Research Article

Association of Anemia with Malaria Incidence in Long-Tailed Macaques: Implications for Zoonotic Disease Control

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Abstract | Long-tailed macaques (*Macaca fascicularis*), a primate species endemic to Indonesia, frequently interact with humans, increasing their risk of contracting zoonotic diseases such as malaria caused by *Plasmodium spp*. This study aimed to evaluate the incidence of malaria, identify the specific *Plasmodium* species involved, and analyze the relationship between anemia and clinical symptoms in long-tailed macaques. This study has significance for primate conservation efforts and zoonotic disease control strategies by addressing the subclinical impact of malaria in macaques. Thin blood smears stained with 5% Giemsa were examined microscopically at 1000x magnification to identify malaria parasites. Statistical analysis using the Chi-square test assessed the association between anemia and clinical symptoms. A total of 30 blood samples collected in June-July 2020 were analyzed, including samples that previously tested positive for *Plasmodium inui* in the 2020 study (40% positive rate). The results revealed an incidence rate of 2.23% in the population studied. Normochromic normocytic anemia was the most commonly observed type, regardless of malaria status. Clinical symptoms included emaciation, anorexia, diarrhea, and asymptomatic cases. However, Chi-square analysis showed no significant association between anemia and observed clinical symptoms ($p > 0.05$). These findings highlight the need for targeted malaria management strategies in long-tailed macaques that take into account subclinical impacts and broader implications for zoonotic disease control. Such an understanding could provide valuable input into both conservation initiatives and efforts to mitigate human-primate disease transmission.

Keywords | Anemia, Malaria, *Macaca fascicularis, Plasmodium inui,* Zoonoses

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INTRODUCTION

Long-tailed macaques (*Macaca fascicularis*), endemic to Indonesia, play vital ecological roles but face increasing threats from habitat destruction caused by human activities such as deforestation, agricultural expansion, and illegal logging (Afnizar *et al*., 2018). These disruptions force macaques into closer contact with humans, heightening risks of crop damage and zoonotic disease transmission (Oriza and Setyawati, 2019).

One significant zoonotic disease is malaria, caused by *Plasmodium spp.* transmitted through the bites of female *Anopheles* mosquitoes. Long-tailed macaques serve as natural hosts for five *Plasmodium* species, including *P. inui, P. knowlesi,* and *P. cynomolgi* (Zhang *et al*., 2016). *Plasmodium knowlesi*, first documented in humans in Sarawak, Malaysia, highlights the zoonotic potential of primate malaria (Singh *et al*., 2004). While epidemiological data on malaria in macaques in Indonesia remain scarce, their proximity to human populations and forest encroachment suggest a high risk of zoonotic transmission (Ambarita, 2014).

Malaria infections in primates often lead to hematological changes, including anemia, thrombocytopenia, and leukopenia, which reflect the severity of parasitemia (Bashawri *et al*., 2002). Examining blood profiles through Giemsa-stained smears provides critical insights into the type of anemia and overall health status of infected macaques. These data are essential for understanding malaria's impact on primates and addressing its zoonotic potential.

Zoonotic malaria cases in Indonesia have been reported in Kalimantan, Aceh, Sumatra, and Nias, primarily driven by increased human-vector contact (Lempang *et al*., 2022). The potential for *Plasmodium spp.* to act as reservoirs for human infections underscores the urgency of understanding their impacts on primates. This study addresses the relationship between anemia and malaria in long-tailed macaques, filling a critical knowledge gap. While Kesumawati *et al*. (2021) focused on molecular identification of *P. inui* in long-tailed macaques, this study uniquely explores the clinical and hematological impacts, particularly anemia, and investigates the association between anemia and clinical symptoms. This approach provides a more comprehensive understanding of the health dynamics and zoonotic risks associated with malaria in macaques.

By evaluating the incidence of malaria, identifying the specific *Plasmodium* species involved, and investigating the association between anemia and clinical symptoms, this research contributes to the broader goals of malaria elimination and primate conservation outlined by the Indonesian Ministry of Health. The study aims to inform both conservation strategies and zoonotic disease prevention, emphasizing the health dynamics of long-tailed macaques in the face of growing environmental and epidemiological pressures.

