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Dear **Editor-in-Chief,**

I herewith enclosed a research article,

Title:

Prevalence and Hematology on the Swamp Eel (Synbranchus bengalensis) that was infected *Trypanosoma* sp. Marketed in Surabaya

Author(s) name:

Dr. Gunanti Mahasri, Ir., M. Si

Address
(Fill in your institution's name and address, your personal cellular phone and email)

Department of Fish and Aquaculture Health Management, Faculty of Fisheries and Marine Resources, Universitas Airlangga, Surabaya, Indonesia
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Surabaya, 05 Juli 2019

Sincerely yours,
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Dr. Gunanti Mahasri, Ir., M. Si

Prevalence and Hematology on the Swamp Eel (*Synbranchus bengalensis*) that was Infected *Tryponosoma* sp. Marketed in Surabaya

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Abstract. This research aims to determine to prevalence and hematology on the swamp eel (*Synbranchus bengalensis*) that was infected *Trypanosoma* sp. marketed in the Surabaya. This research used survey method through sampling directly sampling at two locations directly. Sampling at the first location is in Ambengan, whereas at the second location in Surabaya Karah. The total sample used in this research as many as 60 swamp eel the size of 45-60 cm, with with the main parameter is the prevalence, degree of infection, total erythrocytes count, and leucocytes differential. The results showed that swamp eels infected *Trypanosoma* sp. were found to have a characteristic morphology as their flagella, the core nucleus and undulating membrane. The result of prevalence of *Trypanosoma* sp. on first location (Ambengan) of 23.3% by total of swamp eel infected as many as 7 of 30 swamp eels with an average total erythrocytes count from 0.77 $x10^6$ cells/mm, and the average leucocytes differential monocytes 8,5%, basophils 2.2%, eosinophils 1.4%, lymphocytes 80.8%, and neutrophils 6.8%. Whereas at the second location (Karah) of 30% by number of swamp eel were infected as many as 9 out of 30 individuals with an average total erythrocytes count from $0.80x10^6$ cells/mm^3, and the average leucocytes differential monocytes 8,5%, basophils 2.3%, eosinophils 1.3%, lymphocytes 79.8%, and neutrophils 7.7%. The degree of infection in both locations have the same that category is moderate. Based on the results data analysis prevalence and degree of infection which means that the of *Trypanosoma* sp. among two locations there are no significant differences (P>0.05). Based on data analysis using *Mann-Whitney U-Test* showed result that there wasn't different total erythrocytes count on the swamp eel infected *Trypanosoma* sp. in both locations that is (p≥0,05). Based on data analysis using *Independent T-Test* showed result that there wasn't different in leucocytes differential on the swamp eel infected *Trypanosoma* sp. in both locations that is (p≥0,05).

Key words : degree of infection, leucocytes differential, prevalence, total erythrocytes, swamp eel (*Synbranchus bengalensis*), and *Trypanosoma* sp.

Running title : Prevalence, Hematology Swamp Eel Infected *Trypanosoma* sp.

INTRODUCTION

Fisheries commodities that have high market potential in the community and exports are eel. This is evidenced by the export figures for 2012 data reaching 6.081 tons (BPS, 2012). Exports of eels issued from Indonesia include Asia, Europe, Australia and even the United States (Manurung et al., 2015). Domestically, the demand for eels to meet the needs of the domestic market is high. Demand for eels in Jakarta reaches 20 ton/day, in Yogyakarta as many as 30 tons, (Syarif et al., 2017). Eels have contains nutrients including 75.32% protein, 0.58% fat, and 22.54% carbohydrates and contain various minerals, vitamin A, iron and omega 3 (Astiana et al., 2015).

Type of eels consumed is swamp eel (*Synbranchus bengalensis*). Swamp eel that is difficult to find in traditional markets, but can be found in collectors in the city of Surabaya. Collectors who sell swamp eels in the city of Surabaya are provided in the Ambengan and Karah areas. Khati et al. (2014) stated that in Riau District, there were still many fishermen's catches from swamps or rice fields supported by endoparasites, so that swamp eels were marketed infected *Trypanosoma* sp. The natural habitat of this swamp eel is a swamp or bog which generally contains a lot of organic material. Welcomme (1979) states that the form of muddy marsh ecosystem which has a pH of about 3,6 to 6,5 tends to acid. In addition, much organic material towing swamp waters is the result of the decomposition of organisms. Environmental conditions such as pH tends to be acidic or amount of material at the bottom waters oganik according to Prasetyo *et al*. (2004) allows opportunities diseases that infect aquatic organisms is quite large.

