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Analysis of Plasmodium sp. Parasites in Livestock in Malaria Endemic Areas, Muara Enim Regency

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Abstract. Malaria is still a health problem in developing countries, including Indonesia. Malaria is caused by the bite of the female Anopheles mosquito, the vector of the Plasmodium parasite. Cattle is one of the risk factors as a host for the Anopheles mosquito. The density of Anopheles mosquitoes around the cowshed is higher. The existence of livestock cages in residential areas can cause malaria cases. Lawang Kidul District, Muara Enim Regency is a malaria-endemic area in South Sumatra. The study aimed to detect Plasmodium sp. parasites in cattle in malaria-endemic areas. The method used is analytic observational with a cross-sectional approach. Samples were taken by purposive sampling of 80 samples of cow blood and 80 livestock owners as respondents in Lawang Kidul District, Muara Enim Regency. The results showed that 41.2% of the detected bovine blood samples were found to be Plasmodium sp. The results also showed a significant relationship between cage distance and the habit of using mosquito nets with p-values of 0.001 and 0.003, and it was known that cage spacing was the most dominant risk factor with a p-value of 0.000 and OR=13.21. Based on this, it was concluded that cattle are hosts for the proliferation of Plasmodium sp, so zoonophylaxis can be part of an effective strategy to reduce malaria incidence. Malaria prevention and control efforts are needed for each individual and household.

INTRODUCTION

Malaria is still a significant public health problem, especially in developing countries, including Indonesia [1]. Despite its declining incidence, malaria remains a significant public health challenge where vector control is considered one of the main strategies for disease elimination [2]. Malaria is caused by infection with Plasmodium parasites that live and multiply in infected red blood cells [3]. In Indonesia, malaria is caused by Plasmodium vivax and Plasmodium falciparum [4].

Factors that influence the risk of malaria, namely human behavior and home environmental factors. A change in the factors that cause malaria will affect the risk of malaria. The risk factors for malaria in Indonesia consist of behavioral factors and community attitudes (night activities, use of mosquito repellent, use of mosquito nets), environmental factors, and the physical environment where they live (existence of cattle pens, presence of bushes, presence of watery rice fields, temperature, humidity), the presence of wires in the ventilation, the condition of the ceiling of the house, and the density of the walls of the house) [5]. Since it is known that zoophilic vectors can transmit malaria. Zoonophylaxis has been used to prevent diseases where animals have a role in diverting mosquitoes from biting humans, thereby preventing the occurrence of parasites in humans. The presence of livestock can also increase the possibility of mosquitoes as vectors to breed more and increase malaria transmission (zoopotention). So, the presence of livestock can be an essential variable for the prevalence and risk of malaria. Anopheles mosquitoes tend to like cow blood. The density of Anopheles mosquitoes around cowsheds is higher than in residential houses. They

were catching mosquitoes in cattle pens at a distance of < 50 m, 50 m, 100 m, and 500 m. There are differences in the density of Anopheles mosquitoes based on the distance between the cowshed and residential areas.

There are differences in the density of Anopheles mosquitoes based on the distance between the cowshed and residential areas. Six species of Anopheles vagus were caught, with the number of dilated ovaries of Anopheles vagus mosquitoes as much as one dilated in two, two dilated in one and no dilatation in three [6].

Lawang Kidul District, Muara Enim Regency is a low malaria endemic area with an API value of 1 in the last two years and with the most indigenous cases, namely in 2018 of 0.83 with 56 indigenous cases, in 2019 of 0.31 with 17 indigenous cases, and in 2020 of 0.13 with 8 indigenous cases. Even though there has been a decline, it is still suspected to people who experience positive malaria without symptoms, which does not rule out the possibility of a spike in cases. In addition, until 2020 Muara Enim Regency has not yet received a malaria elimination certificate [7]. The livelihoods of the people there are generally farmers, mining workers, and ranchers, due to environmental conditions including rice fields, mining, and animal husbandry. The existence of livestock cages is one of the risk factors that can cause Anopheles mosquitoes to breed (breeding place) [8].