MATERIALS AND METHODS

Ethic Approval

This study was approved by the research ethics committee at the Faculty of Medicine, Padjadjaran University, Indonesia, under ethical clearance number 843/UN6.KEP/EC/2024 and IPB Ethics Commision, under ethical clearance number PRC-20-E005. All procedures adhered to ARRIVE guidelines to ensure ethical and humane treatment of animals. Measures to ensure animal welfare during blood sampling included minimizing stress through proper handling techniques, use of trained personnel, and adherence to established veterinary protocols. The study was conducted from August to October 2024 at the Primate Research Center (PRC), IPB University, Bogor, Indonesia.

Study Population

The study population comprised 30 Giemsa-stained blood smear samples from long-tailed macaques (*Macaca fascicularis*), collected during routine monitoring at the Primate Research Center, IPB University, from June to July 2020. These samples were part of a routine health examination program aimed at identifying potential zoonotic diseases in primates, as documented in medical records. The samples are stored in slide boxes at room temperature 22-25°C and kept in a dry, dust-free environment to prevent any degradation or contamination until microscopic analysis. All analyses were performed immediately after sampling, but samples have been stored for 2 years.

Study Variables

The variables used in this study include long-tailed macaques (*Macaca fascicularis*), *Plasmodium spp.*, morphological type of anemia (hypochromic microcytic anemia, normocytic normochromic anemia, macrocytic anemia), and presenting clinical symptoms.

Inclusion and Exclusion Criteria

Data included should be clear giemsa-stained blood smear samples from long-tailed macaques. Data will be excluded if the blood slides are of such poor quality that they cannot be read Table 1.

Table 1: Inclusion and exclusion criteria for data selection.

No	Inclusion Criteria	Exclusion Criteria
1.	Giemsa-stained blood smears from long-tailed macaques.	Blood smears that fail to meet quality standards for analysis (e.g., smudging, incomplete staining, physical damage, or significant contamination).
2.	Blood smears from long-tailed macaques free of other diseases as verified in medical records.	Blood smears from macaques diagnosed with other diseases that could affect hematological parameters or confound malaria results.
3.	Complete and accurate medical records accompanying the blood smear samples.	Samples with incomplete, missing, or unreliable medical records critical for data interpretation.

Blood Smear Preparations

Blood smear slides stained with 5% Giemsa were examined and documented under a Nikon Eclipse 80i microscope using 1000x magnification with immersion oil. Observations focused on identifying *Plasmodium spp.* and assessing hematological abnormalities.

Study Design and Data Analysis

This research design uses descriptive quantitative methods with a cross-sectional approach. Data were analyzed descriptively using SPSS Statistics 25. Frequencies and proportions were calculated, and Chi-square tests ($\alpha = 0.005$) assessed the association between clinical symptoms and anemia in malaria-positive cases.

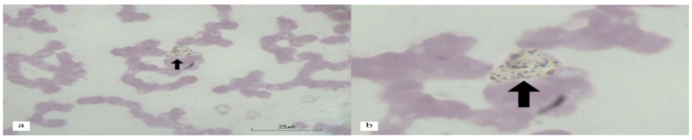

Figure 1: Visualization of *Plasmodium inui* in a blood smear under 1000x magnification.

RESULTS

Previously similar observation with the same thin blood smear samples were made by Kesumawati *et al.* (2021), which identified *Plasmodium inui.* The result of observation of thin blood smear showed that 12 out 30 (40%) samples of long-tailed macaques were morphologically infected with *Plasmodium inui* (Figure 1). The incidence rate of *Plasmodium inui* in this study was calculated at 22.26 per 1000 animals (2.23%) (Table 5), consistent with prior findings in long-tailed macaque populations. However, comparisons to similar studies in different regions or with larger sample sizes are needed to contextualize these findings. For instance, Kesumawati *et al.* (2021) documented a similar prevalence of *P. inui* in long-tailed macaques, while studies in other Southeast Asian populations reported varying rates due to differences in ecological and vector dynamics. Among malaria-positive macaques, normochromic normocytic anemia was the most frequently observed type, occurring in 6 out of 12 cases (50%). Hypochromic microcytic and macrocytic anemia were each identified in 3 cases (25%). These findings are consistent with the hematological changes reported in similar studies on primate malaria but require further comparison to other populations to contextualize the incidence rate. The Chi-square test revealed no significant association between anemia morphology and clinical symptoms such as emaciation, anorexia, or diarrhea (Table 3). The absence of a significant relationship could be attributed to the small sample size, subclinical infections, or other confounding factors such as nutritional status and undiagnosed comorbidities. Moreover, the natural reservoir role of long-tailed macaques and their varying immune responses likely influence the expression of clinical symptoms (Table 2).

