Eco. Env. & Cons. 26 (November Suppl. Issue) : 2020; pp. (S221-S224)
Copyright@ EM International
ISSN 0971–765X

Correlation between muara grouper fish weight (*Epinephelus coioides*) with *Anisakis* worm infection level in Mayangan Indonesia

T. D. Setyaningrum, S. Koesdarto, T. R. Yustinasari, Kusnoto, M. Yunus* and E. B. Aksono

[1]Faculty of Veterinary Medicine, University of Airlangga, Surabaya, Indonesia

(Received 19 March, 2020; Accepted 20 July, 2020)

ABSTRACT

This study aims to determine the relationship between the weight of estuary grouper fish (*Epinephelus coioides*) and the level of larval infection stage III (L3) *Anisakis* sp. at PPP Mayangan Probolinggo. This research is a laboratory exploratory study. The first step is collecting samples, then measuring the weight, length and width of grouper fish, the next step is grouping surgery, and the last step is to identify the larvae of worms by native methods and carmine staining which is observed with a 100x light microscope and drawn with a lucida camera microscope. The results of the study concluded that the weight of groupers affected the level of *Anisakis* sp. larvae infection with a correlation coefficient of 0.635 and a significance value of 0.003 or <0.01.

Key words: *Estuary grouper, Weight, Infection, Anisak*

Introduction

Parasitic diseases are diseases caused by parasitic body infections such as protozoa or metazoa. Parasites more often attack adult fish because more adult fish will accumulate parasites. As a carnivorous fish, groupers have a big role in the process of spreading parasites, one of which is Anisakidae (Kurniawan, 2015). Anisakidae is a parasite that can only be found in carnivorous marine fish. Judging from their eating habits and habitat, it is likely that marine fish are infected with nematode parasitic worms. Parasitic worms found in fish that live freely in nature are not lethal, but infected fish can transmit parasites to other fish through interactions with each other (Indaryanto *et al.,* 2014). *Anisakis* sp. is one of the genus of worms from the family Anisakidae which can be found in the body of the fish, stomach, intestine, liver, body cavity, gonads and kidneys and some are found in the musculus. Larger fish have more food in their bodies so there is almost no parasitic competition for food.

All needs *Anisakis* sp. found in large fish so that the possibility of parasites moves to smaller fish a little (Noble and Noble, 1989). *Anisakis* sp. can cause a disease called anisakiasis in humans who are infected after consuming infected raw fish or those that are not perfectly cooked (Acha and Szyfres 2003). Observations in several Indonesian waters show grouper fish including the high prevalence of Anisakidae larvae, including Probolinggo (10%), and Tuban (80%) (Raharjo *et al.,* 2017). The Mayangan Beach Fishery Port (PPP) is in the city of Probolinggo and is a fairly large fish landing area in East Java. Fisheries production in PPP Mayangan always increases every year, one of which is grouper production which reaches 164.80 kg in July 2018 (Fisheries Port Information Center, 2018). Grouper

is a marine fish commodity that has economic value and high market demand both from within and internationally. Because of the large levels of parasitic infections, especially Anisakidae worms, a study was carried out on the relationship of the weight of estuar grouper fish with the level of *Anisakis* sp. as an effort to prevent anisakiasis in the community.

Materials and Methods

The grouper samples studied were obtained from fishermen who caught fish in the Mayangan Sea, Probolinggo were taken fresh as many as 32 individuals. Preservation of the sample using an ice box filled with blocks of ice, is expected to inhibit sample spoilage. Fish weights are grouped into two intervals, that isless than 250 andmore than 250 grams. The distinction is made to expose the digestive organs and gills. The viscera organ and muscle of the fish to be examined are taken and placed on a petri dish and given a physiological NaCl solution then an examination of the parasitic infection *Anisakis* sp.

Staining was carried out for identification of larval species by the Semichen-Acetic Carmine method using stage III (L3) worm larvae which stored in glycerin alcohol 5% for 24 hours. After that, dipped in dilute carmine solution for four hours, then transferred in an acidic alcohol solution for two minutes. After completion, it is transferred in an alkaline alcohol solution for 20 minutes, then subsequent gradually dehydrated with alcohol 70%, 85%, and 95% respectively for five minutes. Then dip the larvae in the Hung I solution for 20 minutes, the larvae are transferred to a glass object, and dropped with a Hung II solution, then covered with a glass cover.

Stage III (L3) larvae were identified from *Anisakis* sp. with a 100x magnification light microscope. Identification of *Anisakis* sp. was performed based on the identification key of Lorenzo, (2000) (Lorenzo, 2000). Stage III (L3) larvae *Anisakis* sp. then in the image using a lucida camera microscope.

