Proceeding of the International Conference on Public Health, Vol. 7, Issue 1, 2022, pp. 107-118

ISSN 2424 – 6735 online
DOI: https://doi.org/10.17501/24246735.2022.7111

MOLECULAR DETECTION OF *Plasmodium* spp. IN *Anopheles* AND ITS VECTOR POTENTIAL IN LOW-ENDEMIC AREAS IN INDONESIA

Anggraeni YM[1*], Setiyaningsih R[2], Mujiyanto M[1], Trapsilowati W[2], Pujiyanti A[2], Rahardianingtyas E[2], Prihatin MT[2], Hidajat MC[1], Tri Ramadhani T[1], Wigati RA[1], Ipa M[1], Mulyono A[1], Kesuma AP[1] Ristiyanto R[1] and Garjito TA[1]

[1]*Research Centre for Public Health and Nutrition, National Research and Innovation Agency, Indonesia*

[2]*Institute for Vector and Reservoir Control Research and Development, Ministry of Health, Indonesia*

Abstract: Indonesia has 31.1% of regencies/cities categorized as low-endemic areas. The areas are expected to accelerate the malaria elimination status; meanwhile, it is crucial to reduce the vector density in the area. Vector data updates in malaria-endemic areas are essential in anticipating the transmission and an increase in malaria cases. The purpose of the study was to get information on new malaria vector updates in low-endemic malaria areas in Indonesia. The study was conducted from 2015-2018 in Jambi, Riau Islands, Special Region of Yogyakarta, Central Kalimantan, North Kalimantan, and South Sulawesi. Mosquito and larva collection was performed in low-endemic areas of malaria. We used the night landing collection, animal-baited trap, livestock bait, light trap, and resting morning collection. The *Plasmodium* was identified from *Anopheles* samples using the polymerase chain reaction (PCR) with *Plasmodium* genus-specific primers. The results showed a new potential vector in low-endemic malaria areas in Indonesia. They are *An. vagus* (Jambi, Central Kalimantan, South Sulawesi, and Yogyakarta), *An. kochi* (Jambi), *An. dirus* and *An. barbirostris* (Central Kalimantan), and *An. subpictus* (South Sulawesi). The discovery of the suspected *Anopheles* species as malaria vectors suggests a potential transmission, and increased cases may occur. Vector surveillance needs to be strengthened to reduce malaria transmission.

Keywords: *Anopheles*, PCR, low-endemic, malaria, vector

Introduction

Malaria is a vector-borne disease that has become a global problem. An estimated 219 million malaria cases occurred in 2017, with an estimated 435,000 deaths. There was a decrease when compared to 2010 (239 million cases), but it increased when compared to 2016 (217 million cases) (Alonso et al., 2019; World malaria report 2019, 2019). Deaths occur directly or indirectly in large amounts of anaemia. Absenteeism caused by malaria affects productivity and company performance. Malaria prevention can lead to a high reduction in malaria burden and increased productivity (Alonso et al., 2019; Lukwa, Mawoyo, Zablon, Siya, & Alaba, 2019; White, 2018).

Corresponding Author: *yusn004@brin.go.id

The malaria prevention program in Indonesia has emphasized the elimination of malaria achieved gradually until 2030 (Kementerian Kesehatan, 2019). A malaria elimination strategy is implemented by maintaining indigenous cases and preventing transmission from imported cases (Lal, Rajvanshi, Jayswar, Das, & Bharti, 2019). Low endemic regencies are defined as regencies/cities with an Annual Parasite Incidence of less than 1 per 1000 inhabitants. National data showed that in 2019, there were 31.1% of regencies and cities in Indonesia that fall into the category of low-endemic areas (Kementerian Kesehatan, 2019). Java and Bali Province, Indonesia, previously targeted to achieve elimination in 2015 (Murhandarwati et al., 2015), but it has not reached the specified target, so it has been rescheduled for 2023 (Kementerian Kesehatan, 2019).

Indonesia has reported 24 species of *Anopheles* spp. as malaria vectors (Syafruddin et al., 2020). Each species of *Anopheles* spp. has specific bionomic characteristics that affect malaria transmission dynamics and vector control implementation (Laurent et al., 2017). Detailed knowledge of local vector species is required to control and achieve malaria elimination goals (Mishra et al., 2020).

Ability to detect *Plasmodium* spp. sporozoite in the salivary glands of *Anopheles* is necessary for studying malaria. Detection and identification of infected mosquitoes are required to determine mosquito species that act as malaria vectors. The techniques used are microscopic examination, immunology, and molecular assays using the PCR method (Echeverry et al., 2017). Microscopic examination is the gold standard of malaria screening. This method has a sensitivity of 50-500 parasites/µl, is low-cost, and identifies the species and density of parasites. In many endemic areas of malaria, the microscopic diagnosis has limitations, especially the lack of skilled examiners, inadequate quality control, and the possibility of misdiagnosis due to low parasitemia or mixed infections. Microscopic examination has low sensitivity when performed by undertrained personnel in endemic areas, especially in primary and secondary health facilities (Berzosa et al., 2018).