Trypanosoma sp. is one of the endoparasites found in blood. Shahi et al. (2013), reported that *Trypanosoma mukasai* infected *Trilophysa marmorata* is freshwater fish in the Jhelum river, India. Ruszczyk et al. (2008) say that, goldfish that harm *Trypanosoma carassii* cause deaths of 60-100%. These parasites can infect through vectors like a leeches (Maqbool and Ahmed, 2014). Leremenko *et al*. (2014) say that the case *Trypanosomiasis* causes high mortality in populations of freshwater fish. *Trypanosoma* that infect fish *Carassius carrasisus* causing death with a high prevalence is 81% while the prevalence in fish *Esox Lucius* is 73%. If level or degree of infection included in the category of heavy and will cause pathological changes in the body (Shahi *et al*., 2013).

The clinical symptomps of fish caused by the *Trypanosoma* sp. parasite is anemia with a decrease in the number of erythrocytes in the body. Infection of Trypanosoma will to increase the immune response by leucocyte cells as the body's defense cells. *Trypanosoma* sp. will change the amount of leucocytes . When the parasite enters the host's body, it runs against the body's defense system. The body's defense system will recognize parasites and stimulate lymphocytes to form antibodies to phagocytic cells to attack pathogens by phagocytosis. This is the process of the immune system's work against parasites (Gupta, 2006).

Based on the background, this research is expected to add scientific information about prevalence and hematology on the swamp eel (*Synbranchus bengalensis*) that was infected *Trypanosoma* sp. is very important to know early. In addition to the existence of this research can be used as information regarding blood protozoan parasites that attack the swamp eel.

MATERIALS AND METHODS

Tools and materials

The tools used in this research are trays, plastic buckets, microscopes, 1 ml syringes, glass objects, pipettes, coloring tubes, rulers, hand counters, hot plates, micro tubes and scalpels.

The materials used for this researchs swamp eel with a length of between 50-60 cm as many as 30 individuals. Samples were taken from eel collectors on Jalan Ambengan and Jalan Karah, Surabaya City. Other ingredients used to add 2% EDTA anticoagulants, aquadest solution, 5% methanol, 20% giemsa, hayem solution and immersion oil.

Procedures

Sampling

This research was conducted in October - December 2018 at the Microbiology Laboratory, Faculty of Fisheries and Marine Affairs, Airlangga University, Surabaya. This research method uses a survey method. Sampling of swamp eels (*Synbranchus bengalensis*) is done directly from collectors in the city of Surabaya in the Ambengan region, Genteng District, the center of Surabaya as location I and in the Karah region, Jambangan District, south Surabaya as Location II. Based on the results of the survey, swamp eels sold by Ambengan (Location I) from collectors were 300 per day, while Karah region collectors (Location II) from sold 250-280 tails per day.

Sampling of swamp eels (Synbranchus bengalensis) from each location was 10% of the total population, so that 30 samples were taken in each collector with an eel length of 45 - 60 cm. Swamp eels (Synbranchus bengalensis) sold by collectors in the Ambengan area of location I came from Lumajang Regency, while collectors in the Karah region as location II came from Lamongan Regency.

Samples of swamp eels were taken with large plastic and given oxygen to stay alive, then taken to the Microbiology Laboratory of the Faculty of Fisheries and Marine, Airlangga University to examine the presence of Trypanosoma parasites, and total erythrocytes count with hemocytometer and leucocytes differential.

Sample examination and identification parasites

The next procedure is making blood smear preparations for blood begins with taking blood in the caudal (vena caudalis). Taking blood from a vein caudalis using a syringe that had been given EDTA (ethylene diamine tetra Acetid Acid) that functions as an anticoagulant. After the blood was taken then immediately do blood smear manufacture and then fixed with methanol for 3 minutes and dried. Further preparations of blood smear staining using Giemsa for 30 minutes. Preparations are already dyed washed with running water (Prabowo, 2009).

Samples of blood smear that has been staining, examined using stereo microscope with objective lens 100X, and documented using photomicroscope OptiLab. *Trypanosoma* had been identified using literature or references. *Trypanosoma* species identification by Molina *et al*. (2016) based on morphological characteristics of the hemoparasit.

Prevalence and degrees of infection *Trypanosoma*

The prevalence and degree of infection is used to determine the level of parasitic infection. The prevalence represents the percentage of fish infected by parasites in fish populations, while to determine the degree of infection by looking at the intensity that is the total of parasites found in fish were examined and infected (Anshary, 2008). Prevalence can be calculated using a formula :

$$\text{Prevalence} = \frac{\text{Total of fish infected by disease}}{\text{Total of fishes examined}} \text{ x } 100\%$$

While the intensity calculation using a formula :

$$\text{Intensity (ind/tail)} = \frac{\text{Total of parasites found}}{\text{Total of infected fish}} \text{ x } 100\%$$