DISCUSSION

Evaluation of Malaria Incidence

This study observed a 40% infection rate of *Plasmodium inui* among long-tailed macaques (Kesumawati *et al.*, 2021) (Figure 1), with an incidence rate of 22.26 per 1000 animals (2.23%). The incidence of *Plasmodium inui* infection in long-tailed macaques can be calculated using data related to the number of new cases, population, and a

Table 2: Detailed characteristics of blood smear samples of positive long-tailed monkeys, including clinical symptoms and morphology of anemia.

No	Sample	Sex (M/F)	Age (I/J/A)	Weight (Kg)	Clinical Symptoms	Status (L/D)	Types of Plasmodium spp.*	% Parasitemia	Morphological Types of Anemia (MH/NN/M)	% Anemia
1.	J180808A	F	A	2.48	Asymptomatic	L	P. inui	0.1%	NN	49.58%
2.	J030710B	F	J	2.47	Emaciation, anorexia	D	P. inui	1.2%	NN	67.02%
3.	100409	F	J	2.50	Emaciation, diarrhea, anorexia	D	P. inui	0.2%	NN	73.51%
4.	IA3557	F	I	2.24	Emaciation, diarrhea, anorexia	D	P. inui	0.3%	NN	58.85%
5.	IA3585	F	A	3.39	Asymptomatic	D	P. inui	0.6%	M	57.88%
6.	IA3612	F	J	2.18	Asymptomatic	D	P. inui	0.2%	M	67.66%
7.	C7024	F	J	3.39	Asymptomatic	L	P. inui	0.3%	MH	41.03%
8.	121218	F	A	2.63	Emaciation, diarrhea, anorexia	D	P. inui	0.3%	MH	33.79%
9.	T3953	F	I	2.63	Emaciation, diarrhea, anorexia	D	P. inui	0.5%	M	57.79%
10.	100113	F	J	2.77	Emaciation, anorexia	D	P. inui	0.1%	MH	36.21%
11.	130606	F	I	2.46	Emaciation, anorexia	D	P. inui	0.6%	NN	54.74%
12.	IA3560X	F	J	1.91	Emaciation, anorexia	D	P. inui	0.4%	NN	65.66%

M: Male; **F:** Female; **I:** Infant; **J:** Juvenille; **A:** Adult; **Kg:** Kilogram; **D:** Death; **L:** life; **MH:** Microcytic hypochromic; **NN:** Normocytic normochromic; **M:** Macrocytic; *: (Kesumawati *et al.*, 2021).

Table 3: Detailed characteristics of blood smear samples of negative long-tailed monkeys, including clinical symptoms and morphology of anemia.

