



Longitudinal Surveillance of Anopheles Mosquitoes in Kulon Progo, Indonesia

Fardhiasih Dwi Astuti¹✉, Ali Sukamto², Shafira Putri Hermayanti¹

¹ Faculty of Public Health, Ahmad Dahlan University, Yogyakarta, Indonesia

² Health Office of Kulon Progo Regency, Yogyakarta, Indonesia

Article Info

Article History:

Received:

20 April 2026

Revised:

11 June 2026

Accepted:

23 July 2026

Published:

30 July 2026

Keywords:

Anopheles

Entomological Surveillance

Malaria Elimination

Vector Receptivity

Citation:

Astuti, F.D., Sukamto, A., & Hermayanti, S.P. (2026).

Longitudinal Surveillance of Anopheles Mosquitoes in Kulon Progo, Indonesia.

MEDICA (International Medical Scientific Journal),

8(7), 608–618.

<https://doi.org/10.53770/medica.v8i7.961>

Abstract

Sustaining malaria elimination requires continued surveillance in receptive areas where ecological and anthropogenic conditions may still support vector persistence. This study assessed monthly *Anopheles* mosquito density in Kulon Progo Regency, Indonesia, during 2023–2024 as an early warning indicator for malaria re-establishment. A descriptive longitudinal entomological surveillance study was conducted in receptive subdistricts of Kulon Progo. Adult mosquitoes were collected from 18:00 to 06:00 using indoor and outdoor human landing catches and resting collections around houses and livestock shelters. Specimens were identified morphologically, and density was expressed as Man-Hour Density (MHD). *Anopheles* density varied markedly by subdistrict and year. Nanggulan recorded the highest MHD in both years, increasing from 35.21 in 2023 to 69.46 in 2024. Additional measurable vector activity was detected in Kokap (MHD = 6.53) and Sentolo (MHD = 20.00) in 2024. Species data showed that *Anopheles vagus* was the dominant morphologically identified taxon, accounting for 84.7% of all specimens, while *An. anularis*, *An. aconitus*, *An. kochi*, *An. barbirostris*, and several low-frequency taxa were also recorded. Malaria receptivity in Kulon Progo remains heterogeneous and dynamic despite malaria-free status. Routine monthly entomological surveillance can support early detection of increased vector density and guide spatially targeted vector control. The findings should be interpreted as evidence of vector receptivity rather than confirmed transmission because sporozoite infection, vector competence, and blood-meal source were not assessed.

Corresponding Author:

✉ Fardhiasih Dwi Astuti

Faculty of Public Health, Ahmad Dahlan University, Yogyakarta, Indonesia

Email: fardhiasih.dwiastuti@ikm.uad.ac.id

1. INTRODUCTION

Malaria continues to be a significant global health challenge, particularly in tropical and subtropical regions where favorable environmental conditions support the survival and proliferation of *Anopheles* mosquitoes, the primary vectors of *Plasmodium* parasites (Kogan, F., 2020). Globally, an estimated 2.3 billion people—approximately 41% of the world's population—live in malaria-risk areas, with *Plasmodium falciparum* and *Plasmodium vivax* responsible for the vast majority of cases (Battle & Baird, 2021). However, recent insights emphasize that the global burden of *P. vivax* has been underestimated due to latent and subpatent reservoirs, hepatic and sexual latency, and diagnostic limitations. These biological complexities obscure the full scale of transmission and health impacts, particularly in resource-limited settings (Battle & Baird, 2021). In countries approaching or maintaining elimination, surveillance systems must therefore monitor not only human cases but also the receptivity of local environments to vector persistence.

In Indonesia, sustaining malaria elimination remains a public health priority, particularly in previously receptive districts where vector habitats, human mobility, climate variability, and land-use change may permit re-establishment. Vector surveillance is a core component of elimination maintenance because it enables the detection of changes in species distribution, density, and bionomics before epidemiological signals become apparent (Setiyaningsih et al., 2018; Xia Zhi-Gui et al., 2021). Evidence from Indonesia further indicates that heterogeneous *Anopheles* ecology requires locally adapted surveillance and vector control rather than uniform intervention strategies (Nurwidayati et al., 2024; Setiyaningsih et al., 2019).

