

**The histopathological study in C57BL/6 and Swiss Webster mice infected with
Plasmodium berghei ANKA**

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Abstract:

Introduction: Animal models provide important information regarding the pathogenesis, protection, and diagnosis of various diseases. This study aimed to investigate the histopathological alterations in *Plasmodium berghei* ANKA (PbA) infection in C57BL/6 and Swiss Webster mice.

Materials and Methods: Both mouse strains were used in this study. Fifteen mice of each strain were divided into three groups: (1) positive control group (Group 1) infected with 10^7 PbA in 200 μ l RBC and treated with dihydroartemisinin-piperaquine (DHP); (2) negative control group (Group 2) infected with PbA as previously described and treated with aquadest; and (3) normal group (Group 3) without infection and treated with aquadest.

Results: Microscopic examination revealed splenonecrosis, indicated by the depletion of white pulp and megakaryocytes and accumulation of hemosiderin in the spleen; midzonal hepatic degeneration and/or necrosis, followed by infiltration of lymphocytes and macrophages and accumulation of hemosiderin in the liver; pneumonia; and pulmonary edema in the lungs. These lesions were consistently observed in both the mouse strains. Spleen lesions were more severe in Swiss Webster mice than in C57BL/6 mice, whereas hepatic necrosis was more severe in C57BL/6 mice. However, the lung lesions were not significantly different between the two mouse strains, with only mild lesions observed.

Conclusions: Our findings revealed strain-dependent differences in organ pathology during *Plasmodium berghei* ANKA infection. C57BL/6 mice are more prone to hepatic necrosis, whereas Swiss Webster mice exhibit severe splenic damage. These differences clarify the inconsistencies and provide practical guidance for selecting appropriate mouse models for malaria research.

Keywords: Experimental pathology, Hemosiderin accumulation, Hepatic necrosis, Plasmodium, Splenomegaly

1. Introduction:

Malaria is a significant hemoparasitic disease and remains a public health concern in Indonesia. The disease is caused by *Plasmodium* parasites, comprising several species capable of infecting humans, including *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi* [1]. In Indonesia, *P. falciparum* and *P. vivax* are the primary endemic malaria species distributed across various regions [2]. The pathogenicity and clinical severity of malaria vary depending on the *Plasmodium* species and host response. Malaria infection can induce pathological changes in various tissues and organs, particularly in severe cases [1]. However, the mechanisms underlying tissue damage and organ dysfunction during infections are not fully understood. Therefore, the use of experimental animal models is crucial for studying the host response to *Plasmodium* infection and elucidating the mechanisms underlying malaria-induced tissue and organ damage under controlled experimental conditions [3].

Among the various experimental malaria models, *Plasmodium berghei* ANKA (PbA) infection in mice is widely used to study malaria pathogenesis and pathology associated with severe malaria. Although PbA infection in mice does not reproduce the full clinical and pathological spectrum of human malaria, this model can generate several pathological features relevant to severe malaria. These features include inflammatory responses and pathological changes in various organs, including the brain, liver, lungs, and spleen [4].

The pathological response to PbA infection is not determined solely by the parasite but is also influenced by the host's genetic background. Different mouse strains exhibit considerable variations in susceptibility, disease progression, immune response, and pathological manifestations following infection with the same *Plasmodium* parasite [4]. Thus, the combination of parasite and host strains is a key factor in determining the phenotype of experimental malaria in animal models. Direct comparisons between strains under comparable infection conditions are necessary to determine the extent to which the host genetic background contributes to malaria-associated tissue damage.

Swiss Webster and C57BL/6 are laboratory mouse strains widely used in experimental malaria research; however, they possess distinct genetic backgrounds and may exhibit differing responses to PbA infection. Both strains are commonly used as models susceptible to PbA-associated severe malaria and develop pathological changes, including organ lesions [5,6]. However, comparative characterization of organ histopathological responses in Swiss Webster and C57BL/6 mice following identical PbA infections remains limited. Specifically, it is not yet clear whether these two strains exhibit similar patterns and severity of lesions in major organs affected by PbA infection. Addressing this gap is important because inter-strain

histopathological differences may reflect variations in host susceptibility and biological responses to infection rather than simply resulting from differences in the parasite or experimental conditions.

Understanding strain-specific pathological responses has significant implications for malaria research. Such responses can influence the interpretation of disease severity and organ damage and determine the suitability of a specific mouse strain for particular research questions. Understanding how the host genetic background specifically affects organ pathology can assist researchers in accurately selecting and interpreting mouse models for studying malaria-induced pathogenesis and tissue damage. Therefore, a comparative histopathological assessment of Swiss Webster and C57BL/6 mice infected with the same PbA isolate can provide insights into the contribution of the host background to malaria-related pathologies.