No	Sample	Sex (M/F)	Age (I/J/A)	Weight (Kg)	Clinical Symptoms	Status (L/D)	Types of Plasmodium spp.*	% Parasitemia	Morphological Types of Anemia (MH/NN/M)	% Anemia
1.	C4646	M	J	5.44	Diarrhea	D	-	-	NN	91.25%
2.	130912	F	J	2.13	Emaciation, anorexia	D	-	-	M	48.42%
3.	C2951	M	A	6.03	Asymptomatic	L	-	-	NN	60.12%
4.	C6046	F	J	2.86	Bloat	D	-	-	NN	61.92%
5.	C6728	F	J	4.39	Wound	D	-	-	NN	60.18%
6.	W0199	F	J	2.51	Emaciation, anorexia	D	-	-	NN	68.53%
7.	IA3456	F	J	3.53	Asymptomatic	D	-	-	M	62.05%
8.	T3863	F	J	3.70	Weak	D	-	-	M	71.18%
9.	T3932	F	J	2.66	Bloat	D	-	-	M	51.17%
10.	T3943	F	J	2.69	Cough	D	-	-	NN	56.91%
11.	C7456	M	A	6.02	Asymptomatic	L	-	-	NN	66.09%
12.	110405	F	J	2.28	Emaciation, diarrhea, anorexia	D	-	-	NN	58.02%
13.	90728	F	J	2.81	Weak	D	-	-	NN	66.02%
14.	111217	F	J	2.30	Wound	D	-	-	NN	79.85%
15.	IA3594	F	J	2.73	Emaciation, diarrhea, anorexia	L	-	-	NN	72.98%
16.	IA3576	F	J	2.56	Asymptomatic	D	-	-	NN	61.41%
17.	IA3608	F	I	3.30	Emaciation, diarrhea, anorexia	D	-	-	NN	74.18%
18.	IA3495	F	J	3.72	Cough	D	-	-	NN	68.69%

M: Male; **F:** Female; **I:** Infant; **J:** Juvenille; **A:** Adult; **Kg:** Kilogram; **D:** Death; **L:** life; **-:** Negative; **MH:** Microcytic hypochromic; **NN:** Normocytic normochromic; **M:** Macrocytic; ***:** (Kesumawati *et al.*, 2021).

predetermined scale of 1000 (Antunez, 2013) (Table 4). The samples have been analyzed by previous researchers in 2021 using PCR and sequencing methods which also identified *P. inui* (Kesumawati *et al.*, 2021). Although the infection rate appears low, it indicates that malaria remains a common occurrence in macaque populations. Research on *P. inui* infection rates in long-tailed macaques in Indonesia is limited, but globally, *P. inui* infections in long-tailed macaques have been reported in several countries, including Malaysia, Thailand and China (Zhou et al., 2010). Environmental factors, such as the presence of *Anopheles* mosquito vectors and favorable conditions in Indonesia's western regions, play a key role in malaria transmission (Sanjaya and Syafria, 2014). Additionally, the social structure of macaques, characterized by large, close-knit groups, facilitates parasite spread through physical interactions (Darmono et al., 2020).

Table 4: Relationship of anemia incidence to clinical symptoms with malaria

Anemia*Malaria*Clinical Sign	N	%	p-value
	30	100%	0.168

The zoonotic potential of *P. inui* is significant, given its morphological similarities to human malaria parasites. Reports of experimental human infections with *P. inui* further underscore the need for public health vigilance, especially in regions with high human-macaque interactions and *Anopheles* vector populations (Putaporntip et al., 2022). From a conservation perspective, the health of long-tailed macaques is critical as they serve as both ecological indicators and reservoirs for zoonotic pathogens. The persistent threat of malaria in macaque populations, exacerbated by habitat destruction and climate change, could disrupt population dynamics and reduce genetic diversity. Conservation programs should incorporate regular health monitoring and address environmental changes that facilitate the spread of *Plasmodium* spp., such as deforestation and the expansion of human settlements (Darmono et al., 2020) (Table 5). Formula for calculating malaria incidence rates based on observed data.

$$Incidence\ rate = \frac{N}{P} \times K$$

Where;

N: Number of new case.
P: Population at risk.
K: Scale (Antunez, 2013).

Links Researchers

Anemia Type Evaluation

The study identified three anemia types in macaques: normochromic normocytic, hypochromic microcytic, and macrocytic anemia (Figure 2). Normochromic normocytic anemia can be caused by the destruction of erythrocytes due to parasites that carry out their reproductive cycle in erythrocytes and the failure of the bone marrow to produce red blood cells (Chaparro and Suchdev, 2019). Macrocytic anemia can be caused by vitamin B12 deficiency, and folate deficiency during infection can aggravate the condition, as these nutrients are required for maturation. These large red blood cells have inefficient function and a shorter life span, exacerbating the condition of erythrocyte anemia. Microcytic anemia can be caused by iron deficiency, resulting in the bone marrow producing smaller erythrocytes and low hemoglobin, causing microcytic anemia (KT *et al*., 2022).

