Identification and prevalence infection of helminth in the gastrointestinal tract swamp eel (*Synbranchus bengalensis*) which marketed in Surabaya, East Java

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Abstract. Disease is one of the obstacles in cultivation are caused by an imbalance of the interaction between environmental factors, host, and disease agents. This study aimed to determine the prevalence of helminth species and endoparasites which infect the gastrointestinal tract swamp eel (*Synbranchus bengalensis*) is marketed in the city of Surabaya, East Java. Samples was taken 75 swamp eel of (10%) from total population 750. The research method used is a survey method. The collected data were analyzed descriptively. Based on the results of checks are performed on 75 samples of eel swamp tail result 22 positive swamp eels infected with helminth endoparasit *Pingus sinensis* and *Eustrongylides Ignotus*. Based on these results the prevalence values obtained for an average of 29.3%. In this study, found a single L3 *Pingus sinensis* infection with a prevalence rate of 4% and a single infection L4 *Eustrongylides Ignotus* infection rate of 8%. While the prevalence of infection mixture L3 *Pingus sinensis* and L4 *Eustrongylides Ignotus* by 16%. These results indicate that the helminth *Pingus sinensis* and *Eustrongylides Ignotus* often infects the eel.

1. Introduction

Eel is one of the fisheries commodities that have high nutritional value. Rice eel is a good source of animal protein because of the high protein content reaching 81.25% and contains 15 amino acids [1]. Like other types of fish, the digestibility value of protein in eels is also very high, making it very suitable for a source of protein for all age groups. Eels are also rich in iron (20 mg/100 gram), higher than iron in eggs and beef (2.28 mg/100 gram). In addition to high protein content, eels also have a high fat content and have a greater calorie content compared to beef [2]. There are three types of eels known in Indonesia, namely rice eel (*Monopterus albus*), swamp eel (*Synbranchus bengalensis*) and sea eel (*Macrotoma caligans* and *Anguilla*). Eels and sea eels in the export code are not distinguished according to their species, only they are divided into three common names: live eels, fresh eels and frozen eels. The world's need for eels and sea eels is increasing every year. The demand for live, fresh and frozen eels in 2007 reached 2,189 tons, in 2008 it increased to 2,676 tons and in 2009 it could reach 4,744 tons [3].
According to the Surabaya City Government, the Surabaya region is divided into five parts namely, Central, East, West, North and South Surabaya. Eels that are marketed in the city of Surabaya come from Sidoarjo, Lamongan, Bojonegoro, Lumajang, and Probolinggo. Eels which are marketed in the city of Surabaya are generally obtained naturally from rice fields and from swamps, therefore these eels have a great potential for parasitic infection. Swamp is an environment whose water quality depends on nature so that it will affect the life of organisms that live around it. Diseases of the eel are infectious and non-infectious. Infectious disease is a disease caused by parasites, fungi and bacteria [4].

According to Mahasri et al [5], one of the cause of eel disease are parasites. Parasites are organisms that live on the body of other organisms and generally have a negative effect on their host. The parasitic attack makes the fish lose its appetite, then slowly weakens and leads to death. Some previous studies reported that the type of parasite that is often found in eels are worms. One worm that is harmful to humans and vertebrate animals found in the body of Monopterus albus is generally from the class Nematoda, genus Gnathostoma. This organism often infects humans and various animals that consume raw or inadequate eel, where the eel contains larvae. Besides of Gnathostoma worms, Pingus sinensis and Eustrongyldes ignotus worms also found in eels [6].

2. Materials and methods
2.1. Place and time
The sampling place is carried out at the eel agents located in the City of Central Surabaya, East Surabaya, West Surabaya, North Surabaya and South Surabaya. This research was conducted in the education laboratory of the Faculty of Fisheries and Marine Universitas Airlangga in September-November 2015.