Total erythrocytes count with a hemocytometer

Blaxhall and Daisley (1973) stated that the total erythrocyte cells can be count using a haemocytometer which begins by taking eel blood in the tail vein using a 1 ml syringe that has been given EDTA which functions anticoagulant. Then, put on the microtube. Blood samples in the microtube are taken up to 0.5 ml into the erythrocyte pipette. Then the hayem solution is added to the 101 ml line limit and homogenized. Close the haemocytometer using a glass cover, then put homogeneous solution. Hayem solution serves as a diluent solution. Once homogeneous, two drops of blood are released so that air bubbles. Observations were made in a microscope 40x10 magnification with 5 fields in a small block. Total erythrocytes count with a hemocytometer in the small block section of Blaxhall and Daisley (1973), namely:

$$\text{total erythrocyte cells} = n \times 10^4 \text{ sel/mm}^3$$

Leucocyte differential calculation

Blood tests to determine changes in the number of erythrocytes can use haemocytometers and differential fish leucocytes can use blood-pressure preparations. Svobodova and Vykusova (1991) stated that the making of blood-ulcer preparations begins with the taking of eel blood in the caudal vein using a 1 ml syringe which has been given EDTA anticoagulant. Then, stored in the microtube. Take a drop of blood and drop it on the tip of a glass object, and make blood pillow by means of another glass object forming an angle of 30-45º to expand 2/3 of the part of the glass object, then dry it. Furthermore, blood-thinning preparations were put into 5% methanol for 3 minutes. The later preparations are included in 20% giemsa staining for 20 minutes, wash with water and dried. The results of the blood pillow preparations were then observed using a 1000x magnification microscope. Furthermore, blood pressure preparations were carried out by calculating the differential number of leucocytes .

Differential leucocytes calculation with cross sectioned method. This method is carried out by observing a field of microscope which is distinguished between lymphocytes, monocytes, basophils, neutrophils and eosinophils until 100 leucocyte cells are obtained and then expressed as a percentage (Dalimunthe, 2006).

Svobodova and Vykusova (1991) say that, leucocyte differential calculated using formula:

$$\text{Monocytes percentage (\%)} = \frac{\text{monocythes}}{100} \text{ x } 100\%$$

$$\text{Lymphocytes percentage (\%)} = \frac{\text{lymphocytes}}{100} \text{ x } 100\%$$

$$\text{Basophils percentage (\%)} = \frac{\text{basophils}}{100} \text{ x } 100\%$$

$$\text{Eosinophils percentage \%)} = \frac{\text{eosinophils}}{100} \text{ x } 100\%$$

$$\text{Neutrophils percentage (\%)} = \frac{\text{neutrophils}}{100} \quad 100\%$$

Analysis Data

Analysis of the data used in the study for pravelence using Chi-square test, degree of infection and total erythrocytes count using the *Mann-Whitney U-Test* statistical test and for leucocytes differential using the *Independent T-Test* statistical test, and the results of this research are presented in the tables and images.

RESULTS AND DISCUSSION

Results

This research used swamp eels sized 40-60 cm with a total sample of 60 individuals. Sampling from two locations, location I (Ambengan) and location II (Karah). The results showed that eels infected *Trypanosoma* sp. which can be see in Figure 1. *Trypanosoma* sp. were found to have a characteristic morphology as their flagella, the core nucleus and undulating membrane. The morphology of *Trypanosoma* sp. is to have flagella which amounts to one. Flagella serve for the movement. Some *Trypanosoma* sp. have undulating membrane which flagella covering the whole body, and oval-shaped cell nucleus.

The prevalence represents the percentage of fish infected by parasites in fish populations, while to determine the degree of infection by looking at the intensity that is the total of parasites found in fish were examined and infected (Anshary, 2008).

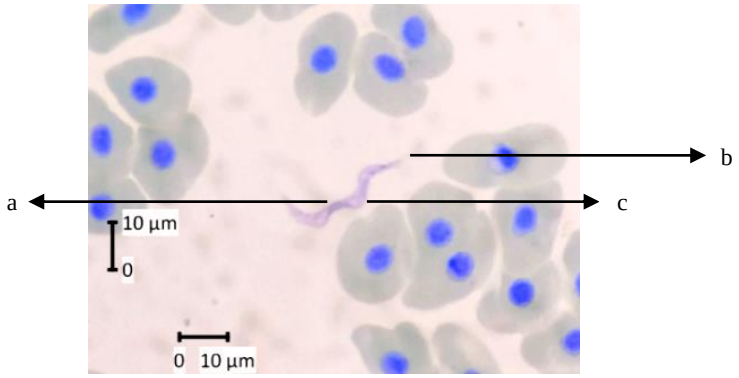

Figure 1. *Trypanosoma* sp. using 1000x magnification microsope.
Description : a.nucleus; b. *undulating membran*; c. flagella

The results showed that the prevalence of *Trypanosoma* sp. on first location (Ambengan) of 23.3% by total of swamp eel infected as many as seven of 30 swamp eels, whereas at the second location (Karah) of 30% by number of swamp eel were infected as many as nine out of 30 individuals. The location I (Ambengan) and location II (Karah) is a center collectors of swamp eel in Surabaya. Furthermore, from the analysis of the prevalence of the Chi-square test showed that there was no significant difference between the prevalence in the location I (Ambengan) and location II (Karah) is P> 0.05. The degree infection that infects the swamp eel can be determined by calculating the intensity is the total of parasites found in fish were examined and infected (Anshary, 2008). The result of the calculation of prevalence can be seen in Table 1.

