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- 2. Balasan dari editor IVJ bahwa artikel telah diterima dan sedang diproses (25 Juni 2018)
- 3. Revisi artikel (3 Agustus 2018)
- 4. Artikel Disetujui untuk Diterbitkan (12 Oktober 2018)

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Artikel yang disubmit:

Peripheral Blood and Histopathology of Koi Fish (*Cyprinus carpio*) Infested by *Argulus japonicus* in Mungkid and Muntilan District, Magelang, Central Java

Kismiyati^{1*}, Rinca Purnamawati², Aldino Giancarlo², and Muhammad Browijoyo Santanumurti¹

¹ Department of Fish Health Management and Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya 60115

² Undergraduate Program of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya 60115

*kismiyati@fpk.unair.ac.id

Abstract

Koi fish (*Cyprinus carpio*) is one of the ornamental fish that has high economic potential. However, *Argulus japonicus* is one of the problems that affect koi fish. In Indonesia, the attack of *Argulus japonicus*. in koi fish is still often happened. *Argulus japonicus* may causes bleeding and damages in Koi fish, even death. The author did the study in Mungkid and Mungkilan since the two districts were the biggest koi production in Central Java, Indonesia. In this study showed that the blood of infected koi fish would decrease (neutrophils, monocytes, eosinophils and basophils), except lymphocytes if the infestation level was increase. In histopathology test, the types of damage that found in this study were congestion, ballooning degeneration and erosion of the epithelium. Keywords: Peripheral Blood, Histopathology, Argulus japonicus, Muntilan, Mungkid

Introduction

Koi fish (*Cyprinus carpio*) is one of the ornamental fish that has high economic potential. The average production of koi fish reaches 72,000 tons per year at a price of Rp. 100.000-200.000 for domestic market and Rp. 1.000.000-25.000.000 for the international market (Sunarto, 2005). Since its high price, many people are culturing and also involved in koi fish market. However, *Argulus japonicus* is one of the problems that affect koi fish.

In Indonesia, the attack of *Argulus japonicus*. in koi fish is still often happened. According to the research conducted in Magelang, the prevalence of *Argulus japonicus* data showed 4.16% (Wahyuni *et al.*, 2013). While in 2014, research in Surabaya, the second largest city in Indonesia, showed the prevalence of *Argulus japonicus*. reached 30.7%-40% (Hermawan, 2014).

Argulus is a major ectoparasite that can cause injury to the host and lead secondary infection caused by bacteria, viruses and fungi through the wounds (Partasasmita, 1978). Argulus attacks on the fins, skin, gills, and the entire surface of the host's body (Walker, 2008). The wounds inflicted by the Argulus in a long period of time will cause bleeding and damage that may result in inflammation followed by other tissue damage (Yildiz and Kumantas, 2002; Notash, 2012). It will affect the growth, production, even death (Alifudin *et al.*, 2002).

Through this study, the authors want to know the peripheral blood and histopathology of *Argulus japonicus*. in the district of Mungkid and Muntilan, Magelang, Central Java. Mungkid and Muntilan are districts in Central Java that have the most koi fish farming business (Badan Pusat Statistik, 2013). By knowing the prevalence level of *Argulus japonicus*, government and farmers will understand the presence of *Argulus japonicus*. since there is not enough information. By understanding the presence of *Argulus japonicus*, they can prevent the harm of the parasite including death.

Materials and Methods

Fish Collection

The research was conducted on 29 February - 7 March 2016. The positively infected koi fishes were collected from two districts, Mungkid (14) and Muntilan (29) with size 5-15 cm. Collected Koi Fish with net and kept alive to be transported to the laboratory for parasitological and histopathological analysis. The analysis was conducted at the Laboratory of Balai Benih Ikan Ngrajek, Magelang; Laboratory of Fisheries and Marine Faculty-Universitas Airlangga, Surabaya and Laboratory of Pathology Faculty of Veterinary-Universitas Airlangga, Surabaya. This study also added positive control of healthy koi fishes from each place to compare them with samples.

Leukocyte collection

The blood was taken by injecting a 1 ml syringe that has been given EDTA (Ethylene Diamine Tetra Acetic) 10% (Syahida *et al.* 2013). The blood was prepared in slide preparation. The dried apical preparations are then fixed in methanol solution for 5-10 minutes. Immersed in a 10% Giemsa solution for 10-15 minutes after dry. Rinsed with aquadest and dried. Thereafter, the slide preparation could be observed under a microscope (Svobodova and Vykusova, 1991)

Histopathology

Histopathology preparations used 5 samples for each organ (gill, fin, and scale) with infestation rate of normal, few and medium. The preparation of histopathologic begins by necropsy, gill taken and preserved in a 10% Buffer Neutral Formalin (BNF) solution for 1-2 days. Next phase is dehydration process which aims to grab water gradually with an auto technical tool for 20 hours. The process is by pouring gradually into 70% alcohol (1 hour), 80% alcohol (2 hours), 90% alcohol (2 hours), 96% alcohol (2 hours) and absolute alcohol (2 hours) (Muntiha, 2001).