Accordingly, this study aimed to compare the histopathological changes in Swiss Webster and C57BL/6 mice following infection with *Plasmodium berghei* ANKA (PbA). Histopathological changes in the liver, spleen, and lungs were comparatively evaluated to characterize the differences in the patterns and severity of tissue lesions between the two mouse strains. By identifying similarities and differences in organ pathology between Swiss Webster and C57BL/6 mice following PbA infection, this study provides information on the influence of the host genetic background on malaria-associated tissue damage and contributes to the appropriate selection and interpretation of mouse models for experimental malaria research.

2. Materials and Methods:

This study was approved by the Health Research Ethics Committee of the National Institute of Health Research and Development (HREC-NIHRD), Indonesian Ministry of Health, in accordance with the Declaration of Helsinki. Ethical approval number: LB.02.01/12/KE.220/2019.

This study used 30 female mice, comprising 15 mice of each Swiss Webster and C57BL/6 strains. Mice weighing 22–25 g were provided by the Experimental Animal Laboratory of the Indonesian Center of Research and Development for Biomedical and Technology, Ministry of Health. The mice were grouped and kept in standard plastic laboratory animal cages. The animals were provided with rodent feed and tap water ad libitum.

Mice were infected with malaria on day 0 and euthanized on day 6 for pathological and histopathological examinations, following the procedure described by Baptista et al. (2010) with minor modifications [7]. Termination was performed under anesthesia, followed by rapid exsanguination of the jugular vein and aorta.

Plasmodium berghei ANKA (PbA) was provided by the Centre of Research and Development for Biomedical Sciences and Technology Agency for Health Research and Development, Indonesian Ministry of Health. PbA was prepared by passaging 10^7 parasites in 0,2 ml of red blood cells per mouse and inoculated intraperitoneally (i.p.) into five donor mice. Parasitemia was microscopically assessed as the percentage of parasitemia at 3 days post-inoculation using thick and thin blood smears to obtain 1–5% parasitemia levels. Donor mice were euthanized to collect red blood samples for use as inoculants in the present study. The inoculum was diluted before dosing the treated mice by diluting 2 μ L of red blood cells into 0.8 mL phosphate-buffered saline (PBS) solution. Red blood cell (RBC) counts were determined using a hemacytometer. The mice were then administered 200 μ L of RBC (parasite 10^7) intraperitoneally [8].

The mice were divided into two groups of 15 mice each: Swiss Webster and C57BL/6 mice. Each group was randomly divided into three treatment groups, each containing five mice: (1) Group 1: positive control group: infected with PbA at 10^7 parasites/200 μ L RBC and treated with DHP (0.52 mg/kg BW: 20.8 mg dihydroartemisinin and 166.4 mg piperaquine); (2) Group 2: negative control group: infected with PBA at 10^7 parasites/200 μ L of RBC and treated with aquadest; and (3) Group 3: normal group, without infection and received only aquadest. (3) Group 3: Normal group: without infection and received only aquades.

Mice were infected with *P. berghei* ANKA intraperitoneally on day 1 at 10^7 parasites/200 μ L RBC. Treatment was performed one day after infection with 1 mL of DHP or aquadest intragastrically for three days. All surviving mice were euthanized on day 6 post-infection following animal ethics using ketamine (80–100 mg/kg BW intraperitoneally) and xylazine (10 mg/kg BW intraperitoneally), followed by rapid exsanguination of both major blood vessels and tissues of the liver, spleen, and lungs, which were collected for histopathological examination. The selection of day 6 as the sampling time point was guided by previous reports showing that peak parasitemia and the most pronounced pathological changes occur between days 5 and 7 post-infection. This was adopted with minor modifications from the published protocols.

The tissues were fixed in 10% buffered neutral formalin (BNF 10%), and histopathological analysis was performed. Histopathological slides were prepared following dehydration, clearing, infiltration, and embedding, and sliced using a microtome to a thickness of 5 μ m. Hematoxylin and eosin staining was performed on all the tissue samples.

Histopathological changes were examined under a light microscope (Nikon Eclipse Ni) at 200 $\times$ magnification, while photomicroscopy was performed using a Nikon DS-Fi2 and Nikon

Digital Sight DS U3. Histopathological scoring was performed independently by two blinded observers. Both evaluators assessed all slides without knowledge of group allocation. Inter-observer reliability was confirmed, and any differences in scoring were resolved through consensus discussions. This approach ensured consistency and minimized bias in the semi-quantitative- assessments.

