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Relative Frequency (Composition) of *Anopheles* sp. Mosquitoes as Malaria Vectors in Kulon Progo District Special Region of Yogyakarta

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ABSTRACT

Malaria is caused by Plasmodium infection transmitted *Anopheles* sp. One of the obstacles in malaria control is the variation of *Anopheles* sp. Species which have various characteristics and behaviour, so it's very important to understand the species of *Anopheles* sp. with aim to develop the disease control program. This study was conducted in Kulon Progo Regency, Yogyakarta, one of the malaria endemic areas in Indonesia. Data collection of *Anopheles* sp. species diversity was carried out through *landing collection* inside and outside people's homes at 18.00 - 06.00 WIB. Observations of abiotics factors in the habitat of *Anopheles* sp. larvae was carried out around the *landing collection* location. The result of research conducted in the Samigaluh, Kalibawang, and Pengasih Sub-Districts showed that the most common species found was *Anopeheles vagus*. The dominance of *Anopheles vagus* occurred in the Kalibawang and Pengasih dictricts, while in the Samigaluh Sub-Districts it became the second most among other species. Measurement of abiotic factors in the habitat of *Anopheles* sp. larvae through the parameters of temperature, air humidity, pH DO of water shows that the location where the larvae were found can support larval breeding and growth. That can be a factor in the large population of *Anopheles* sp., especially *Anopheles vagus*, which is a known vector of malarias based on research and its vectorial capacity.

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INTRODUCTION

Malaria remains a global health issue, especially in tropical and subtropical regions (Utami et al., 2022). Even in Indonesia, Malaria cases still prevail across the country, causing morbidity and mortality in humans (Fitriani et al., 2023). Java Island is the most densely populated area in Indonesia with several areas where malaria transmission cases are still found and even declared as malaria Extraordinary Events (*KLB*), one of which is Kulon Progo Regency in Yogyakarta (Kemenkes RI, 2021), where the number of malaria cases fluctuates across the years. In 2022, 97 cases were recorded, and these were spread in several areas, such as Galur, Pengasih, Kokap, Girimulyo, and Samigaluh (Dinas Kesehatan Kabupaten Kulon Progo, 2023).

Malaria is caused by infection with *Plasmodium* sp. parasites carried by *Anopheles* sp. (Wathon et al., 2023). The genus *Anopheles sp.* is the largest member of the order Diptera, family Culicidae with 80 species including malaria vectors (Choochote et al., 2013). *Anopheles* sp. has diverse morphological features (Senjarini et al., 2020) and different behaviors, which include feeding location (indoors or outdoors), biting behavior (predominantly on humans and animals), geographic distribution, and vectorial abilities (vector or non-vector) (Weeraratne et al., 2017). As such, the exploration and mapping of *Anopheles* sp. species is very important because regional characteristics hold bearing impacts on the species distribution (Rushadi et al., 2021). The diversity of *Anopheles* sp. thence raises concerns as to what effective measures are necessary to improve measures to tackle malaria (Tahir et al., 2016).

The control strategy of *Anopheles* sp. continues to be extensively carried out because of the absence of a malaria vaccine (Wathon et al., 2023). However, efforts to control *Anopheles* sp. have not achieved maximum results, given that the transmission remains high in several regions in Indonesia. The control of malaria vectors so far has been conducted through vector control programs, one of which is fogging to nullify adult mosquitoes and larvae. Nevertheless, this has not been effective in controlling vectors. Fogging can even raise vector resistance to insecticides (Elyazar et al., 2013). In addition, low public awareness of environmental hygiene can increase the number of *Anopheles sp.* breeding sites, leading to increased malaria cases.

The present study argues that vector controls and malaria prevention have not been successful due to the absence of a comprehensive understanding of the characteristics and life behavior of each species of *Anopheles* sp. Such an issue even becomes more complex in Indonesia as there are 25 species of *Anopheles* sp. vectors (Mahdalena & Wurisastuti, 2021). This study explores the diversity of *Anopheles* sp. in a malaria-endemic area, Kulon Progo Regency, Yogyakarta and the environmental condition of the study area. The exploration denotes a urgency step for the development of effective and efficient vector control strategies (Musfirah, 2017). Equally essential is studying the disease epidemiology, because differences in *Anopheles* sp. species determine their competence as vectors of malaria transmission.

MATERIAL AND METHODS

Equipment and Materials

The equipment used in this study were a stereo microscope, optilab camera, GPS, petridish, pipette, flashlight, paper cup, tweezers, scissors, zip plastic, gauze, and rubber. The identification of *Anopheles* sp. was performed by referring to the Anopheline Mosquitoes of Malaya and Borneo (Reid, 1968). The materials in the study were samples of *Anopheles* sp. and larvae, chloroform solution, and 70% alcohol.

Research Time and Location

This study was conducted in Samigaluh, Kalibawang, and Pengasih Sub-Districts, in Kulon Progo Regency from July to October 2023. These Sub-Districts were selected as research sites due to their high rate of malaria cases in 2022. Through purposive sampling, two houses in each Sub-District were chosen as sampling locations and another house as a comparison (Table 1). The selection criteria were the presence of livestock barns and the surrounding environment supporting the breeding of *Anopheles* sp. The comparison house had no livestock barns but the surrounding environment supported the breeding of *Anopheles* sp.