2.2. Work procedure
First, preparing tools such as scissors, tweezers, scalpels and trays. Next prepare the eel samples to be observed. Sampling was carried out at agents or collectors of eels in the city of Surabaya, each sample was taken as much as 10% of the total number of eels sold each day. Approximately, the total sales of all agents per day were 750 eels. Sampling was carried out at five eel agents in Surabaya representing each part of the region namely Karah eel agents representing South Surabaya, Simo eel agents, Banyu Urip representing West Surabaya, Keputran eel agents representing Central Surabaya, Mulyosari eel agents representing East Surabaya and Krembangan eel agents representing North Surabaya. According to the survey, swamp eel (Synbranchus bengalensis) has a body length range of 40-60 cm. At the Karah eel agent (Location A) sells swamp eels with an average of 150 eels every day, 15 samples were taken at this place. Simo eel agent (Location B) sells swamp eels an average of 200 eels per day, 20 samples were taken. Keputran eel agent (Location C) sells swamp eels an average of 150 eels per day, 15 samples were taken. Mulyosari eel agent (Location D) sells swamp eels an average of 100 eels per day, then 10 eel samples are taken and the Krembangan eel agent (Location E) sells swamp eels for an average of 150 eels per day, so 15 samples were taken. Eels that were marketed come from the City of Lamongan, Bojonegoro, Lumajang and Probolinggo, sample taking was done in a living condition, so that the total sample taken was 75 eels. Samples were put into plastic with a living condition and then taken to the Laboratory of the Faculty of Fisheries and Marine Universitas Airlangga for identification.

2.3. Worm identification
Samples were taken and then placed on a tray, then the eels were weighed and measured in length. Surgery was performed by cutting the lower abdomen from the anterior body to the ventral fins, then cut into the dorsal body to the lateral line and then cut out leads to the anal part of the fish. Eel stomach and intestine are cut and then stored in glycerin alcohol 5% for parasitic examination. Examination of the eel digestive tract parasites is carried out by removing the stool in an ordered manner leading to the posterior
end of the intestine. Stools that have come out are then placed on a glass object and dripped with water then observed under a microscope at 100x magnification. Parasite identification was carried out based on [6,7,8,9].

2.4. Staining method
Worm staining uses the Semichen-Acetic Carmine method which refers to Kuhlman [10] and is modified by the way the worm is stored in 5% glycerin alcohol and then fixed between two glass objects and tied both ends with threads. Then put in 70% alcohol for five minutes. After that, remove the worms to the carmine solution that has been diluted with 70% alcohol in a ratio of 1:2, left for 24 hours, then the worms are removed from the glass object, then transferred in an acidic alcohol solution for two minutes (70% alcohol + HCl). After completion, it is transferred in an alkaline alcohol solution for 20 minutes (70% alcohol + NaHCO₃). Next step is dehydration with 70% alcohol for five minutes, 85% alcohol for five minutes and 95% alcohol for five minutes.

2.5. Research parameter
The main parameter observed in this study is to identify and calculate the prevalence of endoparasites of worms that infect marsh eels (Synbranchus bengalensis) which are marketed in the city of Surabaya. The prevalence of worm endoparasites in swamp eels is calculated in the following way [8].

\[
\text{Prevalence} = \frac{\text{Rice eels identified with endoparasites}}{\text{Total rice eels observed}} \times 100\%
\]

2.6. Data analysis
Data from the identification of parasites that infect marsh eels (Synbranchus bengalensis) were analyzed descriptively and presented in the form of pictures and tables. The parasitic prevalence values are calculated and presented in tabular form [11].

3. Result and discussion
3.1. Results
3.1.1. Worms identification
The results of research conducted on 75 samples of swamp eels (Synbranchus bengalensis) which are marketed in the city of Surabaya, East Java were found the presence of endoparasitic worms. The results of identification of endoparasitic worms according to the key identification of parasites by [6,7,8,9] can be seen in Table 1.

| No | Endoparasites   | Type of Infected Organ                  |
|----|----------------|----------------------------------------|
| 1  | Pingus sinensis | Outer surface of intestinal wall (penetration) |
| 2  | Eustrongylydes ignotus | Outer surface of intestinal wall (penetration) |

The results of identification of endoparasitic worms in the digestive tract of swamp eel (Synbranchus bengalensis) which are marketed in the city of Surabaya, were found two types of endoparasitic worms from the Nematoda class namely L3 Pingus sinensis and L4 Eustrongylydes ignotus. L3 Pingus sinensis and L4 Eustrongylydes ignotus were found to infect the outer surface of the intestinal wall (penetration) of swamp eels.
3.1.2. The 3rd stadium larvae (L3) *Pingus sinensis*

Worm identification is based on morphological characteristics. Worms found in the digestive tracts of swamp eels are worms that are classified as Nematodes because they have an elongated cylindrical body shape, an esophagus, ventriculus, and nerve rings. L3 *Pingus sinensis* found in this study was reddish milky white, papillae, elongated cylindrical body shape, body length of 5.0-5.6 mm and body width of 0.12 mm while esophagus length of 0.54-0.59 mm, L3 *Pingus sinensis* has a nerve ring length of 197-250 μm from the anterior end of the body, and has a spicula length of 0.06 mm.