International experience supports the operational value of longitudinal vector monitoring in post-elimination and near-elimination settings. Continuous surveillance in China, India, Thailand, and other settings has shown that temporal vector monitoring can identify seasonal peaks, residual receptivity, and geographic areas requiring targeted responses (Feng et al., 2022; Rahi et al., 2024; Walshe et al., 2024; Zhang et al., 2018). Such evidence is consistent with integrated vector management principles, which emphasize the use of locally generated entomological intelligence to guide timely, site-specific public health actions (Otolorin et al., 2025).

Bioecological mapping of *Anopheles* spp. The importance of adapting these tools to local vector behavior has been further exemplified across endemic and previously endemic regions (Utami et al., 2022). For example, the emergence of *Anopheles barbirostris* as a potential vector in peri-urban areas necessitates enhanced entomological coverage in zones historically considered low-risk. The strategic alignment of such surveillance activities with elimination objectives strengthens health systems' resilience against the risk of reintroduction.

Existing studies have documented the benefits of vector surveillance in endemic and post-elimination regions. However, there is a limited amount of longitudinal subdistrict-level data available that represent the monthly dynamics of *Anopheles* density in Indonesia. Although research in Purworejo led the way in understanding species composition and distribution (Setiyaningsih et al., 2018), this is a sorely needed step to address intra-annual variations and their representation in risk assessment frameworks for early intervention.

At national and regional levels, vector control strategies have largely been reactive, with insufficient incorporation of entomological intelligence in forward planning for public health (Messenger et al., 2023). The lack of routine vector data impedes timely responses, especially in areas where malaria is no longer prioritized. This study aimed to characterize the monthly density and species composition of *Anopheles* mosquitoes in Kulon Progo Regency during 2023–2024. Specifically, the study assessed spatial and temporal

variation in Man-Hour Density across receptive subdistricts and described the dominant and low-frequency *Anopheles* taxa detected through routine entomological surveillance. The findings are intended to inform local early warning systems and strengthen public health strategies to sustain malaria elimination.

2. METHODS

This study used a descriptive longitudinal entomological survey design to characterize the monthly density and species composition of *Anopheles* mosquitoes in Kulon Progo Regency, Yogyakarta Special Region, Indonesia, during 2023 and 2024. The survey was conducted in malaria-receptive areas across six subdistricts: Kalibawang, Samigaluh, Nanggulan, Temon, Kokap, and Sentolo. These sites were selected on the basis of local malaria risk classification and historical programmatic records indicating receptivity to malaria transmission.

Monthly surveillance was conducted when operationally feasible. Because collection effort varied across site-year combinations, mosquito abundance was standardized using Man-Hour Density (MHD), allowing comparison across sites with different numbers of collectors and collection hours. Environmental variables such as rainfall, temperature, humidity, and land-use indicators were not measured concurrently in this study; consequently, seasonal interpretations were treated as ecological inferences and not as formal environmental associations.

Adult mosquito collections were undertaken at three selected houses in each survey location. Night collections were performed from 18:00 to 06:00 using a combination of human landing catch (HLC) and resting collection in nearby livestock shelters. HLC was conducted both indoors and outdoors to capture host-seeking mosquitoes, while additional collections were made from walls and surrounding animal shelters to increase the likelihood of detecting *Anopheles* mosquitoes present in the local environment.

Collections were conducted hourly throughout the night. Within each hour, approximately 40 minutes were allocated for human landing catches using an aspirator, followed by 10 minutes of resting collections from walls and adjacent livestock shelters. This standardized schedule was applied to allow comparison of mosquito density across sites and survey periods. All collected adult mosquitoes were sorted and identified morphologically to species level using standard taxonomic characteristics for *Anopheles*.

This research utilized a descriptive quantitative design. The vector surveys were conducted in malaria-risk areas across Kulon Progo. Observations were conducted hourly from 18:00 to 06:00. Each hour included 40 minutes of human-bait collection using an aspirator and 10 minutes of wall and surrounding livestock shelter collections. The aspiration process targeted both indoor and outdoor wall surfaces, as well as nearby animal enclosures, to maximize vector detection and density estimation.

