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Research Article

Bed-nets Hour Density after a Usage of Three Years of Long-lasting Insecticide-treated Nets in Malaria Pre-elimination Areas in Ngadirejo Village, Purworejo District, Indonesia

Didik Sumanto[1], Wahyu Aji Safrudin[2], Wahyu Handoyo[2,3], Sayono[1]

[1]Department of Epidemiology, Faculty of Public Health, Universitas Muhammadiyah Semarang.
[2]Faculty of Public Health, Universitas Muhammadiyah Semarang.
[3]Public Health Centre, Semarang.
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I N F O

Corresponding Author:
Didik Sumanto, Department of Epidemiology, Faculty of Public Health, Universitas Muhammadiyah Semarang.
E-mail Id:
didik.24272@gmail.com
Orcid Id:
https://orcid.org/0000-0003-4714-4901
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A B S T R A C T

Background: Malaria, caused by the genus *Plasmodium,* is still a global health problem. Its transmission and spread are strongly influenced by the presence of the *Anopheles* vector. One of the efforts to reduce cases is the distribution of long-lasting insecticide-treated nets to the residents of malaria-endemic areas. This study aims to measure the density of mosquitoes perched on insecticide-treated bed nets after three years of usage.

Method: A cross-sectional study was conducted to observe mosquitoes perching on insecticide-treated bed nets at night and calculation of mosquito density data in units of mosquitoes/LLINs/hour.

Results: The number of mosquito bites before bedtime in respondents reached 72.5%, while the number of bed nets infested with mosquitoes reached 74.4%. The density of mosquitoes perched varied between 1 and 10 mosquitoes/LLINs/hour with the highest density proportion of 3 mosquitoes/LLINs/hour.

Conclusion: LLINs that have been used for three years were found to be less effective at killing and preventing mosquitoes. It is advisable to re-dip with the insecticide doses to match the local vector susceptibility level by officer assistance so that the mosquito nets still function properly.

Keywords: Mosquito Density, LLINs, Pre-elimination, Malaria

Introduction

Malaria is caused by a blood parasite of *Plasmodium* spp. which is transmitted through the *Anopheles* mosquito vector.[1] The Kaligesing Subdistrict, Purworejo Regency, which borders Kulonprogo Regency, is located in the Menoreh Hills area, which is a malaria-endemic area. Until 2014, the Kaligesing area in the Village of Jatirejo, Kaligono, and Kaliharjo still had annual parasitic incidence (API) values of 1.46‰, 0.87‰, and 0.81‰ respectively.[2] Two years later, Jatirejo Village has succeeded in reducing cases to 0.32‰.[3] The last indigenous malaria cases were recorded

in the Village of Jatirejo, Ngadirejo, and Hardimulyo in 2018 with the most cases found in Ngadirejo Village.[4]

Controlling the *Anopheles* population in the home is a good effort to reduce the potential bites in family members. Prevention efforts can be made by increasing awareness of mosquito bite risk at night, including the use of insecticide-treated bed nets. The distribution of Long Lasting Insecticide-treated Nets (LLINs) to the Ngadirejo Village community in 2018 had a positive impact on controlling the population of *Anopheles*. This is evidenced by the absence of new indigenous cases until the first quarter of 2021.[5] This means for almost three years, Ngadirejo Village had managed to maintain zero indigenous malaria cases. This result can serve as an example for the Bener sub-district Purworejo which reported an increase in malaria cases in the second quarter of 2021.[6]

LLINs are long-lasting insecticide-treated nets with an effective insecticide level that lasts a minimum of three years. They have the power to kill perched mosquitoes and are resistant to 20 washes in laboratory testing.[7] After being washed, LLINs still maintain their level of effectiveness in reducing the Man Hour Density (MHD) of mosquitoes.[8] The effectiveness of LLINs is influenced by people's behaviour in their use, such as sleeping habits outside the home, perceptions of their reliability[9], duration of use[10], the type of insecticide added[11], and the support from the community to use them[12]. This study aims to evaluate the effectiveness of LLINs in killing and repelling mosquitoes after three years of use by counting the number of mosquitoes perched.