Result of morphological identification found that worms are described as follows: Species *Pingus sinensis*, Genus *Pingus*, Family Quimperiidae, Order Spirurida, Class Nematoda, and Phylum Nemathelminthes. From the description above, this endoparasitic worm belongs to the *Pingus sinensis* species which is in accordance with the description of Moravec et al. [6] who discovered *Channa argus* infected with L3 *Pingus sinensis* in Boa’an lake, Hubey-China. Morphology of L3 *Pingus sinensis* drawn using a binocular microscope equipped with a Lucida camera is shown in Figure 1.

![Figure 1. 3rd stage *Pingus sinensis* larvae.](image)

**Note:** A. anterior part of L3 *Pingus sinensis* with lucida camera, B. posterior part of L3 *Pingus sinensis* with lucida camera, 100x (bar scale: 10 μm), C. anterior part of L3 *Pingus sinensis* with Semichen Acetic Carmine coloring, D. posterior part of L3 *Pingus sinensis* with Semichen Acetic Carmine 100x (bar scale: 10μm); a. mouth, b. esophagus, c. nerve ring, d. ventriculus, e. anal, f. Tail.

3.1.3. The 4th stage larvae (L4) *Eustrongylides ignotus*

The stage four larvae of *Eustrongylides ignotus* found in the swamp eel intestine are nematode-class worms because they have a long, unsegmented upper body. The posterior part of the worm is seen to have three cuticle layers, while the anterior part of the worm has a labial papillae with each of the six papillae, equipped two on the lateral part, two on the subventral part and two on the subdorsal part so that the worms found include IV stadia larvae.

The examination of worms in the swamp eel digestive tract, L4 *Eustrongylides ignotus* was found with an elongated cylindrical with a size of 50-60 mm, blackish red, had an esophagus length of 8.704 mm, and had a spicula. Male worms have a posterior spicula, which distinguishes male and female worms. The digestive tract of this worm looks like a DNA chain. Fish is the host between the two, in the body of the fish development occurs (L3) to (L4). The result of morphological identification found that the worms can
be described as follows: Genus *Eustrongylides*, Family Dioctophymatoidea, Order Aenophorea, Class Nematoda, and Phylum Nemathelminthes. From the description above, this endoparasitic worm belongs to the species *Eustrongylides ignotus* which is in accordance with the description of Hoffman [8] The results of worm images using a binocular microscope equipped with a lucida camera can be found in Figure 2.

![Figure 2](image-url)  

**Figure 2.** 4th stage larvae of *Eustrongylides ignotus* Note: A. anterior part of L4 *Eustrongylides ignotus* with lucida camera, B. posterior part of L4 *Eustrongylides ignotus* with lucida camera, 100x (bar scale: 100 μm), C. anterior part of L4 *Eustrongylides ignotus* with Semichen Acetic Carmine staining, D. posterior part of L4 *Eustrongylides ignotus* with Semichen Acetic Carmine 100x (bar scale: 100μm); a. Labial papillae, b. osephagus, c. ventriculus, d. spicules, e. three cuticle layers.

3.1.4. Worm Prevalence in the swamp eel digestion (*Synbranchus bengalensis*)  
The prevalence of *Pingus sinensis* and *Eustrongylides ignotus* worms that infect the digestive tract of swamp eels (*Synbranchus bengalensis*) can be seen in Table 2.
Table 2. Prevalence of Pingus sinensis and Eustrongylides ignotus worms in the digestive tract of swamp eels (Synbranchus bengalensis) which are marketed in the city of Surabaya, East Java

| Location of Sampling | Number of Eels Checked (Tail) | Number of Eels Infected (individual) | Number of Eels Infected with Worms (individual) | Prevalence (%) |
|----------------------|------------------------------|-------------------------------------|-----------------------------------------------|----------------|
| Agen Karah (A)       | 15                           | 5                                   | -                                             | 33.3           |
| Agen Simo (B)        | 20                           | 6                                   | -                                             | 30             |
| Agen Keputran (C)    | 15                           | 4                                   | -                                             | 26.7           |
| Agen Mulyosari (D)   | 10                           | 3                                   | -                                             | 30             |
| Agen Kerembangan (E) | 15                           | 4                                   | 4                                             | 26.7           |
| **Total**            | **75**                       | **22**                              | **4**                                         | **Mean 29.3**  |

