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RESEARCH ARTICLE

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Leukocyte Differential Study in *Macaca nemestrina* infected by *Plasmodium* spp.

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Abstract
The differential count of leukocytes in *Macaca nemestrina* (pig-tailed macaques) infected by *Plasmodium* is a way to determine the pattern of the immune response that is increased by the presence of a disease in the body in the form of a parasite in the blood, namely *Plasmodium* spp. *Plasmodium* is a parasitic protozoan that can cause malaria and is transmitted through infected mosquito bites. This parasitic protozoan can infect birds, reptiles, rodents, primates, and humans. This study also distinguished the percentage of white blood cells (leukocytes) between infected and uninfected macaques with *Plasmodium* spp, with the number of samples taken by as many as 24 monkeys. Whole blood was made a thin review on a glass preparation. After drying, it was fixed using methanol by soaking it for 15 minutes and then soaking it in Giemsa ▪ s solution for 30 minutes. The number of leukocytes and differential leukocytes was counted using a light microscope. Polymerase Chain Reaction (PCR) examination as confirmation of microscopic examination. The calculation results showed the percentage of neutrophils and monocytes in monkeys infected by *Plasmodium* spp. Greater than the uninfected macaque, basophils were still in normal numbers for lymphocytes and eosinophils.

Key words: *Plasmodium* spp., *Macaca nemestrina*, differential leukocyte

1. Introduction

Indonesia is one of the countries that have a rich diversity of wildlife species, especially primates. Pig-taild macaques (*Macaca nemestrina*) is one of the primates whose population in nature is currently decreasing. The threat to the existence of apes in nature is the destruction of tropical forests as a place to live. The macaques have lost about 49% of their habitat, which was originally an area of 354,115 km^2 to only about 179,140 km^2, so the macaques are classified as vulnerable primates on the IUCN list and included in Appendix II of CITES (Ilyas *et al*. 2016). One of the diseases that can attack animals, especially primates, is *Plasmodium* sp. or malaria. Malaria in primates has long been known, namely in 1907. A scientist named Martin Mayer found this disease in the blood of *Macaca fascicularis* originating from Java where *Plasmodium* spp. Morphologically has a shape similar to *P. vivax* in humans, which was later named and converted to *P. cynomolgi* (Shinta 2001). Several cases of zoonotic infection from *Plasmodium* sp. monkeys (chimpanzees, gorillas, orangutans, gibbons) have been reported in several countries, namely *P. knowlesi* (1965) in humans in America, *P. simium* (1966) in squirrel monkeys in Brazil, *P. inui* (1971) in Pahang, Malaysia, *P. cynomolgi* (1973) in America and *P. brasilianum* in Sao Paulo (1966, 1995). *P. knowlesi,* in 2008, was detected in Malaysia and misdiagnosed in humans as *P. malariae*. If this disease is not treated properly and quickly, the patient will experience complications such as liver and kidney failure and death. (Wijayanti 2012).

Leukocytes are white blood cells in the blood that help the body fight infection or disease. Leukocytes are blood cells that contain a nucleus, also called white blood cells. Leukocytes are the body›s defence against foreign objects entering the body (Guyton 2011). Leukocytes are generally divided into agranular and granular. Agranular leukocytes have a homogeneous-looking cytoplasm and a spherical nucleus consisting of lymphocytes and monocytes. Granular leukocytes contain specific granules in their cytoplasm consisting of neutrophils, eosinophils, and basophils (Ganong 2012). Each type of leukocyte has various functions related to non-specific and specific immunity, making leukocytes have a very important role in the body›s defence against antigens and

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infections. Observed in blood smear preparations. The main function of leukocytes is as an immune system, but there are different mechanisms for each type of leukocyte (Wulandari *et al*. 2014).

Pig-tailed macaques are a primate species characterized by a large body and greyish-brown to slightly golden hair; around their face is lighter and lighter brown hair (Lekagul and Neely 1977). The distribution areas of these primates in the territory of Indonesia include Central Kalimantan, East Kalimantan, South Kalimantan, Lampung, Bengkulu, South Sumatra, Jambi, West Sumatra, North Sumatra, Aceh, and Bangka Island (Supriatna and Wahyono 2000). This study aims to examine the effect of *Plasmodium* infection on *Macaca nemestrina* on white blood cells as a self-defence mechanism.

2. Materials and Methods

2.1. Making a Blood Smear

Blood samples were taken from 24 macaques in one captive population, then continued with Polymerase Chain Reaction examination. PCR examination was carried out to detect the presence of *Plasmodium* spp. in monkey blood. PCR results showed as many as 12 blood samples infected with *Plasmodium* spp. (positive). Furthermore, the other 12 samples were not infected (negative). Making a blood review by taking 10 l of blood samples using a micropipette, then dripping 1 drop on the object glass. The blood that has been placed on the object glass is then pushed at an angle of 45° using the edge of the side of the object glass until a thin layer of blood is formed. The blood smear preparations were then fixed with methanol solution for 15 minutes, stained with Giemsa stain for 30 minutes, rinsed with running water, and allowed to dry.