Material and Method

The study site, Ngadirejo Village, was one of the malaria pre-elimination areas. Indigenous malaria cases were still here until 2018. The uniqueness of malaria cases here is only localised in Kembangsoko Hamlet. The location is directly adjacent to the tea garden area in the Kulonprogo Regency. This village is part of the expanse of Menoreh Hill and has been known as a malaria-endemic area for a long time. All of the respondent's families received 2 units of LLINs each in 2018. The bed nets' size distributed was 200 cm x 200 cm x 200 cm. Data collection was carried out between April and September 2021. Ethical approval was issued by the Ethics Commission of the Faculty of Medicine, Universitas Muhammadiyah Semarang number 029/EC/FK/2020.

A cross-sectional study was conducted to directly observe the density of mosquitoes on insecticide-treated bed nets, condition of the bed nets, and to obtain information about the usage and care of bed nets during the period of use. The research sample was all Kembangsoko Hamlet community in Ngadirejo Village, totalling 55 families, but only 40 families were willing to become respondents. Fifteen families were reluctant to become respondents due to direct observations in the calculation of the number of mosquitoes perched on the bed nets being done during nighttime sleep which they felt might interfere with the resting time.

The density of mosquitoes in mosquito nets (BHD) was conceived as a measure of mosquito density to estimate the potential risk of mosquito bites on people indoors. The basis for calculating this density is the Man Hour Density (MHD) calculation formula.[13] The BHD calculation is based on the number of mosquitoes that land on a mosquito net installed and used for sleeping by family members at night. The respondent's family sleeping in the mosquito net is assumed to be someone's bait. The types of mosquitoes observed were only differentiated by genus by observing the perch behaviour of adult mosquitoes. The position of the mosquito's body when perched[14] is the basis for the identification of the mosquito genus grouped into *Anopheles sp* and non-*Anopheles sp*. The mosquito number calculation was done every hour with a duration of observation of 45 minutes and 15 minutes of rest. The mosquitoes were observed from 7.00 pm-04.00 am. The final result was the average obtained from the sum of all observations of the number of *Anopheles* mosquitoes perched divided by the number of hours of observation, expressed in units of mosquitoes/LLINs/hour. Mosquito density is calculated as Bed-Nets Hour Density (BHD).

Results

Most of the respondents were husbands. They were the head of the family with the most common type of work being farming, although the topography of the land in this village is relatively hilly and not flat (Table 1). The level of welfare of the respondents lies majorly in the middle group because there are no more soil-floored houses. All of them had tiled floors or at least cement plaster. All the walls of the house were made of cement bricks, nothing was made of wood or bamboo. Almost half of the respondents raised livestock in their home environment. Most of them did not like to use mosquito repellent at night.

In 2018, the research site was the target of a programme for distributing long-lasting insecticide-treated nets (LLINs). Each family received two mosquito nets to reduce the threat of *Anopheles* bites at night. It was found that one family did not receive the insecticide-treated bed nets because it was a new family which at the time of the programme was still at their parents' house. The two insecticide-treated mosquito nets distributed to each family turned out to be not all used by family members for sleeping. As many as 76.9% of respondents said that some family members slept without mosquito nets for various reasons, including feeling hot and not sleeping freely. All respondents had used the bed nets, but not all family members slept in bed nets. The longest usage of a bed net for a night's sleep was nine hours. All respondents stated that they had washed

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their bed nets. The first washing was mostly done after using it for a duration of 6 months. The method of washing bed nets used by respondents was to soak them in soapy water before washing and rinsing them with clean water. Most of the respondents were drying the bed nets in direct sunlight (Table 2).

The attack of mosquito bites before sleeping in the respondent's family is still relatively high, reaching 72.5%. These data were obtained by asking respondents and their families before observing mosquitoes on mosquito nets. This high incidence of mosquito bites could occur because the respondent's family members had not yet entered the mosquito net. An interesting phenomenon was found in this observation, namely between the availability of blood feed and mosquitoes landing on mosquito nets. Even though the mosquito blood feed source is abundant from family members who sleep without mosquito nets, the proportion of mosquito nets infested by mosquitoes is still relatively high, reaching 74.4% (Figure 1). The freedom of the mosquitoes to land on the nets is an indication that the insecticide content in the nets has decreased. It is also possible that the insecticides in the nets have disappeared due to the washing carried out by the respondents. The limitation of this study is that the bioassay test for insecticide content in mosquito nets was not carried out.