The hypothesis about the ability of Plasmodium sp. in livestock is exciting, as a first step it is necessary to detect parasites in livestock blood in malaria endemic areas. This has never been done before in South Sumatra, so researchers are interested in analyzing the presence of Plasmodium sp. parasites in cattle in malaria endemic areas, Muara Enim Regency.

METHODS

The area in this study is in Lawang Kidul District, Muara Enim Regency, which consists of 7 villages, namely Darmo, Keban Agung, Tanjung Enim, South Tanjung Enim, Tanjung Market, Tegal Rejo, and Lingga. The cross-sectional study was conducted from June 2022 to July 2022. The sampling technique used in this study was purposive sampling. The calculation results of the sample obtained a total sample of 80 samples consisting of 80 samples of cow blood that had been >3 months in Lawang Kidul District, Muara Enim Regency, and 80 livestock owners respondents. The variables analyzed included the distance between the cage and the residence, the breeding place for mosquitoes, smoking habits, the habit of using mosquito nets, the habit of going out at night, and the number of animals in the cage. The sample size is calculated using the sample formula (1) derived from [9] to test the two different hypothesis proportions.

$$n = \frac{z_{1-\frac{\alpha}{2}}\sqrt{2P(1-P)} + z_{1-\beta}\sqrt{P1(1-P1) + P2(1-P2)}}{(P1 - P2)^2} \quad (1)$$

Note:

- n : Number of samples
- $z_{1-\alpha/2}$: Standard derivative ($z = 1.96$ for $\alpha = 0.05$)
- $z_{1-\beta}$: Standard derivative β test strength 80 % = 0.84
- α : Level of significance (5 %)
- $1 - \beta$: Test strength (80%)
- P1 : Proportion of Plasmodium sp. of parasites in livestock based on respondents using mosquito nets (P1 = 0.125) [13]
- P2 : Proportion of Plasmodium sp. of parasites in livestock based on respondents not using mosquito nets (P2 = 0.013) [13]
- P : $\frac{P1+P2}{2} = \frac{0.125+0.013}{2} = 0.069$

The dependent variable was the presence of Plasmodium sp. parasites in livestock (Plasmodium sp. was found or not). This cow blood sample was made into thick and thin blood slides and then microscopically examined which is the gold standard for malaria examination. The examination was carried out at the Palembang Health Laboratory Center, which is an accredited national reference laboratory for malaria testing. The dependent variable was summarized as a category with a value of one if Plasmodium sp. parasites were found in livestock. Before collecting information using a valid and reliable questionnaire, the validity and reliability of the questionnaire were tested.

The independent variables in this study consisted of the distance from the cage to the residence, the breeding place for mosquitoes, smoking habits, the habit of using mosquito nets, the habit of going out at night, and the number of

animals in the cage. All independent variables were coded 1 as the risk category and code 2 as the no risk category. The distance between the cage and residence is 10 meters (code 1) and >10 meters (code 2), the presence of breeding places exists (code 1) and does not exist (code 2) around the cage, the respondent's habit of smoking around the cage if not (code 2) code 1) and yes (code 2). The respondent's habit of using a mosquito net is good and does not tear when sleeping if it is not (code 1) and yes (code 2). The respondent's habit of going out at night if yes (code 1) and no (code 2), and the number of animals in the cattle pen is >10 heads (code 1), and ≤10 heads (code 2).

Univariate Analysis

Univariate analysis was used to describe the data performed on each variable and research results. The data are presented in the frequency distribution table. The univariate analysis in this study was to analyze the frequency distribution of each research variable, namely the distance from the cattle pen to the residence, the presence of breeding places for mosquitoes, smoking habits, the habit of using mosquito nets, the habit of going out at night, the number of animals in the cage. and the presence of Plasmodium sp. parasites in livestock.