The PCR-based method shows a higher sensitivity to *Plasmodium* detection than other methods. The PCR method detects low parasitemia with a sensitivity of 2-5 parasites/µl. However, PCR is not appropriately used for malaria screening in the field. This method has limitations in terms of relatively expensive costs and complex processes (Berzosa et al., 2018)

Vector surveillance with more sensitive methods can examine more samples to determine vector suspects. Therefore, the vector suspect examination method with PCR needs to be done for surveillance in low-endemic areas because it is more sensitive and specific, especially in low-endemic areas. The study aims to update new malaria vectors in six low-endemic provinces in Indonesia. The discovery of new suspected vectors is crucial in preventing widespread malaria transmission, so it is necessary to update malaria vector data every specific period.

Materials and Methods

This research is a descriptive study with a cross-sectional design, conducted from 2015 – 2018. The study population is all mosquitoes and larvae *Anopheles* spp. found in low endemic areas in six provinces in Indonesia. Study samples were mosquitoes and larvae of *Anopheles* spp. found in six provinces: Jambi (Sarolangun Regency, Tanjung Jabung Barat, and Bungo), Special Region of Yogyakarta (Kulon Progo Regency), Central Kalimantan (Gunung Mas, Murung Raya, and Pulang Pisau), North Kalimantan (Bulungan and Nunukan), South Sulawesi (East Luwu and Pangkajene

Islands) and Riau Islands (Lingga and Bintan). We collected the sample in each regency on six ecosystems: settlements (in the forest, non-forest, coastal ecosystems), remote forest, non-forest, and coastal areas. The instruments used were mosquito collection equipment and mosquito identification keys (Sudomo et al., 2017).

Mosquito collection was performed by the night landing collections using human bait and animal bait, resting morning collection, and light traps. Larvae were collected in places that have the potential to breed malaria vector mosquitoes. Some breeding places were observed, including rice fields, rivers, lagoons, springs, etc. Mosquitoes were identified using a mosquito identification key (O'Connor & Soepanto, 1999; Panthusiri, Rattanarithikul, & Prachong, 1994; Reid, 1966). The head and thorax of identified mosquitoes were cut and detected for the presence of Plasmodium by polymerase chain reaction (PCR) method (Setiyaningsih et al., 2018).

The total DNA from mosquito samples was extracted using DNeasy Blood & Tissue Kits (Qiagen, Hilden, Germany) and detected using nested-PCR. We used genus-specific primers PLU1 (5'- TCA AAG ATT AAG CCA TGC AAG TGA – 3'), PLU5 (5' - CCT GTT GTT GCC TTA AAC TCC – 3'), PLU3 (5' - TTT TTA TAA GGA TAA CTA CGG AAA AGC TGT – 3'), and PLU4 (5' - TAC CCG TCA TAG CCA TGT TAG GCC AAT ACC – 3') (Singh et al., 1999)

The nested PCR refers to the GoTaq Green Master Mix protocol standard (Promega, Madison, WI, USA) with PCR cycle setting conditions as follows: first nested: 95ºC for 15 minutes, followed by 35 denaturation cycles of 94ºC for 30 seconds, annealing 55ºC for 1 minute and extension temperature of 72ºC for 1 minute. Final extension at a temperature of 72ºC for 1 minute. The PCR amplification process ends after the temperature is at a temperature of 4ºC. For the second nested PCR, we used a similar setting as the first nested, with modification in the annealing step (62ºC for 1 minute).

Results and Discussion

The results of the study obtained 36 species of *Anopheles*. The highest species diversity of *Anopheles* was found in Central Kalimantan (23 species), while the Special Region of Yogyakarta had the least number of *Anopheles* species (7 species). Figure 1 displays the research location, while Table 1 presented the number of *Anopheles* species found.