Table 1.Characteristics of Respondents (N=40)

Observation Variable (n)	Frequency	Percentage
Gender		
Male	34	85
Female	6	15
Profession		
Not yet working	1	2.5
Labourer	10	25
Farmer	25	62.5
Private	3	7.5
Odd job	1	2.5
House floor material		
Ceramic	27	67.5
Plaster	13	32.5
House wall material		
Cement wall	40	100
Wood/ bamboo	0	0
Presence of livestock		
Yes	18	45
No	22	55
Use of mosquito repellent in the room		
Yes	4	10
No	36	90
Use of mosquito repellent lotion at night		
Yes	2	5
No	38	95

Table 2.Usage of Long-lasting Insecticide-treated Nets (LLINs)

Variable	Frequency	Percentage
Distribution of LLINs (n=40)		
Received	39	97.5
Not received	1	2.5
LLINs usage (n=40)		
Yes	39	97.5
No	1	2.5
The habit of sleeping in LLINs (n=39)		
All family members	9	23.1
Some family members	30	76.9
Usage duration of LLINs (n=39) (hours)		
7	1	2.6
8	9	23.1
9	12	30.8
10	6	15.4
11	5	12.8
12	2	5.1
13	1	2.6
15	3	7.7
LLINs washing (n=39)		
Yes	39	100
No	0	0
Washing the LLINs for the first time (n=39) (month)		
First	2	5.1
Second	4	10.3
Third	5	12.8
Fourth	1	2.6
Sixth	26	66.6

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Twelfth	1	2.6
Method of washing (n=39)		
Soaking with soap	39	100
No soaking with soap	0	0
Frequency of LLINs washing (n=39)		
Every 3rd month	11	28.2
3-6 months	23	59.0
More than every 6th month	5	12.8
Method of drying LLINs (n=39)		
Direct sunlight	36	92.3
Aerated	3	7.7

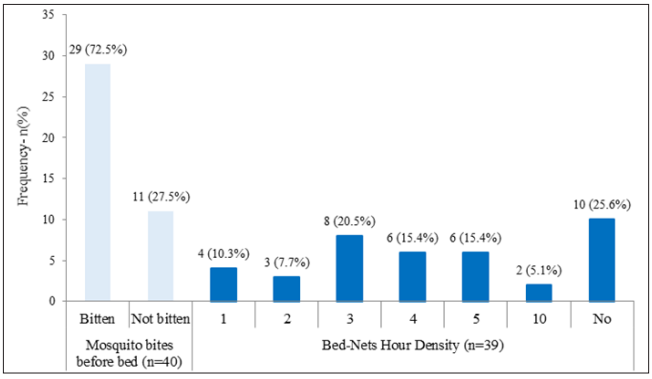

Figure 1.Mosquito Bites and Mosquitoes Perched on LLINs

Discussion

Farmer's profession, the most common work done by the participants, requires working in the fields and gardens during the day hence during working hours, it was difficult to meet the head of the family. Re-confirmation could be done at night together with the observation of mosquitoes on the LLINs, but the respondent had already considered the answers given by other family members who were interviewed during the day so there was no need to repeat the interview with the head of the household.

The socio-economic conditions of respondents were already in the middle category. Based on the *Badan Kependudukan dan Keluarga Berencana Nasional* (BKKBN) classification, the description of the respondent's residence was included in the category of a prosperous family,[15] meaning that no respondents came from poor families. This does not mean that the condition of the house was very good in terms of sanitation and spatial planning. The life of rural people involves giving relatively less attention to the spatial layout so the bedroom looks less well-organised. A good level of welfare will certainly have an impact on good room conditions, especially in terms of sanitation.[16] Room sanitation is important concerning the presence of mosquitoes. Houses made of cement walls are very good at preventing the entry of mosquitoes because they are not perforated like walls made of wood or bamboo.

One of the risk factors for malaria is outdoor activities at night.[17] All respondents said that there were family members who often left the house at night for certain activities with the most common being night patrols and religious activities regularly scheduled in Yasin-Tahlil recitations. All respondents were Muslims, making religious activities in this village lively at night. Religious activities of the Yasin-Tahlil recitation were a routine agenda as followed by most of the respondents. This activity was held once a week every Thursday night. A small number of respondents went out at night to work the night shift and the others to feed livestock. The greatest potential risk of *Anopheles* bites was in the case of respondents who went out at night to feed livestock. The cattle pen position outside of the house required the respondents to go out to feed or add cattle feed. In previous studies, it was reported that zoophilic *An. maculatus* was caught in the cattle cage area[18] even though this species was one of the vectors of malaria in Purworejo.[19]