The primary entomological indicator used in this study was Man-Hour Density (MHD), which was used to standardize mosquito density across collection sites and survey periods. MHD was calculated as follows:

$$\text{MHD} = \text{Number of mosquitoes collected} / (\text{Number of collectors} \times \text{Total collection hours})$$

In addition to MHD, the number of mosquitoes collected by species and by collection site was summarized to describe species composition and temporal variation in *Anopheles* abundance. Because the objective of this study was to describe vector density and species composition under routine surveillance conditions. Data were analyzed descriptively to summarize mosquito density and species composition by month, year, and collection site. Monthly MHD values were compared across subdistricts to identify temporal trends, seasonal increases in mosquito density, and areas with persistently elevated vector

abundance. Species counts were tabulated to describe the relative distribution of *Anopheles* taxa across sites and years. The findings were presented in tables and figures to support interpretation of spatial and temporal patterns relevant to malaria receptivity monitoring.

Because HLC involves human collectors, field activities were conducted with attention to participant safety and informed participation. Collectors were adults who received information about field procedures and potential exposure risks and participated voluntarily. Permission for household and peridomestic collections was obtained from household representatives before sampling. The surveillance activity was reviewed by an ethics committee at Ahmad Dahlan University with ethical approval No.012408236.

3. RESULTS AND DISCUSSION

Table 1 summarizes *Anopheles* mosquito density by collection site in 2023 and 2024. In 2023, Nanggulan recorded the highest MHD (35.21), followed by Temon (12.46), Samigaluh (4.54), and Kalibawang (4.41). In 2024, Nanggulan remained the highest-density site, with MHD increasing to 69.46. Sentolo also showed substantial vector activity (MHD = 20.00), followed by Kalibawang (10.41), Kokap (6.53), and Samigaluh (3.63). These data demonstrate marked spatial heterogeneity in vector density and suggest that malaria receptivity in Kulon Progo is not evenly distributed across subdistricts.

Table 1. *Anopheles* mosquito density by collection site in Kulon Progo Regency, 2023–2024.

Year	Collection Site	Number of Mosquitoes	Number of Collectors	Collection Hours	Man-Hour Density (MHD)
2023	Kalibawang	5.185	14	84	4.41
2023	Samigaluh	1.961	9	48	4.54
2023	Nanggulan	845	2	12	35.21
2023	Temon	299	2	12	12.46
2024	Samigaluh	2.092	12	48	3.63
2024	Kalibawang	2.498	8	30	10.41
2024	Nanggulan	1.667	2	12	69.46
2024	Kokap	705	6	18	6.53
2024	Sentolo	972	4	12	20.00

The species composition of Anopheles mosquitoes collected is detailed in Table 2.

Table 2. Species density of *Anopheles* Mosquitoes by collection site in Kulon Progo, 2023–2024.

Year	Collection Site	<i>An. maculatus</i>	<i>An. barbirostris</i>	<i>An. aconitus</i>	<i>An. vagus</i>	<i>An. kochi</i>	<i>An. anularis</i>	<i>An. balabensis</i>	<i>An. indinatus</i>	<i>An. tessellatus</i>
2023	Samigaluh	9	13	18	1.806	74	41	0	0	0
	Kalibawang	11	120	200	4.359	135	350	9	1	0
	Nanggulan	0	13	8	770	6	48	0	0	0
	Temon	0	0	0	296	0	0	0	3	0
2024	Samigaluh	65	72	84	1.478	123	266	4	0	0
	Kalibawang	5	2	133	2.076	114	165	0	2	1
	Nanggulan	0	0	13	1.414	3	236	0	1	0
	Kokap	10	18	55	612	6	0	0	4	0
	Sentolo	0	5	8	937	1	2	0	19	0

Species composition is presented in Table 2. Across all sites and years, *Anopheles vagus* was the dominant morphologically identified taxon, representing 13.748 of 16.224 collected mosquitoes (84.7%). Other recurring taxa included *An. anularis* (6.8%), *An. aconitus* (3.2%), *An. kochi* (2.8%), and *An. barbirostris* (1.5%). Low-frequency taxa, including *An. maculatus*, *An. balabacensis*, *An. indivinitus*, and *An. tessellatus*, were also recorded.

