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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 infection disease because of *Plasmodium Sp.* and it is spread by Anopheles mosquito. Anopheles lives and breed in the field, forest, and river/beach. People who can be suffered from malaria are about 41% in the world. One of development of malaria research can be done in animal model. This research's goal was making 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. Research method was experimental using post test only with control group design and time series. Number of *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 paracite in 0.2 ml of blood. Evaluation of parasite number and bodyweight of control group and infection group were done every day since day 0 until day 4. Examanation of paracite used thick and thin blood smear. Bodyweight of *Mus musculus* was examined by 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 was in day 4 (25.44). There was significant different of bodyweight in control group ($p = 0.000$), an 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 infection disease which is caused by *Plasmodium Sp.* It is contagioused 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 area in Indonesia. *Annual Paracite Insidence* (API) devided 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 the death. Malaria complications were affecting kidney, lung, severe anemia, icterus, seizure, hypoglikemia, and metabolic asidosis [3]. Development of malaria still need more researches in every aspects. Sometimes the research can not be done directly in human. So, we need animal model which can be infected by malaria. After that, the research can be done in 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 placenta in mice, that was signed by placental histology and apoptosis expression [5, 6].

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Research about *P. berghei's* infection in rodent must be detected its success first before continuing the next advance research. Infection of *P. berghei* successed if the parasite was found in 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 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 the similar clinical manifestation like 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. Blood smear is token from tail blood [7, 8]. Erythrocytes invasion is an essential process for the survival of malaria parasite. All Plasmodiums that enfected animals had 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 strong preference to retyculocytes.

All of dose of infected *P. berghei* (10^2-10^7) could cause death in 30 days. 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. Infected animal model had low haemoglobin [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 infected animal model was splenomegali dan hepatomegali [7].

B. *The Changes in of P. berghei Infection*

P. berghei infection caused inflammation. Inflammation cytokine inceased from day 3 after infection. It induced boody defense to increase anti inflammation after day 3-5 post infection [7].

P. berghei infection also caused oxidative stress. Both of inflammation and oxidative stress destabilized cell membrane, and after that damaged erythrocytes and hepatocytes. That damage made jaundice in all of 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*

Parasites size was very small and only could be seen by microscope. Parasite could be detected by making blood smear and coloued by Giemsa. Giemsa blood smear was dropped by imertion oil and checked by microscope with 100× magnification. If in blood smear was found parasite, it was definitely diagnose 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 made preparation on object glass became thick and thin Giemsa blood smear. Blood smear were checked to calculate the number of infected red blood cells with microscope.

Examination of bodyweight was also evaluated everyday by digital scale. Evaluated was done in day 0 until day 4. The results of this research was analyzed by distinguishing in day 0 until day 4.

IV. DATA ANALYSIS

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

A. *Number of Parasite*

Table 1 showed that the mean of plasmodium number increased everyday with p value $p = 0.031$.

It meant there was significant different 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 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			