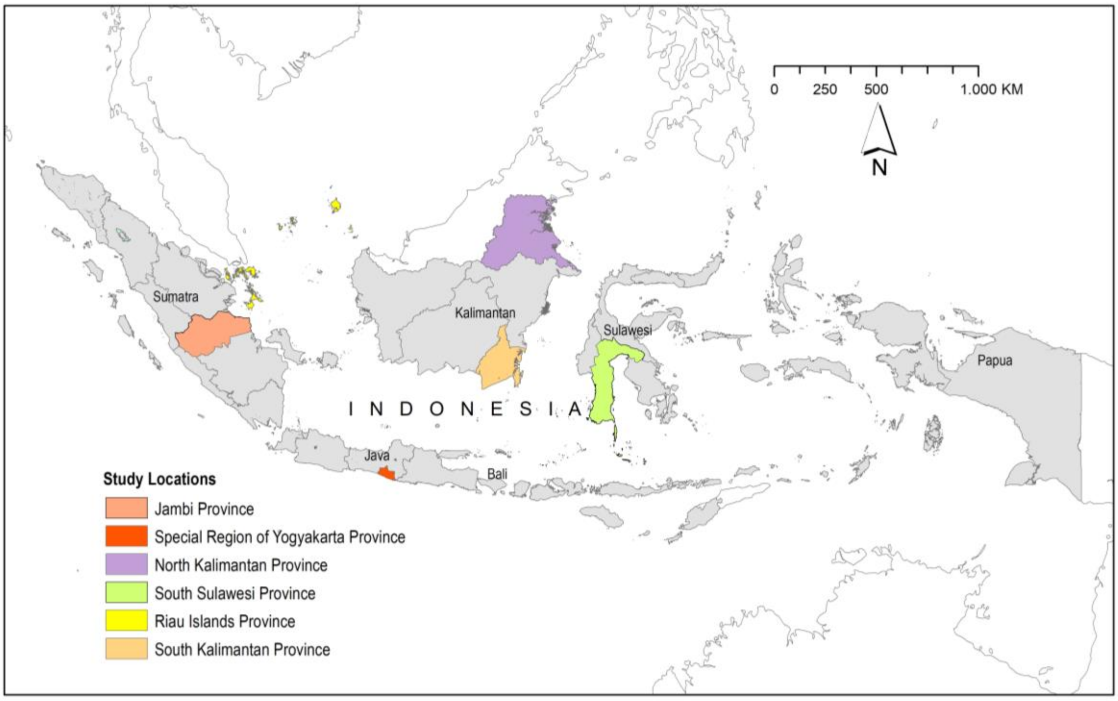

Figure 1. Research location map

Table 1. Number of Anopheles species at the research site

Province	Number of *Anopheles* species found	Number of vector species found	Number of new suspected vector
Jambi	17	5	1
Central Kalimantan	23	2	3
South Sulawesi	17	5	2
Special Region of Yogyakarta	9	2	1
Riau Islands	7	-	-
North Kalimantan	15	-	-

New suspected malaria vectors were found in four out of six provinces. Details of PCR detection results are presented in Table 2. Central Kalimantan is the province with the highest number of suspected malaria vector species (three species). Table 2 shows the results of an update of malaria vector species tested using PCR.

Table 2. Malaria vector species update results with PCR

Province	Reported malaria vectors (Elyazar, Sinka, Gething, Tarmidzi, Surya, Kusriastuti, Baird, et al., 2013)	Species of *Anopheles* found 1: confirmed as malaria vector 2: PCR positive	Positive PCR Results
Jambi	*An. sundaicus,* *An. letifer,* *An. maculatus,* *An. nigerrimus,*	*An. annularis* *An. argyropus,* *An. balabacensis*[1] *An. barbirostris*	*An. kochi*

	An. balacensis, *An. sinensis*	*An. barbumbrosus* *An. crawfordi* *An. kochi*[2] *An. letifer*[1] *An. maculatus*[1] *An. nigerrimus*[1] *An. nitidus* *An. peditaeniatus* *An. philippinensis* *An. separatus* *An. sinensis*[1] *An. tesselatus* *An. umbrosus*	
Central Kalimantan	*An. letifer,* *An. nigerrimus*	*An. aitkenii* *An. baezai* *An. baimaii* *An. barbirostris*[2] *An. barbumbrosus* *An. dirus*[2] *An. hackeri* *An. hodgkini* *An. indefinitus* *An. kochi* *An. latens* *An. letifer*[1] *An. leucosphyrus* *An. limosus* *An. maculatus* *An. nigerrimus*[1] *An. nitidus* *An. peditaeniatus* *An. sinensis* *An. subpictus* *An. tesselatus* *An. umbrosus* *An. vagus*[2]	*An.vagus* *An.dirus* *An.barbirostris*
South Sulawesi	*An. nigerrimus,* *An. sundaicus,* *An. barbirostris,* *An. flavirostris,* *An. ludlowae,* *An. subpictus*	*An. aconitus* *An. argyropus* *An. barbirostris*[1] *An. barbumbrosus* *An. crawfordi* *An. flavirostris*[1] *An. indefinitus* *An. karwari* *An. kochi* *An. limosus* *An. ludlowae*[1]	*An.vagus* *An.subpictus*

