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Evaluation of Parasite Number and Bodyweight in *Mus musculus* which was Infected by *Plasmodium berghei*

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Abstract: Malaria is an infectious disease because of *Plasmodium Sp.*, and the Anopheles mosquito spreads it. Anopheles lives and breeds in the field, forest, and river/beach. People who can be suffered from malaria are about 41% in the world. One of the developments of malaria research can be done in an animal model. This research's goal was to make an animal model of malaria infection by analyzing the differences of parasite number and bodyweight of *Mus musculus* from day 0 until day 4 of infection. The research method was experimental using post-test only with control group design and time series. Several *Mus musculus* was 18/group. Infection of *P. berghei* was injected intraperitoneal in *Mus musculus*; it was called day 0. The dose was 10^7 of the parasite in 0.2 ml of blood. The control group and infection group's parasite number and body weight were done daily from day 0 until day 4. Examination of parasite used thick and thin blood smear. Bodyweight of *Mus musculus* was examined by a digital scale. The result was significant differences of parasite number in infected in *Mus musculus* among day 0, 1, 2, 3 and 4 ($p = 0.031$). The highest parasite numbers were on day 4 (25.44). There was significant difference of body weight in the control group ($p = 0.000$), and also in infection group ($p = 0.000$). The conclusion was *P. berghei* infection could be used to induce malaria infection in *Mus musculus*.

Keywords: *Parasite number, bodyweight, P. berghei*

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I. INTRODUCTION

Malaria is an infectious disease which is caused by *Plasmodium Sp.* It is contagious by Anopheles mosquito. Anopheles mosquito lives and breed in field, hill, forest, beach and river. Distribution of malaria consists of tropical and subtropical countries including Indonesia.

Percentage of risky citizens who can suffer from malaria was 41%. Malaria can be found in all of the area in Indonesia. *Annual Parasite Incidence* (API) divided Indonesia became some categories. High category of malaria was in east Indonesia. Middle category of malaria was in Kalimantan, Sulawesi and Sumatera. Low category of malaria was in Java and Bali [1, 2]. Malaria can cause death. Malaria complications were affecting kidney, lung, severe anemia, icterus, seizure, hypoglycemia, and metabolic acidosis [3]. Development of malaria still needs more researches in every aspects. Sometimes the research can not be done directly in human. So, we need an animal model which can be infected by malaria. After that, the research can be done in an animal model to produce new information and technology of malaria.

Malaria in rodent can be infected by *Plasmodium berghei* (*P. berghei*). It is used as model organism of malaria infection. That infection could produce parasite life cyclic in blood and was found in body tissue such as lung, adipose and brain [4]. Infection of *P. berghei* also caused oxidative stress and abnormality of the placenta in mice, that was signed by placental histology and

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apoptosis expression [5, 6].

Research about *P. berghei's* infection in rodent must be detected its success first before continuing the next advance research. Infection of *P. berghei* succeed if the parasite was found in a blood smear. Another indicator of inflammation and infections process is bodyweight. This indicator is the simple one to know the early process of body defense. The aim of this research was making an animal model of malaria infection with analyzing the differences of parasite number and bodyweight of *Mus musculus* in day 0 untill day 4 of infection.

II. LITERATURE REVIEW

A. *Infection of P. berghei*

P. berghei can be used for malaria infection in rodent because it has similar clinical manifestation like a human. Malaria infection in animal model relates to the accumulation of infected red blood cell. Infected red blood cell can be found in brain, heart, hepar, lien, lung, and kidney [7]. Parasitemia in animal model is evaluated by Giemsa thin blood smear. Parasitemia is counted by calculation of infected red blood cell in 5 lapping of views. A blood smear is token from tail blood [7, 8]. Erythrocytes invasion is an essential process for the survival of malaria parasite. All Plasmodium that infected animals had a complex life cycle. Characteristics of *Plasmodium berghei* were: [9].

