epinephelus fuscoguttatus-lanceolatus*) is hybridized a grouper of female tiger groupers (*E. fuscoguttatus*) with male kertang groupers (*E. lanceolatus*) [2].

In 2014, mass mortality occurred in cantang groupers cultivated on floating net cages at Lamong Bay. The body of the dead fish has a lot of mucus and there were wounds on some parts of the body. In addition, the growth of the fish during cultivation became slower. The mortality in the cantang groupers was suspected to be related to infectious diseases caused by parasites. One of the effects of the spread of endoparasites in the fish is the presence of invertebrates around the floating net cages that act as intermediate hosts of some endoparasite species [3]. Therefore, carnivorous fish including groupers are more likely to be infected by endoparasite worms than herbivores and omnivorous fish [4].



The endoparasite worms found in the gastrointestinal of several species of groupers are: *Prosorhynchus lafii* [5], *Prosorhynchus maternus* [6], *Callitetrarhynchus gracilis* [7], *Echinostoma* [8], *Camallanus carangis*, *Procamallanus variolae* [9], *Capillaria plectropomy* [10], *Neoechinorhynchus* sp. and *Serrasentis sagittifer* [11].

The results of sea water monitoring in Lamong Bay Surabaya, Indonesia in 2014 showed that the sea water quality is below the seawater quality standard for biota; it also showed the existence of pollution [12]. Decreased water quality can be a trigger of infectious diseases in fish. The development of endoparasites in the fish body can be influenced by environmental factors, such as temperature and chemicals in waters [13]. Research on the prevalence and intensity of endoparasite worms in the gastrointestinal of cantang groupers (*Epinephelus fuscoguttatus-lanceolatus*) is important to determine the prevalence and intensity of endoparasite worms infecting cantang groupers.

2. Methodology

Life specimens of *E. fuscoguttatus-lanceolatus* were taken from floating net cages at Lamong Bay Surabaya, Indonesia on March to April 2016. The samples used in this study were 100 groupers (three months old with a length of 10-20 cm). This research used survey method and the data were analyzed descriptively. The fish were examined for endoparasite under a dissecting microscope. The staining of endoparasite used Semichen-acetic Carmine method, which refers to Kuhlmann's modification [14]. The illustrations used a lucida camera. The key identifications of endoparasite worms are Amin et al. [15], Chen and Shih [16], Grabda [17], Hoffman [18], Kabata [19] and Pavlovskaya [20]. The prevalence of endoparasite worms infecting the gastrointestinal of cantang groupers was calculated based on Bush et al. [21] and the intensity was examined according to Dogiel et al. The category of prevalence and intensity refers to Williams and Williams [23].

3. Results and discussion

From the identification of endoparasite on gastrointestinal of cantang groupers (*Epinephelus fuscoguttatus-lanceolatus*) on floating net cages at Lamong Bay Surabaya, Indonesia, two species of endoparasite worms attached to the intestinal wall were found, which are third stadia larvae of *Anisakis physeteris* and adult worm of *Neoechinorhynchus longnucleanus*. The two species of endoparasite worms can be seen in figure 1 to figure 4.

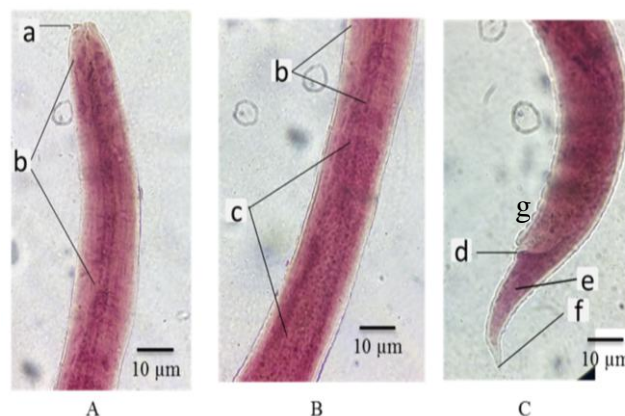


Figure 1. Third Stadia Larvae of *Anisakis physeteris*, scale bar = 10 µm. Coloring images based on binoculars microscope magnification 400x. Description; (A) Anterior part, (B) Mid body part, (C) Posterior part, (a) Booring tooth, (b) Esophagus, (c) Ventriculus, (d) Anal, (e) Appendix, (f) Tail, (g) Vulva.