The Captures of *Anopheles* sp. and Measurement of Abiotic Factors

Anopheles sp. was captured from 6:00 p.m. to 6:00 a.m., which followed the World Health Organization Manual on Practical Entomology in Malaria (World Health Organization (WHO), 1975). This was done through several capture methods, including the indoor human landing catches (IHLC) and outdoor human landing catches (OHLC). Following these methods, a collector remained seated with open arms and hands. Mosquitoes that landed on the collector's body were captured by another collector using an aspirator. Each method was implemented for 40 minutes at each capture time. In this study, resting mosquitoes were also captured through the resting inside house (RIH), resting around barn (RAB), and livestock landing catches (LLC) methods. Mosquito capture through these methods was conducted by collectors who observed and captured resting mosquitoes by using aspirators. Each capture method was conducted for 10 minutes at each capture time. The captured *Anopheles* sp. from each method was put into paper cups and separated based on capture time and method for identification. The abiotic factors, including air temperature and humidity parameters, were measured around the capture sites. The measurement results served as supporting data in the diversity analysis of *Anopheles* sp.

The Identification of *Anopheles* sp.

Mosquito identification was based on its morphological characteristics. Body parts of *Anopheles* sp. that were key to identification included the palpus, proboscis, wing venation, and hind leg tarsus. The body parts of *Anopheles* sp. were observed under a stereo microscope. The identified body characteristics were compared with the information in *the Anopheline Mosquitoes of Malaya and Borneo* (Reid, 1968) and articles from relevant journals.

Table 1. Coordinates of the research sampling

Sampling Location	Samigaluh	Kalibawang	Pengasih
House 1	7°40'57.26" S 110°10'12.32" T	7°43'13.73" S 110°12'37.46" T	7°51'15.44" S 110°08'37.71" T
House 2	7°40'47.07" S 110°10'03.56" T	7°43'13.09" S 110°12'35.01" T	7°51'13.96" S 110°08'37.30" T
House 3	7°40'56.75" S 110°10'01.01" T	7°43'13.90" S 110°12'39.49" T	7°51'15.93" S 110°08'38.51" T

The Collection of *Anopheles* sp. Larvae and Measurement of Abiotic Factors

Larvae were collected around each house which was considered as potential larvae breeding sites. The larvae were then taken for rearing until they became adult mosquitoes. Abiotic factors at the larval sites were also measured, including temperature, air humidity, pH level, and dissolved oxygen (DO) in water parameters. The measurement results aided in analyzing the habitat of *Anopheles* sp.

The Analysis of *Anopheles sp.* proportion

Data on mosquito diversity were counted during the 12-hour study and presented in diagrams to see the diversity of mosquito species, while abiotic factor measurements were presented in tables. Data in both regards were analyzed based on the research site. Parameter analysis was calculated per species, which included relative abundance, frequency of mosquitoes caught, species dominance, and mosquito species density. Relative abundance is the ratio of the number of certain mosquito species expressed in percent.

$$\textbf{Relative abundance} = \frac{\sum Species\ captured}{\sum Total\ number\ of\ captures} \times 100\%$$

The frequency of mosquitoes caught was calculated by comparing the number of mosquito species caught with the total number of mosquitoes caught using the same method.

$$\textbf{Frequency of mosquitoes captured} = \frac{\sum Species\ captured}{\sum Total\ number\ of\ captures}$$

Species dominance corresponds to the relative abundance multiplied by the frequency of mosquitoes caught. To calculate the dominance, the relative abundance is divided by 100.

Species dominance = (Relative abundance/100) x Frequency of mosquitoes captured

(Rushadi et al., 2021)

The density of *Anopheles sp.* was determined based on the Man Hour Density (MHD) value

which is the number of mosquitoes obtained divided by the number of catchers multiplied by the capture duration using the following formula:

$$MHD = \frac{The\ number\ of\ mosquitoes}{The\ number\ of\ catchers\ x\ capture\ duration}$$

Note: MHD: *Man-Hour Density* (mosquito density/catcher/hour) (Wahono et al., 2022).

RESULTS AND DISCUSSION

The Relative Frequency of *Anopheles* at the Research Site

The research was conducted in three Sub-Districts in Kulon Progo Regency, namely Samigaluh, Kalibawang, and Pengasih. These were malaria-endemic areas in 2023 (Dinas Kesehatan Kabupaten Kulon Progo, 2023). The research was carried out in several locations including Manggis Urban Village of Gerbosari Village in Samigaluh Sub-District. The second site was in Ngentak Urban Village of Banjararum Village in Kalibawang Sub-District. The last site was Kopat Urban Village of Karangsari Village in Pengasih Sub-District. The species diversity is presented in the following table.

The total number of *Anopheles* mosquitoes found was 380. There were 7 mosquito species found in the three sites. These species included *Anopheles vagus*, *Anopheles maculatus*, *Anopheles kochi*, *Anopheles barbirostris*, *Anopheles balabacencis*, *Anopheles aconitus*, and *Anopheles annularis*. Based on Table 2, the most dominant species are *Anopheles vagus* and *Anopheles kochi*. *An. vagus* was found dominant in Kalibawang and Pengasih Sub-Districts, while *An. kochi* was found to dominate in Samigaluh Sub-District. Other *Anopheles* species captured were *Anopheles maculatus*, *Anopheles barbirostris*, *Anopheles* balabacensis, *Anopheles aconitus*, and *Anopheles annularis*.