The second stage is purification which aims to transfers and replaces the alcohol solution from the tissue. The process of putting the tissue into solution of xylol 1 (1 hour), xylol 2 (2 hours), and xylol 3 (2 hours). The next step is impregnation that aims to equate the state of the network with the embedding material. The tissue is put into liquid paraffin with temperature of 56 - 60° C for 2 hours. The next process is the embedding. The result is inserted into the water bath with a temperature of $\pm 40^{\circ}$ C. Next stage is tissue staining by using HE (Haematoxylin-Eosin). The result was inserted successively into xylol 1, xylol 2, and xylol 3 each for 5 minutes to remove paraffin from the tissues (Muntiha, 2001).

The next process of hydration is inserting sequentially into an absolute alcohol (4 minutes), 96% alcohol (3 minutes), 90% alcohol (3 minutes), 80% alcohol (2 minutes), 70% alcohol (2 minutes), then input into the water flows for 10 minutes. The main paint on this coloring is Mayer's Hematoxylin with a long immersion for 5 minutes. For composite paint use Eosin $1\% \pm 3-5$ minutes. The next process is dehydration by inserting sequentially into 70% alcohol (2 min), 80% alcohol (2 min), 90% alcohol (3 min), 96% alcohol (4 min), and absolute alcohol for 5 min. Insert the tissue into xylol 1, xylol 2, and xylol 3 every 5 minutes. Preparations are dried and covered with a glazed glass cover (Muntiha, 2001).

Observation of hispathological organs of gill, fins and skin

Observations were done by using microscope to determine the histopathological changes in gill organs, fins, and skin of koi fish (*Cyprinus carpio*). Scoring is done to determine the damage level of the gill organs, fins, and skin of koi fish. Histopathologic changes observed were congestion, ballooning degeneration, infiltration, hemorrhage and erosion of the epithelium (Pantung *et al*, 2008).

Each type of damage is scored in the same way that is with the following categories:

- normal: no damage or histopathological change at all in one field of view.
- few: if there is damage or histopathological changes less than 30% in a single field of view.
- medium: if there is damage or histopathologic changes 30-70% in one field of view.
- high: if there is histopathological damage or change of more than 70% in one field of view.

Results

Water quality

Water quality measurements were conducted in Mungkid and Muntilan. Water quality parameters included temperature, ph, DO, and ammonia. Water quality measurement results could be seen in Table 1.

Parameter	Mungkid		Muntilan		Normal (SNI,	
	Pond 1	Pond 2	Pond 1	Pond 2	2000)	
Temperature (°C)	26,71	27,49	27,52	27,17	25-30	
рН	7,04	8,25	8,02	8,31	6,5-8	
DO (mg/L)	4,9	4,74	4,86	4,69	>4	
Ammonia (mg/L)	0	0,5	1	1	0,02	

Table. 1 Data water quality in Mungkid and Muntilan.

Leukocytes

The results showed the average percentage of lymphocytes from normal koi fish in Mungkid District was 89.5%. The average percentage of lymphocytes of koi infested with Argulus (few category) in Mungkid sub-district was 84.5%. It was lower than normal. The average percentage of lymphocytes from infected koi fishes (medium category) in the district of Argungkeng was 79.7%. It was lower than normal and few category.

The average percentage of neutrophils of normal koi in Mungkid was 4.8%. The average percentage of infected koi neutrophils infected by Argulus (few category) in Mungkid was 5.7%. It was higher than normal category. The average percentage of koi's (medium category) neutrophils in Mungkid was 8.25%. It was higher than normal and few category.

The average percentage of monocytes of normal koi fish in Mungkid was 4%. The average percentage of monocytes of infected koi (few category) was 5%. It was higher than normal category. The average percentage of infected koi was 7%. It was higher than normal and few category.

The average percentage of normal koi fishes' eosinophils in Mungkid was 1.4%. The average percentage of eosinophils of infected koi (few category) in Mungkid was 4.2%. It was higher than normal category. The average percentage of koi fishes (medium category) eosinophils in Mungkid was 4.5%. It was higher than normal and few category.

