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

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

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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. It may even causes death in Koi fish. The study was carried out in Mungkid and Mungkilan districts were the biggest koi production in Central Java, Indonesia. This study showed that the neutrophils, monocytes, eosinophils and basophils of infected fish would decreases, except the lymphocytes.

Key words: Peripheral Blood, koi fish, *Argulus japonicus*, infestation.

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). However, *Argulus japonicus* is one of the problem that affect koi fish. The prevalence of *Argulus japonicus* was recorded at 4.16% (Wahyuni *et al*., 2013).

Argulus attacks on the fins, skin, gills, and the entire surface of the host's body (Walker, 2008). The wounds inflicted by the Argulus will cause bleeding and damage that may result in inflammation, followed by other tissue damage (Notash, 2012). It will affect the growth, production, and even death (Alifudin *et al*., 2002).

Through this study, on the peripheral blood and histopathology of *Argulus japonicus,* in the district of Mungkid and Muntilan, Magelang, Central Java was done to create awareness among the fish farmers (Badan Pusat Statistik, 2013).

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Materials and Methods

The research was conducted from 29 February to 7 March 2016. The positively infected koi fishes with size 5-15 cm were collected from two districts, Mungkid (14) and Muntilan (29). 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 infected samples.

All parts of the skin and fins were scrapped carefully for Parasitological examination, and were examined under a microscope (Ebrahimi *et al*., 2018). Parasite identification was performed by using article of diagnostic keys (Walker *et al*., 2004). The prevalence level was calculated by using prevalence formula (Kabata, 1985).

The blood was drawn by 1 ml syringe coated with EDTA (Ethylene Diamine Tetra Acetic) 10% (Syahida *et al*. 2013). The dried blood smear preparations were 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. The slides were observed under a microscope to record the changes in the leukocytes in the different locations under study (Svobodova and Vykusova, 1991).

Results and Discussion

Argulus was found in the mandibles, labial spines and stylets of the mouth, in the fin and skin of koi fish (*Cyprinus carpio*) (Walker *et al*., 2004) Fig 1.

Fig 1. Koi fish infected with *Argulus japonicus*

Table I. The prevalence level of koi fish in Mungkid and Muntilan

No. of samples tested	Region	
	Mungkid	Muntilan
Total	13	29
Positive	4	16
Prevalence (%)	30.77	55.17

The prevalence level of koi fish infested by Argulus in Mungkid and Muntilan Districts are presented in Table I.

The leukocytes examined in this study consisted of lymphocytes, monocytes, neutrophils, eosinophils and basophils (Fig 2). Leukocytes were examined under 1000x magnification.

The leukocytic distribution in the healthy, medium level of infected koi fish is presented in Table II. 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) recorded that lymphocytes in goldfish ranged from 76 to 97.5%. The average percentage of infected koi lymphocytes in Mungkid and Muntilan decreased according to the severity of infestation of *Argulus*.

The decrease in the number of lymphocytes is due to the infection with *Argulus japonica* and the concomitant decrease in the lymphocytes since they are 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 were 5.7% and 5.4% respectively. The average percentage of infected koi neutrophils of medium category infection in Mungkid and Muntilan were 8.25% and 8.5% respectively. According to Harikrishnan *et al*., (2010), the increase in neutrophils acts as the first line of defense. The main function of neutrophils was

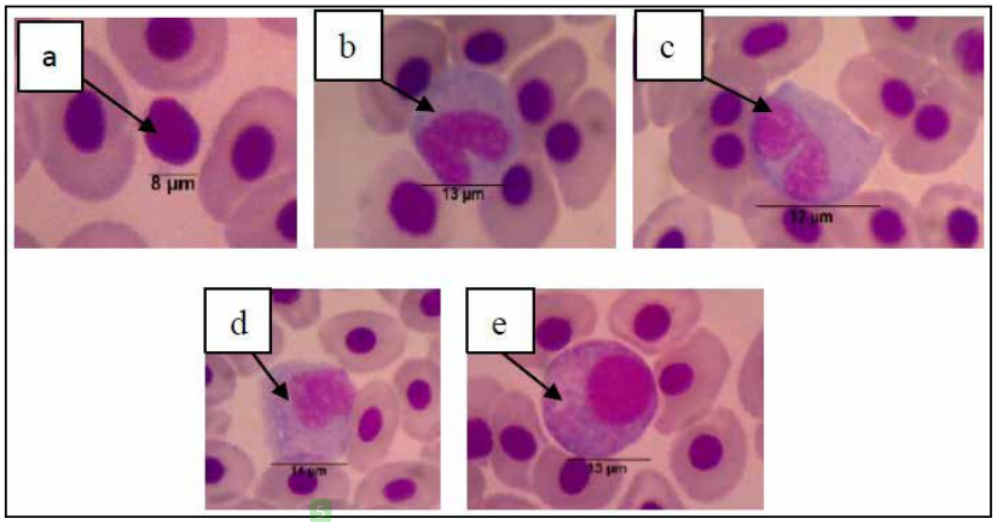

Fig 2. Leukocytes examination a. Lymphocytes; b. Monocytes; c. Neutrophils; d. Eosinophils; e. Basophils