Histopathological changes and morphometric analyses were performed by scoring the lesions (Table 1).

Table 1. Scoring system of histopathological lesions in the liver, spleen, and lungs of C57BL/6 and Swiss Webster Mice infected with *Plasmodium berghei* ANKA

Score	Parameter of lesions		
	Liver	Spleen	Lungs
–	No specific lesions	No specific lesions	No specific lesions
+	Degeneration of epithelial cells with the distribution of lesions $\leq 25\%$	Depletion of red/white pulp with a lesion distribution of $\leq 25\%$.	Peribronchitis with a distribution of lesions $\leq 25\%$.
++	Necrosis of epithelial cells with a lesion distribution of 25–50%.	Depletion of red/white pulp, necrosis of the interseptum and megakaryocyte cells, and accumulation of hemosiderin with a lesion distribution of 25 – 50%.	Pneumonia, pulmonary edema, and peribroncheolitis had lesion distributions of 25 – 50%.
+++	Necrosis of epithelial cells and haemorrhages with the distribution of lesions 50 – 75%	Depletion of red/white pulp, necrosis of the interseptum, megakaryocyte cells, and accumulation of hemosiderin with the distribution of lesions 50 – 75%	Pneumonia, pulmonary edema, hemorrhages, and peribroncheolitis with lesions were distributed in 50 – 75% of cases.
++++	Necrosis of epithelial cells, hemorrhages, and infiltration of mononuclear cells with the distribution of lesions $> 75\%$	Depletion of red/white pulp, necrosis of the interseptum and megakaryocyte cells, and accumulation of hemosiderin with lesion distribution $> 75\%$.	Pneumonia, pulmonary edema, hemorrhage, and peribroncheolitis with lesion distribution $> 75\%$.

Notes: No specific lesion (–), mild lesion (+), moderate lesion (++), severe lesion (+++), and very severe lesion (++++).

3. Results:

Macroscopically, mild swelling of the spleen was observed in Group 1, with a moderately blunt capsular surface. A moderately to severely swollen spleen with a blunt edge was observed in Group 2 of both mouse strains. No changes were observed in Group 3, which showed a sharpened edge on the capsular surface (Figure 1).

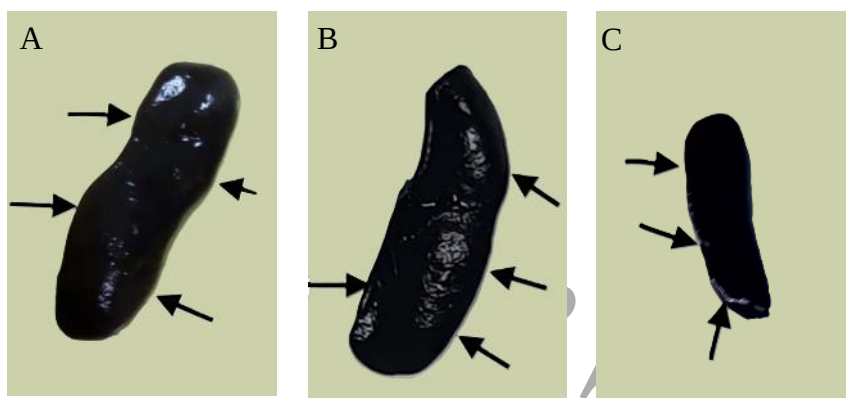


Figure 1. Macroscopic changes of the spleen in normal and *Plasmodium berghei* ANKA - infected mice

Notes: A, Group 1 (infected + DHP treatment) shows a moderately swollen spleen with a moderately blunt edge; B, Group 2 (infected + aquadest) shows a severely swollen spleen with a blunt edge; and C, Group 3 (non-infected + aquadest) shows a normal spleen with a sharp edge.

Microscopically, some lesions were due to PbA infection, such as an increase in megakaryocyte activity, necrosis of the intercept, necrosis of the interstitium, accumulation of hemosiderin, and depletion of the red pulp in the spleen. The distribution and severity of splenic lesions in both strains are shown in Table 2. Severity was scored according to the system shown in Table 1.

Table 2. Histopathological changes in the spleen of C57BL/6 and Swiss Webster Mice infected with *P. berghei* ANKA

Pathological changes	C57BL/6 strain			Swiss Webster strain		
	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
Increased activity of megakaryocytes	+	+	-	+	+	-
Necrosis of intersepta	-	-	-	+	++	-
Necrosis of interstitial	-	+	-	-	-	-
Accumulation of hemosiderin	-	+	-	-	++	-
Depleasia of red pulp	-	-	-	-	++	-

Notes: No specific lesion (-), mild lesion (+), moderate lesion (++), severe lesion (+++), and very severe lesion (++++)

Microscopic examination revealed splenonecrosis, hemosiderin accumulation, and red-pulp depletion (Figure 2).