The dominance of a species makes it easier to find in an area. In this case, *Anopheles* sp. mosquito species that have high dominance become potential in contact with humans and infect them so that they can cause malaria. The research locations in Kalibawang and Pengasih Sub-Districts with the dominant species found were *Anopheles vagus* because of its suitable habitat, namely settlements with livestock pens around them. Each research location has a different species dominance according to its environmental conditions. *An. vagus* is a species that is spread especially on the islands of Sumatra and Java (Wahyuni *et al*., 2018).

All captured species were confirmed as malaria vectors. *An. vagus* was a species found in many areas in Indonesia and confirmed as a malaria vector because it contains *Plasmodium vivax* (Novianto et al., 2022). The discovery of *An. vagus* in Purworejo also indicates that the species is a malaria vector because it contains *Plasmodium falciparum* (Karo et al., 2021). Another species was *An. kochi*, a zoophilic mosquito confirmed as a malaria vector in Indonesia (Aida et al., 2022; Dalilah et al., 2022).

An. maculatus is also confirmed as a malaria vector due to the finding of *Plasmodium* reported in a study in Purworejo (Karo et al., 2021). *An. barbirostris* was confirmed as a malaria vector because it carried *Plasmodium* (Pahlepi et al., 2022). This species was also confirmed as a malaria vector in Riau. This mosquito is a zoophilic and endophilic species and can be found sucking blood inside the house (Lesmana et al., 2021). *An. aconitus* is another malaria vector in Indonesia. Finally, *An. annularis* is also confirmed as a malaria vector in Indonesia (Aida et al., 2022).

The presence of *Anopheles* mosquitoes confirmed as malaria vectors calls for serious attention because they can spread malaria and increase the number of malaria cases. One way to prevent malaria cases is setting up barns as a barrier to humans. Placement of barns at a certain distance can be an option for female *Anopheles* mosquitoes in conducting blood feeding. If the human blood source is difficult to reach, the mosquito will locate alternative blood sources, such as livestock. This can be one of the community prevention efforts in handling malaria cases (Nisrina et al., 2020). In general, both vector and non-vector *Anopheles* sp. prefer the blood of livestock at night which is usually found in livestock barns around houses (Azkiyah et al., 2021). This also evidenced that most of the *Anopheles* species found were active around livestock barns at night.

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The Diversity of *Anopheles* Based on Research Site

The captured *Anopheles* species were identified and grouped by species and research site to determine the species diversity at each research site. The results of the diversity analysis are shown in Figure 1.

Figure 1A shows the diversity of *Anopheles* species in Samigaluh Sub-District where *An. kochi* accounted for 51% of the total capture. This species tends to be exophilic and zoophilic and likes livestock barns, such as cows and goats (Dalilah et al., 2022). Another study has shown that *An. kochi* is also anthropozoophilic (Yahya et al., 2020). *An. kochi* in the research site was mostly found resting around livestock barns and on livestock, indicating that *An. kochi* is a zoophilic species that sucks the blood of livestock. *An. kochi* is mostly found in hilly areas in contrast to *An. vagus* which inhabits lowland and hilly areas (Yuniawan et al., 2019). Samigaluh Sub-District is one of the hilly areas where *An. kochi* was found prevalent. Gerbosari Village is a highland of Manoreh hills. This area is mostly characterized by slopes, so it is rarely explored as a place to live. The slopes are utilized as plantation or forestry sites. In addition, most residents have livestock barns, making it a potential breeding place for *Anopheles* sp.

Figures 1B and 1C show that *An. vagus* was dominant in Kalibawang and Samigaluh Sub-Districts, where the species was found at a proportion of 89%. and 97%, respectively. *An. vagus* is an exophilic species and prefers the blood of livestock or zoophilic. This species has been confirmed as a malaria vector in some areas because it carries *Plasmodium falciparum* and *Plasmodium vivax* (Karo et al., 2021; Novianto et al., 2022). Based on the research results, this species was also found to rest around livestock barns, indicating that the *An. vagus* species were zoophilic and exophilic. The research site in Kalibawang Sub-District is mostly characterized by Manoreh hills. The research site was adjacent to a comprehensive rice field. Unlike the site in Kalibawang, the one in Pengasih Sub-District is situated in a lowland.

The research site was a residential area with several barns and large gardens. *An. vagus* is a confirmed malaria vector in Kulon Progo Regency because it carries *Plasmodium falciparum.* It was mostly found in residential areas that had livestock barns around them and was often found outdoors at high densities. Rice field is a perfect location where *An. vagus* can be found although *An. vagus* can live in other areas such as highlands, lowlands, and brackish waters (Suryaningtyas et al., 2022).

Table 2. Types of *Anopheles* species based on capture locations

Species	Sampling Location			Rearing Result	Total
	Samigaluh	Kalibawang	Pengasih		
Anopheles vagus	33	78	182	1	294
Anopheles maculatus	5	-	-	5	10
Anopheles kochi	51	3	-	-	54
Anopheles barbirostris	6	2	-	-	8
Anopheles balabacencis	1	-	-	-	1
Anopheles aconitus	4	5	3	-	12
Anopheles annularis	-	-	1	-	1
Total	100	88	186	6	380