2.2. Leukocyte Differential Calculation

A total of 24 blood smear preparations were observed using a light microscope with a magnification of 100x. Every 100 leukocytes found were counted and grouped into each type of leukocyte: basophils, eosinophils, neutrophils, lymphocytes, and monocytes. The leukocyte count was carried out using an analogue cell counter, and the results were compared from the two types of preparations.

2.3. Data Analysis

Two kinds of data analysis were used: descriptive analysis to determine the morphological description of *Plasmodium* spp. Using a digital microscope and statistical analysis using the Statistical Product and Service Solutions (SPSS) application to test the difference/effect of *Plasmodium* spp. on the leukocyte count. The SPSS method used is the Independent Sample T-test method.

3. Result

3.1. Morphology of *Plasmodium* spp.

Observation of the blood review preparations from 12 samples found 12 positive and 12 negative samples infected with *Plasmodium* spp. The parasites detected in the blood review were only found in the schizont phase (Figure 1).

Observation of blood reviews for the presence of parasites, only *Plasmodium* spp. in the schizont phase. According to Rasita (2019), the parasite developed from the trophozoite stage to become a schizont. This asexual development process is called schizogony, then the infected erythrocytes (schizonts) rupture and the merozoites inside will come out and infect other red blood cells.

Plasmodium spp. can be seen in red blood cells resembling bluish black spots, which can be oval, round, or ring in shape depending on the cycle going on at the time of blood collection (Rosmanah 2015). The parasitic form of *Plasmodium* spp. in the young trophozoite phase (ring phase), according to Lee *et al*. (2019), is characterized by a ring-like cytoplasmic appearance that covers the vacuole with rounded chromatin dots protruding from the cytoplasm. The mature trophozoite phase is characterized by a thicker cytoplasm than the young trophozoites. The schizont phase is characterized by 2 to 5 portions of the nuclear chromosomal mass and pigment granules that make up two-thirds of the infected erythrocytes. The gametocyte phase is characterized by a spherical parasite occupying most of the infected erythrocytes, with scattered and irregular dark brown chromatin

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3.2. Differential Leukocytes

Leukocyte differentiation is a grouping of leukocytes based on cell size, the presence or absence of granules, and the number of nuclei observed on blood smear preparations. The main function of

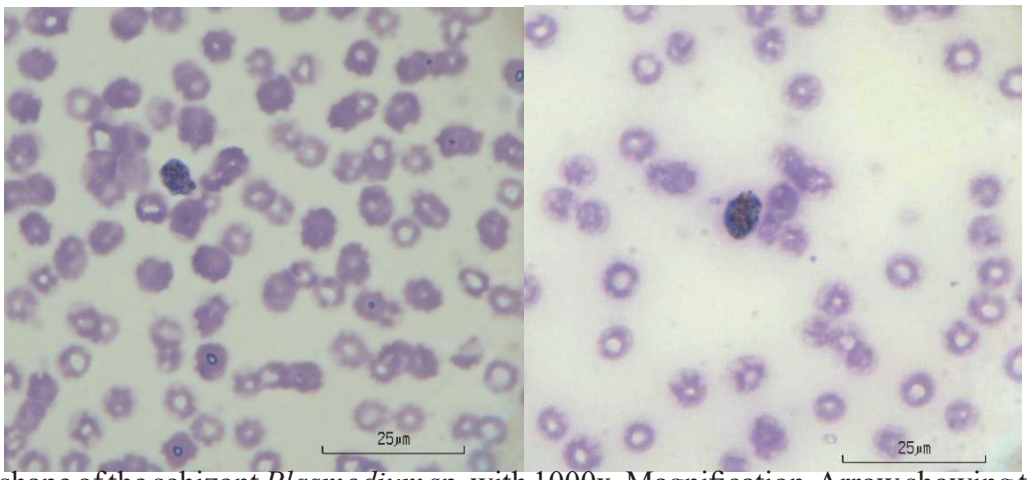

Figure 1. The shape of the schizont *Plasmodium* sp. with 1000x. Magnification. Arrow showing the *Plasmodium* sp.

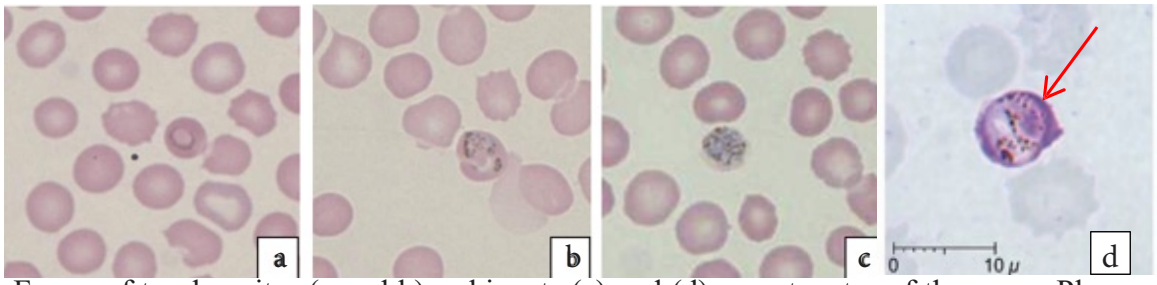

Figure 2. Forms of trophozoites (a and b), schizonts (c) and (d) gametocytes of the genus Plasmodium with Giemsa staining (1000 times magnification) (Rosmanah 2015; Lee *et al*. 2009)

Table 1. Average percentage of leukocytes between positive and negative samples of *Plasmodium* sp.