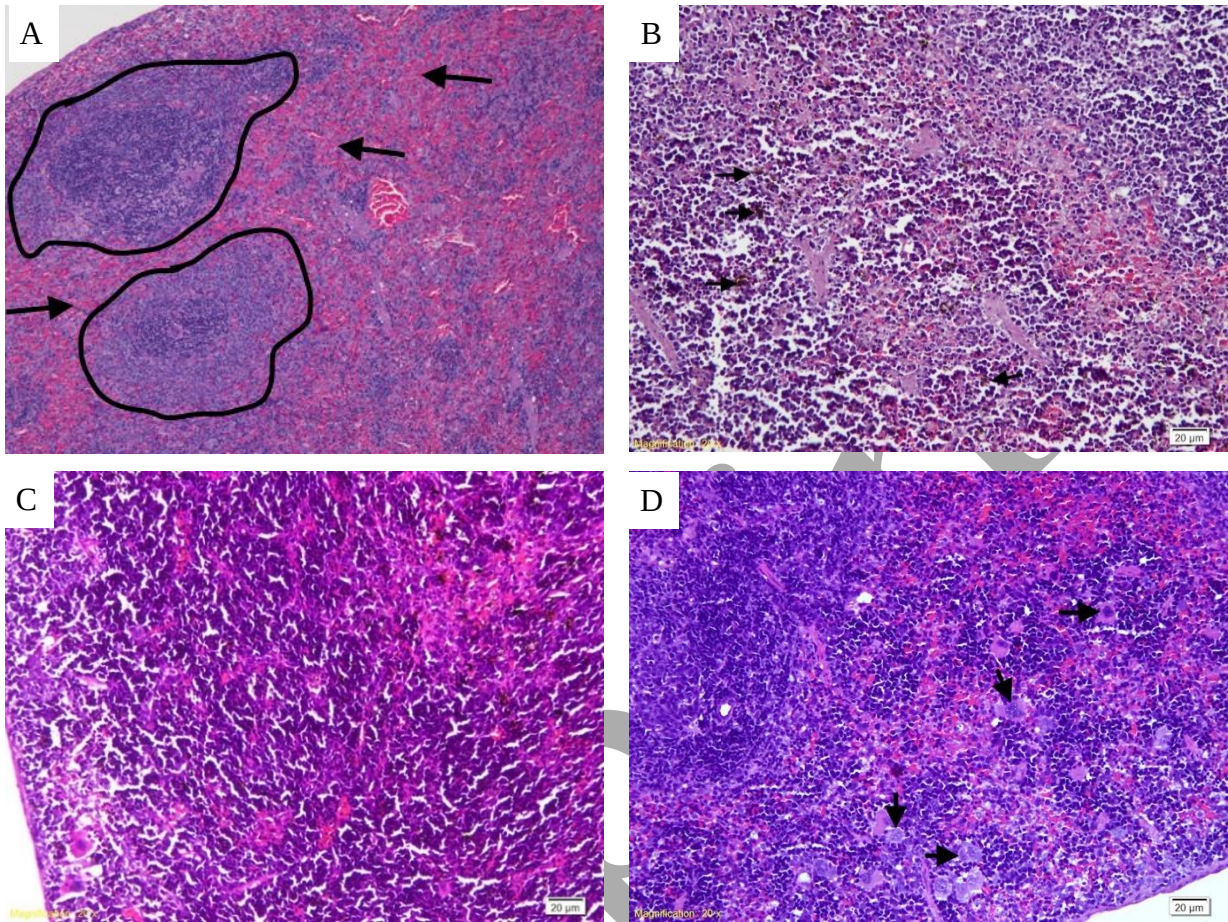


Figure 2. Histopathological changes in the spleens of normal and *P. berghei* ANKA-infected mice

Notes: A. Normal spleen. The white pulp of the spleen (circled) is clearly demarcated from the red pulp (arrows); B. Spleen showing splenonekrosis and hemosiderosis (brown pigment); C. Spleen showing depletion of red pulp (unclear demarcation of white pulp). D. Spleen showing increased megakaryocyte activity/megakaryosis (arrows). HE x200.

The liver showed midzonal hepatic necrosis, mononuclear cell infiltration, karyopyknotic vacuolation of epithelial cells, hemorrhage, dilatation of blood vessels and sinusoids, and hemosiderin accumulation. The representative hepatic lesions and their severity scores are presented in Table 3.