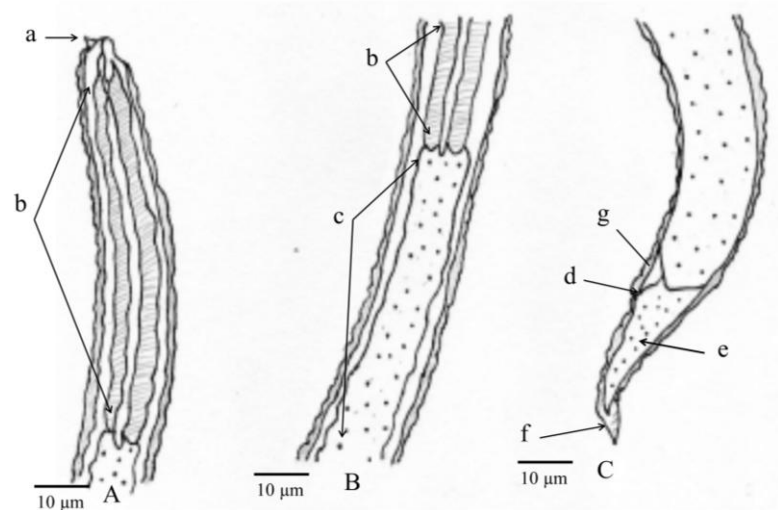


Figure 2. Third Stadia Larvae of *Anisakis physeteris*, scale bar = 10 µm. Images with binocular microscope equipped with camera lucida. Description; (A) Anterior part, (B) Mid body part, (C) Posterior part, (a) Boring tooth, (b) Esophagus, (c) Ventriculus, (d) Anal, (e) Appendix, (f) Tail, (g) Vulva.

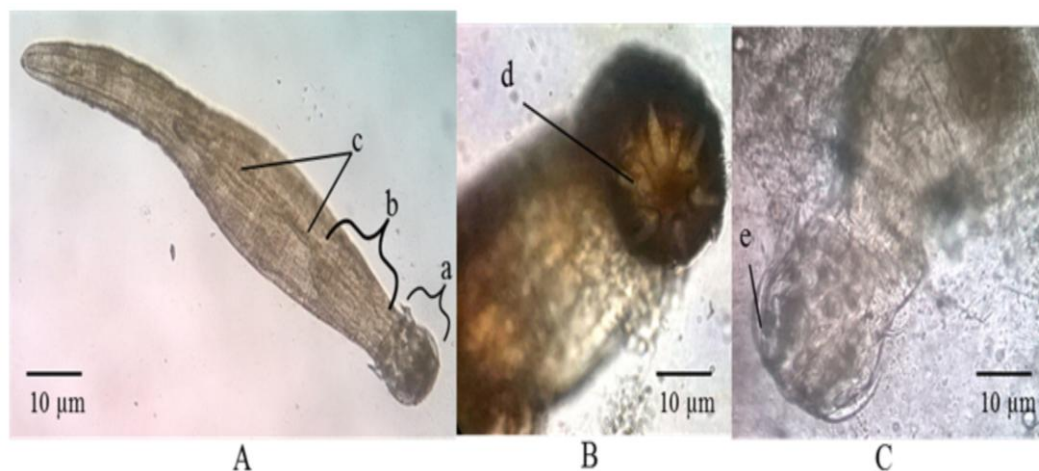


Figure 3. Adult worm of *Neoechinorhynchus longnucleanus*, scale bar = 10 µm. Native on a magnification binocular microscope magnification 100x (in figure A) and magnification 400x (in figures B and C). Description; (A) whole body, (B) Anterior end portion, (C) Proboscis, (a) Proboscis, (b) Proboscis receptacle, (c) Lemnisci, (d) Hook the first line, (e) Hook.