Table II. The leucocyte distribution in normal, low and medium level of infected koi fishes in Mungkid and Muntilan (%)

Location	Argulus japonicus parasite	Lymphocytes	Neutrophils	Monocytes	Eosinophils	Basophils
Mungkid	Normal (positive control)	89.5	4.8	4	1.4	0.14
	few (1-5-5 *Argulus*)	84.5	5.7	5	4.2	0.5
	medium (6-10 *Argulus*)	79.7	8.25	7	4.5	0.5
Muntilan	Normal (control)	89.6	4.6	3.9	1.5	0.2
	few (1-5 *Argulus*)	85.1	5.4	4.9	4.1	0.35
	medium (6-10 *Argulus*)	78	8.5	6.5	6.5	0.5

the destruction of foreign matter through the phagocytic process by chemotaxis and 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 in low level of ion infect koi fish in Mungkid and Muntilan were 5% and 4.9%, respectively. The medium category of infection in Mungkid and Muntilam showed 7% and 6.5% monocytes in the two regions. The inflammatory process during tissue damage by infection or antigen-antibody reaction, which would increase monocyte production to two times more (Maftuch, 2007). The main function of monocytes was phagocytosis. Monocytes swallow and destroy organisms which could not be controlled by neutrophils, especially fungi. Increased monocytes showed chronic infections or inflammatory responses, in addition, the increased monocytes might also indicate an acute-phase of recovery (Voigt and Swist, *loc. cit*).

The eosinophil percentage from normal koi fish in Mungkid was 1.4%, while in Muntilan it was 1.5%. The percentage of eosinophils in normal fish blood ranged from 0.78-2.00% (Affandi and Tang, 2002). The percentage of infected eosinophils of infected koi fish low category in Mungkid and Muntilan were between 4.2% and 4.1%. In the medium category of infected fish in Mungkid and Muntilan were 4.5 and 6.5%. The eosinophils were a type of leukocytes associated with parasitic infections, thus signifying the presence of parasites (Robert, 1989).

The study showed that 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, *loc. cit*). Basophil images were rarely found with Giemsa staining and whose function is not known clearly (Bijanti, 2005) Table II.

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In vitro Evaluation of Acaricidal Property of *Acalypha Indica* (Kuppaimeni) against Ticks Infesting Sheep

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Abstract

Laboratory tests were carried out to determine the toxicity of the ethanolic extract of *Acalypha indica* on engorged females and larvae of sheep tick *Haemaphysalis intermedia* using the Adult Immersion Test (AIT) and the Larval Packet Test (LPT). It was observed that the ethanolic extract of *A. indica* at 30% concentration showed acaricidal activity. In AIT, egg laying was lower in ticks exposed to different concentrations of *A. indica* compared with those exposed to 30% ethanol used as control. In LPT, there was increasing mortality of tick larvae with increasing concentrations of *A. indica* as opposed to no mortality in the control.

Key words: *Acalypha indica*, Acaricide and *Haemaphysalis intermedia*.

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Haemaphysalis intermedia ticks are considered to be the most prevalent sheep tick species in Tamil Nadu, causing direct damage such as blood loss, hide injuries, irritation and inoculation of toxins and indirect damages by transmission of *Babesia* spp., *Anaplasma* spp. and *Theileria* spp. to the host. Tick control mainly depends on synthetic acaricides but has been complicated by the emergence of drug resistance (Miller *et al.,* 2007). Hence the use of extracts from various plants is being studied because they are potentially less toxic to the animals and are safer for the environment. Phytoacaricides can be developed from many compounds isolated from plants. These can act on ticks by reducing their development, survival and reproductive rate (Bagavan *et al.,* 2009).

The *Acalypha indica* (Kuppaimeni) is

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RUBRIC: 6TH-8TH SCIENCE ARGUMENT (CER)

CLAIM

Take an arguable position on the scientific topic and develop the essay around that stance.

ADVANCED	The essay introduces a precise, qualitative and/or quantitative claim based on the scientific topic or text(s), regarding the relationship between dependent and independent variables. The essay develops the claim and counterclaim fairly, distinguishing the claim from alternate or opposing claims.
PROFICIENT	The essay introduces a clear, qualitative and/or quantitative claim based on the scientific topic or text(s), regarding the relationship between dependent and independent variables. The essay effectively acknowledges and distinguishes the claim from alternate or opposing claims.
DEVELOPING	The essay attempts to introduce a qualitative and/or quantitative claim, based on the scientific topic or text(s), but it may be somewhat unclear or not maintained throughout the essay. The essay may not clearly acknowledge or distinguish the claim from alternate or opposing claims.
EMERGING	The essay does not clearly make a claim based on the scientific topic or text(s), or the claim is overly simplistic or vague. The essay does not acknowledge or distinguish counterclaims.

