Morphology and Parasitaemia Development of *Plasmodium berghei* in Balb/ c Mice (*Mus musculus*)

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Abstract
Malaria is one of the most severe public health problems worldwide. It is a leading cause of death and disease in many developing countries, where young children and pregnant women are the groups most affected. Malaria disease caused by *Plasmodium* parasite have symptoms that typically include fever, fatigue, vomiting and headaches. In severe cases, it can cause yellow skin, seizures, coma or death. The present study is aimed to monitoring parasitemia level and percentage of parasite morphology as parasitaemia progresses. This research used *Plasmodium berghei* NK strain obtained from National University of Malaysia which originally from MR4, USA. Design used in this research was completely randomized design, with 2 treatments which were mice without infection and mice with infection of *P. berghei* parasite. Method used in this research was staining method of thin smear of blood using Giemsa stained and observed by microscopic. Parameter of observation were morphology and development of *P. berghei* at different level of parasitaemia (10%, 20%, 30% and 40%). The result showed from microscopic examination of blood slides prepared from the study animals indicated that the ring form was dominant stage obtained at all different stages of infection followed by trophozoite stage. Meanwhile schizont stage was the lowest stage obtained at all different stages observed.
Key words: Malaria, *Plasmodium berghei*, parasitaemia, trophozoite

Introduction

Malaria is a deadly disease that can lead to death (Philips, 2001; WHO, 2004). Children and pregnant women are the most vulnerable group of victims (CDC 2004). The disease is caused by infection with protozoan parasites of the genus *Plasmodium* (Carvalho et al., 2007). The World Health Organization (WHO) has reported about 4 billion people from 90 different countries are at risk of contracting the disease and as many as 500 million cases of malaria are reported each year (Carvalho et al., 2007; Esmark et al. 2006), and the death of at least 1 up to 2.5 million people each year (Curtidor et al., 2006; Esmark et al., 2006; WHO, 2009).

Most of deaths occurred in south Africa of the Sahara desert that is 80 percent of the total deaths from malaria worldwide (Pettersson 2005). In children, death is usually caused by cerebral malaria, acute anemia and respiratory disorders, meanwhile deaths in adults other than respiratory distress syndrome, the symptoms are more prevalent such as kidney damage and lung damage (Becker et al. 2004). In addition, the disease can cause an enlarged spleen and liver (Engwerda et al. 2004). This condition is caused by a buildup of malaria pigment granules. *Plasmodium* that attack small mammals such as mice among which are *P. berghei* (Cruz et al. 2000; Lau et al. 2001; Sherman 1998). *Plasmodium* has been used in studies to replace the use of *P. falciparum* in many aspects such as drug resistance, the diversity of antigens, the mechanisms of entry into the erythrocyte and study of organelles plastids. The results of an assessment of the rodent parasite and the host is very helpful in assessing the relationship between *Plasmodium* in human (Leiden University, 2005).Intraeritrosit stage is the most violent stage of infection (Menard, 2000; Vaid, 2007). Merozoites are released from the infected erythrocyte will be in plasma and bind erythrocyte and entering new ones. In circulation, merozoites quickly attaches and enters erythrocytes and replicate to produce new infective merozoites (Cowman and Crabb, 2006). This cycle continues until create clinical symptoms in patients (Greenwood et al. 2005). The transition stage in different cells and tissues in the mosquito vector and the mammalian host shows a high ability of malaria parasite to adapt to its environment (Matuschewski 2006; Vaughan et al., 2008). Merozoites are released into the bloodstream to infect erythrocytes either repeat the cycle intraeritrosit through the establishment of schizont-merozoites, or undergo a gametogenesis process for the formation of gametocytes (Bannister & Mitchell, 2003; Fujiako and Aikawa, 1999).

Materials and Methods

The study began with the isolation and preservation of *in vivo* culture parasite *P. berghei* in mice stocks and make infection in mice with a predetermined dose of 1 x 10^6 infected erythrocytes/ mice. The research was conducted at the Laboratory of Microtechnique, FMIPA, Unsyiah. The materials used

in this study are the cryopreservation of parasite *Plasmodium berghei*, methanol, Giemsa dye and physiologycal saline solution.

Inoculation of *P. berghei* Parasite

White male mice Balb / c for about 8 weeks old were distributed in two treatments, the mice without infection with *P. berghei*-infected mice as controls and *P. berghei infected mice*. Furthermore, *P. berghei*-infected mice were divided into 4 groups of sampling at 10% parasitemia, 20% parasitemia, 30% parasitaemia and 40% parasitaemia. Each sampling involves 5 mice.

P. berghei which cryopreserved in liquid of nitrogen first heated in the bath 37 ° C for 10 minutes. Furthermore, the solution containing the parasite is mixed with NaCl NaCl physiological saline solution in the ratio 1: 1 and centrifuged for 10 minutes at speeds of 650 x g. Pellets composed of *P. berghei*-infected erythrocytes then mixed again with physiological NaCl in the ratio of 1: 1. The pellets are then injected in healthy mice as stocks by intraperitoneal route. *P. berghei* inoculums are then prepared from stock mice which have parasitemia about 30%. The number of erythrocytes calculated using Haemasitometer and level of parasitaemia observed throughout thin blood smear of slide with Giemsa staining (Field & Shute 1955). Furthermore, parasitic blood was taken from the tail end of a small piece of mice stock and diluted with physiological saline solution at the dose of 1 x 10^6 erythrocytes infected with malaria parasites/mice and then injected into mice in each treatment.