Table 1. The prevalence of the swamp eel infected *Trypanosoma* sp. are marketed in the Surabaya.

Location	The total number of swamp eels examined (tails)	The total number of swamp eels infected *Trypanosoma* sp. (tails)	Total *Trypanosoma*	Prevalence (%)	Intensity	Degree of Infection
I (Ambengan)	30	7	88	23.3	12.57	Moderate
II (Karah)	30	9	53	30	5.88	Moderate

The results of the average total erythrocytes count on the swamp eel infected *Trypanosoma* sp. marketed in the Surabaya city which can be see in Table 2. The average total erythrocytes count on the swamp eel infected *Trypanosoma* sp. from both locations showed below normal. Roberts (2012) that the normal total erythrocytes of teleostei is between 1.05-3.0x10^6 cells/mm3, but the total erythrocytes cells on the swamp eel infected with *Trypanosoma* sp. at location I (Ambengan) is 0.77x10^6 cells/mm^3 of seven tails, and in location II (Karah) of 0.80x10^6 cells/mm^3 of nine tails.

The results of data analysis of total erythrocytes count on the swamp eel infected *Trypanosoma* sp. from both locations using the Mann-Whitney U-Test showed no significant differences ($p \geq 0.05$).

Table 2. The results of total erythrocytes count on the swamp eel infected *Trypanosoma* sp. are marketed in the Surabaya.

Location	The total number of swamp eels infected *Trypanosoma* sp. (tails)	Degree of Infection	Total erythrocytes ($x10^6$ cel/mm^3)	Normal erythrocytes Roberts (2012)
I (Ambengan)	7	Moderate	0,77 $x10^6$	1,05 - 3,0$x10^6$ cel/mm^3
II (Karah)	9	Moderate	0,80 $x10^6$	