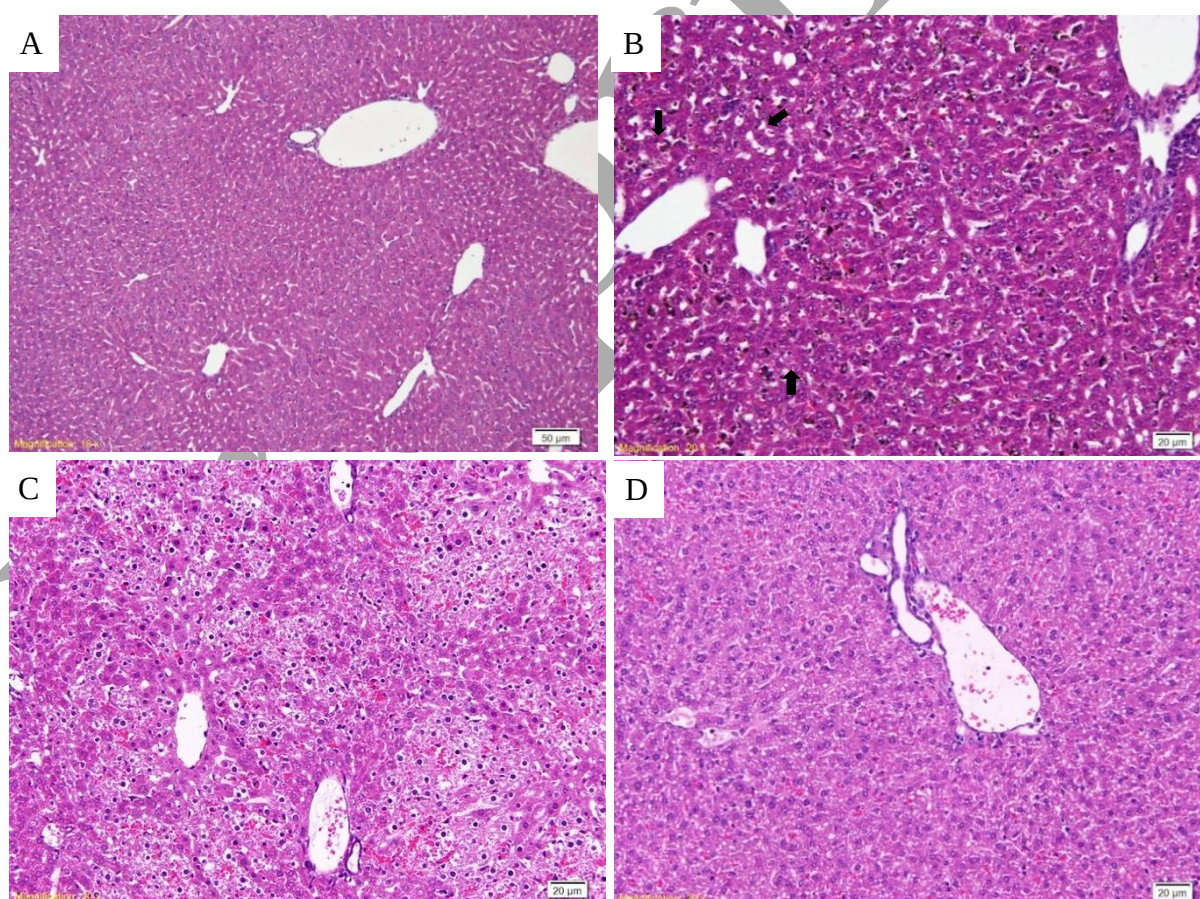
Table 3. Histopathological changes in the liver of C57BL/6 and Swiss Webster Mice infected with *P. berghei* ANKA

Pathological changes	C57BL/6 strain	Swiss Webster strain
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	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
Midzonal hepatic degeneration	-	-	-	+	-	-
Midzonal hepatic necrosis	++	+++	-	-	++	-
Infiltration of mononuclear cells	+	++	-	+	++	-
Karyopyknosis	-	-	-	+	-	-
Vacuolation of epithelial cells	++	+	-	+	+	-
Haemorrhagy	+	+++	-	+	++	-
Accumulation of hemosiderin	-	++	-	-	++	-
Dilatation of blood vessels	-	-	-	-	+	-
Dilatation of sinusoid	+	+	-	+	-	-

Notes: No specific lesion (-), mild lesion (+), moderate lesion (++), severe lesion (+++), and very severe lesion (++++).