Bivariate Analysis

Bivariate analysis was used to determine the relationship between the independent variable and the dependent variable, which was analyzed using the Chi-Square test by comparing the probability value (p- value) to the value of = .05. If the p-value is .05, then Ho means that it is rejected, meaning there is a significant relationship between the independent and dependent variables. If the p-value is > 0.05, then Ho is accepted or fails to be rejected, meaning there is no significant relationship between the independent and dependent variables

Multivariate Analysis

Multivariate analysis was used to determine the relationship between variables to obtain the most dominant variable influencing the presence of Plasmodium sp. parasites in livestock using multiple logistic regression. If the bivariate analysis results show a p-value of .25, the research variables can be included in the multivariate analysis modeling. On the other hand, if the bivariate analysis results show a p-value > .25, then the variable cannot be included in the multivariate modeling.

RESULT AND DISCUSSION

Result

Univariate analysis was used to examine each research variable: the presence of Plasmodium sp. parasites in livestock, the distance from the cattle pen to their residence, the presence of breeding places for mosquitoes, smoking habits, the habit of using mosquito nets, the pattern of going out at night, and the number of animals in the house. In the next step, bivariate analysis was used to examine the association between the distance between the cattle shed and the place of residence, the presence of breeding places for mosquitoes, smoking habits, the habit of using mosquito nets, the pattern of going out at night, and the number of animals in the cage for the presence of parasites—Plasmodium sp. in livestock. Multivariate analysis was also carried out to determine the dominant factor for the existence of Plasmodium sp. parasites in livestock.

Regency, it was found that more blood samples did not find Plasmodium sp. parasites (58.7%) than those found (41.3%). The results also showed that 33 (41.3%) of the 80 respondents observed had a residence 10 meters from the cattle barn, and 47 (58.7%) respondents lived >10 meters. The observed results also showed that the majority of the cages were found to be breeding places (76.2%), and the majority of respondents carried out smoking habits around the cells (85.0%). The interviews and observations also showed that most respondents did not use mosquito nets while sleeping (88.8%), and more did not go out of the house at night (58.8%). Based on observations, most animals in the cattle pens amounted to 10 heads (68.8%).

TABLE 1. Univariate and Bivariate Analysis (n=80).

Research variables	n=80	95% CI	PR; 95% CI	p-value
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Presence of Plasmodium sp. in livestock				
Found	41.3			
Not found	58.7			
Cage Distance				
≤10 meters	41.3	3.917-32.364	3,798 (2.036-7.085)	.000
>10 meters	58.7			
Breeding place				
There is breeding place	76.2	.441-3.681	1.157 (0.600-2.231)	.857
There isn't breeding place	23.8			
Smoking habit				
No	15.0	.185-2.450	.782 (0.335-1.822)	.752
Yes	85.0			
Habit of using mosquito nets				
No	88.8	.008-0.575	.396 (0.268-0.586)	.003
Yes	11.2			
Habit of going out at night				
Yes	41.2	.828-5.120	1.513 (0.902-2.539)	.183
No	58.8			
Number of Animals				
>10 tails	31.2			
≤10 tails	68.8	.354-2.430	.957 (0.540-1.695)	1.000

The results of the bivariate analysis showed a significant relationship between cage spacing (p-value 0.000) and the habit of using mosquito nets (p-value 0.003) with the presence of Plasmodium sp. parasites in livestock in Lawang Kidul District, Muara Enim Regency.

TABLE 2. Multivariate Analysis (n=80).

Research variables	Simple logistic regression PR (95% CI) ^a	on analysis P-value	Multiple logistic regressi PR (95% CI) ^b	on analysis P-value
Cage Distance				
≤10 meters	11.259 (3.917-32.364)	.000	13.210 (3.577-48.783)	.000
>10 meters				
Habit of using mosquito nets				
No	.068 (.008-0.575)	.002	0.392 (0.038-4.005)	.430
Yes				
Habit of going out at night				
Yes	2.059 (.828-5.120)	.118	3.679 (1.045-12.957)	.043
No				

The results of multivariate analysis showed that the most dominant risk factor was cage distance with a p-value of 0.000 and PR value of 13.21.