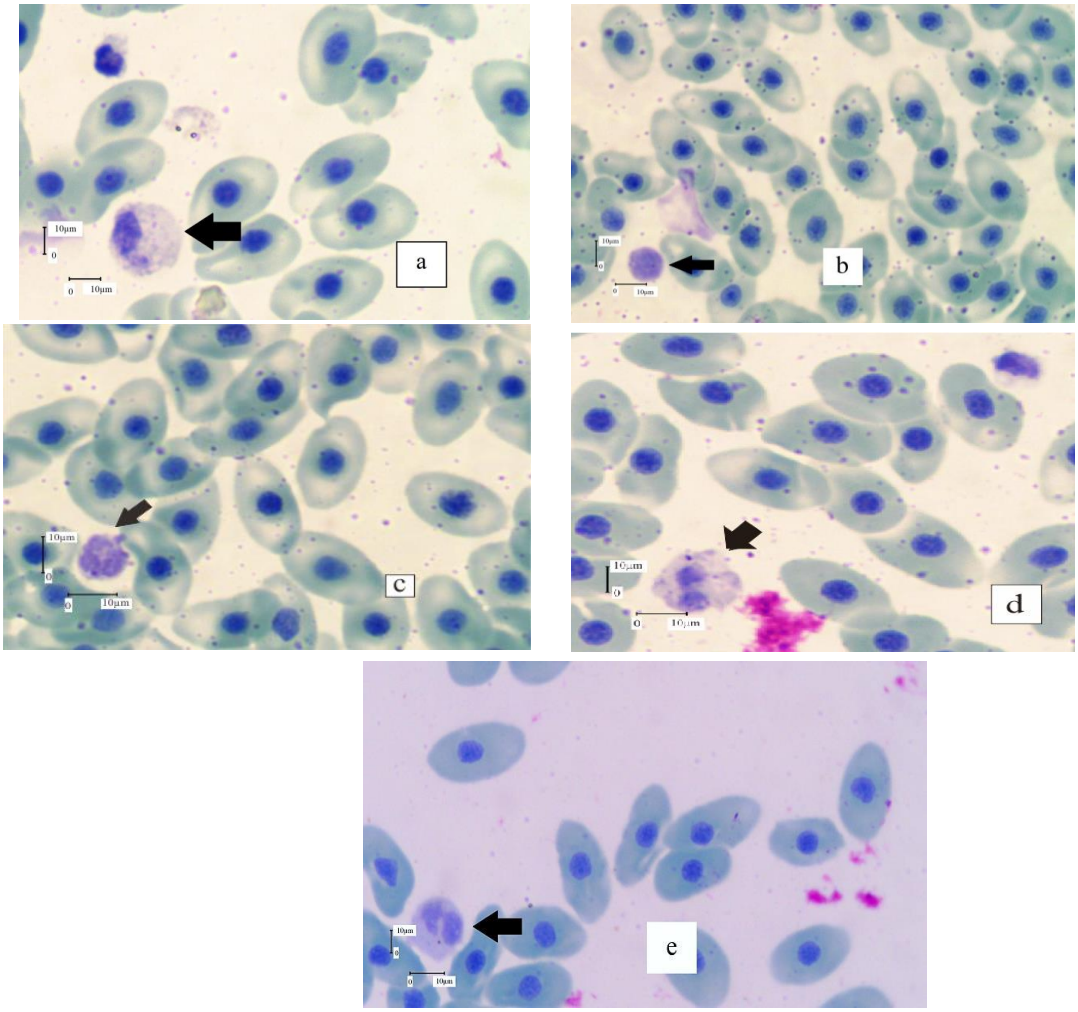

Figure 2. Leucocytes cells swamp eels using 1000x magnification microscope
Description : a. Monochytes; b. Lymphochytes ; c. Basophils; d. Eosinophils; e. Neutrophils

Differential leucocytes swamp eel using 1000x magnification can be see in Figure 2. The results of the calculating avarage differential leucocytes of swamp eel infected *Trypanosoma* sp. marketed in Surabaya can be seen in Table 3.

The average monocytes percentage of swamp eel infected *Trypanosoma* sp. for both locations in Table 3. shows results above normal, which is 8.5%, whereas Campbell and Ellis (2013) that the normal percentage monocytes of fish is <5%.

The average lymphocytes percentage of swamp eel infected *Trypanosoma* sp. from location I (Ambengan) was 80.8% and location II (Karah) was 79.8%. These results are still in the normal range, Salasia et al. (2001) the normal percentage lymphocytes in fish is between 65.20-86%.

The average basophils percentage of swamp eel infected *Trypanosoma* sp. in both locations showed above normal, location I (Ambengan) of 2.2% and location II (Karah) of 2.3%. Affandi and Tang (2002) said that the normal percentage basophils in fish ranged from 0.17 to 0.19%.

The average eosinophil percentage of swamp eels infected *Trypanosoma* sp. in both locations showed above normal results, location I (Ambengan) of 1.4% and location II (Karah) of 1.3%. Svobodova and Vykusova (1991) said that the normal percentage of eosinophils in fish is between 0-1%.

The average neutrophils percentage of swamp eels infected *Trypanosoma* sp. from location I (Ambengan) was 6.8% and location II (Karah) was 7.7%. The average percentage of these neutrophils is still in a normal percentage. Roberts (2012) that the normal percentage neutrophils in fish is 6-8%.

Analysis data of monocytes, lymphocytes, basophils, eosinophils and neutrophils swamp eels infected *Trypanosoma* sp. from location I (Ambengan) and location II (Karah) using the *Independent T-Test* statistical test the results showed that were not significantly different ($p \geq 0.05$).

Table 3. The results of differential leucocytes swamp eel infected *Trypanosoma* marketed in the Surabaya.

Location	Total of swamp eel infected by *Trypanosoma* sp. (tail)	Degree of Infection	Monochyte (%)	Lymphochyte (%)	Basophil (%)	Eosinophil (%)	Neutrophil (%)
I (Ambengan)	7	Moderate	8.5	80.8	2.2	1.4	6.8
II (Karah)	9	Moderate	8.5	79.8	2.3	1.3	7.7
Percentage normal of leucocytes differential			<5 Campbell and Ellis (2013)	65.20-86 Salasia et al. (2001)	0.17-0.19 Affandi and Tang (2002)	0-1 Svobodova and Vykusova (1991)	6-8 Roberts (2012)

Discussion

The results showed that the swamp eel (*Synbranchus bengalensis*) which marketed in Surabaya, in the location I (Ambengan) and location II (Karah) found their endoparasit *Trypanosoma* sp. *Trypanosoma* sp. is a parasite that attacks the blood cells (haemoflagellata). In general, the habitat of swamp eel (*Synbranchus bengalensis*) will affect their *Trypanosoma* sp. infection. The natural habitat of swamp eel that is shallow waters and muddy banks of rivers, canals and marshes. According Welcomme (1979) that the form of muddy marsh ecosystem which has a pH of about 3,6 to 6,5 tends to acid.

The amount of organic material the bottom waters of the swamp is the decomposition of organisms. Environmental conditions such as pH tends to be acidic or amount of material at the bottom according to Prasetyo *et al.* (2004) allows opportunities diseases that infect aquatic organisms is quite large. Soil organic matter too much towing will cause low dissolved oxygen levels due to the breakdown in need of dissolved oxygen. The composition of the organic material can cause a decrease in the pH of the water, this can happen because the organic matter decomposition process can produce acids. Most aquatic biota sensitive to changes in pH (Yuningsih *et al*., 2014).