Representative micrographs highlight midzonal necrosis, midzonal degeneration, sinusoidal dilatation, hepatitis, and hemosiderosis (Figure 3).



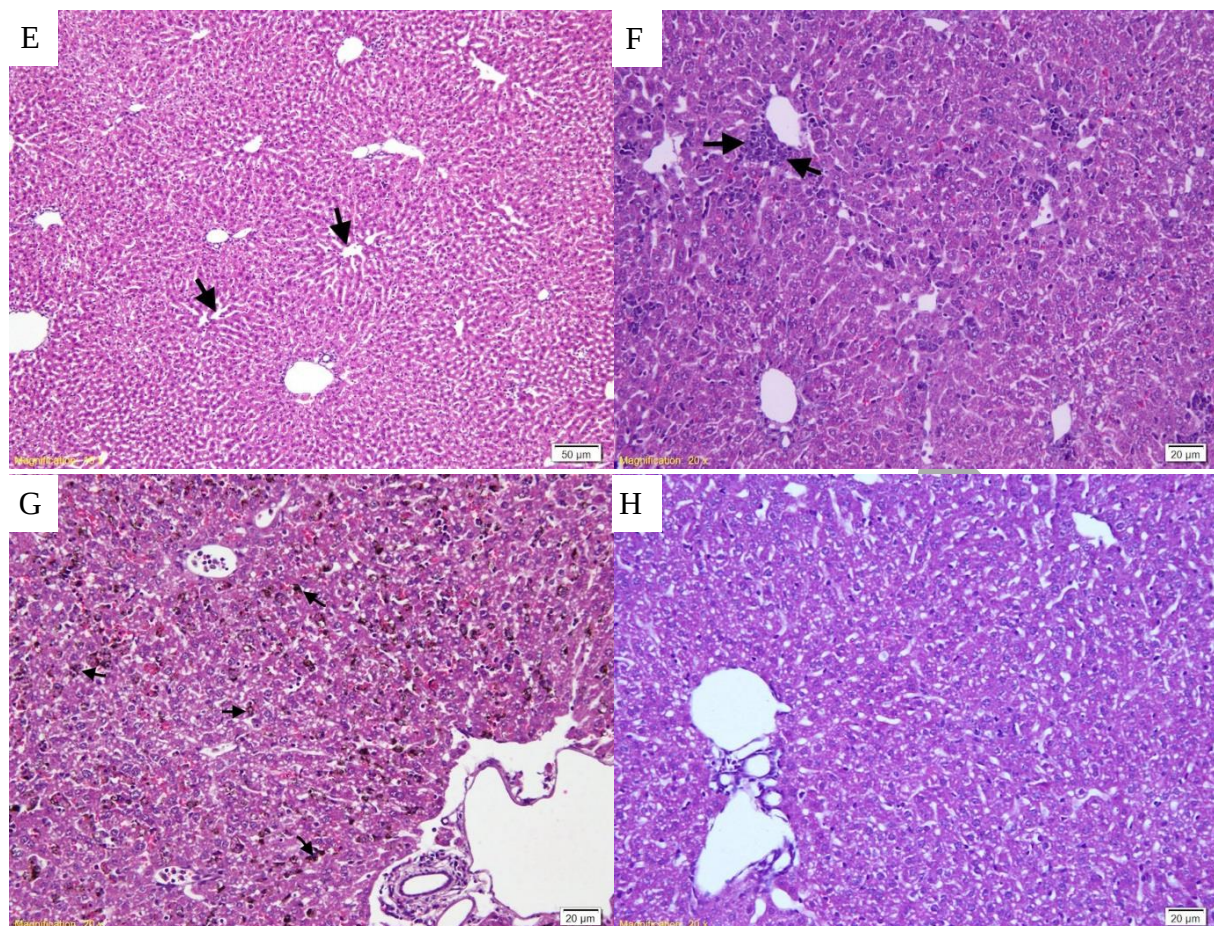


Figure 3. Histopathological changes in the livers of normal and *P. berghei* ANKA-infected mice

Notes: A. Normal liver: Epithelial cells of the liver are arranged radially with a central vein in the middle. No specific lesions. 100x; B. Midzonal necrosis, 200x; C. Necrosis, 200x; D. Midzonal degeneration, 200x; E. Sinusoidal dilatation, 100x; F. Hepatitis, 200x; G. Hemosiderosis (brownish pigment marked with arrows), 200x; H. Hepatocyte degeneration 200x.

The lungs showed lymphocyte infiltration in the peribroncheolus, edema pulmonum, and pneumonia (Table 4). The degree of the lesions was determined using a scoring system.