1. Exoerythrocytic stages occurred in parenchymal cells of the liver and matured 48 hours after the introduction of sporozoites.

2. Erythrocytic stages took 22-24 hours and it was a synchronous.

3. Merozoites had a strong preference to reticulocytes.

All of the doses of infected *P. berghei* (10^2-10^7) could cause death in 30 days. A blood smear showed positive infection before day 5, and change of body temperature (hypothermia) happened since day 5. Bodyweight of infected *P. berghei* started decreasing since day 3. The infected animal model had low hemoglobin [7]. One of the signs to show Plasmodium infection was messy hair. Inflammation process of plasmodium infection started in day 3. Inflammation cytokine was found in blood and organ. Organ evaluation in an infected animal model was splenomegaly dan hepatomegaly [7].

B. *The Changes of P. berghei Infection*

P. berghei infection caused inflammation. Inflammation cytokine increased from day 3 after infection. It induced body defense to increase anti-inflammation after day 3-5 post-infection [7]. *P. berghei* infection also caused oxidative stress. Both inflammation and oxidative stress destabilized cell membrane, and after that damaged erythrocytes and hepatocytes. That damage made jaundice in all of the body [10].

C. *Inflammation and Bodyweight*

Weight loss could happen because of inflammation cytokines production such as TNF-alpha and interleukin-6. Those inflammation cytokines production suppressed appetite and promoted muscle and fat breakdown. That process induced inefficient energy expenditure. The decreasing of energy intake would cause decreasing of energy consumption, gluconeogenesis, lactate recycling, and protein turn over [11].

D. *Diagnose of Malaria*

Parasite's size was very small and only could be seen by a microscope. The parasite could be detected by making a blood smear and colored by Giemsa. Giemsa blood smear was dropped by immersion oil and checked by a microscope with 100× magnification. If in blood smear was found parasite, it was definitely diagnosed for malaria [12].

III. RESEARCH MODEL

This research used true experimental with post test only with control group design using time series. This research used *Mus musculus*. The number of *Mus musculus* were 18/group. Groups of research were K1 (*P. berghei* infection) and K0 (without infection). Infection of *P. berghei* was done by injecting 10^7 of *P. berghei* in 0.2 ml of blood intraperitoneal. The day when *P. berghei* was injected was called day 0. After that, tail blood was taken by puncturing with lancet needle. The blood was prepared on object glass became thick and thin Giemsa blood smear. Blood smear was checked to calculate the number of infected red blood cells with microscope.

Examination of bodyweight was also evaluated every day on a digital scale. Evaluation was done in day 0 until day 4. The results of this research were analyzed by distinguishing in day 0 until day 4.

IV. DATA ANALYSIS

Data in this research were a number of parasite in K1, since day 0 until day 4, bodyweight in K1 and K0 in day 0 until day 4. All of data were analyzed by the normality test, but they were not in normal distribution, so the test was used Friedmann test.

A. *Number of Parasites*

Table 1 showed that the mean of plasmodium number increased every day with p-value $p = 0.031$. It meant

there was significant difference of plasmodium number in day 0 until day 4. The infected red blood cell was showed in Figure 1.

TABLE 1
NUMBER OF PARASITE IN A BLOOD SMEAR

Day	Number of plasmodium	Deviation standard	p-value	Statistic analysis	α value
0	0	0	0.031	Friedmann Test	0.05
1	1.56	0.915			
2	6.06	3.161			
3	15.44	7.504			
4	25.44	11.102			