The results of the prevalence calculation on location II (Karah) is 30% including the criteria for the prevalence of infection is commonly while further prevalence of the location I (Ambengan) is 23.33% including the criteria often. According to Williams and Williams (1996) states that the category is commonly describe that these parasites infect fish with a percentage of the usual 30-49%, while often illustrates that these parasites often infect fish with a percentage of 10-29%. Diba (2009) adds that the level of prevalence rates caused by factors such as durability or condition of the host body that is able to adapt to changes in water quality or for their attacks parasitic infection.

The intensity or degree of infection is very important known to predict the health condition of the fish. Some of the factors that affect the intensity of parasitic infections in fish, that is the environment, fish, and parasites. These three factors are mutually bound to each other (Ningsi, 2010). The results of research showed that each of *Trypanosoma* sp. endoparasites which infect swamp eel have the same degree of infection between the two locations, at the location I (Ambengan) and location II (Karah). The intensity at location I (Ambengan) is 12.57 ind/tail and location II (Karah) is 5.88 ind/tail with the criteria of degree of infection the same that category is moderate.

To determine whether the total of different *Trypanosoma* sp. found or not between the two sampling location, the analysis Mann Whitney U Test. Based on the results of Mann Whitney U Test note that the value of the sign. 0,695, therefore $P>0.05$, fail to reject H0, which means that the degree of infection of *Trypanosoma* sp. between location I (Ambengan) and location II (Karah) were not significantly different. It is thought the quality of the waters swamp eels from the town of Lumajang and Lamongan relatively the same.

Ohoiulum (2002) states that the value of the prevalence and intensity of a parasite of the fish is determined by the age of the fish, the possibility increases with age of the fish. Alifuddin *et al*. (2003) also states that the older the fish, the

higher the value of the prevalence and intensity of a parasite, the body surface area of the parasites that infect fish is rising. Age older fish have a tendency to have a larger body size and have a longer time in the water to contact with parasites, so that the presence of these conditions, the fish susceptible to the parasite infection.

The results of the average total eryhrocyhtes count of swamp eel infected *Trypanosoma* sp. showed below normal, well swamp eels from location I (Ambengan) and location II (Karah), that is equal to $0.77x10^6$ cells/mm3 and $0.80x10^6$ cells/mm3. This is accordance with Roberts (2012) that the normal total eryhrocyhtes count of teleostei fish erythrocytes must be in the range of 1.05 - $3.0x10^6$ cells/mm3. The average total eryhrocyhtes count of swamp eel infected *Trypanosoma* sp. shown under normal showed anemia. Wedemeyer and Yatsuke (1977) say that, total eryhrocyhtes count below normal is a symptom of anemia, while the high total eryhrocyhtes count shows fish in a stressful state. Islam and Woo (1991) say that anemia that occurs due to *Trypanosoma* sp. infection is caused by two factors, hemolytic and hemodilution factors.

Hemolytic factor is a where *Trypanosoma* sp. produces toxins as a result of secretions or excretions that can cause hemolysis in erythrocyte cells (Islam and Woo, 1991). Mbaya et al. (2012) also said that *Trypanosoma* sp. produces in vitro active chemicals or enzymes such as proteases and neuraminidase when they are in fish blood which can damage erythrocyte cell membranes that cause lysis. The hemodilution factor is an increase in blood volume. Increased blood volume occurs when the peak phase of parasitemia, where more and more parasites, blood volume will increase (Islam and Woo, 1991). Igbokwe (1995) that during the peak phase of *Trypanosoma* sp. parasitemia requires a lot of glucose in the blood of the host to meet the metabolism in the body.

Mbaya et al. (2012) said that *Trypanosoma* sp. can damage erythrocyte membranes mechanically. This destruction by whipping flagella into the erythrocyte membrane so that discontinuities occur on the erythrocyte membrane surface. In addition, Shahi et al. (2013) said that anemia experienced by fish infected with *Trypanosoma* sp. can also be caused by leeches that attach to the body to get food by sucking blood. According to Anshary et al. (2016) which are generally leech species *Piscicola* sp. and *Hemiclepsis* sp. acts as a vector of *Trypanosoma* sp. in the Cyprinidae group of fish.

The average monocytes percentage of swamp eel infected *Trypanosoma* sp. from location I (Ambengan) and location II (Karah) showed normal results above 8.5% in both locations. This is not in accordance with Campbell and Ellis (2013) who say that the normal monocytes percentage in fish is > 5%. An increase in the percentage of monocytes shows that the process of parasitic phagocytosis works, where monocytes will go to infected areas by penetrating blood vessels and tissues and differentiating into macrophage cells (Affandi and Tang, 2002). Monocytes have the function to repeatedly phagocytosis of large cells.