EVIDENCE

Include relevant facts, definitions, and examples to back up the claim.

ADVANCED	The essay supplies sufficient relevant, accurate qualitative and/or quantitative data and evidence related to the scientific topic or text(s) to support its claim and counterclaim.
PROFICIENT	The essay supplies relevant, accurate qualitative and/or quantitative data and evidence related to the scientific topic or text(s) to support its claim and counterclaim.
DEVELOPING	The essay supplies some qualitative and/or quantitative data and evidence, but it may not be closely related to the scientific topic or text(s), or the support that is offered relies mostly on summary of the source(s), thereby not effectively supporting the essay's claim and counterclaim.
EMERGING	The essay supplies very little or no data and evidence to support its claim and counterclaim, or the evidence that is provided is not clear or relevant.

REASONING

Explain how or why each piece of evidence supports the claim.

ADVANCED	The essay effectively applies scientific ideas and principles in order to explain how or why the cited evidence supports the claim. The essay demonstrates consistently logical reasoning and understanding of the scientific topic and/or text(s). The essay's explanations anticipate the audience's knowledge level and concerns about this scientific topic.

PROFICIENT	The essay applies scientific reasoning in order to explain how or why the cited evidence supports the claim. The essay demonstrates logical reasoning and understanding of the scientific topic and/or text(s). The essay's explanations attempt to anticipate the audience's knowledge level and concerns about this scientific topic.
DEVELOPING	The essay includes some reasoning and understanding of the scientific topic and/or text(s), but it does not effectively apply scientific ideas or principles to explain how or why the evidence supports the claim.
EMERGING	The essay does not demonstrate clear or relevant reasoning to support the claim or to demonstrate an understanding of the scientific topic and/or text(s).

FOCUS

Focus your writing on the prompt and task.

ADVANCED	The essay maintains strong focus on the purpose and task, using the whole essay to support and develop the claim and counterclaims evenly while thoroughly addressing the demands of the prompt.
PROFICIENT	The essay addresses the demands of the prompt and is mostly focused on the purpose and task. The essay may not acknowledge the claim and counterclaims evenly throughout.
DEVELOPING	The essay may not fully address the demands of the prompt or stay focused on the purpose and task. The writing may stray significantly off topic at times, and introduce the writer's bias occasionally, making it difficult to follow the central claim at times.
EMERGING	The essay does not maintain focus on purpose or task.

ORGANIZATION

Organize your writing in a logical sequence.

ADVANCED	The essay incorporates an organizational structure throughout that establishes clear relationships among the claim(s), counterclaims, reasons, and evidence. Effective transitional words and phrases are included to clarify the relationships between and among ideas (i.e. claim and reasons, reasons and evidence, claim and counterclaim) in a way that strengthens the argument. The essay includes an introduction and conclusion that effectively follows from and supports the argument presented.
PROFICIENT	The essay incorporates an organizational structure with clear transitional words and phrases that show the relationship between and among ideas. The essay includes a progression of ideas from beginning to end, including an introduction and concluding statement or section that follows from and supports the argument presented.
DEVELOPING	The essay uses a basic organizational structure and minimal transitional words and phrases, though relationships between and among ideas are not consistently

	clear. The essay moves from beginning to end; however, an introduction and/or conclusion may not be clearly evident.
EMERGING	The essay does not have an organizational structure and may simply offer a series of ideas without any clear transitions or connections. An introduction and conclusion are not evident.

LANGUAGE

Pay close attention to your tone, style, word choice, and sentence structure when writing.

ADVANCED	The essay effectively establishes and maintains a formal style and objective tone and incorporates language that anticipates the reader's knowledge level and concerns. The essay consistently demonstrates a clear command of conventions, while also employing discipline-specific word choices and varied sentence structure.
PROFICIENT	The essay generally establishes and maintains a formal style with few possible exceptions and incorporates language that anticipates the reader's knowledge level and concerns. The essay demonstrates a general command of conventions, while also employing discipline-specific word choices and some variety in sentence structure.
DEVELOPING	The essay does not maintain a formal style consistently and incorporates language that may not show an awareness of the reader's knowledge or concerns. The essay may contain errors in conventions that interfere with meaning. Some attempts at discipline-specific word choices are made, and sentence structure may not vary often.
EMERGING	The essay employs language that is inappropriate for the audience and is not formal in style. The essay may contain pervasive errors in conventions that interfere with meaning, word choice is not discipline-specific, and sentence structures are simplistic and unvaried.