Sample	Basophil	Eosinophil	Band neutrophil	Segment Neutrophil	Lymphocytes	Monocytes
Positive	0,33±0,89[a]	1,83±2,37[a]	0,58±2,02[a]	52,92±9,54[a]	42,92±10,51[a]	1,42±1,93[a]
Negative	0,00±0,00[a]	2,67±2,46[a]	1,50±1,78[a]	46,75±14,59[a]	48,00±15,70[a]	0,33±0,65[a]

leukocytes is as an immune system, but there are different mechanisms for each type of leukocyte (Wulandari *et al*. 2014). The average number of leukocytes from each sample is shown in Table 1.

4. Discussion

The results of the leukocyte count show the number of numbers that are significant to each other (not statistically significant), which also affects their function in the body. Basophils are leukocytes that function in hypersensitivity reactions by releasing heparin when an allergic reaction occurs (Ganong 2012). The percentage of basophils in the two data was not significantly different and was still in normal condition. The average percentage of normal basophils in monkeys› blood ranged from 0.0 to 3.0% (Rahlmann *et al*. 1967). The percentage of basophils that were low and not significantly different was due to the nature of basophils that did not respond to the presence of parasites.

Eosinophils are a type of leukocyte that plays a protective role against malaria by triggering the killing of parasites (Irmayanti *et al*. 2017). The average percentage of normal eosinophils in monkeys› blood ranged from 0.0 to 5.1% (Rahlmann *et al*. 1967). The percentage of eosinophils in both data shows a normal number and is not significantly different. The high mean value of eosinophils is caused by exposure to parasites in animals (Paden *et al*. 2014)

Neutrophils are leukocytes that act as the body›s defence against parasitic or bacterial infections (Wulandari *et al*. 2014). The average percentage of

neutrophils in monkeys› blood ranged from 44.8-58.3% (Rahlmann *et al*. 1967). The results showed that the percentage of neutrophils in monkeys infected by *Plasmodium* sp. larger than the uninfected macaques. This finding aligns with the percentage of neutrophils in the blood, increasing when a bacterial or parasitic infection occurs (Napirah *et al*. 2013).

Lymphocytes are leukocytes that respond to antigens (foreign objects) by increasing the circulation of antibodies in the blood and the development of the immune system (Bikrisima *et al*. 2013). The average percentage of lymphocytes in monkeys› blood ranged from 37.0-51.2% (Rahlmann *et al*. 1967). the percentage of lymphocytes displayed in the data shows a normal number and is not significantly different between positive and negative data. According to Mau and Mulatsih (2017), the increase in lymphocytes occurs as a sign of parasites in the patient›s body. Foreign bodies such as *Plasmodium* will trigger antigen-presenting cell (APCs) formation. APC will stimulate the body to form T lymphocyte cells and produce Interleukin-2 (IL-2). IL-2 will stimulate cytotoxic T cells to destroy foreign objects (*Plasmodium* spp.) that enter the body (Intan *et al*. 2016).

Monocytes are leukocytes that act as the body›s second defence after neutrophils against parasitic infections. The average percentage of monocytes in monkeys› blood ranged from 0.5-8.0% (Rahlmann *et al*. 1967). The increasing number of monocytes (Dila *et al*. 2020) indicates the occurrence of parasitic phagocytosis as a body defence mechanism.

The data results in Table 3 show the average range of leukocyte percentage of monkeys which are differentiated according to the presence or absence of *Plasmodium* spp. Infection. Statistical analysis using the Independent Sample T-test method showed no significant difference between positive and negative data ($p>0.05$). The results also showed the number of some leukocytes in positive *Plasmodium* spp. Lower than the negative apes. Eosinophils and lymphocytes, as examples, show that these are mutually exclusive. These results were obtained because the leukocyte count factor using the field of view method caused some leukocytes to be counted more. It can also be caused by other diseases experienced by monkeys, such as flu or tuberculosis, affecting the number of leukocytes. The value of all types of leukocytes but still within the normal range. These results are supported by research by Rosmanah (2015), that the presence of *Plasmodium* spp. in monkeys does not cause hematological abnormalities so that it does not impact their health. This study shows the percentage of neutrophils and monocytes in monkeys infected by *Plasmodium* spp. Greater than the uninfected macaque, basophils were still in normal numbers for lymphocytes and eosinophils.

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