		An. nigerrimus[1] *An. peditaeniatus* *An. subpictus*[1] *An. sulawesi* *An. tesselatus* *An. vagus*[2]	
Special Region of Yogyakarta	*An. aconitus,* *An. sundaicus,* *An. maculatus,* *An. balabacensis*	*An. aconitus*[1] *An. annularis* *An. balabacensis*[1] *An. barbirostris* *An. indefinitus* *An. kochi* *An. subpictus* *An. tesselatus* *An. vagus*[2]	*An.vagus*
Riau Islands	*No Data*	*An. karwari* *An. kochi* *An. nigerrimus* *An. peditaeniatus* *An. separatus* *An. sinensis* *An. umbrosus*	PCR negative
North Kalimantan	*No Data*	*An. argyropus* *An. baimaii* *An. balabacensis* *An. barbirostris* *An. barbumbrosus* *An. kochi* *An. latens* *An. letifer* *An. litoralis* *An. maculatus* *An. peditaeniatus* *An. separatus* *An. sundaicus* *An. tesselatus* *An. vagus*	PCR negative

1: confirmed as malaria vector
2: PCR positive

The collection of *Anopheles* mosquitoes showed that some species of *Anopheles* are found in all provinces *(An. kochi* and *An. tesselatus*), but some species are locally specific.

*Anopheles vagus, An. diru*s and *An. barbirostris* are suspected new malaria vectors by PCR method in Central Kalimantan Province. *Anopheles* mosquitoes previously confirmed as malaria vectors in Central Kalimantan Province are *An. letifer* and *An. nigerrimus* (B2P2VRP, 2017; Elyazar, Sinka, Gething, Tarmidzi, Surya, Kusriastuti, Baird, et al., 2013; Kementerian Kesehatan RI, 2014). *Anopheles vagus* is also suspected of new malaria vectors in South Sulawesi and the Special Region of Yogyakarta. The previous data of malaria vectors in South Sulawesi Province were *An.nigerrimus, An. sundaicus, An.barbirostris, An. flavirostris, An. ludlowae,* and *An. subpictus.* while in Yogyakarta were *An. aconitus, An. sundaicus, An. maculatus,* and *An. balabacensis* (Elyazar, Sinka, Gething, Tarmidzi, Surya, Kusriastuti, Baird, et al., 2013).

Density, abundance, and preference for a human bite of vector-confirmed *Anopheles* spp. effect on the occurrence of malaria (Montoya, Correa, Bascuñan, & Zabala, 2017; Mosha et al., 2020). Vector behavior is closely related to environmental changes that affect the increase in temperature, acclimation, and accumulation of carbon dioxide (Le, Kumar, Ruiz, Mbogo, & Muturi, 2019). Also, population dynamics and bionomics characteristics need to be considered to conduct malaria prevention efforts and effective interventions toward malaria elimination in Indonesia, which is targeted to be achieved by 2030 (Sitohang et al., 2018). The presence and variety of species in a location and season need to be known continuously to determine appropriate vector control efforts with surveillance activities (Sitohang et al., 2018; Montoya et al., 2017). The surveillance system is crucial in achieving a malaria elimination status in a region. Well-conducted surveillance can ensure the availability of the correct data as an instrument for malaria control and elimination (Sitohang et al., 2018; Visa et al., 2020).

The Wallacea and Weber lines influenced the distribution of *Anopheles* spp. in Indonesia. The Weber and Wallacea lines divide the distribution of mosquito species into three parts: the oriental, transitional, and Indo-Australian regions of Indonesia. The division of this region causes a tendency for different species in each area.

Anopheles kochi

Anopheles kochi found in Jambi was assumed to be a new malaria vector. The discovery of *An. kochi* as a malaria vector in Jambi adds to the list of malaria vectors in Jambi province. *Anopheles kochi* is generally reported as a vector in Sumatra Province (Elyazar, Sinka, Gething, Tarmidzi, Surya, Kusriastuti, Baird, et al., 2013). Some studies showed that *An. kochi* is confirmed as a malaria vector by the CSP-ELISA method. *Anopheles kochi* in Bangladesh and Thailand were found positive for *Plasmodium vivax* and *Plasmodium falciparum* (Al-Amin et al., 2015; Jatuwattana et al., 2020). *Anopheles kochi* is also positive for *Plasmodium vivax* in South Halmahera North Maluku Islands (Laurent et al., 2017).

Anopheles kochi as a malaria vector is supported by its behavior. Sucking blood is one of the behaviors that affect the potential of a mosquito as a vector, especially when a species has multiple-sucking blood in a night.

Based on the results in Halmahera, Molucca, *An. kochi, An. farauti, An. hackeri, An. hinesorum, An. indefinitus, An. punctulatus, An. tessellatus, An. vagus*, and *An. vanus* have this multiple host-feeding behavior. It can be seen from the results of mosquito collection by positive animal-baited trap method containing *Plasmodium vivax* (Laurent et al., 2017).

The variety of breeding places is another factor that supports *An. kochi* as a vector. *Anopheles kochi's* habitats are easily found in nature and artificial. Some types of natural breeding places include swamps, ponds, and rivers, while the artificial habitats are rice fields, fish ponds, buffalo potholes, wells, and trenches (Elyazar, Sinka, Gething, Tarmidzi, Surya, Kusriastuti, Baird, et al., 2013; Ohba, Van Soai, Van Anh, Nguyen, & Takagi, 2015).