Discussion

Based on the results of univariate analysis, it showed that from 80 samples of cow blood located in Lawang Kidul District, Muara Enim, The existence of large livestock such as cows is used as hosts because they can provide sufficient food (blood) for malaria vectors, thereby reducing the frequency of

vectors biting humans. As an alternative, the presence of livestock can also increase the availability of food (blood), and mosquitoes can survive longer, thereby increasing the risk of malaria transmission [10]. Based on the results of research from 80 large livestock, as many as 33 tails (41.3%) were found to be Plasmodium sp. parasites consisting of 30 cows at the trophozoite stage of Plasmodium falciparum (Pf) and three cows found to be at a combined trophozoite stage of Plasmodium falciparum and Plasmodium vivax (mix Pf+Pv). Trophozoites appear morphologically similar to Plasmodium sp. in humans. Previously, research on Plasmodium sp. parasites on livestock (cows, sheep, goats) had been carried out in Iraqi Livestock, with as many as 40 infected livestock, and Plasmodium

sp. was found in their blood smears [11]. In Indonesia, *P.falciparum* and *P.vivax* were found in 270 animals from the villages of Gaura and Fakfak [19].

The identification of the *Plasmodium* sp. parasite is carried out using the microscopic method which is the gold standard for malaria examination, by competent and WHO certified personnel. The trophozoites found were morphologically similar to *Plasmodium* sp. in humans. Based on the results, *Plasmodium falciparum* trophozoites were most commonly detected in livestock, which was the cause of the most severe infection and resulted in the highest mortality rate. This proves that in Lawang Kidul District there are still many vectors of malaria.

Based on bivariate analysis showed that there was a significant relationship between cage spacing and the presence of *Plasmodium* sp. parasites in livestock (p -value $< .05$). The results of this analysis are following research conducted in Eastern Indonesia which refers to the 2010 Riskesdas analysis, which states that there is a relationship between respondents who live around large farms and the incidence of malaria [12]. In the examination of blood preparations, as much as 4.12% found *Plasmodium* sp. in the blood of Etawa goats whose cage was located <10 meters away, while not found at a distance of >10 meters [13].

The existence of cattle pens is one of the factors that influence the transmission of malaria. Meanwhile, in a study conducted in Lahat Regency, South Sumatra, it was written that there was no relationship between the presence of livestock around the house and the incidence of malaria [14]. The laying of cattle pens ≤ 10 meters around the residence proves that cows which are cattle barriers, can function as zoophylaxis where *Plasmodium* sp. mosquitoes like cow blood so that they divert rather than bite human blood. However, special attention needs to be paid to maintaining the cages and livestock so that they do not have the impact of becoming a zoopotential [4].

Based on bivariate analysis, there was a significant relationship between mosquito nets and the presence of *Plasmodium* sp. parasites in livestock (p -value $< .05$). This is in line with previous studies that the level of coverage of using mosquito nets independently was associated with a 19.1% reduction in the risk of *Plasmodium falciparum* ($p < .001$) in residences compared to those who did not use mosquito nets [15]. Another study also stated a relationship between the prevalence of malaria cases in livestock rearing and using mosquito nets [16]. In contrast to research in Lahat Regency, South Sumatra, there is no relationship between mosquito nets and malaria incidence [14]. The same study in Sangaji Village showed that there was no relationship between the use of mosquito nets and the incidence of malaria (p -value =0.935). Based on observations, the mosquito nets used by the respondents were ordinary, not insecticide-treated. Meanwhile, from the results of interviews for respondents who do not use mosquito nets, because they feel uncomfortable and "hot". Respondents prefer to use a fan. In line with research in the working area of the Kedungmundu Health Center that all residents sleep using a fan that aims to repel mosquitoes [17]. This is also the same as the theory which says that to avoid contact with mosquitoes' various ways can be done, such as using mosquito rackets, turning on fans, using mosquito repellent, and using mosquito nets while sleeping. Mosquitoes as zoophilic vectors, and large livestock feeds are very effective in reducing human exposure to mosquito bites [18].