Table 5: Incidence rate of malaria infection in blood smear preparations.

Total Population(June-July 2020)	Plasmodium spp. Species Infection	Number of Infected Animals	Scale	Incident	%
539	*Plasmodium inui**	12	1000	22.26	2.23

***:** (Kesumawati *et al.,* 2021).

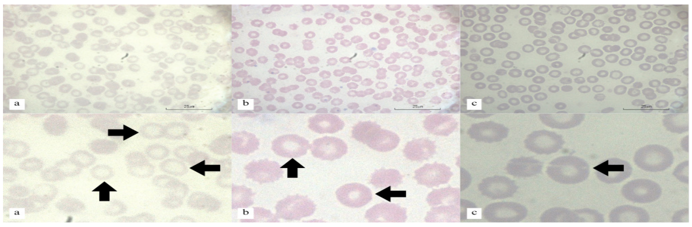

Figure 2: Morphological types of anemia identified in Giemsa-stained blood smears; **a:** Hypochromic microcytic anemia; **b:** Normochromic normocytic anemia; **c:** Macrocytic anemia.

Normochromic normocytic anemia was the most prevalent, occurring in both malaria-positive (50%) and malaria-negative (78%) samples. This suggests that anemia in macaques may arise from factors unrelated to malaria infection, such as malnutrition or other systemic conditions (Sharief, 2023). This study shows that normochromic normocytic anemia is widespread among long-tailed macaques populations, regardless of the presence of microscopically detected parasitic infection.

Interestingly, no morphological differences were observed between *P. inui*-infected and uninfected normochromic normocytic anemia cases (Figure 3). This finding aligns with prior studies indicating that *Plasmodium spp.* infections may not directly influence certain hematological parameters (Rosmanah, 2016). In addition, it is possible that the inflammatory effects of malaria have not been seen due to the early stages of infection. The study conducted by Imwong *et al*. (2019), that *Plasmodium spp.* infection can take place without showing significant signs of inflammation in the early stages.

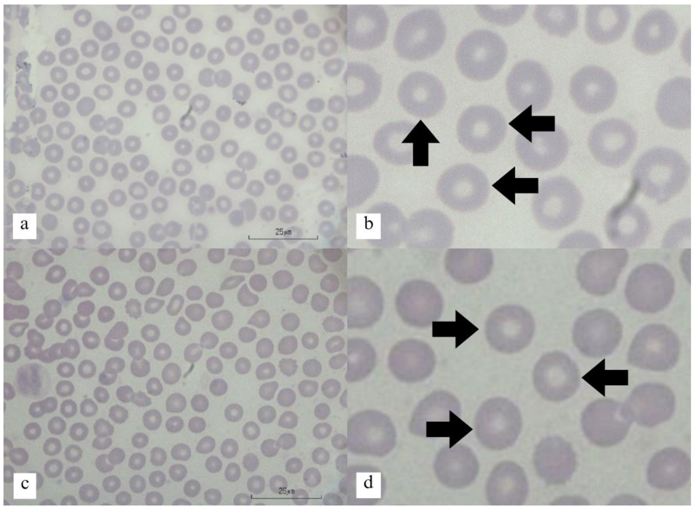

Figure 3: Normocytic anemia identified from positive and negative blood smear preparations by giemsa staining and 1000x magnification; **a, b:** Normochromic normocytic anemia of positive preparations; **c, d:** Normochromic normocytic anemia of negative blood smear preparations.

Evaluation of the Relationship Between Anemia Incidence, Types of Anemia and Clinical Symptoms Associated with Malaria

The clinical presentation of long-tailed macaques infected with *P. inui* varied, ranging from asymptomatic conditions to emaciation, diarrhea, and anorexia. The majority of infected macaques exhibited no specific symptoms, making the infection difficult to recognize. This observation aligns with Sanchez *et al.* (2022), who reported that 85% of *P. pitheci*-positive orangutans showed no clinical symptoms despite prolonged infections.