Table 4. Histopathological changes in the lung of C57BL/6 and Swiss Webster Mice infected with *P. berghei* ANKA

Pathological changes	C57BL/6 strain			Swiss Webster strain		
	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
Peribroncheolitis		+	-	-	+	-
Oedema pulmonum	+	+	-	+	-	-
Pneumonia	-	-	-	-	+	-

Notes: No specific lesion (-), mild lesion (+)

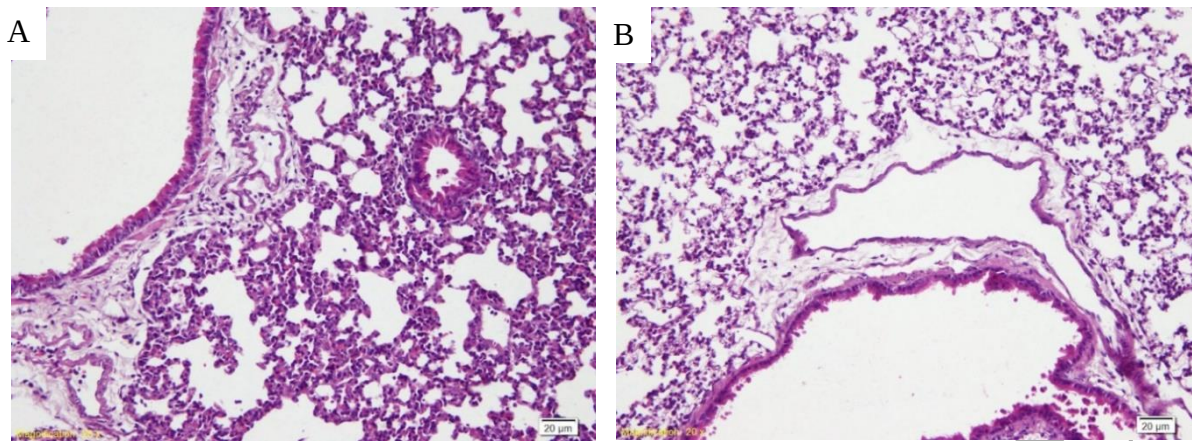


Figure 4. Histopathological changes in the lungs of normal and *P. berghei* ANKA-infected mice
Notes: A. Lung with no specific lesions, 200x; B. Lung edema 200x

4. Discussion:

In contrast to the typical cycle of infection in humans, *P. berghei* ANKA infection in this group of laboratory animals did not go through the sporozoite phase because *P. berghei* ANKA was administered intraperitoneally (ip) and entered the bloodstream without passing through the exoerythrocytic phase in the liver cells [9].

This study observed histopathological changes in the livers, spleens, and lungs of two strains of mice, Swiss Webster and C57BL/6, infected with *Plasmodium berghei* ANKA. The parasite's capacity to adhere to the endothelium of the lungs, liver, brain, and other organs can cause obstruction of small blood vessels, resulting in complications in malaria patients and leading to pathological alterations in the organs afflicted with the disease [10].

The spleen plays a crucial role in the immune response to parasites and in the purification of blood to kill pRBCs during malaria infection [11]. Hemosiderin accumulation was also observed in the present study. This is because the elimination of pRBCs during malaria infection results in pigmentation and the buildup of hemozoin in the tissues, which can also contribute to spleen weight increase [12]. Hemozoin, primarily found outside the hepatocyte cytoplasm and black and brown, is the most significant malaria pigment. Hemosiderin pigment, which contains iron, is formed when hemoglobin breaks down. The spleen, liver, and bone exhibit hemosiderin, followed by phagocytosis of red blood cells, which turn brown and are dispersed in the cytoplasm [13].

Increased megakaryocyte activity was also observed in this study. This is due to the release of pro-inflammatory mediators by splenic macrophages and monocytes, which are exposed to significant amounts of these mediators from the pRBCs that they phagocytose [14].

The spleens of *P. berghei*-infected mice in this study exhibited edema (swelling) caused by the host immune response to malaria infection [15]. This investigation also revealed red pulp depletion. Cells in bursal lymphoid follicles degenerate, a process known as cell depletion. The medulla is typically where the process of cell reduction and decay in the follicle starts, moving to the cortex until it reaches total decay, which causes atrophy and loss of lymphoid follicles [16]. The red and white pulps, which have distinct boundaries (marginal zones), constitute the normal histological structure of the spleen. However, the distinction between the red and white pulps became unclear throughout the infection until the red pulp disappeared.

