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by Linayati, B D Madsuari

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³Prevalence and distribution of *Anisakis* sp worms in internal organs of Tuna (*Euthynnus affinis*) at fish auction in Pekalongan city

Linayati Linayati^{1,2} and B D Madusari¹

¹Fisheries Faculty Of Pekalongan University, Indonesia Sriwijaya Street 3rd, Pekalongan

⁸E-mail: pattyana95ina@yahoo.co.id

Abstract. The purpose of this paper was to determine and show the result of the prevalence of the presence of *Anisakis* worms as well as the distribution of *Anisakis* worms in parts of the body in Tuna. This research was conducted in May 2017 at the Fish auction in Pekalongan City. The tuna used in this study are grouped into 2 sizes, group I with sizes 20 - 26 cm and group II with sizes 27-33 cm. The number of fish used was 60. Furthermore, observations were made on internal organs. The research method is a cross-section or one sampling. Data were then tabulated and analyzed by t-test to test the differences between the two groups. The results showed that the fish size group gave a significant effect on the number of *Anisakis* in the body of fish ($p < 0.05$). The prevalence of fish in group II was 85% while the group I was 70%. The intestine becomes the most internal organ found as much as 120 *Anisakis*, followed by 75 stomachs, muscle 20 and abdominal cavity 45 in group II. While group I found 70 of *Anisakis* sp in the intestine, 50 in the stomach and 40 abdominal cavity and 30 muscle tissue.

Keyword : prevalence, distribution, *anisakis*, worm, Pekalongan,

1. Introduction

Pekalongan City is located on the north coast of Java which has potential of fishing industry. Fish auction of Pekalongan City site is one of the central market of marine fish to the people of Pekalongan and its surroundings. Some economical types of fish include red snapper, tuna (Mackarel) and grouper. Marine fish itself is a food source with high nutritional value in the form of protein with a content of 17-24% of its weight (Fardiaz, 1995) [4]. In addition to protein, the content of omega 3 that is owned by marine fish has a positive effect on health. Tuna is a type of marine fish that is widely consumed by many peoples. This fish is included in the Scrombidae class which is found in many waters in Indonesia and is a type of carnivorous fish that has the potential to be infected with parasitic worms that have a life cycle in the sea. This is because the carnivorous fish feed on chepalophoda, small crustacea and small fish that often host of parasitic organisms that live in the sea. That some free-living marine fish, especially those that are carnivorous, are often found to be infected with *Anisakis* (Sardjito, and Desrina, 2005) [17].

Parasites which found in fish have become one of the obstacles in the development of the fishing industry. The presence of *Anisakis* worms can be a factor in decreasing the quality of fish health. This

²To whom any correspondence should be addressed (pattyana95ina@yahoo.co.id)



also affects production of commercial fishing industry which uses Scrombidae as its main raw material. Humans who consume accidentally Tuna are infected with Anisakis will experience health problems such as anemia, anorexia, inflammation and lethargy (Gunawan et al, 2008)[6] abdominal pain, vomiting and nausea, diarrhea (Ivanovic et al, 2017)[8]. Anisakiasis is a disease caused by Anisakis worms, especially *Anisakis* sp and including dangerous zoonotic problem. Although this worm has an effect on human health, the study of Anisakis worms in marine fish is still limited.

The purpose of this study was to find out the prevalence of the presence of *Anisakis* and its distribution in the internal organs of the body of the tuna in different lengths.

2. Material And Methods

The samples were collected from 60 fishes which is sold at Fish auction. This study uses the cross-section method with the main data is the number of Anisakis worms that are processed using the independent t test to determine the differences in the 2 groups of sizes. Furthermore, observations were made to see the distribution of Anisakis worms on the body of fish. The study was conducted in the early month of May 2017 in Fish auction of Pekalongan city. The fish are grouped into 2 lengths, namely group I with a range of 20-26 cm and group II with a range of 27-33 cm. In this study also calculated the prevalence of Anisakis worms with the following formula:

$$\text{Prevalence} = \frac{\text{total of infected fish}}{\text{total of checked fish}} \times 100\%$$

The fish samples obtained are stored in an ice box containing dry ice which functions to slow down the decay process. Observation of the presence of Anisakis worms was carried out at the Biology Laboratory of the University of Pekalongan. The data obtained is then tabulated to be statistically analyzed. Data on the prevalence and distribution of Anisakis in internal organs are reported descriptively.