The average lymphocytes percentage of swamp eel from location I (Ambengan) and location II (Karah) was infected with *Trypanosoma* sp. at 80.8% and 79.8%. The average lymphocytes percentage still within the normal range in teleostei fish between 65.20-86% (Salasia et al., 2001). The lymphocyte percentage is still within normal limits but has decreased from normal eels to not being infected. This decrease is related to the increase of phagocytic cells such as monocytes, neutrophils and eosinophils which are responsible for phagocytosis of pathogens (Lestariningrum et al., 2012). Lymphocyte cells are one of the most abundant components of leukocytes and have the function of producing antibodies (Fujaya, 2002).

The average basophils percantage of swamp eel infected with *Trypanosoma* sp. from location I (Ambengan) and location II (Karah) showed a percentage above the normal of 2.2% and 2.3%. The normal percentage basophils in fish is between 0-0.5% (Scobodova and Vykusova, 1991). The basophils percentage above normal is related to the presence of allergies and parasitic infections which result in histamine release and are included in the category of acute disease (Santoso et al., 2013).

The average eosinophils percentage of swamp eel from location I (Ambengan) and location II (Karah) infected *Trypanosoma* sp. showed results above the normal percentage of 1.4% and 1.3%. The normal percentage eosinophils in fish is between 0-1% (Scobodova and Vykusova, 1991). Effendi (2003) said that the high percentage of eosinophils proved the presence of parasitic infections that carried out phagocytosis of the antigen complex. Eosinophils have a function for phagocytosis of parasites that are more selective but slower than neutrophils. Neutrophil cells migrate to pathogens, pathogenic digestion and destruction by lysozyme enzymes in phagolysosomes (Rahma et al., 2015).

The average neutrophils percentage of swamp eel infected *Trypanosoma* sp. from Ambengan and Karah locations showed normal results, namely 6.8% and 7.7%. According to Roberts (2012) that the normal neutrophils percentage in fish ranges from 6-8%. Afifah et al. (2014) argue that the number of normal neutrophil cells can be because neutrophils are not able to phagocytosis of pathogens. So, cells that function in the immune system specific to pathogenic infections are eosinophil cells. Saad et al. (2017) said that the immune system is specifically produced by the lymph if there are antigens that enter the body.

ACKNOWLEDGEMENTS

The study by the Department of Fish and Aquaculture Health Management Faculty of Fisheries and Marine Resources, Airlangga University, Surabaya, Indonesia

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SUBMISSION CHECKLIST

Ensure that the following items are present:

The first corresponding author must be accompanied with contact details:	**Give mark (X)**
• E-mail address	**X**
• Full postal address (incl street name and number (location), city, postal code, state/province, country)	**X**
• Phone and facsimile numbers (incl country phone code)	**X**

All necessary files have been uploaded, and contain:

• Keywords	**X**
• Running titles	**X**
• All figure captions	**X**
• All tables (incl title and note/description)	**X**

Further considerations

• Manuscript has been "spell & grammar-checked" Better, if it is revised by a professional science editor or a native English speaker	**X**
• References are in the correct format for this journal	**X**
• All references mentioned in the Reference list are cited in the text, and vice versa	**X**
• Colored figures are only used if the information in the text may be losing without those images	**X**
• Charts (graphs and diagrams) are drawn in black and white images; use shading to differentiate	**X**

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[biodiv] Editor Decision

2 pesan

Smujo Editors <smujo.id@gmail.com> 16 Agustus 2019 01.36
Balas Ke: Smujo Editors <editors@smujo.id>
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Gunanti Mahasri, Koesdarto, , Wulan Sari, Santanumurti, Kandi, Savia Fitri:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Prevalence and Hematology on the Swamp Eel (Synbranchus bengalensis) that was Infected Tryponosoma sp. Marketed in Surabaya: Prevalence and Hematology".

Our decision is: Revisions Required

Smujo Editors
editors@smujo.id

Reviewer D:

This manuscript has very poor english. In many part were meaningless. It seems translated directly from Indonesian version. Therefore, major re write of the manuscript is needed to more concise and follows academic writing rule.

1. The title of the manuscript need to be more concise (see review)

2. Abstract need to be re write with better english (passive voice, concise)

3. Introduction : add one paragraf about the imprtant and the benefit of this research

4. Methodology : more information on sampling methods(random? purposive? etc)

5. Results :

Average and SD of the samples as well as the blood profiles should be added, because I believe that tables were not extracted from single data..... but an average.

put references in the table where classification/status (moderate, acute etc) were stated

6. Discussion : More references related to role, indication, impact of Trypanosoms to human health, health status of the eels, food safety.

Recommendation: Resubmit for Review

Reviewer R:

The manuscript “Prevalence and hematology on the swamp eel (*Synbranchus bengalensis*) that was infected *Tryponosoma* sp. marketed in Surabaya” have main objective to evaluate the swamp eel (*Synbranchus bengalensis*) hematological parameters that was infected with Trypanosoma sp. bought in two different markets.