Although generally asymptomatic, weight loss and anorexia can serve as indirect indicators of malaria. For example, emaciation may stem from decreased appetite due to systemic effects of infection (Sanchez *et al*., 2022; Stokes *et al*., 1983). Similarly, diarrhea may result from intestinal inflammation caused by malaria-induced gastroenteritis (Nopratilova *et al*., 2023). These findings underscore the subtle and multifactorial nature of clinical symptoms in malaria-positive primates.

Juvenile macaques showed higher susceptibility to malaria, with increased parasitemia and more severe anemia compared to adults. This vulnerability is likely due to their immature immune systems and limited erythrocyte regeneration capacity. For instance, sample IA3557, an infant male, exhibited severe anemia (0.3% parasitemia) accompanied by emaciation, diarrhea, and anorexia. These findings align with Peterson *et al*. (2021), who noted that young primates generate stronger pro-inflammatory cytokine responses, potentially exacerbating anemia severity. Another factor is that long-tailed monkeys live in large, tight-knit groups, facilitating the spread of parasites through physical interactions (Darmono et al., 2020).

Interestingly, severe anemia was also observed in malaria-free macaques, particularly in adults (e.g., samples C4646, T3863). This highlights the role of non-malarial factors, such as malnutrition and environmental stressors, in anemia development. Such findings reinforce the importance of considering anemia as a multifactorial condition rather than attributing it solely to malaria.

Statistical analysis using the Chi-square test (df = 6, χ^2 = 9.107, ρ = 0.168) revealed no significant relationship between anemia incidence and clinical symptoms in malaria cases. For instance, sample 121218 exhibited moderate anemia (33.79%) and clinical symptoms of underweight and diarrhea despite a parasitemia level of 0.3%. This lack of correlation may reflect the complex interplay of factors such as malnutrition, comorbidities, or the role of macaques as asymptomatic reservoirs of malaria (Siregar *et al*., 2015; Putaporntip *et al*., 2022).

The results of this study have a limited number of samples (n=30), so it is not necessarily able to represent the long-tailed monkey population in the Bogor City area as a whole. Research in different ecological environments could provide more comprehensive insights. Nevertheless, the data obtained from this study has covered individuals relevant to the research objectives so that the results of the study can be scientifically accounted for because they are based on valid and systematic analysis.

CONCLUSIONS AND RECOMMENDATIONS

This study identified *Plasmodium inui* infection in 40% of analyzed blood smears from long-tailed monkeys, with an incidence rate of 22.26 per 1000 animals (2.23%). Statistical analysis (ρ = 0.168, α = 0.005) showed no significant association between anemia and clinical symptoms in positive malaria cases. The high prevalence of normochromic normocytic anemia suggests multifactorial causes, such as malnutrition or other systemic factors, beyond malaria. These results show that more research needs to be done with bigger samples and over longer periods of time to look into the effects that aren't obvious and come up with better ways to protect primates and stop the spread of zoonotic diseases.

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NOVELTY STATEMENTS

This study examines the impact of Plasmodium inui on anemia in long-tailed macaques (Macaca fascicularis) and its correlation with clinical symptoms, a rarely explored area. Findings reveal no signifi cant correlation, suggesting other contributing factors. Through retrospective and epidemiological analysis, this study enhances understanding of primate malaria in Indonesia and its implications for conservation and zoonotic control.

AUTHOR'S CONTRIBUTIONS

Atria Destriana Fath, Afiat Berbudi, Huda Shalahudin Darusman and Shafia Khairani, concept the research. Atria Destriana Fath, conducted the research under supervision. Huda Shalahudin Darusman, Lis Rosmanah, Upik Kesumawati Hadi, Susi Soviana, Uus Saepuloh, Afiat Berbudi, Shafia Khairani and Atria Destriana Fath, wrote the manuscript. Shafia Khairani, Afiat Berbudi and Huda Shalahudin Darusman, proofread. All authors discussed the results and contributed to the final manuscript. All authors approved for submitting.

Data Availability

The data supporting this study's findings are included within the article and its supplementary materials.

Conflict of Interest

Authors declare that they have no conflict of interest.

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