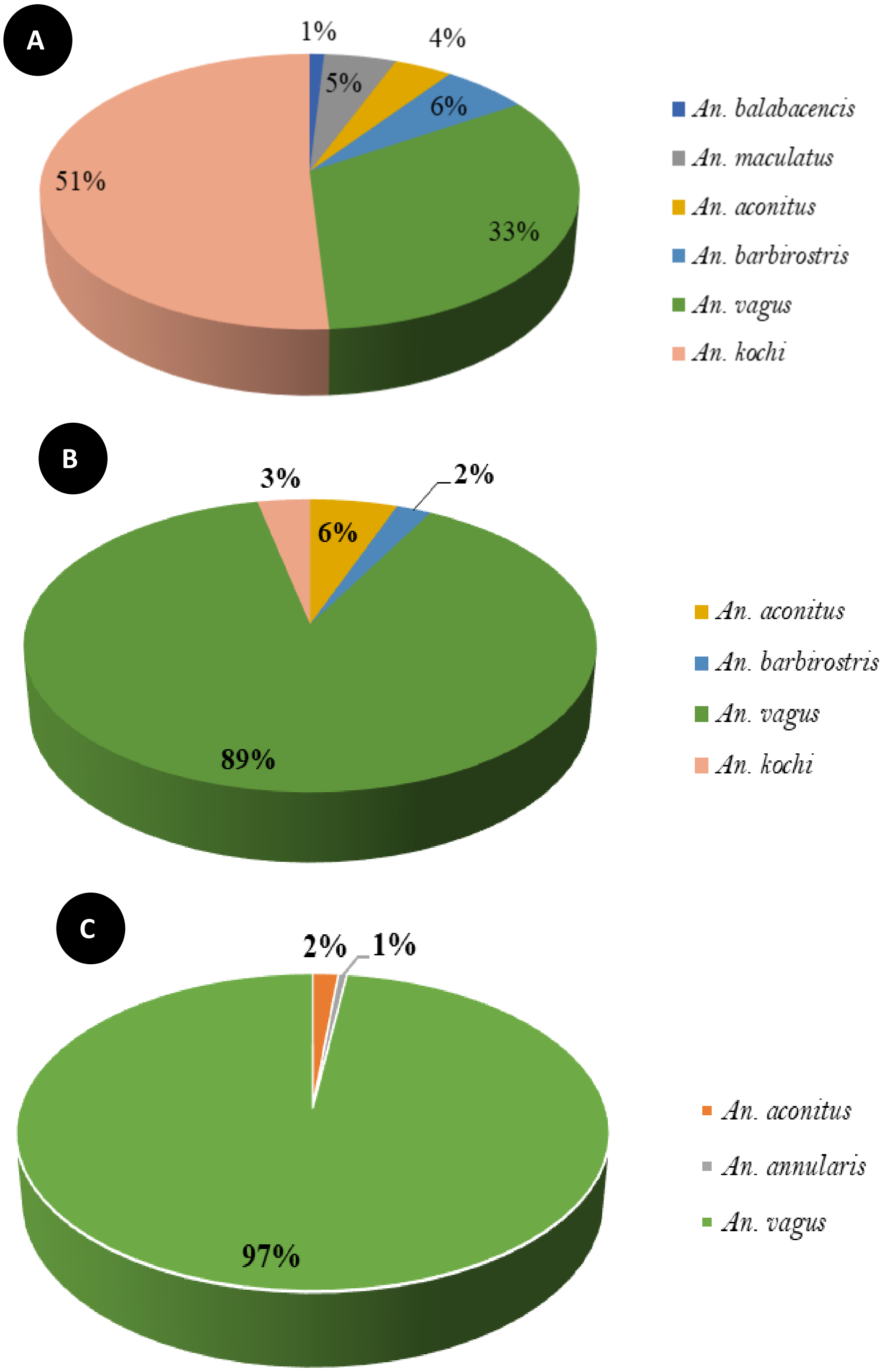

Figure 1. The Proportion of *Anopheles sp.* found in the research sites: (A) Samigaluh; (B) Kalibawang; (C) Pengasih

The transmission potential of malaria vectors is influenced by two important factors related to their life cycle. An infected mosquito can transmit if it has the necessary time periods for the pathogen to develop or the incubation period, and once infected, the mosquito needs *blood feeding* to transmit the infection. It can be concluded that the vectorial capacity of mosquitoes does not depend on their life cycle but depends on the increase in the number of infectious mosquito bites. The younger the mosquito when infected, the more likely it is to transmit the infection (Iacovidou *et al.*, 2022).

The Morphological Characteristics of *Anopheles* sp.

Anopheles species found in the study were identified morphologically. The results in the three sites indicate that many of the captured mosquitoes were found resting around livestock barns or perching on livestock.

1. *Anopheles vagus*

Diagnostic Characters: a. The palpus proboscis has a pale band that is approximately 3 times the length of the dark band underneath, b. The tarsus of the hind legs has a dark color.

2. *An. maculatus*

Diagnostic Characters: a. The palpus has three pale bands with one narrow band and two pale bands of almost equal width, b. The third pale band is separated by a dark band approximately ¼ the length of the pale band. The tarsus of the hind legs has a pale color.

3. *An. Kochi*

Diagnostic Characters: a. The palpus color is mostly pale, and the pale band has golden hues, b. The tarsus of the hind legs is mottled with three pale bands.

4. *An. barbirostris*

Diagnostic Characters: a. Palpus and proboscis appear entirely dark, b. The femur tibia joint has a slight pale band.

5. *An. balabacensis*

Diagnostic Characters: a. Palpus with 2 pale bands appears wide, b. Tarsus of the hind legs has a clear pale band at the fourth internode.