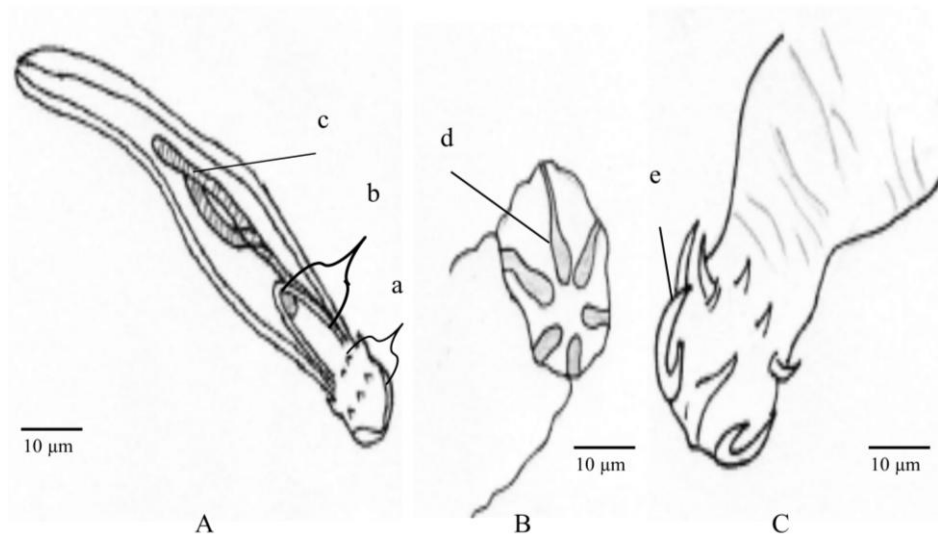


Figure 4. Adult worm of *Neoechinorhynchus longnucleanus*, scale bar = 10 µm. Images with binocular microscope equipped with camera lucida. Description; (A) whole body, (B) Anterior end portion, (C) Proboscis, (a) Proboscis, (b) Proboscis receptacle, (c) leminisci, (d) Hook the first line, (e) Hook.

Anisakis physeteris are included in Phylum Nematelminthes, Class Nematoda, Order Ascaridida, Suborder Ascaridata, Family Anisakidae, and Genus *Anisakis* [12]. This worm has an elongated cylindrical body, with a total length of 4.113 mm and width of 0.13 mm. There were cuticle, booring tooth (larval tooth), esophageal with a length of 0.63 mm, ventriculus with a length of 0.13 mm, intestine, conical tail and on the tail, there was no mucron. This is in accordance with the statement of Anshary [24] and Hoffman [18] that the worm *Anisakis* sp. could be distinguished from other Anisakidae parasites by looking at the anterior end (booring tooth) and the ventricular shape that looks elongated and looks like white spots when observed with a binocular microscope. *Anisakis physeteris* worms were included in *Anisakis* type II which has a conical tail without mucron, according to [24, 16, 17]. *Anisakis physeteris* found was female larvae of third stadia larvae characterized by the presence of vulva in the posterior body. The life cycle of *Anisakis* starts from eggs which develop to second stadia larvae that infect the first intermediate host (small crustaceans), then ingested by the fish, developed into third stadia larvae in the fish body and adult in marine mammals. Nematode worms usually infect the gastrointestinal of fish whereas larvae was found in almost every organ of fish [24, 25, 18]. The study of Chen and Shih [16] found third stadia larvae of *Anisakis physeteris* infecting the gastrointestinal of *Scomber australasicus*.