The average percentage of basophils of normal koi fishes in Kecamatan Mungkid was 0.14%. The average percentage of basophils of infested koi in few category and medium category was 0.5%. The average leucocyte of koi fishes in this study from Mungkid could be seen in Table 3.

Location	Argulus	Lymphocytes	Neutrophils	Monocytes	Eosinophils	Basophils
	japonicus	(%)	(%)	(%)	(%)	(%)
Mungkid	Normal	89,5	4,8	4	1,4	0,14
	(positive					
	control)					
	few (1-5	84,5	5,7	5	4,2	0,5
	Argulus)					
	medium	79,7	8,25	7	4,5	0,5
	(6-10					

Table 3. The average of leucocyte of koi fishes in Mungkid.

Argulus)

The result showed the average percentage of lymphocytes from 15 normal koi fish in Muntilan was 89.6%. The average percentage of lymphocytes from infected koi fishes (few category) in Muntilan was 85.1%. It was lower than normal category. The average percentage of lymphocytes from infected koi fishes (medium category) in the Muntilan was 78%. It was lower than normal and few category.

The average percentage of neutrophils of normal koi fishes in Muntilan was 4.6%. The average percentage of neutrophils of infected koi fishes (few category) in Muntilan subdistrict was 5.4%. It was higher than normal category. The average percentage of neutrophils of infected koi fishes (medium category) in Muntilan was 8.5%. It was higher than normal and few category.

The average percentage of monocytes in normal koi fishes in Muntilan was 3.9%. The average percentage of monocytes of infected koi fishes (few category) was 4.9%. The average percentage of monocytes of infected koi fishes (medium category) was 6.5%. It was higher than normal and few category.

The average percentage of eosinophils of normal koi in Muntilan District was 1.5%. The average percentage of eosinophils of infected koi (few category) in Muntilan was 4.1%. It was higher than normal. The average percentage of eosinophils of koi fish (medium category) in Muntilan was 6.5%. It was higher than normal and few category.

The average percentage of basophils of normal fishes in Muntilan was 0.2%. The average percentage of basophils of infested koi (few category) was 0.35. It was higher than normal. The average percentage of basophils of infested koi was 0.5%. It was higher than normal and few category. The average leucocyte of koi fishes (*Cyprinus carpio*) in Muntilan could be seen in Table 4.

Location	Argulus	Lymphoc	Neutrophil	Monocyte	Eosinophil	Basophil
		ytes (%)	s	s	S	S
			(%)	(%)	(%)	(%)
Muntilan	Normal (control)	89,6	4,6	3,9	1,5	0,2
	few (1-5	85,1	5,4	4,9	4,1	0,35
	Argulus)					
	medium (6-10	78	8,5	6,5	6,5	0,5
	Argulus)					

Table 4. The average of leucocyte of koi fishes in Muntilan

Histopathology

Observations on pectoral fins indicated a score range of 0-1.4. This study showed histopathologic damage less than 30%. It could be concluded as minor damage. *Argulus japonicus*. infestations on pectoral fins were indicated as few category and histopathologic changed in the form of congestion, ballooning degeneration, and erosion of the epithelium. Congestion was shown by the presence of thickened blood vessels with a darker red color. It was due to the accumulation of blood cells and forming special patterns of rounds. In the change of ballooning degeneration, the cell looks enlarged and there was empty space inside

like balloon cells. Erosion of the epithelium was detected by the soft tissue erosion of the pectoral fins.

The observation on caudal fin organs showed scores range 0-0.8. Scoring results on caudal fin indicated that there was a histopathological damage or change less than 30%. It was indicated as minor damage.

Argulus infestations on caudal fin were indicated as few and medium category. The few category showed the histopathological changes of erosion of the epithelium while the medium category showed the form of inflammatory cell infiltration and erosion of the epithelium. Erosion of the epithelium was indicated by the erosion of tissue on the caudal fin. Inflammatory cells were indicated by the presence of red leukocytes by eosin staining.

The results of observation of skin showed scaling range of 0-0.2. Scores on the skin showed that in one field of vision there was a histopathological damage or change less than 30% and indicated as minor damage.

Argulus japonicus infestation on the skin was indicated as few category with histopathological changes in the form of hemorrhage. Hemorrhage was indicated by the presence of red blood cells that come out in the tissues. The histopathologic figures of infested koi fish by *Argulus japonicus* was shown in Figure 1.