The initial step in this research is that each fish is given a sign of several labels so that no errors occur in the observation. The next step is observing the external conditions of the fish such as scales, eyes and tail to see the physical damage to the fish. All samples in good condition are then taken to the laboratory. Then do an incision in the cloaca section towards the direction towards the operculum and continue towards the lower part of the operculum so that the belly of the fish can be opened. Observations are made on the contents of internal organs such as the intestine, stomach, liver, body cavity and fish muscle tissue. For fish muscle samples, slicing is carried out horizontally so that the shape of the filet can be obtained. Digestive organs such as the stomach, intestines, and liver are lifted out to be examined carefully so that the existence of Anisakis in the organ organs above is known. The organ is placed in a petri dish and given a physiological nucleus and then observed inside. The worms obtained were collected in a separate petri dish and then observed for their characteristics. Previously, Anisakis worms were performed with multilevel dehydration procedures using 75% - 95% alcohol (Hibur et al, 2016)[7]. This is to facilitate the morphological observation of the Anisakis under a microscope and a magnifying glass. Identification of Anisakis worms based on characteristic by observing the ventriculus and mucron. The third of stage of Anisakis had ventriculus, a booring tooth and mucron located on the posterior part. According to Fukuda et al (1998)[5] mentioning that the important characteristic of Anisakis and other nematode is Ventriculus which is directly connected to intestine.

3. Results and Discussion

Based on observations on two groups of tuna size data obtained data on the number of different Anisakis worms. Data on the prevalence of Anisakis worms are presented in Figure 1 (Amount of Anisakis worms in 2 size groups). In the first group there was a prevalence of 70% or 42 fish infected with Anisakis worms from 60 fish samples. In the second group, the prevalence of 85% or 51 infected fishes from 60 samples was observed. Furthermore, the number of Anisakis worms in the first group was 190 and the second group was 260. After the independent test, the t test shows the results of a P value of 0.00 < 0.05 P table so that it can be concluded that there are significant differences between the two groups. Based on these data it is clear that the number of Anisakis in the size of the fish that is longer will be larger than the smaller size fish. In this study, Anisakis worms were identified as white worms

with elongated round shapes and measuring between 1 cm to 1.7 cm. This refers to the opinion of Awik et al (2007)[2] that *Anisakis* worms have a white, rounded length with sizes ranging from 10 mm to 20 mm.

3.1. Prevalence

Prevalence is the percentage value of the number of fish infected with the disease in one population. The prevalence value in fish with groups of lengths 27 - 33 cm showed higher results of 85% compared to the first group. This is consistent with the results of the t test which stated that the size of the test had a significant effect on the number of worms ($P < 0.05$). The longer size of fish shows the greater number of *Anisakis*. This can be caused by larger fish having the ability to survive longer, causing fish to be exposed to *Anisakis* for longer periods of time compared to small fish. Noble and Noble (1989)[14] stated that there are several factors that influence the number of parasites in fish, including body size, age, season and climate. Some studies also show that the larger the size of the fish, the higher the number of parasites found. Muttaqin et al (2013)[13] stated that the red snapper (*Lutjanus marabarcus*) with a longer size in TPI in Lamongan showed a higher intensity of *Anisakis* which reached 80% compared to smaller fish with only 66%. Furthermore, in layur fish (*Trichiurus lepturus*) it was also found that the same size of the longer layur fish was obtained, the number of *Anisakis* worms was greater than that of the short size fish (Samarariana et al, 2012)[16].

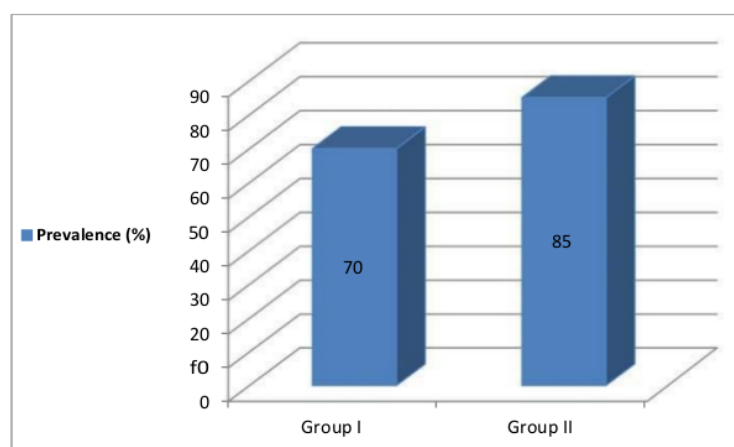


Figure 1. Prevalence *Anisakis* Worms

In addition to the size, there are other factors that cause a high prevalence of *Anisakis*, which is the size of the fish's mouth. The larger the size of the fish's mouth, the greater the size of food swallowed. The dimensions of the fish's mouth will depend on the age and length of the body so that the more fish the body will display the bigger the dimensions of the mouth it will have. A larger mouth allows more food and food size to enter. Fish with a larger mouth size will eat smaller fish and crustaceans more so that the accumulation of *Anisakis* worms is also higher. Kamal et al (2006)[10] stated that the larger the size of the fish the greater the food he swallowed. The life cycle of *Anisakis* worms begins with the egg grazing in the sea and the larvae of the worm will be eaten by crustaceans. Furthermore, infected crustaceans will be eaten by small fish and then enter the intestinal wall of the fish. Carnivorous fish such as tuna will eat small fish and crustaceans that have been infected so that they accumulate in the fish's body. This is in accordance with the opinion of Kimple et al (2004)[11] that piscivorous will experience the accumulation of simplistic *Anisakis* worms in the body if the fish or crustaceans that they eat have been infected by *Anisakis simplex* through the food chain. In addition, the greater food