Neoechinorhynchus longnucleanus belongs to the Phylum of Acanthocephala, Class of Eoacanthocephala, Order of Neoechinorhynchida, Family of Neoechinorhynchidae and Genus of *Neoechinorhynchus* [15]. This worm has a short round proboscis equipped with three row hooks; each line has six hooks. The hook on the first row is the largest compared to those on the second and third rows (fig 3B,C; 4B,C). According to Hoffman's, the genus *Neoechinorhynchus* has a small, bowed or straight, short proboscis body equipped with three rows of hooks with each row of six hooks; the anterior hook is longer and greater than the others. *Neoechinorhynchus longnucleanus* has an anterior hook with a simple root shape, two leminisci of different lengths of 0.81 mm and 0.67 mm (fig 3A,C; 4A,C). This is in line with the statement of Amin et al. that *Neoechinorhynchus longnucleanus* has an anterior hook root with a simple shape and has two different lengths of leminisci and a large nuclei. The worm was an adult *Neoechinorhynchus longnucleanus*. The life cycle of the genus *Neoechinorhynchus* developed into adulthood in marine and freshwater fish, frogs and turtles; larvae developed in the crustacea. Adult worms of *Neoechinorhynchus* were attached to the small intestine and some of them in the peritoneal cavity [18,26]. Amin et al. found *Neoechinorhynchus*

longnucleanus infecting the intestine of *Strongylura strongylura* and Ruckert et al. found it in the gastrointestinal of tiger grouper (*E. fuscoguttatus*).

3.1. Prevalence of endoparasite worms

Prevalence refers to the percentage of fish infected by endoparasites in the population. The prevalence of cantang groupers infected by endoparasite worms could be seen in table 1.

Table 1. Prevalence of cantang groupers infected by endoparasite worms on floating net cages at Lamong Bay Surabaya, Indonesia (100 samples).

| No | Species of Endoparasite | Infected fish (individual) | Prevalence (%) | Infection Category (Williams and Williams, 1996) | Predilection of endoparasites |
|-------|--|----------------------------|----------------|--|-------------------------------|
| 1 | <i>Anisakis physeteris</i> | 1 | 1 | <i>Occasionally</i> | Intestinal wall |
| 2 | <i>Neoechinorhynchus longnucleanus</i> | 3 | 3 | <i>Occasionally</i> | Intestinal wall |
| Total | | 4 | 4 | | |

The total prevalence of endoparasite worms infecting the gastrointestinal of cantang groupers was 4%. The highest prevalence was a single infection of *Neoechinorhynchus longnucleanus* with a prevalence rate of 3 % and the lowest prevalence was a single infection of *Anisakis physeteris* with a prevalence rate of 1 %. According to Williams and Williams [23], the prevalence value was included in the occasional category. The prevalence of cantang groupers infected by *Anisakis physeteris* and *Neoechinorhynchus longnucleanus* was low compared with some studies that found some prevalences of *Anisakis* and *Neoechinorhynchus* worm such as third stadia larvae of *Anisakis physeteris* infecting *Strongylura strongylura* from Taiwanese waters with prevalence of 4 % [16], the prevalence of *Anisakis* sp. in gastrointestinal of *Lutjanus malabaricus* on the auction of fish at Brondong Lamongan was 67 % for fish with the size of 21-24 cm and 80 % for fish with the size of 25-37 cm [27], while *N. longnucleanus* was found to infect the intestinal wall of cultured *E. fuscoguttatus* in Lampung Bay, which was 2.9 % [11], the prevalence of *N. agilis* in *Cheon labrosus* in Beymelek Lagoon Lake in Atalya, Turkey was 24% for male and 8.3 % for female [28] and the prevalence of *N. villaldoi* in *Austrolebias bellottii* from Punta Indio, Argentina was 80% [19].