Meanwhile, for the results of bivariate analysis on breeding place variables, smoking habits in around the cage, the habit of going out at night, and the number of animals in the cage had no relationship to the presence of the *Plasmodium* sp. parasite in livestock (p -value > 0.05). This research is in line with the highest endemic areas in Central Java where there is no relationship between the distance between breeding places and the incidence of malaria [20]. Lawang Kidul District, Muara Enim Regency has many breeding places for *Anopheles* sp. (breeding places). Malaria vector breeding places such as stagnant water, dry forest, ditches, swamps, shrubs [21]. Based on the results of observations, it was found that there were breeding places for mosquitoes around the cage, namely standing water, shrubs, swamps, and dry forest. At the location of livestock pens, almost half of the respondents raised fish that eat larvae independently in large ponds, these fish help eat mosquito larvae or larvae. However, there have been no spraying and vector control activities in the past year.

Respondents in Lawang Kidul District have a habit of burning wood, dry leaves, straw, aiming to make efforts to repel mosquitoes. It can be seen that there are remains of wood burning places and dry leaves around the cattle pens. This is justified by the respondents that they do the smoking habit every day in the afternoon or evening. Fumigation is one way to control the rate of mosquito spread, if done correctly [22]. The fumigation that is carried out around the cattle pens in Lawang Kidul District is not accompanied by fumigation around the residence, so we can watch out for the return of mosquitoes to residential areas. Chemical control such as spraying (fogging) around cages and residences as well as applying insecticide to large livestock will be more appropriate in controlling the spread of *Anopheles* sp.

Then these results are also in line with research in Lahat Regency, South Sumatra that there is no relationship between the habit of leaving the house at night and the incidence of malaria [14]. Similar studies state that the habit of going out at night is not significantly related to the incidence of malaria ($p=0.439$) [23]. Based on the results of the interviews, respondents who had house habits at night carried out activities in the cattle pens such as cleaning the stables, fumigating and feeding the livestock. Some of the respondents who carried out their activities in the cattle

sheds wore closed clothes when leaving the house, long pants and long-sleeved shirts so as to prevent malaria mosquito bites. This is consistent with research in one of the sub-districts in Mandailing Natal Regency, which is an endemic area, which states that there is a relationship between the density of clothes and the incidence of malaria ($p = 0.013$) [24].

Many factors can affect the density of *Plasmodium* sp. mosquitoes, causing the high incidence of malaria. A sparsely populated place to live, but the presence of livestock means there will be fewer mosquitoes that feed on human blood. Vector population density is one of the key factors that affect the intensity of infection and the prevalence of this disease [12]. Studies in Africa, the zoophylactic effect of livestock found two situations where the vector population density did not increase with the presence of livestock or when the number of livestock and vector availability was high enough that the increase in vector density was counteracted by the transfer of bites from humans to animals. The most appropriate conditions for testing on livestock given insecticides [25]. In a study in Jatirejo, Purworejo, the livestock bait method found more density of *Plasmodium* sp. mosquitoes than bait for people leaving the house [26].

The results of the multivariate analysis found that the dominant risk factor affecting the presence of *Plasmodium* sp. parasites in livestock was the distance between the cage and the place of residence. The OR value stated that the cage distance of <10 meters was at risk of increasing the risk of *Plasmodium* sp. parasites found in livestock by 13.21 (CI: 3.577-48.783) times greater. In this study, most respondents lived within 100m of the cattle barn. *Anopheles* mosquitoes tend to like cow's blood, so the percentage of malaria in people who keep cows is lower than in people who do not. A study in the cattle breeding area of Montong Beter Village, Sakra Barat District, East Lombok Regency, found a difference in *Anopheles* mosquito density based on the distance between the cowshed and residential areas [6].

Based on interviews and observations, it was found that the majority of respondents and residents of the settlements lived close to the cattle shed. Research conducted in the Kaligesing District of Purworejo found that 4.12% of blood preparations were *Plasmodium* sp. in the blood of Etawa goats whose cages were located <10 meters away, while none were found at a distance of >10 meters [13]. The existence of residences around large animal farms is a risk factor for the incidence of malaria [12]. Figure 1 and Figure 2 show microscopic picture of the trophozoite stages of *Plasmodium falciparum* and *Plasmodium vivax* in cattle.