availability factor in fish causes smaller competition between parasites. The absence of competition means that the number of parasites in large size fish is higher. This is supported by the opinion of Noble and Noble (1989)[14] which states that Anisakis parasitic infection in larger fish has a high value. The high prevalence of Anisakis in Tuna which reaches 70-85% shows lower flesh quality and can cause human health implication if its consumed as a raw or uncooked fish. According to Zubaidy (2010)[19] that the prevalence rate of 41 - 100% is included in the high category.

3.2. Anisakis Distribution

The distribution of Anisakis in the body of tuna is presented in Figure 2 (Distribution of Anisakis worms in several organs in Tuna). According to the data produced by Anisakis worms, most are found in the digestive organs, namely the intestine compared to the stomach, abdominal cavity and muscle tissue. This is supported by the statement of Afrirudin and Abdulgani (2009)[1] that the digestive tract is the most infected organ by Anisakis worms. The results of the study showed that the intestine was the most commonly found Anisakis, followed by the stomach, abdominal cavity, and finally in the muscles of the body. The intestine is a part of the body that serves to accommodate the food that enters so that in this section there are lots of nutrients compared to other organs in the body. The presence of Anisakis worms in the intestine is related to food needs to survive. Jun Suzuki et al (2009)[9] states that the structure and physiological conditions in the intestine can affect the number of parasites in it. In the intestine can be easily found tissue cells, blood body fluids and food extracts. Environmental factors that can influence the presence of parasites, one of which is the availability of food. The more food availability, the greater the number of parasites found. Habitat which is supporting for parasite is a place with full of food, oxygen and other factor such as competition between species (William and Jones (1993)[18]. The intestine is the body part with the greatest availability of food because it is a major part of the digestive organs of food. Food that enters the esophagus will go to the intestine and then go to the stomach. This causes the stomach to become the second internal organ to find the most Anisakis. The stomach is a place for food to be digested before it is absorbed by the body so that Anisakis can be found in this organ. Furthermore, Anisakis is also found in muscle tissue because muscles also contain many nutrients such as proteins, fats and carbohydrates which are also needed by living organisms including parasitic parasites. Similarly, the body cavity of a fish that contains a lot of blood causes Anisakis worms to live in that part. This is supported by the statement of Rohde (1994)[15] that Anisakis can be found in other body tissues of fish other than the digestive tract.

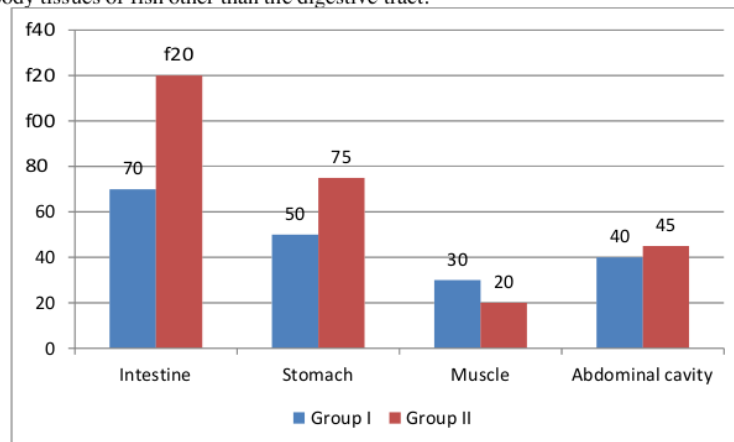


Figure 2. Number of Anisakis Worms in internal organ

Anisakis becomes very potential as endoparasites in the body of the fish due to its very supportive for life of Anisakis. Furthermore that worm has specific protection from digestive enzymes on intestine. Epidermis of anisakis has a protector from digestive enzymes in the intestine, namely in the form of cuticles (Lorenzo, 2000)[12]. Cuticle is an outer layer that is strong and flexible and has a protective function.

4. Conclusion

The difference in the length of the tuna affects the number of anisakis found. In Group II with fish lengths of 27 - 33 cm, Anisakis was found in the amount of 260 while group I with a smaller size of 20-26 cm had Anisakis of 190. The prevalence of Anisakis in group II fish was also higher at 85%, while group I by 70%. The intestine is the most internal organ found Anisakis compared to the stomach, muscle tissue, liver and abdominal cavity. Based on these data it can be concluded that larger fish are more susceptible to infection with Anisakis worms .

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