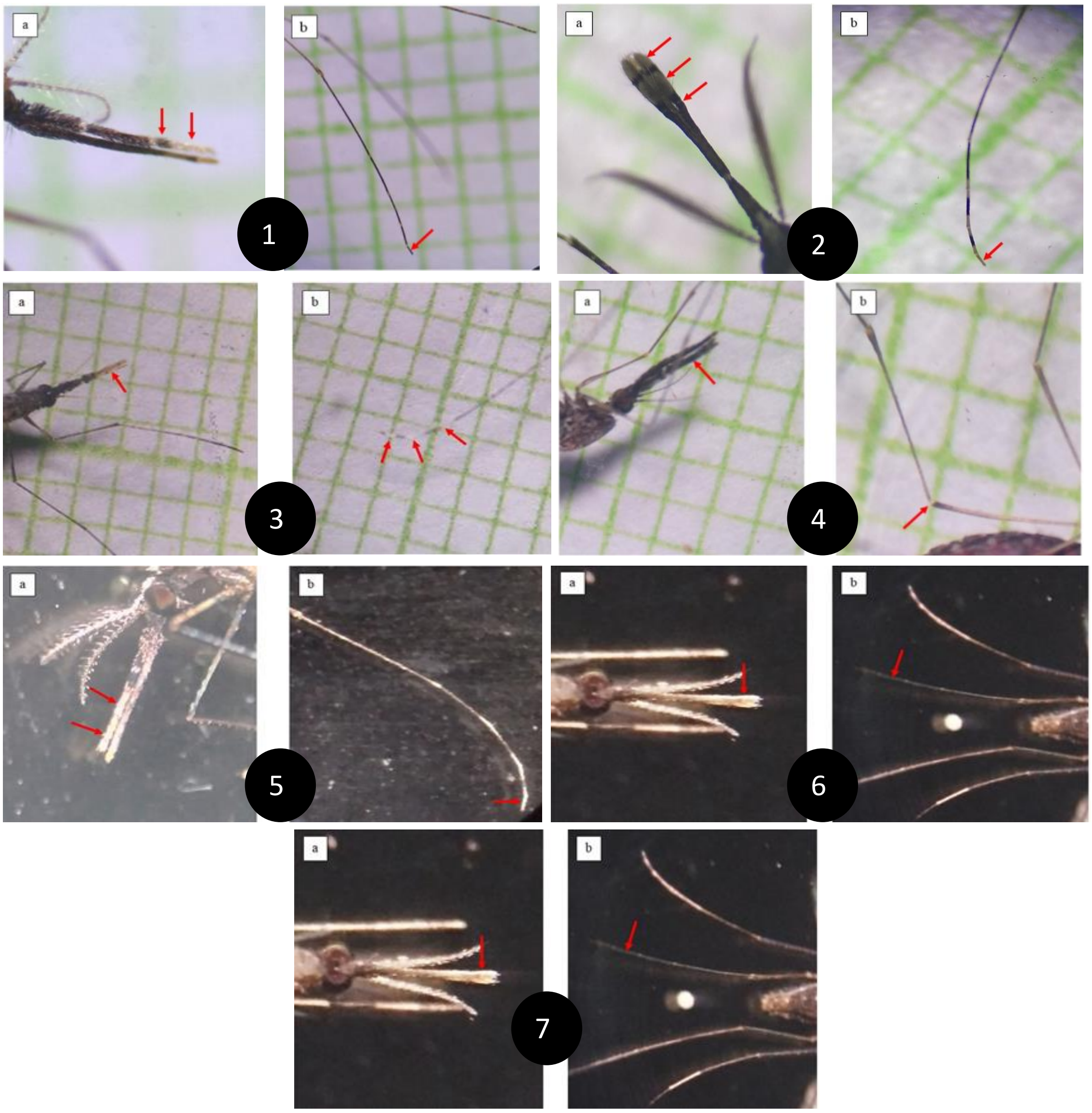

Figure 2. Morphological Characteristics of *Anopheles* sp. 1. *Anopheles vagus*, 2. *Anopheles Maculatus*, 3. *Anopheles Kochi*, 4. *Anopheles barbirostris*, 5. *Anopheles balabacensis*, 6. *Anopheles aconitus*, 7. *Anopheles annularis*

6. *An. aconitus*

Diagnostic Characters: a. The palpus has pale bands that tend to appear wide, b. The tarsus has no pale bands.

7. *An. annularis.*

Diagnostic Characters: a. Palpus with 3 pale bands, b. The tarsus of the hind leg appears pale at the first segment. Those caught in Samigaluh Sub-District were also found resting on livestock. These included one *An. kochi* and one *An. barbirostris. An. vagus* was the dominant species in Kalibawang and Pengasih, while *An. kochi* was dominant in Samigaluh. *An. vagus* generally prefers livestock and is exophilic. It was found between 18.00 - 19.00. As a malaria vector, this species has been widely spread throughout Indonesia (Novianto et al., 2022). *An. kochi* is also a malaria vector in Sumatra and Sulawesi. It is widely found outdoors after sucking blood before searching for breeding habitat (Mahdalena & Wurisastuti, 2021).