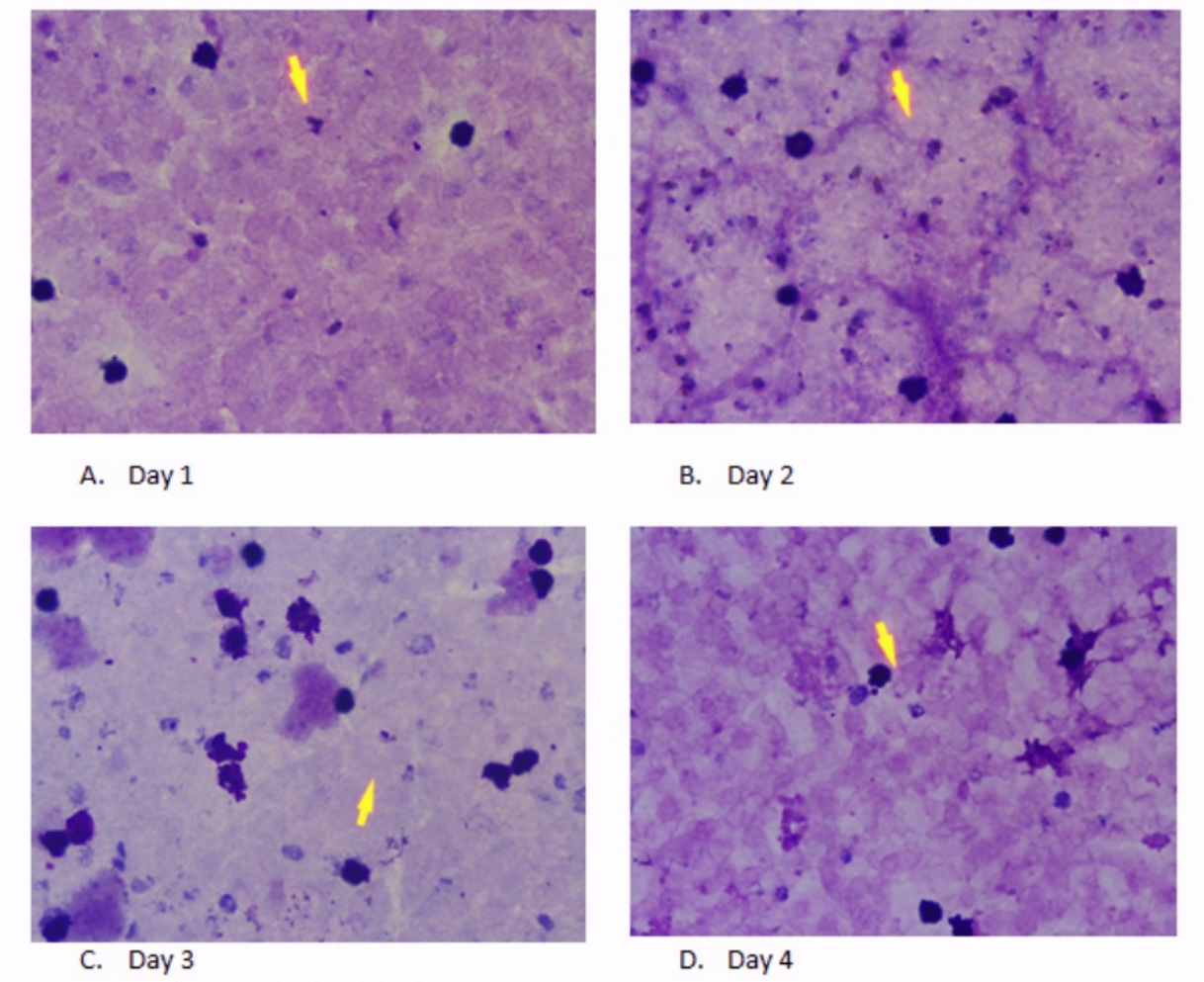

Fig. 1. Infected red blood cell (Giemsa blood smear)

B. *Bodyweight of Mus musculus in K0*

Table 2 showed that bodyweight of *Mus musculus* in K0 (without infection) increased everyday 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 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 umber increased everyday, and the most number was in day 4, and the data was significant different.

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

Generally, 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 2 examanations. The results were PCR had 100% sensitivity but 60% specificity, 83.33% for positive expected value, and 100% for negative expected value. Those values was comparation with thin blood smear. PCR's specificity was only 60%, so in this research still used thin blood smear to evaluate the prasite's numbers.

Data of bodyweight of *Mus musculus* showed significant different 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 didn't use for fighting inflammation. Bodyweight of *Mus musculus* in *P. berghei* infection tend to decreased and unstable. It was caused by 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 tend to unstable or decreasing. Process of defense from inflammation caused gluconeogenesis, lactate recycling and protein turn over [11]. Gluconeogenesis is pathway to convert non carbohydrate such as lactate, glycerol and amin acids become glucose. Its process in liver. It is regulated by hormone and nutritional cuse [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 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 an initial research it had showed 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 is applicable to make research in animal model if the variable will be difficult to be examined in human.

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