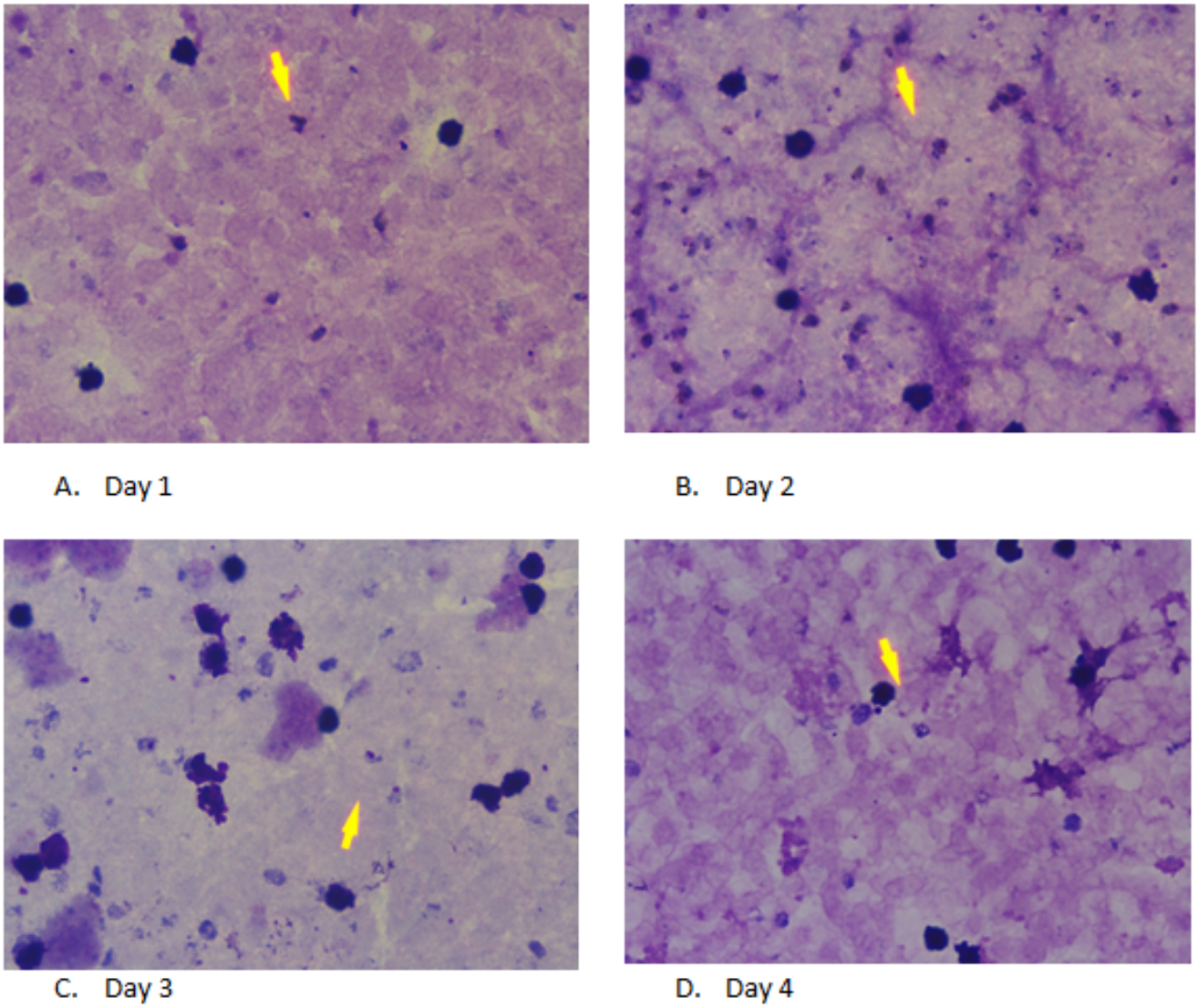

Fig. 1. Infected red blood cell (Giemsa blood smear)

B. Bodyweight of Mus musculus in K0

Table 2 showed that bodyweight of *Mus musculus* in K0 (without infection) increased every day with p-value $p = 0.000$. It showed there was significant difference of bodyweight of K0 in day 0 until day 4.