The programme for distributing insecticide-treated mosquito nets does not take into account the level of welfare of the population. It is aimed at at-risk populations living in malaria-endemic areas, especially with respect to the location of the patient and their receptive areas. Residents who live within *Anopheles*' flight distance from the patient's house must receive top priority. The size of the distributed LLINs was 200 cm x 200 cm x 200 cm and 2 pieces were given to each family. The distribution of two pieces of LLINs was intended so that all family members could make maximum use of them. All families only installed one LLIN while the other was a spare for replacement when the used one was washed. All respondents had used the distributed LLINs, but their utilisation had not been maximised. Due to the installation of only one LLIN, some family members did not sleep in the bed nets. There were still less than a quarter of the respondents whose entire family members slept in the bed nets. The lack of obedience of some of the respondents' family members sleeping in the mosquito nets was better than the compliance by the population in Kulonprogo where almost half of the respondents did not sleep in the bed nets because they were uncomfortable and felt hot.[20] The low level of community compliance in the use of LLINs was also reported more broadly in the results of a national survey in seven districts in Indonesia.[21]

The duration of maximum usage of LLINs was 9 hours every night. All respondents slept under bed nets during

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the *Anopheles* peak hour activity, except for some family members. The data provided information on the maximum effort of the respondents to break the chain of parasitic transmission through the bite of *Anopheles*. A small number of respondents used LLINs for 10-15 hours a day, especially those with children and toddlers. The effectiveness of using LLINs to prevent *Anopheles* bites can be achieved if the duration of the use is in line with the time of activity of female mosquitoes in search of blood feed. Previous data reported the activity of *Anopheles* mosquitoes in the study area between 06.00 pm and 04.00 am with peak hours being 07.00-12.00 pm.[18]

Most of the respondents had washed their LLINs for the first time after 6 months of usage. Washing bed nets by soaking them in soapy water before washing and drying them directly in the sun can cause a decrease in the efficacy of LLINs in killing or repelling mosquitoes.[12] The lack of knowledge on how to care for LLINs needs to be taken into account considering that its effect was seen in a study conducted in Sorong, Papua.[22] The number of LLINs that were perched by mosquitoes as per this study illustrated that their efficacy had decreased. Laboratory testing for LLINs showed that they should still be effective after 20 washes,[7] but field use may give different results. A study conducted in Sungai Nyamuk Village stated that LLINs could only maintain their effectiveness in killing mosquitoes for 6 months.[23] This finding is corroborated by a report from Sumare Village, Mamuju Regency, West Sulawesi Province which revealed that the efficacy of LLINs only lasted for one year.[24] The main factors causing the decreased efficacy of these LLINs are incorrect washing and drying methods under direct sunlight.[10]

The use of mosquito repellent by respondents was relatively very low. This can provoke the arrival of *Anopheles* looking for blood feed as evidenced by the presence of mosquito bites before bedtime. The density of perched mosquitoes in the range of 1-10 mosquito/LLINs/hour can still be a threat. The important information obtained from this survey was that LLINs had lost their efficacy after 3 years of usage, so an insecticide re-immersion programme was needed. The reappearance of a malaria outbreak in the sub-district adjacent to the survey location[6] should be treated as an alert regarding the threat of malaria transmission still existing.

Conclusion

Mosquitoes that freely perch on mosquito nets show low insecticide efficacy of LLINs. The LLINs after being used for three years without re-immersion function only as ordinary mosquito nets. The mosquitoes are not killed because the insecticide efficacy of the mosquito nets decreases. Most of the LLINs used by the community are still in good condition after three years of use, so it is necessary to re-immerse them in insecticides so that they can restore their function. The BHD calculation can be a benchmark for the potential for mosquito bites in the house for people in malaria-endemic areas, as a predictor of potential disease transmission. For people in a non-endemic area, it can be used as anticipation of mosquito bites. Calculating the density of mosquitoes with BHD is safer than MHD because it can minimise the negative impact on officers who are exposed to mosquitoes. The mosquitoes will not be sucking the officer's blood but will only perch on mosquito nets. The officers can make observations freely from inside the mosquito net. In the future, BHD can be used as a reference for measuring vector density indoors and also developed for outdoor areas such as livestock barns.

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Conflict of Interest: None

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