Liver damage and inflammation are common in *Plasmodium* spp. infection. during malaria development [17,18]. The histopathological changes in the liver in this study included midzonal hepatic degeneration, necrosis, mononuclear cell infiltration, karyopiknosis, vacuolization of liver epithelial cells, hemorrhage, accumulation of hemosiderin, and dilatation of blood vessels.

This is consistent with recent studies that observed hepatic necrosis, leukocyte infiltration, and increased production of pro-inflammatory cytokines in the livers of malaria model mice infected with *P. chabaudi*, *P. yoelli*, and *P. berghei* [19–21]. Importantly, Table 3 shows that C57BL/6 mice developed more severe hepatic necrosis than Swiss Webster mice, highlighting the strain-specific susceptibility to liver injury. Furthermore, the host genetic background influences organ susceptibility; C57BL/6 mice are more prone to severe hepatic necrosis, whereas Swiss Webster mice exhibit more pronounced splenic pathology [22]. This difference strengthens the evidence that the host genetic background influences the severity of hepatic pathology and suggests that C57BL/6 mice are a more suitable model for studying hepatic complications of malaria.

In this study, parasites in the erythrocytic cycle stage of circulation were responsible for the histological alterations in the liver. The parasite first infects hepatocytes in the early erythrocytic phase, then multiplies asexually to release merozoites, leaves the liver, and enters the bloodstream to spread the infection. When parasites enter the bloodstream, liver macrophages multiply and kill infected red blood cells by phagocytosing and destroying hemoglobin. This causes iron to build up in the liver (haemosiderosis), which is typically linked to anemia and can result in cirrhosis of the liver. Necrosis may result from rapid damage to the liver parenchyma that occurs quickly [23].

The response to infection and tissue damage, which results in liver inflammation or hepatitis, is characterized by the production of pro-inflammatory cytokines, such as TNF- α , IL-1, and IL-6[24]. This condition can protect the host against infection but can also worsen liver damage in some cases.

Vacuolization of liver epithelial cells was also observed in this study. A similar finding was reported in another study, in which the development of vacuoles in the liver parenchyma of infected mice was attributed to cell damage and morphological alterations as a natural response of liver tissue to this infection [25].

Although lymphocyte infiltration, pneumonia, and pulmonary edema were identified as lung alterations, Table 4 shows that both C57BL/6 and Swiss Webster mice developed only mild pulmonary lesions, in contrast to the more severe splenic and hepatic pathologies. This relative sparing of the lungs may reflect organ tropism and immune distribution in malaria, where the spleen and liver act as the primary sites of parasite clearance and iron deposition [26]. Nevertheless, pulmonary involvement can occur during *P. berghei* ANKA infections. Previous studies in C57BL/6 mice have shown that *P. berghei* ANKA infection can induce malaria-associated acute lung injury (ALI), characterized by disruption of the alveolar–capillary barrier

and pulmonary inflammation. The severity of ALI is associated with peripheral parasite burden and pulmonary parasite sequestration and varies among mouse strains [27]. Similar changes were observed when Swiss Webster mice were infected with *P. berghei* ANKA[15]. These findings suggest that the extent of pulmonary lesions may be influenced by the degree of parasite involvement in the lungs and the host's susceptibility. Therefore, the mild pulmonary lesions observed in both strains in the present study may reflect relatively limited pulmonary involvement, although the underlying mechanism could not be confirmed based on the parameters assessed.

5. Conclusion:

This study revealed that *Plasmodium berghei* ANKA infection drives strain-dependent organ pathology shaped by immune and metabolic responses. C57BL/6 mice showed more severe hepatic necrosis and inflammation, indicating their suitability for modelling liver complications. Swiss Webster mice exhibited greater splenic damage, making them more representative of splenic pathologies. The lung lesions were mild and similar in both strains. These findings highlight the predictive value of the host genetic background in selecting appropriate models for malaria research.

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Compliance with ethical guidelines

This study was approved by the Health Research Ethics Committee of the National Institute of Health Research and Development (HREC-NIHRD). Ethical approval number: LB.02.01/12/KE.220/2019.

Data availability:

All data analyzed in this study are included in this manuscript.

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Author Contributions:

PRI: Study concept and design, data acquisition, data analysis and interpretation, manuscript drafting, statistical analysis, administrative and technical support, and supervision of the study. YS: acquisition, analysis, and interpretation of data; drafting of the manuscript. SA, SSM, FE, RR, HF, IF, SA, JHS, and RMD: acquisition of data; drafting of the manuscript; and technical and material support.

Conflict of Interest: The authors declare no conflicts of interest in this study. Grammarly was used solely for the English language editing of the manuscript.

Preprint

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