Anopheles vagus

Some studies showed that *An. vagus* is known to suck the blood of animals and has multiple blood-sucking behavior. This multiple biting behavior allows for contact with humans so that the potential for malaria transmission can occur (Keven et al., 2017; Laurent et al., 2017).

The distribution of *An. vagus* as a suspected malaria vector has been reported in several locations in Indonesia. *Anopheles vagus* detection of sporozoite using PCR method was found positive for *Plasmodium vivax* in Lebak and Pandeglang, Banten with a sporozoite rate of 1.02% (Astuti, Ipa, Prasetyowati, Fuadzy, & Dhewantara, 2016), Central Sulawesi (Maksud, 2017), and Pangkajene, South Sulawesi (Setiyaningsih et al., 2018). The discovery of various types of breeding places is one of the factors that caused *An. vagus* plays a role as a suspect vector. Several studies showed the type of breeding place that is positive for *An. vagus* include riverbanks, swamps, ponds, rice fields, irrigation, sewers, former wheel treads, ditches, artificial container containers such as drums, and boats (Elyazar, Sinka, Gething, Tarmidzi, Surya, Kusriastuti, Baird, et al., 2013; Mading & Sopi, 2014; Maksud, 2017; Ohba et al., 2015). *Anopheles vagus* is also known to be distributed in various ecosystems both forest, non-forest and coastal ecosystems (Setiyaningsih et al., 2018).

The potential of *An. vagus* as a malaria vector can also be seen in its density and behavior. Some studies showed that *An. vagus* is found dominant in a location. (Alam et al., 2018; Astuti et al., 2016; Cooper, Edstein, Frances, & Beebe, 2010; Nguyen et al., 2021; Pinontoan, Supadmanaba, Manuaba, Sukrama, & Manuaba, 2017; Sugiarto, Hadi, Soviana, & Hakim, 2017).

Anopheles dirus

*Anophel*es dirus has been confirmed as a malaria vector in some areas. Previously, *An. dirus* is as a malaria vector in West Talk Thailand Province (Tananchai et al., 2019). *Anopheles dirus* has also been positive for *Plasmodium vivax* in the Mekong of Vietnam (Boonkaew et al., 2020).

Anopheles dirus can act as a vector because of its human blood-sucking indoor and outdoor behavior. Studies in Thailand showed that *An. dirus* suck blood in the house with peak density from 01.00-02.00. Peak density of *An. dirus* suck blood outside the house from 23.00 to 24.00. The time of sucking blood increases the chances of malaria transmission due to human rest The potential of *An. dirus* as a vector is also supported because it is found both in the rainy season and in summer (Marcombe et al., 2020; Tananchai et al., 2019).

Anopheles barbirostris

Anopheles barbirostris has been confirmed as a malaria vector in South Sulawesi, North Sulawesi, Central sulawesi, Southeast Sulawesi, and East Nusa Tenggara. It found that *An. barbirostris* positive of *Plasmodium* adds an *Anopheles* species that are suspected vectors in Central Kalimantan and West Nusa Tenggara (Elyazar, Sinka, Gething, Tarmidzi, Surya, Kusriastuti, Baird, et al., 2013). Based on studies in western Thailand, An. barbirostris is reported to be a suspected vector of malaria by using

ELISA (Sriwichai et al., 2016). *Anopheles barbirostris* in Indonesia tend to suck blood outdoors. *Anopheles barbirostris* in Thailand are also known to suck a lot of blood outdoors, and peak density is found in the transition of rainy and dry seasons. Blood-sucking behavior outside the house increases the chances of outdoor malaria transmission (Setiyaningsih et al., 2020; Sriwichai et al., 2016).

Anopheles subpictus

Anopheles subpictus is also a suspected new malaria vector in South Sulawesi by PCR detection. It has previously been confirmed in Bengkulu, Java, Bali, NTB, NTT and Sulawesi (Elyazar, Sinka, Gething, Tarmidzi, Surya, Kusriastuti, Winarno, et al., 2013; Kementerian Kesehatan RI, 2014) (Iqbal R.F. Elyazar et al., 2013; Ministry of Health, 2014). Studies in urban areas of West India showed that *An. subpictus* detected *Plasmodium falciparum* and *Plasmodium vivax* in salivary glands and intestines. *Anopheles subpictus* can act as a malaria vector due to its behavior that sucks human blood. The results showed that *An. subpictus* known to suck 27% of human blood (Kumar et al., 2016).

Conclusion

New suspected malaria vectors are found in some low-endemic malaria areas in Indonesia. *Anopheles kochi* is a suspected new malaria vector in Jambi. *Anopheles vagus, An.dirus,* and *An.barbirostris* are suspected of malaria vectors in Central Kalimantan. *Anopheles vagus* and *An. subpictus* are suspected malaria vectors in South Sulawesi. *An. vagus* is a malaria vector in the Special Region of Yogyakarta. Meanwhile, in Riau Islands and North Kalimantan Province, there are no suspects of new malaria vectors with the PCR method.