Note:  
- **a**: single infection Pingus sinensis  
- **b**: single infection Eustrongylides ignotus  
- **ab**: mixed infection Pingus sinensis dan Eustrongylides ignotus

Sample examination results showed that the prevalence rate of L3 Pingus sinensis and L4 Eustrongylides ignotus infection at location A was 33.3%, location B was 30%, location C was swamp eel 26.7%, location D was 30% and location E was 26.7%. The highest prevalence of L3 Pingus sinensis and L4 Eustrongylides ignotus was found to be 33.3%. Pingus sinensis and Eustrongylides ignotus worms are found on the outside of the intestinal wall (Penetration) of swamp eel. The average prevalence of swamp eels marketed in Surabaya City infected with L3 Pingus sinensis and L4 Eustrongylides ignotus was 29.3% (22 infected samples from a total of 75 samples). The results showed that the prevalence rate of each sampling is varied.

The results of examination of the samples showed that in one sample, one worm could be infected with more than one endoparasitic worm. From 75 samples examined, there were 6 samples that were infected with a single worm Eustrongylides ignotus and 4 samples that were infected with a single worm Pingus sinensis. In addition, there were also swamp eels infected with a mixture of Pingus sinensis and Eustrongylides ignotus worms for 12 out of 75 eels examined.

In addition, the calculation of the prevalence of single and mixed infections is performed. The prevalence of swamp eels infected with single Pingus sinensis worms was 5.3% and swamp eels infected with single Eustrongylides ignotus by 8%, while swamp eels infected with mixed Eustrongylides ignotus and Pingus sinensis were 16%.
3.2. Discussion

Based on the results of the examination of the two worms identified based on morphology, two endoparasitic worm species infecting the swamp eel digestive tract are marketed in Surabaya, East Java, namely L3 *Pingus sinensis* and L4 *Eustrongylides ignotus*. The worm was found to infect swamp eels according to Moravec et al [6] who discovered *Channa argus* infected with *Pingus sinensis* in Boaan Lake, Hubey-China and *Monopterus albus* infected by *Eustrongylides ignotus* in Liangzi Lake, Hubey-China.

Endoparasite worms found in this study are included in the Phylum Nemathelminthes, Nematode Class, Order Spirurida, Family Quimperiidae, Genus *Pingus*, *Pingus sinensis* Species. *Pingus sinensis* worms found in this study were reddish milky white in color, have a papillae, elongated cylindrical body shape, body length of 5.0-5.6 mm and body width of 0.12 mm while esophagus length of 0.54-0.59 mm. While L3 *Pingus sinensis* has a nerve ring length of 197-250 μm from the anterior end of the body, and has a spikula length of 0.06 mm. This is in accordance with Bykhovyskaya et al [9] which stated that L3 *Pingus sinensis* was found to have an elongated cylindrical body shape, in the mouth there was papillae, in male worms having a body length of 4.9-5.8 mm and having a body width of 0.12 mm while the length of the esophagus in male worms 0.54-0.59 mm. *Pingus sinensis* male has a nerve ring length of 195-256 μm from the anterior end of the body, and has a spicule length of 0.06 mm. Female worms have a body length of 5.8-6.2 mm and have a body width of 0.165-0.250 mm, while female worm esophagus size is 0.64-0.74 mm and female *Pingus sinensis* has a nerve ring length of 195-218 μm from the anterior end body.

This worm has a life cycle starting from eggs issued by adult worms with feces into the water. The eggs hatch into stage 1 (L1) larvae. L1 moves freely in the water, if swallowed by a host, then in the body of the host between I develops into larval stage II. If the intermediate host I is eaten by the intermediate host II (freshwater fish), L2 will develop into L3 in the body of the fish. If the intermediate host II is eaten by the definitive host (bird) the larvae will develop into adult worms [12].

*Pingus sinensis* worm infections generally do not show any clear clinical symptoms in fish. This makes it difficult to detect the presence of parasites on the body of the fish, but if surgery is performed and observations are made on the internal organs, the presence of endoparasitic larvae can be seen. In the marsh eel examined not only found the larval stadia III *Pingus sinensis* but also the IV stadia larvae *Eustrongylides ignotus* was found attached to the outer surface of the intestinal wall.