Below there are some considerations:

The manuscript should be rewritten using a more scientific writing. Grammar and spelling should be revised, too.

In the introduction, I suggest that the authors, in addition to explaining the economic issue, describe in a paragraph what (*Synbranchus bengalensis*) is.

In another paragraph, the question of Trypanosoma could be introduced, explaining what type of trypanosome is found in this eel. All these topics substantiated by references.

In materials and methods, scientific writing must be use.

There is no need to enter the basic formulas for counting blood elements, nor for prevalence or intensity.

Results:

1. In my opinion, authors should compare their findings with uninfected eels in the same collection.
2. What are the parameters used to state that the infection was moderate in both locations?

3 I suggest showing a map of the locality

4- The authors state that they have identified Trypanosoma. There is no data showing this. What is the *Trypanosoma* specie? Could it be *T. granulosum*? How can authors make this identification?

5- Why use multiple authors to cite reference values for eel blood haematology? Is there this information in only one author?

6- Figures: Subtitles should be more complete and self-explanatory.

7-Why show figure 2? What does she add to the article?

Discussion:

Scientific writing should be used. It could be more succinct and clear, trying to describe the observed changes in the host haematology regarding *Trypanosoma* sp infection, based on references.

Recommendation: Resubmit Elsewhere

Biodiversitas Journal of Biological Diversity

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[Kutipan teks disembunyikan]

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[biodiv] Editor Decision

2 pesan

Smujo Editors <smujo.id@gmail.com> 15 September 2019 18.35
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Biodiversitas Journal of Biological Diversity

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Gunanti Mahasri <mahasritot@gmail.com> 16 September 2019 06.15
Kepada: Smujo Editors <editors@smujo.id>

Dear Editor
The manuscript is currently under revision, and we're going to send it back as soon as it's ready.
Best Regards
Dr Gunanti Mahasri
[Kutipan teks disembunyikan]

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[biodiv] Editor Decision

1 pesan

Smujo Editors <smujo.id@gmail.com> 13 Oktober 2019 00.05
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Our decision is: Revisions Required

Smujo Editors
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Reviewer A:

This paper provides information to the public that eel can also be parasitized by Trypanosoma, so we can be aware of this. However, some information should be provided and some statements should be clarified in this paper:

1. Please discuss why Trypanosoma has a risk to consumers. Trypanosoma is a genus consisting of a large species. Different species of trypanosomes infect a variety of different vertebrates including animals and humans. Trypanosoma spp. infect humans and eels but both of them are different species.

Some statements in this paper are like stating that Trypanosoma which infects humans and eels is the same species so that it can be transmitted from eels to humans.
However, are there any previous studies that state the Trypanosoma from eel can be transmitted to humans?

2, Many data presented in the table were repeated in the text.

3. So many statements use “may/ might”. If you have data and references that support your statements please don’t use “may”. You have to confidently say so.

4. Please add the conclusion in the end part of the discussion.

5. the discussion is not deep enough for the full paper so I consider adding the discussion, otherwise, I recommend this paper published as short comm.

My other comments and suggestion were placed in the text

Recommendation: Resubmit for Review

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[biodiv] Editor Decision

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Smujo Editors <smujo.id@gmail.com> 21 Oktober 2019 22.21
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GUNANTI MAHASRI, SETIAWAN KOESDARTO, KISMIYATI, DESI P.W. SARI, MUHAMMAD B. SANTAMURTI, IQYU W. KANDI, SELVI D.S. FITRI, MUHAMAD AMIN:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Prevalence and intensity of Trypanosoma sp. in wild swamp eels (Synbranchus bengalensis) marketed in Surabaya, Indonesia".

Our decision is to: Accept Submission

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GUNANTI MAHASRI, SETIAWAN KOESDARTO, KISMIYATI, DESI P.W. SARI, MUHAMMAD B. SANTAMURTI, IQYU W. KANDI, SELVI D.S. FITRI, MUHAMAD AMIN:

The editing of your submission, "Prevalence and intensity of Trypanosoma sp. in wild swamp eels (Synbranchus bengalensis) marketed in Surabaya, Indonesia," is complete. We are now sending it to production.

Submission URL: https://smujo.id/biodiv/authorDashboard/submission/4129

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[Kutipan teks disembunyikan]

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[biodiv] Editor Decision

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We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Prevalence and intensity of Trypanosoma sp. in wild swamp eels (Synbranchus bengalensis) marketed in Surabaya, Indonesia".

Our decision is to: Accept Submission

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Biodiversitas Journal of Biological Diversity

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The editing of your submission, "Prevalence and intensity of Trypanosoma sp. in wild swamp eels (Synbranchus bengalensis) marketed in Surabaya, Indonesia," is complete. We are now sending it to production.

Submission URL: https://smujo.id/biodiv/authorDashboard/submission/4129

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[Kutipan teks disembunyikan]