TABLE 2
BODYWEIGHT OF *MUS MUSCULUS* IN K0

Day	Bodyweight (gram)	Deviation standard	*p*-value	Statistic analysis	α value
0	24.22	0.152	0.000	Friedmann Test	0.05
1	24.44	0.166			
2	25	0.162			
3	25.61	0.118			
4	25.83	0.146			

C. Bodyweight of Mus musculus in K1

Table 3 showed that bodyweight of *Mus musculus* which were infected by *P. berghei* decreased in day 1, and after that increased slowly. *P* value was $p = 0.000$, there was significant different of bodyweight in K1 from day 0 until day 4.

TABLE 3
BODYWEIGHT OF *MUS MUSCULUS* IN K1

Day	Bodyweight (gram)	Deviation standard	*p*-value	Statistic analysis	α value
0	23.67	0.352	0.000	Friedmann Test	0.05
1	23.50	0.355			
2	23.72	0.441			
3	23.78	0.461			
4	24	0.42			

V. DISCUSSION

This research observed the development of *P. berghei* infection that was injected intraperitoneal in *Mus musculus*. Evaluation of parasite number and examination of bodyweight were done everyday. Table 1 showed that parasite number increased everyday, and the most number was in day 4, and the data was significantly different.

That result showed there was a reproduction of *P. berghei* in *Mus musculus*. In this research, life cyclic of Plasmodium that was observed was only in the host. *P. berghei* had power to invade reticulocyte. In the beginning, all of infection invated restriction reticulocyte until 0.5-2% of parasitemia. After that, it has infected *Mus musculus* invated normocyte until 15-25% parasitemia in 2 days [13].

Generally, a parasite in erythrocyte produced 6-12 merozoites per schizont. This nuber was less than in reticulocyte (12-18 merozoites per schizont). Infection of *P. berghei* in *Mus musculus* caused death in 1-3 Minggu [13]. At the end of Plasmodium replication cycle, infected red blod cells ruptured and released merozoite and hemozoin into bood circulation. If there was hemozoin, so it could be parasitemia [14].

Actually, examination of Plasmodium could use thin blood smear or Polymerase Chain Reaction (PCR). [15] compared those two examinations. The results were PCR had 100% sensitivity but 60% specificity, 83.33% for positive expected value, and 100% for negative expected value. Those values were comparison with thin blood smear. PCR's specificity was only 60%, so in this research still used thin blood smear to evaluate the parasite's numbers.

Data of bodyweight of *Mus musculus* showed significant difference in everyday evaluation in K0 and K1 (Table 2 and Table 3). Bodyweight of *Mus musculus* in control without infection increased everyday. It was caused by good immunity of the body, so the energy of metabolism did not use for fighting inflammation. Bodyweight of *Mus musculus* in *P. berghei* infection tend to decreased and unstable. It was caused by the immunity of infected *Mus musculus* was used to fight the inflammation. In infection of malaria, body produced more cytokine more than in healthy condition. Plasmodium

infection caused inflammation that was more severe in more parasite number [7]. Body metabolism was used to fight the infection, so the bodyweight tends to unstable or decreased. The process of defense from inflammation caused by gluconeogenesis, lactate recycling and protein turn over [11]. Gluconeogenesis is a pathway to convert non carbohydrate such as lactate, glycerol and amino acids become glucose. It's a process in the liver. It is regulated by hormone and nutritional cues [16]. Body with inflammation process lost fat and muscle mass. Inflammation could induce protein catabolism especially muscle protein, so it induced weight loss [17].

VI. CONCLUSION

The conclusion were significant differences of parasite number and bodyweight of the control group and infection group in *Mus musculus* among day 0, 1, 2, 3 and 4. So, *P. berghei* infection could be used to induce malaria infection in *Mus musculus*.

Limitation of this research was only examining parasite's numbers and bodyweight without organ preparation, but as initial research, it had shown the success of *P. berghei* infection dose.

Recommendation for the next research is this infection dose can be used for malaria infection, so this way applies to do research in the animal model if the variable will be difficult to be examined in human.

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