Relative Abundance, Frequency, Dominance, and Density of *Anopheles* Mosquitoes

The data on relative abundance, frequency of mosquitoes caught, species dominance, and mosquito density were calculated for the species under investigation (Table 3). The highest figure of each indicator corresponds to its presence at the respective site. Table 3 reports that *An. vagus* dominates in each of the analysis parameters. *An. vagus* is reported at relative abundance of 77.37% and frequency of 0.7% (resting). The largest dominance is also identified in *An. vagus* at 0.61 in the resting state, with the highest density of 12.25 catcher/ person/hour. *An. vagus* is found in almost all major islands in Indonesia, and this species is more prevalent in high-density areas, compared to other species (Novianto et al., 2022). The second largest relative abundance, frequency, dominance, and density is found in *An. kochi.* Its abundance is marked at 14.21%, with a frequency of 0.14% (resting) and 0.5% (landing). The largest dominance is also found in *An. vagus* at 0.019 in resting and 0.071 in landing, and the highest density is 2.25 catcher/hour. *An. kochi* is dominant in Samigaluh Sub-District, a highland. This affirms a report noting that *An. kochi* inhabits hilly areas (Yuniawan et al., 2019).

The highest relative abundance, frequency of mosquitoes caught, and dominance rate in a species can indicate that the species has the highest density and can be stated as a potential vector of disease in an area (Rushadi et al., 2021). The species found to be malaria vectors, i.e., *An. vagus* and *An. kochi*, have the highest density, and they are often found around livestock barns and bite livestock barns. To a large extent, that the research site is close to livestock barns is strongly associated with the increase in the mosquito population. Another important point to note is that *An. vagus* possesses better adaptability compared to other species (Azkiyah et al., 2021).

Table 3. Relative Abundance, Frequency, Dominance, and Density of *Anopheles* Mosquitoes

Species	Relative Abundance (%)	Frequency of Mosquitoes Caught		Species Dominance		MHD
		Resting	Landing	Resting	Landing	
An. vagus	77.37	0.792	0	0.61311	0	12.25
An. maculatus	2.63	0.013	0	0.00035	0	0.417
An. kochi	14.21	0.14	0.5	0.01992	0.071	2.25
An. barbirostris	2.11	0.022	0.25	0.00045	0.005	0.333
An. balabacencis	0.26	0.003	0	0.00001	0	0.042
An. aconitus	3.16	0.03	0.25	0.00094	0.008	0.5
An. annularis	0.26	0.003	0	0.00001	0	0.042

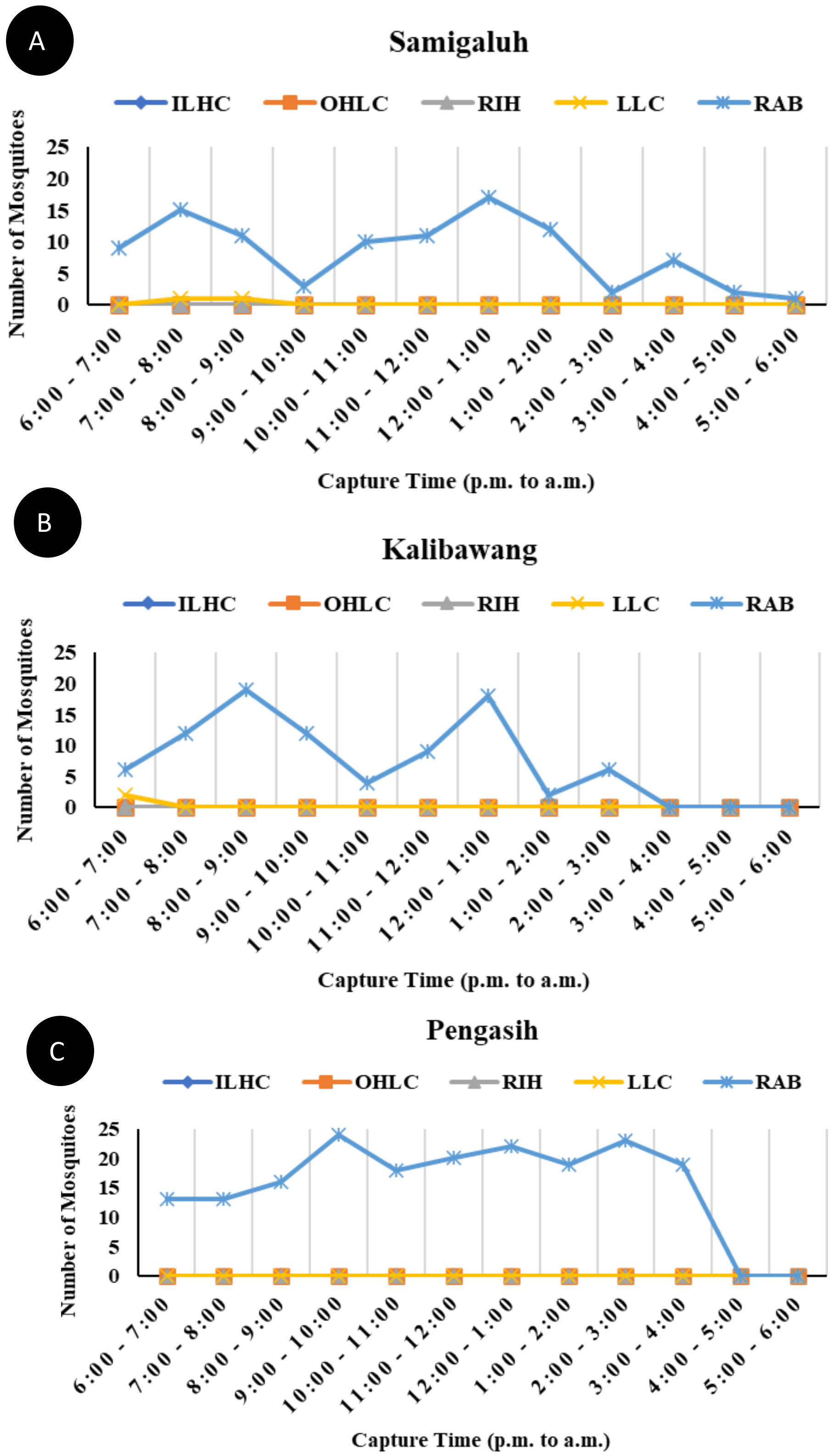

Figure 2. The number of *Anopheles* sp. captured in each capture time with different methods