*Eustrongylides ignotus* worms include Phylum Nemathelminthes, Class Nematoda, Order Aenophorea, Family Dioctophymatoidea, and Genus *Eustrongylides*. From the description above, this endoparasitic worm belongs to the species *Eustrongylides ignotus* which is in accordance with the description of Hoffman [8]. The worm is elongated cylindrical, blackish red, the posterior part of the worm is seen to have three layers of cuticle, while the anterior part of the worm has a labial papillae with each of the six papillae, equipped two on the lateral part, two on the subventral part and two on the subdorsal part so that worms found included IV larval stadia. This is in accordance with Xiong et al [13] who certify the IV larvae of *Eustrongylides ignotus* have Cephalic characteristics of this cone-shaped worm that appears to have 12 labial papillae, arranged in two circles with each of the six papillae, completed by two on the lateral part, two on the subventral section and two on the cone in the subdorsal section.

In the examination of worms in the swamp eel digestive tract, L4 *Eustrongylides ignotus* was found elongated cylindrical with a size of 50-60 mm, blackish red, had an esophagus length of 8.704 mm, and had a spicula. This is in accordance with Xiong et al [13] which states that adult *Eustrongylides ignotus* female worms have a size of 53-70 mm while male *Eustrongylides ignotus* worms have a size of 40-42 mm. According to Hoffman [8] male worms have posterior spicules, this is what distinguishes male and female worms and the intestinal tract looks like a DNA chain. Based on research conducted by Moravec et al [6] *Eustrongylides ignotus* infects *Monopterus albus* in Liangzi Lake, Hubey - China.
A characteristic feature of L4 Eustrongylides ignotus is that it has three posterior cuticle layers [13]. In its life cycle the worm needs copepods as intermediate hosts I and requires fish as intermediate hosts II, then develops into adults in the bodies of rabbits, cats, chickens, ducks, and causes inflammation of the stomach lining of cranes [8].

Based on the results of examinations conducted on 75 samples of swamp eels, 22 positive swamp eels were infected with endoparasitic worms. Based on these results the prevalence value obtained by an average of 29.3%. In this study, a single infection of Pingus sinensis L3 was found with a prevalence rate of 4% and a single infection of L4 Eustrongylides ignotus infection rate of 8%. While the prevalence rate of mixed L3 Pingus sinensis and L4 Eustrongylides ignotus infections is 16%. These results indicate that the Pingus sinensis and Eustrongylides ignotus worms often infect eels in accordance with the statement of Williams et al [14] which states that the category often or often describes that the parasite often infects fish if the average prevalence is 10-29%. The variation in prevalence in each sampling can be influenced by differences in the size of the eel. The frequency of swamp eels infected with endoparasitic worms is influenced by many things including the environmental conditions of these waters. In addition, the presence of small organisms such as crustaceans as food for eels as well as the first intermediate host also influences the presence of endoparasitic worms in fish bodies. Parasites enter the surrounding environment carried by currents or aquatic organisms. Some aquatic organisms can act as hosts between parasites so that when eaten by swamp eel the parasites that are in it also infect the eel. This is in accordance with Yamaguti [15] which states that the presence of endoparasites in the body of fish is also influenced by the presence of invertebrate organisms such as crustaceans and molluscs in the vicinity of aquaculture sites that act as hosts between parasite carriers.

Thus to prevent the spread of worm disease in other regions, it is necessary to examine samples that will be sent outside the region or abroad so that there is no spread of worm infections.

4. Conclusion
Based on the results of identification of worms in the swamp eel digestive tract (Synbranchus bengalensis) which are marketed in the city of Surabaya, East Java found positive larvae of Pingus sinensis and Eustrongylides ignotus and the prevalence value of swamp eels (Synbranchus bengalensis) marketed in Surabaya City, East Java positive swamp eel larvae and Eustrongylides ignotus and the prevalence value of swamp eels (Synbranchus bengalensis) which is marketed in Surabaya City, East Java infected with endoparasitic worms. Based on these results, a prevalence value of an average of 29.3% was obtained, indicating that the Pingus sinensis and Eustrongylides ignotus worms were categorized as Often or frequently infecting eels.

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