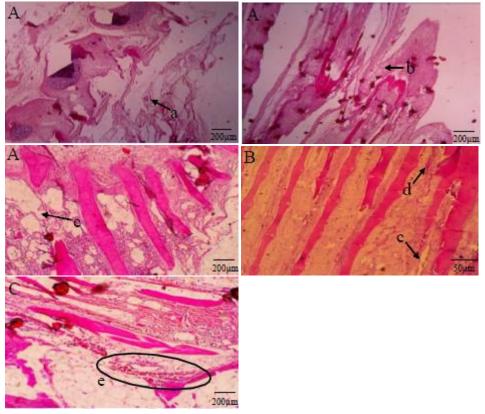


Figure 1. histopathological figure of koi fishes infested by Argulus

A. Pectoral fin of few category (100x); B. Caudal fin of medium category (40x);

C. Skin of few category (40x); a. congestion; b. ballooning degeneration; c. erosion of epithelium

d. infiltration of inflammatory cells; e. hemorrhage

Discussion Blood The examination of fish blood is used to help diagnose a disease, understand the pathway, know the effect of a treatment, examine the immune system and the health status of the fish (Noercholis et al., 2013). The health problems and changes can be known through the components of blood.

The results showed that the average percentage of lymphocytes from normal koi fish in Mungkid and Muntilan was 89.5% and 89.6%. Svobodová & Vykusová (1991) explained that lymphocytes in goldfish ranged from 76 to 97.5%. The average percentage of infected koi lymphocytes in Mungkid and Muntilan decreased. This study showed that more infestation of *Argulus* decreased the lymphocytes.

The decrease in the number of lymphocytes is influenced by the presence of foreign antigens which can cause the immune system to be disturbed by the entry of infection because lymphocytes are the main cells involved in the immune response (Voigt and Swist, 2011).

The study showed that the average percentage of neutrophils from normal koi fish in Mungkid and Muntilan were 4.8% and 4.6%. The average percentage of neutrophils from infected koi fish of low category in Mungkid and Muntilan increased to 5.7% and 5.4%. The average percentage of infected koi neutrophils of medium category in Mungkid and Muntilan also increased to 8.25% and 8.5%. According to Harikrishnan et al., (2010), that neutrophils in the blood would increase in case of infection because neutrophils acted as the first defenses in the body. The main function of neutrophils was the destruction of foreign matter through the phagocytic process of chemotaxis by migrating cell paths to particles or attachment of particles to cells, particle cell ingestion and particle destruction by lysosomal enzymes in phagolysosomes.

The study showed that the average monocyte percentage of normal koi fish in Mungkid was 4%, while in Muntilan was 3.9%. The average percentage of monocytes from infected koi fish category low in Mungkid and Muntilan increased to 5% and 4.9%. The average percentage of monocyte of infected koi fish category medium in Mungkid and Muntilam increased to 7% and 6.5%. The inflammatory process during tissue damage by infection or antigen-antibody reaction, it would increase monocyte production to two times more (Maftuch, 2007). The main function of monocytes was phagocytosis. Monocytes swallow and destroy organisms that could not be controlled by neutrophils, especially fungi. Increased monocytes might also indicate an acute-phase recovery (Voigt and Swist, 2011).

The result of research on average of eosinophil percentage from normal koi fish in Mungkid was 1.4%, while in Muntilan was 1.5%. The percentage of eosinophils in normal fish blood ranged from 0.78-2.00% (Affandi and Tang, 2002). The average percentage of infected eosinophils of infected koi fish low category in Mungkid and Muntilan were between 4.2% and 4.1%. The average of eosinophils percentage of koi medium category fish in Mungkid and Muntilan were 4.5 and 6.5%. Eosinophils in fish were needed for immunity against parasitic infections. The eosinophils were a type of leukocytes associated with parasitic infections, thereby increasing eosinophils signifying the presence of parasites (Robert, 1989).

The study showed the average of basophil percentage of normal koi fish in Mungkid was 0.14%, while Muntilan sub-districts was 0.2%. The average percentage of basophil from infected koi fish category low in Mungkid and Muntilan were 0.5% and 0.35. The average percentage of basophil infected koi fish in Mungkid and Muntilan was 0.5%. Basophil

percentages in normal koi fish ranged from 0 to 0.5% (Svobodová & Vykusová, 1991). Basophile images were rarely found to tend not to appear with Giemsa staining and it was not yet known clearly the function (Bijanti, 2005).

Histopathology

The result of Kolmogorov Smirnov test 1 sample showed bigger number than the value of D table, so there was difference degree of Argulus infection on koi fish from Mungkid and Muntilan, Magelang, Central Java. The infection of low category was the highest among all category. Low category was indicated one to five parasites in each fish (Kismiyati, 2009).

In this study, no Argulus was found infecting the gill organs of koi fish. Infection area of Argulus was a wide surface, such as skin or fins (Taylor *et al.*, 2005). In the histology picture of the gill of koi fish, no structural changes were found.