Figure 2 shows the highest number of captured mosquitoes for each location is RAB. In Samigaluh Sub-District, the highest density is marked at 24:00 - 01:00, while in Kalibawang and Pengasih Sub-District, the highest density is marked at 20:00 - 21:00 and 21:00 - 22:00, respectively. Following the peak density, the graph tends to decrease and demonstrate another rise at certain times. Livestock barns can be a potential location for mosquitoes to rest after blood feeding. The barns around the house can prevent *Anopheles* mosquitoes from biting humans directly. The placement of livestock barns can distract mosquitoes from humans because *Anopheles* mosquitoes tend to favor livestock blood (Krismahardi, 2023).

Another method that found the presence of *Anopheles* mosquitoes is livestock perching. *Anopheles* mosquitoes can be found perching on livestock, but the number is determined by the activity of the catcher and the livestock. When livestock is not at rest, it is very difficult to find *Anopheles* mosquitoes. OHLC, IHLC, and RIH methods failed to capture *Anopheles* mosquitoes in all sites. This can be caused by community behaviors, such as the use of insecticides, mosquito repellents, and mosquito nets that affect the blood feeding on humans (Azkiyah et al., 2021). *Anopheles vagus* has been widely found in Indonesia and is a malaria vector because it contains *Plasmodium vivax* (Novianto *et al.*, 2022). The results showed that *An. vagus* eggs only take about one to four days to hatch after contact with water if the water temperature is warmer then the breeding will be faster (Lobo & Laumalay, 2019). The extensive *breeding places* ranging from rice fields to puddles make *An. vagus* very easy to find in high dominance (Karo *et al.*, 2021).

The Number of *Anopheles* Mosquitoes at Each Hour of Capture

Figure 2 shows that most of the mosquitoes are found resting around livestock barns. The total number of *Anopheles* mosquitoes caught is 374. The data on the abundance of *Anopheles* mosquitoes per hour of capture is presented in Figure 3. Figure 3 shows that the dominant species are *An. vagus* in Kalibawang and Pengasih Sub-Districts, and *An. kochi* in Samigaluh Sub-District.

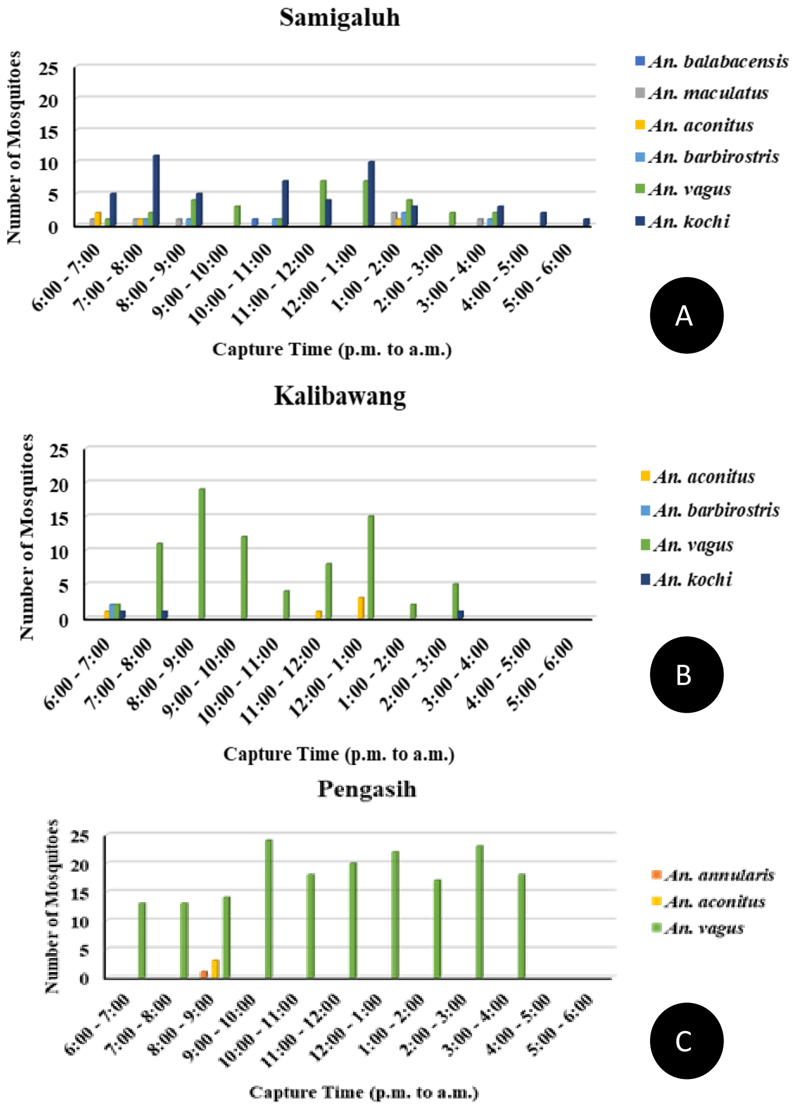

Figure 3. Number of *Anopheles* mosquitoes caught per hour at each location

Figure 3 shows that the dominant species are *An. vagus* in Kalibawang and Pengasih Sub-Districts, and *An. kochi* in Samigaluh Sub-District. Both were found resting around livestock barns. This suggests that the dominant species are exophilic and exophagic. Figure 3 shows the peak biting of *Anopheles* mosquitoes occurs from 19:00 to 22:00, after which the biting rate declines. An increase in *Anopheles* biting activity is marked at 24.00 - 01.00.