Fish could act as a definitive host of *Neoechinorhynchus* [30, 18] and as an intermediate host of *Anisakis* [24, 31, 18]. The presence of *Anisakis physeteris* and *Neoechinorhynchus longnucleanus* infections on the intestinal wall of cantang groupers is due to the eating habit of the fish as predators, such as eating trash fish and small shrimp (which could act as intermediate hosts of the endoparasite worms). The above statement was confirmed by Heemstra and Randall [16] that *Epinephelus* were epibenthic predators feeding on macro invertebrates (mainly crustaceans). It was also supported by Ruckert et al. who stated that fish could be attacked by food-borne diseases and the existence of invertebrates around the floating net cage as an intermediate host of some endoparasite worms. Therefore, these may affect the spread in fish. Fidyandini et al. also stated that low prevalence rates were due to parasite adaptation abilities in host body and host compatibility for parasitic survival and environmental quality.

3.2. Intensity of endoparasite worms

Intensity refers to the endoparasites that infect each individual. The intensity of cantang groupers infected by *A. physeteris* and *N. longnucleanus* could be seen in table 2.

Table 2. Intensity of cantang groupers infected by *A. physeteris* and *N. longnucleanus* on floating net cages at Lamong Bay Surabaya, Indonesia.

| No. | Species of Endoparasite Worm | Number of Infected fish (%) | Number of Endoparasite Worm | Intensity (Individual/fish) |
|-------|------------------------------|-----------------------------|-----------------------------|-----------------------------|
| 1. | <i>A. physeteris</i> | 1 | 1 | 1 |
| 2. | <i>N. longnucleanus</i> | 3 | 3 | 1 |
| Total | | 4 | 4 | 1 |

The total intensity of endoparasite worms found in the gastrointestinal of cantang groupers was 1 individual/fish, that was regarded as mild infection [23]. The intensity of cantang groupers infected by *Anisakis physeteris* and *Neoechinorhynchus longnucleanus* was low compared with some research that found some intensity of *Anisakis* and *Neoechinorhynchus* worms such as; the intensity of *Anisakis* sp. in the gastrointestinal of *Lutjanus malabaricus* on the auction of fish at Brondong Lamongan was 5 individual/fish (fish size 21-24 cm) and 18 individual/fish (fish size 25-37 cm) [27]. The intensity of *Anisakis* sp. in *Chanos chanos* on the ponds of Ketapang Village, Mauk, Tangerang District, Province of Banten was 4 individual/fish [20], while the intensity of *N. agilis* in *Cheon labrosus* in Beymelek Lagoon Lake in Atalya, Turkey was 2 individual/fish (male) and 4 individual/fish (female) [28] and the mean intensity of *N. villaldoi* in *Austrolebias bellottii* from Punta Indio, Argentina was 3.13 individual/fish [29].

The intensity value was also influenced by the endoparasite egg survival factor [35, 36]. This study found that endoparasite worms only infected the intestinal wall of cantang groupers. This is in line with the statement of Murata et al. that the small intestine and lumen of the small intestine provide nutrients. The structure and physiology of the intestine could be parasitic microhabitat affecting the presence of parasites. The seawater quality at Lamong Bay was not much different from the standard of seawater quality for biota (based on the Decree of the Minister of Environment No. 51 of 2004 on the Quality Standard of Sea Water). The pH of 7.85 is within the normal range (pH 7-8.5) and the temperature of 29.1⁰C is in the normal range of sea water temperature (28-30⁰C). Meanwhile, the salinity was 29.3 ppt, less than the normal of sea salinity of 33-34 ppt. The low salinity of this study is due to seasonal changes. Komarawidjaja explained that water quality degradation can be a driving force for the development of pathogens in fish.

4. Conclusions

The endoparasite worms infecting cantang groupers were *Anisakis physeteris* and *Neoechinorhynchus longnucleanus*. The total prevalence of endoparasite worms infecting the gastrointestinal of cantang groupers was 4% with total intensity of 1 individual/fish. The predilection of both species of worms was on the intestinal wall of cantang groupers.

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