Argulus japonicus encountered the fin organs of koi fish. Argulus chose fin as a predilection because the movements of the fin of the fish were very slow (Pramujirini *et al.*, 2015). The slow flipping of the fish fins made it easier for Argulus to escape. In addition, the fins' structure was soft, so *Argulus japonicus* easily attached and sucked the blood. The types of damage that occurred in pectoral fins were congestion, ballooning degeneration and erosion of the epithelium.

In the histopathology of pectoral fins, blood vessels were thickened with darker reds and form special patterns such as cycles or rounds. Congestion was found on pectoral fins of low category infection. The accumulation of blood cells caused the blood flow to slow down and eventually the blood cells accumulated in certain areas. Blockage allowed the inhibition of blood flow so that the distribution of food and oxygen into the tissue would decrease (Resang, 1984).

The other damage found in pectoral fins was ballooning degeneration. Ballooning degeneration was found at the tip of pectoral fins at low category. The cell became enlarged like a cavity on the pectoral fin. Ballooning degeneration was characterized by a swelling of cells in the epidermal layer that occurred due to intracellular edema. The cells looked enlarged and there were empty spaces like the balloon cells (Battenay and Hargis, 2006)

The type of damage found in caudal fin was infiltration of inflammatory cells. The inflammatory cell was a condition in which inflammatory cells entered the tissues in response to disease or toxic agents. Infiltrate of inflammatory cells was found in the medium category of infected koi fish. The inflammatory cell was characterized by increased permeability of blood vessels, fluids, and cells that came out of the blood vessels and the presence of neutrophils in inflamed tissues (Underwood, 1992). The results from histopathology of pectoral fins and caudal fin showed the erosion in the epithelium. This change was found in low category infection in pectoral fins, while in caudal fin was found in low and medium infection. Argulus ectoparasites entered the stylet into the epidermis until the underlying tissue layer. This indicated the erosion of epidermal tissue was caused by penetration of stylet that penetrated epidermal layers by piercing (Sharma *et al.*, 2012).

The histopathological damage found on the skin of koi fish infected with Argulus was hemorrhage. Hemorrhagic was found in low category. The study found the presence of red blood cells in the tissue caused by the discharge of blood cells from the blood vessels. Hemorrhage occurred due to a stylet puncture by Argulus causing bleeding on the infected area. The hemorrhage was the rupture of blood vessels, thus making the color of certain body parts become red (Suprapto, 2007). Changes in the pathology of koi fish anatomy caused by the infection of Argulus was seen in broken pectoral fins. The fins were caused by penetration of stylet that broke epidermis layer by piercing causing erosion of pectoral fin. In caudal fin, the altered anatomic pathology that occurred due to Argulus infection was the appearance of bleeding. Bleeding occurred due to the mechanism of hooks and stylet owned by Argulus (Stackler and Yanong, 2012). The anatomical changes showed in the skin of koi fish were lose the fish scales. An ectoparasite-infested fish on its skin would rub the body around objects that often cause injury (Wiyatno *et al.*, 2012). Damage to the skin and tissues was also directly related to the effects of *Argulus*'s stylet mechanisms that could cause skin and tissue injuries (Walker, 2008).

The measurement of water quality in koi fish ponds showed that water quality was in good condition. In koi fish ponds, water temperature ranges from 26-27°C. The pH ranged from 7-8. The dissolved oxygen concentration (DO) was 2,7-4,43 mg / 1. The Ammonia concentration was 0 mg / 1. Koi fish could live in the 28-30°C temperature range. A good water pH for koi fish maintenance was 7 (Effendie, 2003).

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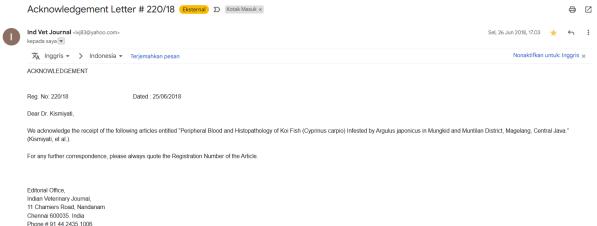
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I wish to submit my article for intended publication in The Indian Veterinary Journal.

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 Name
 : Kismiyati

 Names of all the author (s) as in publication:

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	1. The parasite was not identified by parasitological examination. 2. All the histopathological image showed the out section of parasite either freely or within the fasue sections. 3. No histopathological image showed the out section of parasite either freely or within the fasue sections. 4. The study was another the presence of examples in the All section and the additional of the parasite in the All sections. 5. You are requested to refursh the manuscript with all the necessary details.	
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