Data analysis techniques used in this study are comparative analysis using the Mann-Whitney analysis test and correlational analysis using the Spearman Rho analysis test. Data analysis was performed using SPSS v 21.0 for windows.

Results and Discussion

Larva Identification

The results found 21 stage III (L3) larvae of the genus *Anisakis* sp. which is still included in the Anisakidae family. Stage III (L3) larvae *Anisakis* sp. found in the abdomen of two, in the viscera of 13, and in the musculus of six. The number of worm larvae found in viscera is greater because visceral organs, especially the small intestine, provide a source of nutrition for nematodes including blood, tissue cells, body fluids, and food juices contained in the small intestinal lumen (Roberts, 2000). The presence of larvae in the musculus may be due to migratory larvae to the musculus. Live larvae that are in the intestines or other internal organs can migrate into the flesh of fish after the fish die (Noble and Noble, 1989).

Identification is carried out by the native method and observed with a microscope with a magnification of 100x, visible shape of a transparent white elliptical body with a length of 3-30 mm, anterior has a boring tooth, found ventriculus in the medial sec-

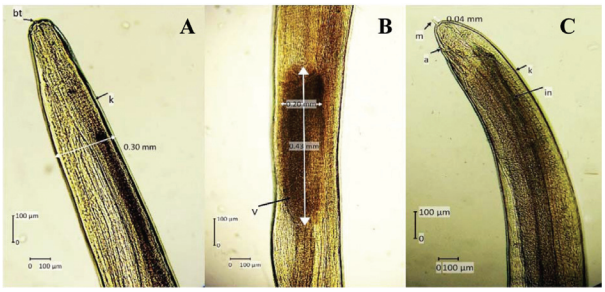

Fig. 1. Stage III larvae (L3) *Anisakis* sp. with native methods using a 100x light microscope. (A) anterior part, bt = booringtooth; k = cuticle. (B) Medial portion, v = ventriculus. (C) the posterior part, a = anus; k = cuticle; m = mukron; in = intestinal.

Table 1. Number of stage III (L3) larvae infections *Anisakis* sp. in estuary groupers (*Epinephelus coioides*) based on weight intervals.

Fish Weight (g)	The number of groupers (pcs)	*Anisakis* infection (pcs)
Less than 250	24	5
More than 250	8	16

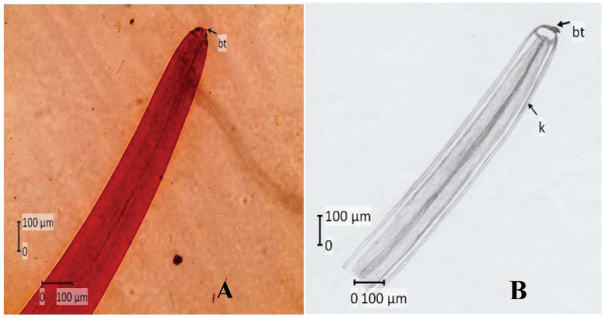

Fig. 2. Anterior part of stage III (L3) larvae *Anisakis* sp. with carmine staining (A) Identification with a 100x light microscope, (B) Image with a 100x lucida camera microscope, bt = booring tooth; k = cuticle.

tion with a length of 0.43 mm and a width of 0.20 mm , and in the posterior part there are anus and mucron measuring 0.04 mm. Morphologically, the parasite *Anisakis* sp. can be distinguished from other Anisakidae parasites by looking at the anterior part (booring tooth), and the ventricular shape using a light microscope (Anshary, 2011). Typical cuticles are found on his body. Cuticle layer functions to protect the body from digestive enzymes in the intestine (Lorenzo, 2000).

The infection rate *Anisakis* sp.

The results of 32 grouper fish found that stage III (L3) larvae *Anisakis* sp. as many as 21 animals with an average length of 1.36 mm. Five stage III (L3) larvae *Anisakis* sp. found in groupers weighing less than 250 g and 16 stage III (L3) larvae *Anisakis* sp. found in groupers weighing more than 250 g.

Noble and Noble (1989) states that the type of food eaten by fish affects the amount of *Anisakis* sp. in the body of a fish. The bigger the fish, the more food both types and quantities it consumes. This results in the accumulation of parasites *Anisakis* sp. on the body of a fish.

Table 2. Comparison of weights of estuary groupers (*Epinephelus coioides*) with infection rates of *Anisakis* sp. stage III (L3)

Weight	Means ± SE
Less than 250	$13.79^{a} \pm 0.08$
More than 250	$24.63^{b} \pm 0.21$

Table 3. Correlation of body weight of estuary groupers (*Epinephelus coioides*) with infection rates of *Anisakis* sp. stage III (L3)

	Weight	Infection rate
Weight	1	0.635**
Infection rate	0.635**	1

Comparative test and correlation test

The results of statistical analysis to determine whether there is influence of estuarine grouper weight (*Epinephelus coioides*) with larval infection rates stage III (L_3) *Anisakis* sp. done by test SPSS Mann-Witney.