Giemsa staining

Thin blood smear of slide treated and control mice were stained by Giemsa. Before stained, slides were fixed with methanol and allowed to dry for 1 minute in room temperature. After that, slide colored with Giemsa dye which diluted in a volume of 1: 9 with a phosphate buffer solution (PBS), pH 6.8, and left for 20 to 30 minutes. The slide is then washed with tap water flows slowly and dried at room temperature. The percentage of parasitaemia observed further under the light microscope with 100x magnification.

Determination of Parasitemia Level

A drop of infected blood of mice were obtained aseptically from the tail end of mice and smeared on sterile glass objects. Thin blood smear of slide then stained by Giemsa and observed under a light microscope with a magnification of 100 times. Parasitaemia level calculated by the formula of Hauda et.al (1993) as follows:

Results and Discussion

Monitoring of the development of parasitaemia in the infected mice was done after inoculation of parasite *P. berghei*. Treated mice that have reached percentage of parasitaemia at 10%, 20%, 30% and 40% was monitored by making thin blood smear of slide as mentioned above. The morphology of the parasite at every stage parasitaemia was also monitored in each group of animals. Figures and tables below showed the morphological forms of ring and trophozoite stages of *P. berghei*.

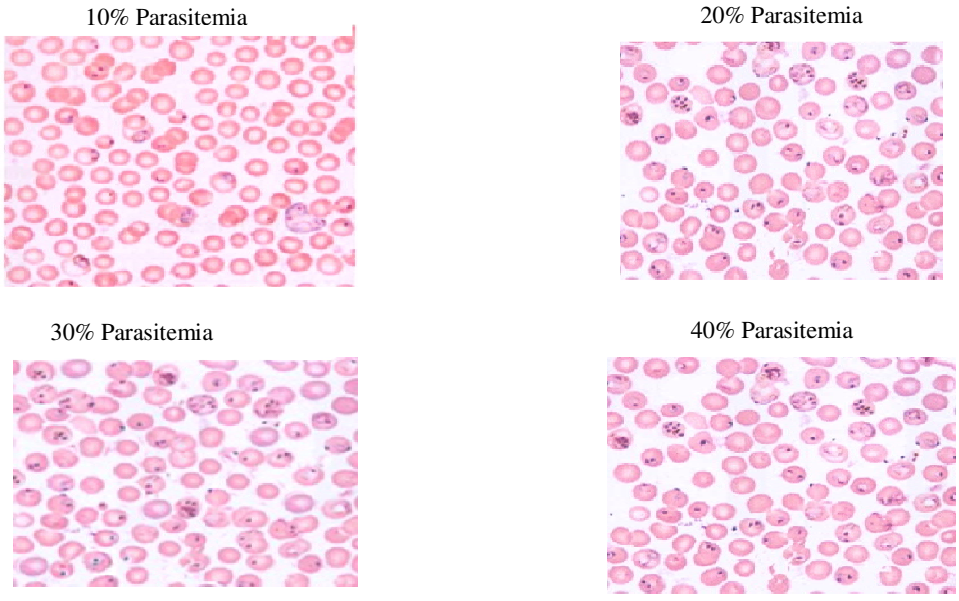

Figure 1. Morphology of intraerythrocyte *P. berghei* parasite at 10%, 20%, 30% and 40% parasitaemia which observed using light microscope at 100 x magnification.

Table 1. Percentage of *P. berghei* morphology in infected erythrocyte (%)

Parasitaemia (%)	Morphology of *P. berghei* in infected erythrocyte (%)		
	Ring form (n=5)	Trophozoite (n=5)	Schyzon (n=5)
10	72.15 ± 2.11	24.26 ± 2.10	0.34 ± 0.17
20	55.72 ± 2.34	40.37 ± 2.15	0.50 ± 0.22
30	54.76 ± 2.45	40.23 ± 3.05	1.05 ± 0.45
40	53.18 ± 3.22	44.56 ± 3.45	0.29 ± 0.12

Both of these stages were always present highest at every level of parasitaemia, while the schizont stage was the lowest stage presents and only can be detected less than 1% of all group of parasitic morphology. The lowest level of intraeritrosit schizont stage was thought to occured because the parasite-infected erythrocytes have been lost at this stage in the blood circulation system and then gathered in organ capillaries such as the lungs, liver and spleen to undergo the process of intraeritrosit schyzogoni (Jansen & Waters, 2002). The release of merozoites in schizont stage causing the attendance of early stage of infection which is a ring and trophozoite forms in erythrocytes. Most of the parasite infected erythrocytes containing ring form that is 53-73% and 23-44% of trophozoite form. The presence of more ring forms at the 10% level of parasitaemia compared with 20% to 40% parasitaemia was also thought to be an early-phase release of merozoites in erythrocytes infected by *P. berghei* parasites. This process resulted in the presence of more rings and young trophozoites stage were observed in thin smear of blood on each level of parasitaemia.

Conclusions

The development of parasitaemia in the infected mice observed from microscopic examination of blood slides prepared from the study animals indicated that the ring form was dominant stage obtained at all different stages of infection followed by trophozoite stage. Meanwhile schizont stage was the lowest stage obtained at all different stages observed.

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