Acknowledgements

We express thanks the National Institute of Health Research and Development director and IVRCRD Salatiga, who encouraged the research and article writing. We also show gratitude to the team involved in collecting data, all field investigators and coordinators during the Rikhus Vektora project, and the local government who allowed us to conduct the research.

Declaration of Interest Statement

The authors declare that they have no conflict of interests.

References

Al-Amin, H. M., Elahi, R., Mohon, A. N., Kafi, M. A. H., Chakma, S., Lord, J. S., … Alam, M. S. (2015). Role of Underappreciated Vectors in Malaria Transmission in an Endemic Region of Bangladesh-India border. Parasites and Vectors, 8(1), 1–9. https://doi.org/10.1186/s13071-015-0803-8

Alam, M. S., Al-Amin, H. M., Elahi, R., Chakma, S., Heel Kafi, M. A., Khan, W. A., … Norris, D. E. (2018). Abundance and dynamics of *Anopheles* (Diptera: Culicidae) larvae in a malaria endemic area of Bangladesh. *Journal of Medical Entomology,* 55(2), 382–391. https://doi.org/10.1093/JME/TJX196

Alonso, S., Chaccour, C. J., Elobolobo, E., Nacima, A., Candrinho, B., Saifodine, A., … Zulliger, R. (2019). The economic burden of malaria on households and the health system in a high transmission district of Mozambique. *Malaria Journal,* 18(1), 1–10. https://doi.org/10.1186/s12936-019-2995-4

Astuti, E. P., Ipa, M., Prasetyowati, H., Fuadzy, H., & Dhewantara, P. W. (2016). Kapasitas Vektor dan Laju Inokulasi Entomologis *Anopheles* vagus dari Wilayah Endemis Malaria di Provinsi Banten.

Vektora : Jurnal Vektor Dan Reservoir Penyakit, 8(1), 23–30. https://doi.org/10.22435/vk.v8i1.5089.23-30

B2P2VRP. (2017). Pedoman Pengumpulan Data Vektor (Nyamuk) di Lapangan. Salatiga.

Berzosa, P., De Lucio, A., Romay-Barja, M., Herrador, Z., González, V., García, L., … Benito, A. (2018). Comparison of three diagnostic methods (microscopy, RDT, and PCR) for the detection of malaria parasites in representative samples from Equatorial Guinea 11 Medical and Health Sciences 1108 Medical Microbiology. *Malaria Journal,* 17(1), 1–12. https://doi.org/10.1186/s12936-018-2481-4

Cooper, R. D., Edstein, M. D., Frances, S. P., & Beebe, N. W. (2010). Malaria vectors of Timor-Leste. Malaria Journal, 9, 40. https://doi.org/10.1186/1475-2875-9-40

Echeverry, D. F., Deason, N. A., Makuru, V., Davidson, J., Xiao, H., Niedbalski, J., … Lobo, N. F. (2017). Fast and robust single PCR for Plasmodium sporozoite detection in mosquitoes using the cytochrome oxidase I gene. *Malaria Journal,* 16(1), 1–8. https://doi.org/10.1186/s12936-017-1881-1

Elyazar, I. R. F., Sinka, M. E., Gething, P. W., Tarmidzi, S. N., Surya, A., Kusriastuti, R., … Bangs, M. J. (2013). The distribution and bionomics of *Anopheles* malaria vector mosquitoes in Indonesia. In Advances in Parasitology (Vol. 83, pp. 173–266). Academic Press. https://doi.org/10.1016/B978-0-12-407705-8.00003-3

Elyazar, I. R. F., Sinka, M. E., Gething, P. W., Tarmidzi, S. N., Surya, A., Kusriastuti, R., … Bangs, M. J. (2013). The Distribution and Bionomics of *Anopheles* Malaria Vector Mosquitoes in Indonesia. In Advances in Parasitology (1st ed., Vol. 83, pp. 173–266). Elsevier Ltd. https://doi.org/10.1016/B978-0-12-407705-8.00003-3

Jatuwattana, W., Saeung, A., Taai, K., Srisuka, W., Thongsahuan, S., Aupalee, K., … Maleewong, W. (2020). Systematic Studies of *Anopheles* (Cellia) kochi (Diptera: Culicidae): Morphology, Cytogenetics, Cross-mating Experiments, Molecular Evidence and Susceptibility Level to Infection with Nocturnally Subperiodic Brugia malayi. *Acta Tropica,* 205. https://doi.org/10.1016/j.actatropica.2019.105300

Kementerian Kesehatan. (2019). Situasi Terkini Perkembangan Program Pengendalian Malaria di Indonesia tahun 2018. Jakarta.