The species prevalent throughout the night at all hours of capture is *An. vagus*. This species is mostly active at 18.00 and reaches its peak density at 20.00 - 22.00. This aligns with research (Novianto et al., 2022), which shows that *An. vagus* is active from 18:00. Another dominant species is *An. kochi* in Samigaluh Sub-District. It is identified with its peak activity at 19.00 - 20.00 and 24.00 - 01.00. *An. kochi* is a zoophilic mosquito that feeds on livestock, such as cows and goats, and has a peak activity at 18.00 - 20.00 (Dalilah et al., 2022; Yahya et al., 2020).

The Influence of Abiotic Factors on the Presence of *Anopheles* Mosquitoes

The abiotic factors were measured at the research site from 18.00 to 06.00. Abiotic factors can affect the presence of *Anopheles* mosquitoes. Table 3 describes the temperature (20.7° C) and humidity (82.6%) measured at the research site in Samigaluh Sub-District. The temperature and humidity measured in Kalibawang Sub-District are marked at 24.4° C and 70.04%. At the research site in Pengasih Sub-District, the same parameters are found at 26.4° C and 81.7%.

The research site in Samigaluh Sub-District is found to have the lowest average temperature and the highest humidity compared to other sites. This is because the site in Samigaluh Sub-District is a highland, characterized by generally low average temperature and high humidity. By contrast, the site in Pengasih Sub-District is a lowland that features high average temperature and low humidity.

Air temperature is strongly influenced by the altitude and location of an area. Air humidity affects the presence of *Anopheles* mosquitoes. Humidity below 60% causes mosquito body fluids to dry out and die while any humidity rate above 60% will prolong the life of mosquitoes and speed up reproduction (Sinum et al., 2023). Temperature and humidity play an important role in mosquito breeding. The optimum temperature threshold is between 25 ° -27° C, and the optimum humidity is above 60% (Azkiyah et al., 2021).

The Effect of Abiotic Factors on *Anopheles* Larvae

Larvae at the research site were taken for rearing and identification. Afterward, abiotic larval data were measured, including air temperature and humidity, using a thermohygrometer. The water pH level was measured using a pH meter. Wind speed was also measured using an anemometer. In addition, DO was measured using a DO meter by bringing water samples from the larval location to the Ecology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences. The abiotic data are shown in the following table.

Table 3. Measurement of abiotic factors affecting *Anopheles* mosquitoes

Measurement Indicators	Research Location		
	Samigaluh	Kalibawang	Pengasih
Temperature (°C)	20.7	24.4	26.4
Air Humidity (%)	82.6	70.04	81.7

Table 4. Abiotic measurements of *Anopheles* larvae

Abiotic Factors	Mean Measurement		
	Samigaluh	Kalibawang	Pengasih
Temperature (°C)	18.7	28.1	31.3
pH	8.8	8.1	8.3
Air Humidity (%)	88.2	63.5	67.8
DO (5 days) (mg/l)	8.8	7.6	5.1

The measurement of larval abiotic factors helps to determine the characteristics of the habitat in which the larvae are bred. Measurements at the research site in Samigaluh Sub-District show the temperature at 18.7° C, air humidity at 88.2%, pH of 8.8, and DO level of 8.8 mg/l. Abiotic factor measurements in Kalibawang Sub-District document the temperature at 28.1° C, air humidity at 63.5%, pH of 8.1, wind speed at 0.9 m/s, and DO level of 7.6 mg/l. Another measurement in Pengasih Sub-District marks a temperature at 31.3° C, air humidity at 67.8%, pH of 8.3, wind speed at 1.4 m/s, and DO level of 5.1 mg/l.

Anopheles breeding grounds are characterized by temperatures ranging from 20° to 38° C, humidity between 52-96%, water pH of 4.7-9.1, and salt content of 0-53 ‰. *An. vagus* and *An. barbirostris* can adapt to certain salt levels. The presence of larvae is also influenced by the presence of flora which can serve as shelter from predators (Aida et al., 2022). The larvae were reared until they became adult mosquitoes. Once they grew up, these adult mosquitoes were then analyzed in the laboratory. Based on Table 2, two species have been identified from the larvae found, namely *An. vagus* and *An. maculatus*. The analysis confirmed that 6 larvae have successfully turned into adult mosquitoes is 6.

CONCLUSION

Anopheles mosquitoes are prevalent vectors in several regions in Indonesia. One of the malaria endemic areas is Kulon Progo District in Yogyakarta, Central Java. This study collects data from three research sites in Kulon Progo District, i.e., Samigaluh, Kalibawang, and Pengasih Sub-Districts. The results have corroborated that the species most commonly found across the research is *An. vagus*. Besides, the results also acknowledge that *An. vagus* has the potential to become a malaria vector.

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