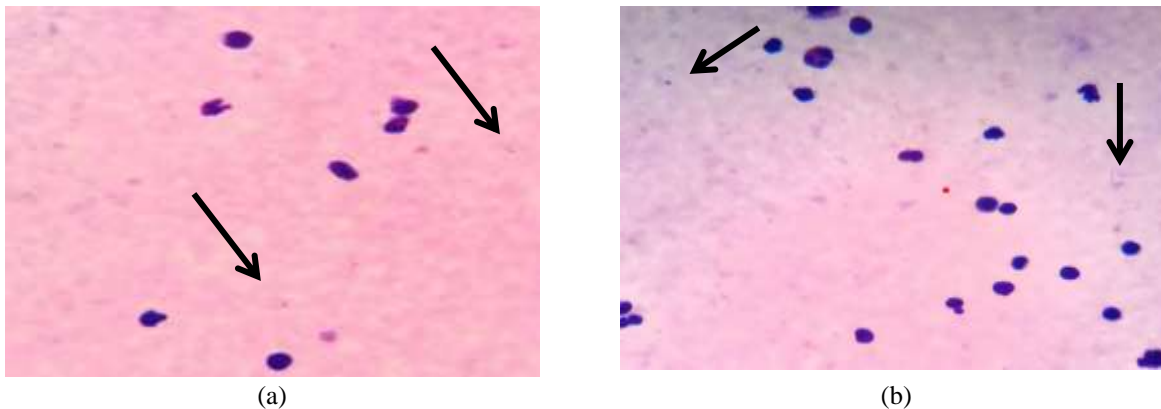


FIGURE 1. Microscopic results of thick preparations.

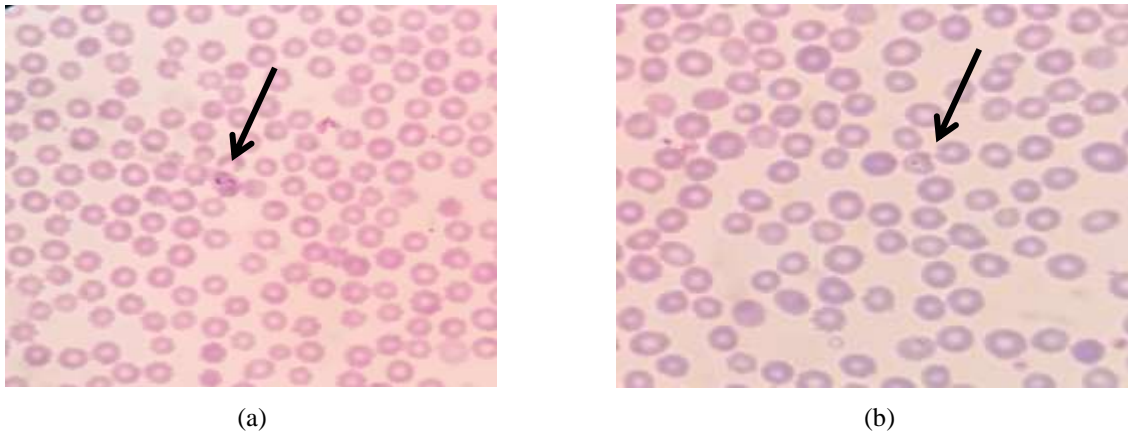


FIGURE 2. Microscopic results of thin preparations: Trophozoite *Plasmodium vivax* (a), Trophozoite *Plasmodium falciparum* (b).

CONCLUSION

The cage distance is the dominant risk factor for *Plasmodium* sp. parasites in livestock in Lawang Kidul District, Muara Enim Regency. Zooprophylaxis can be part of an effective strategy to reduce malaria transmission under specific ecologies and geographies. For this reason, malaria prevention and control efforts are needed for each individual and household. Using mosquito nets when sleeping and mosquito repellent can reduce the frequency of malaria mosquito bites in the house. Houses adjacent to cattle pens need protection, such as installing wire netting on the ventilation section and eliminating gaps between the roof and the walls of the house so that it becomes a barrier to the entry of malaria mosquitoes into the house. I wore clothes and trousers to protect against mosquito attacks at night. Applying insecticides to livestock can contribute to the integrated control of malaria in Muara Enim Regency.

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