Kementerian Kesehatan RI. (2014). Pedoman Pengendalian Vektor Malaria. Jakarta: Kemenkes RI.

Keven, J. B., Reimer, L., Katusele, M., Koimbu, G., Vinit, R., Vincent, N., … Walker, E. D. (2017). Plasticity of Host Selection by Malaria Vectors of Papua New Guinea. *Parasites & Vectors,* 10(1), 95. https://doi.org/10.1186/s13071-017-2038-3

Kumar, A., Hosmani, R., Jadhav, S., Sousa, T. De, Mohanty, A., & Naik, M. (2016). *Anopheles* subpictus Carry Human Malaria Parasites in An Urban Area of Western India and May Facilitate Perennial Malaria Transmission. *Malaria Journal,* 15(24), 1–8. https://doi.org/10.1186/s12936-016-1177-x

Lal, A. A., Rajvanshi, H., Jayswar, H., Das, A., & Bharti, P. (2019). Malaria elimination: Using past and present experience to make malaria-free India by 2030. *Journal of Vector Borne Diseases,* 56(1), 60–65. https://doi.org/10.4103/0972-9062.257777

Laurent, B., Burton, T. A., Zubaidah, S., Miller, H. C., Asih, P. B., Baharuddin, A., … Lobo, N. F. (2017). Host attraction and biting behaviour of *Anopheles* mosquitoes in South Halmahera, Indonesia. *Malaria Journal,* 16(1), 1–9. https://doi.org/10.1186/s12936-017-1950-5

Le, P. V. V., Kumar, P., Ruiz, M. O., Mbogo, C., & Muturi, E. J. (2019). Predicting the direct and indirect impacts of climate change on malaria in coastal Kenya. *PLoS ONE,* 14(2), 1–18. https://doi.org/10.1371/journal.pone.0211258

Lukwa, A. T., Mawoyo, R., Zablon, K. N., Siya, A., & Alaba, O. (2019). Effect of malaria on productivity in a workplace: The case of a banana plantation in Zimbabwe. *Malaria Journal*, 18(1), 1–8. https://doi.org/10.1186/s12936-019-3021-6

Mading, M., & Sopi, I. I. P. . (2014). Beberapa Aspek Bioekologi Nyamuk *Anopheles* vagus di Desa Selong Belanak Kabupaten Lombok Tengah. *Spirakel*, 6(1), 26–32.

Maksud, M. (2017). Aspek Perilaku Penting *Anopheles* vagus dan Potensinya sebagai Vektor Malaria di Sulawesi Tengah : Suatu Telaah Kepustakaan. *Jurnal Vektor Penyakit*, 10(2), 33–38. https://doi.org/10.22435/vektorp.v10i2.6256.33-38

Marcombe, S., Maithaviphet, S., Bobichon, J., Phommavan, N., Nambanya, S., Corbel, V., & Brey, P. T. (2020). New insights into malaria vector bionomics in Lao PDR: a nationwide entomology survey. *Malaria Journal*, 19(1), 1–17. https://doi.org/10.1186/s12936-020-03453-9

Mishra, A. K., Bharti, P. K., Vishwakarma, A., Nisar, S., Rajvanshi, H., Sharma, R. K., … Lal, A. A. (2020). A study of malaria vector surveillance as part of the Malaria Elimination Demonstration Project in Mandla , Madhya Pradesh. *Malaria Journal,* 1–13. https://doi.org/10.1186/s12936-020-03517-w

Montoya, C., Correa, M. M., Bascuñan, P., & Zabala, J. R. (2017). Abundance, composition and natural infection of, 37, 98–105.

Mosha, J. F., Lukole, E., Charlwood, J. D., Wright, A., Rowland, M., Bullock, O., … Protopopoff, N. (2020). Risk factors for malaria infection prevalence and household vector density between mass distribution campaigns of long-lasting insecticidal nets in North-western Tanzania. *Malaria Journal*, 19(1), 1–11. https://doi.org/10.1186/s12936-020-03369-4

Murhandarwati, E. E. H., Fuad, A., Sulistyawati, Wijayanti, M. A., Bia, M. B., Widartono, B. S., … Hawley, W. A. (2015). Change of strategy is required for malaria elimination: A case study in Purworejo District, Central Java Province, Indonesia. *Malaria Journal*, 14(1), 1–14. https://doi.org/10.1186/s12936-015-0828-7

Nguyen, T. Q., Nguyen, M. D., Pham, V. X., Ro, H. M., Edstein, M. D., Chow, W. K., … Motoki, M. T. (2021). Entomological survey in two communes with residual malaria transmission in Gia Lai Province in the central highlands of Vietnam. *Malaria Journal*, 20(1). https://doi.org/10.1186/S12936-021-03941-6

O'Connor, C. T., & Soepanto, A. (1999). Kunci Bergambar Nyamuk *Anopheles* Dewasa di Indonesia. Jakarta: Direktorat Jenderal Pemberantasan Penyakit Menular dan Penyehatan Lingkungan, Departemen Kesehatan RI.