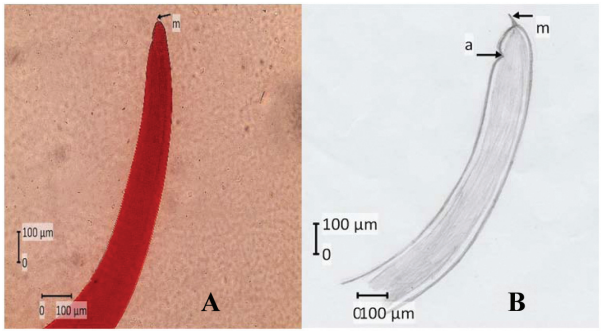

Fig. 3. Posterior stage III larvae (L3) *Anisakis* sp. with carmine staining (A) Identification with a 100x light microscope, (B) Image with a 100x lucida camera microscope, a = anus; m = mukron.

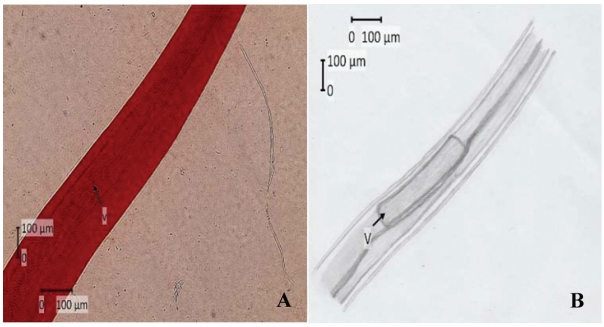

Fig. 4. The medial part of stage III (L3) larvae *Anisakis* sp. with carmine staining. (A) Identification with a 100x light microscope. (B) Image using a 100x lucida camera microscope. v = ventricular.

Statistical analysis to determine the relationship between grouper fish weight and stage III (L3) infection rates of *Anisakis* sp. performed with the Spearman Rho SPSS test.

Interpretation of the correlation coefficient according to Sugiyono in 2012 that the coefficient interval 0.60 to 0.799 means the value of the correlation is strong. So the results of the analysis show a correlation number of 0.635 which means there is a

strong correlation between the weight of estuary grouper fish (*Epinephelus coioides*) with the level of stage III (L3) *Anisakis* sp.

Conclusion

The results showed that there was a very significant influence between the weight of estuary grouper fish (*Epinephelus coioides*) on the level of stage III (L3) infection of *Anisakis* sp. with a significance value of 0.003 or <0.01 and it is known that the greater the weight of estuary groupers (*Epinephelus coioides*) the higher the level of stage III (L3) larvae *Anisakis* sp. with a correlation coefficient of 0.635 or a correlation of 63.5%.

Acknowledgment

We would like to thank the parties or colleagues involved as long as the research process is conducted and do not forget the high appreciation of the support that has been given.

References

Acha, P.N. and Szyfres, B. 2003. *Zoonosis and Communicable Diseases Common to Man and Animals.* Volume III: Parasitosis. 3rd Ed. Washington DC: Pan American Health Organization.

Anshary, H. 2011. Molecular Identification with PCR-RFLP *Anisakis* spp (Nematode: Anisakidae) Larvae on *Auxis thazard* and *Rastrelliger kanagurta* fish from Makassar waters. *Journal of Fisheries, Faculty of Marine Sciences and Fisheries, Hasanuddin University.*

Fisheries Port Information Center, (2018).

Indaryanto, F.R., Wardianto, Y. and Tiuria, R. 2014. Spread of parasitic worms in Female *Rastrelliger brachysoma* in Java. *Journal of Fisheries and Maritime.* 4(4): 215-220.

Kurniawan, A. 2015. Prevalence, Intensity and Molecular Identification of Ectoparasites in Groupers in the floating net cages in Pegametan Bay, Buleleng Regency [Thesis]. Gadjah Mada University. Yogyakarta.

Lorenzo, S. 2000. Usefulness of Currently Available Methods for the Diagnosis of *Anisakis* sp simplex allergy. 55 : 627-633.

Noble, E.R. and Noble, G.A. 1989. Parasitology- The Biology of Animal Parasites, Lea and Febiger. USA.

Raharjo, H.M., Koesdarto, S., Sumarsono, Q.C.K.N., Permatasari, F.D., Wastomi, Z.N. and Sari, N.S.A. 2017. Anisakidae as a Bioindicator Candidate in Response to Environmental Damage. 98 : 374-376.

Roberts, 2000. *Foundation of Parasitology.* 66th edition, University of Miami, McGraw Hill. 369-392.