Ohba, S. Y., Van Soai, N., Van Anh, D. T., Nguyen, Y. T., & Takagi, M. (2015). Study of Mosquito Fauna in Rice Ecosystems Around Hanoi, Northern Vietnam. *Acta Tropica*, 142, 89–95. https://doi.org/10.1016/j.actatropica.2014.11.002

Panthusiri, Rattanarithikul, R., & Prachong. (1994). Illustrated Keys to the Medically Important Mosquitoes of Thailand. Thailand.

Pinontoan, O. R., Supadmanaba, I. G. P., Manuaba, I. B. A., Sukrama, I. D. M., & Manuaba, I. B. P. (2017). Local Diversity and Biting Pattern of *Anopheles* Species in Southern Minahasa. Interdisciplinary Perspectives on Infectious Diseases, 2017. https://doi.org/10.1155/2017/6313016

Reid, J. A. (1966). Anopheline Mosquitoes of Malaya and Borneo. Government of Malaysia.

Setiyaningsih, R., Prihatin, M. T., Mujiyono, M., B, L., Marjiyanto, M., Susilo, D., … Garjito, T. A. (2020). Informasi Terkini *Anopheles* barbirostris dan Potensi Penularan Malaria pada Beberapa Provinsi di Indonesia. Media Penelitian Dan Pengembangan Kesehatan, 30(2), 119–134. https://doi.org/10.22435/mpk.v30i2.3240

Setiyaningsih, R., Widiarti, W., Prihatin, M. T., Nelfita, N., Anggraeni, Y. M., Alfiah, S., … Garjito, T. W. A. (2018). Potensi Penyakit Tular Vektor di Kabupaten Pangkajene dan Kepulauan Propinsi Sulawesi Selatan. Buletin Penelitian Kesehatan, 46(4), 247–256. https://doi.org/https://doi.org/10.22435/bpk.v46i4.38

Singh, B., Bobogare, A., Cox-Singh, J., Snounou, G., Abdullah, M. S., & Rahman, H. A. (1999). A genus- and species-specific nested polymerase chain reaction malaria detection assay for epidemiologic studies. *American Journal of Tropical Medicine and Hygiene,* 60(4), 687–692. https://doi.org/10.4269/ajtmh.1999.60.687

Sriwichai, P., Samung, Y., Sumruayphol, S., Kiattibutr, K., Kumpitak, C., Payakkapol, A., … Sattabongkot, J. (2016). Natural Human Plasmodium Infections in Major *Anopheles* Mosquitoes in Western Thailand. *Parasites and Vectors,* 9(1), 1–9. https://doi.org/10.1186/s13071-016-1295-x

Sudomo, M., Boewono, D. T., Suharjono, Y. R., Hadi, U. K., Anwar, C., Satoto, T. B. T., … Martiningsih, I. (2017). Pedoman Pengumpulan Data Vektor (Nyamuk) di Lapangan. Angewandte Chemie International Edition, 6(11), 951–952. (2nd ed.). Jakarta: Lembaga Penerbit Badan Penelitian dan Pengembangan Kesehatan.

Sugiarto, Hadi, U. K., Soviana, S., & Hakim, L. (2017). Bionomics of *Anopheles* (Diptera: Culicidae) in a malaria endemic region of Sungai Nyamuk village, Sebatik Island – North Kalimantan, Indonesia. *Acta Tropica,* 171, 30–36. https://doi.org/10.1016/J.ACTATROPICA.2017.03.014

Syafruddin, D., Lestari, Y. E., Permana, D. H., Asih, P. B. S., Laurent, B. S., Zubaidah, S., … Lobo, N. F. (2020). *Anopheles* sundaicus complex and the presence of *Anopheles* epiroticus in Indonesia. *PLoS Neglected Tropical Diseases,* 14(7), 1–16. https://doi.org/10.1371/journal.pntd.0008385

Tananchai, C., Pattanakul, M., Nararak, J., Sinou, V., Manguin, S., & Chareonviriyaphap, T. (2019). Diversity and biting patterns of *Anopheles* species in a malaria endemic area, Umphang Valley, Tak Province, western Thailand. *Acta Tropica,* 190, 183–192. https://doi.org/10.1016/j.actatropica.2018.11.009

White, N. J. (2018). Anaemia and malaria 11 Medical and Health Sciences 1108 Medical Microbiology 11 Medical and Health Sciences 1103 Clinical Sciences. *Malaria Journal*, 17(1), 1–17. https://doi.org/10.1186/s12936-018-2509-9

World malaria report 2019. (2019). World malaria report 2019. WHO Regional Office for Africa. Retrieved from https://www.who.int/news-room/fact-sheets/detail/malaria