The dominance of *An. vagus* was consistent across high-density areas, including Kalibawang and Nanggulan, while the detection of additional taxa in 2024, particularly in Kokap and Sentolo, indicates a heterogeneous vector community. This species diversity should be interpreted as evidence of ecological receptivity and potential vector complexity rather than proof that all taxa contribute equally to malaria transmission.

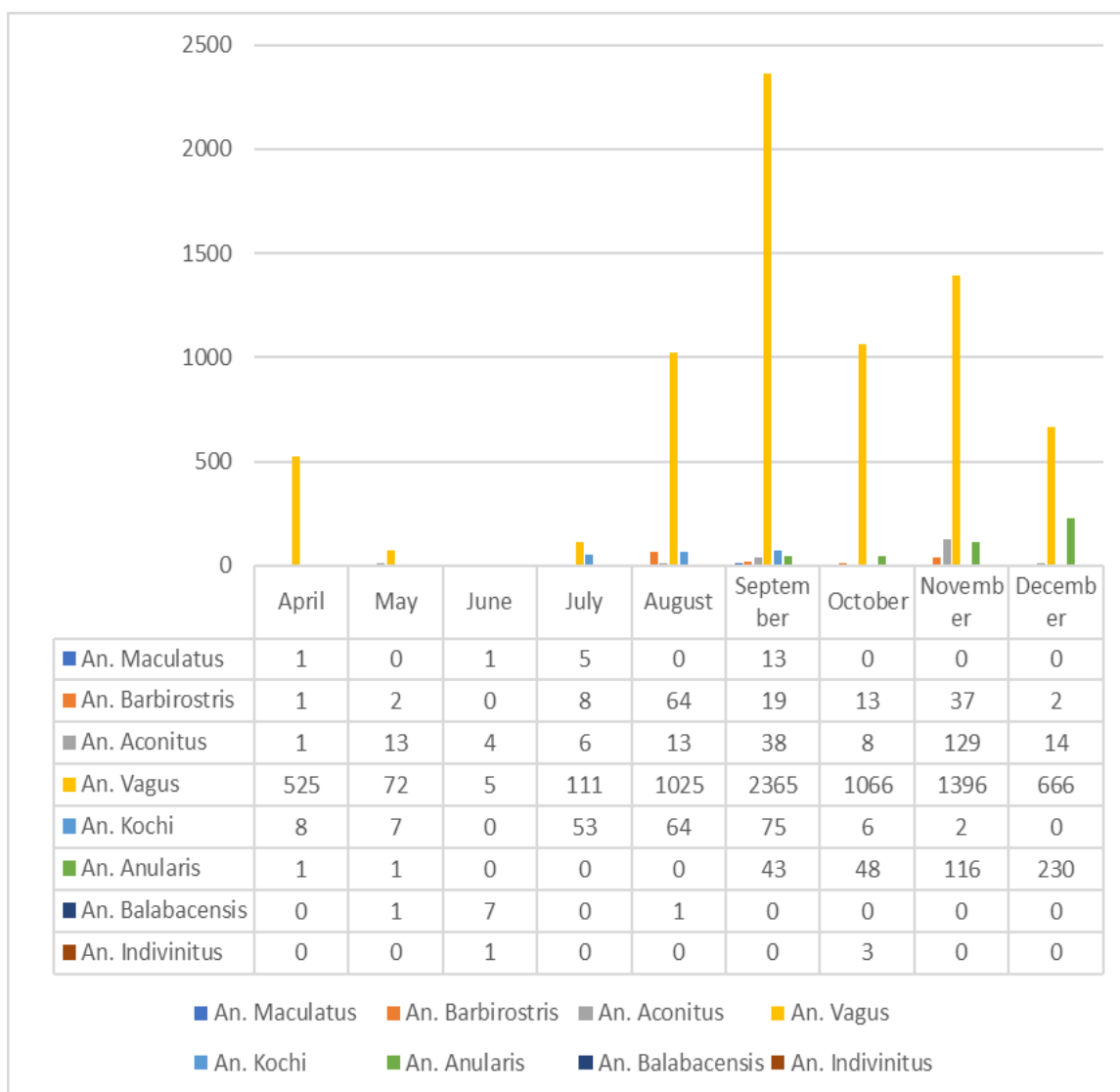


Figure 1. Monthly density of *Anopheles* species in Kulon Progo Regency, 2023.

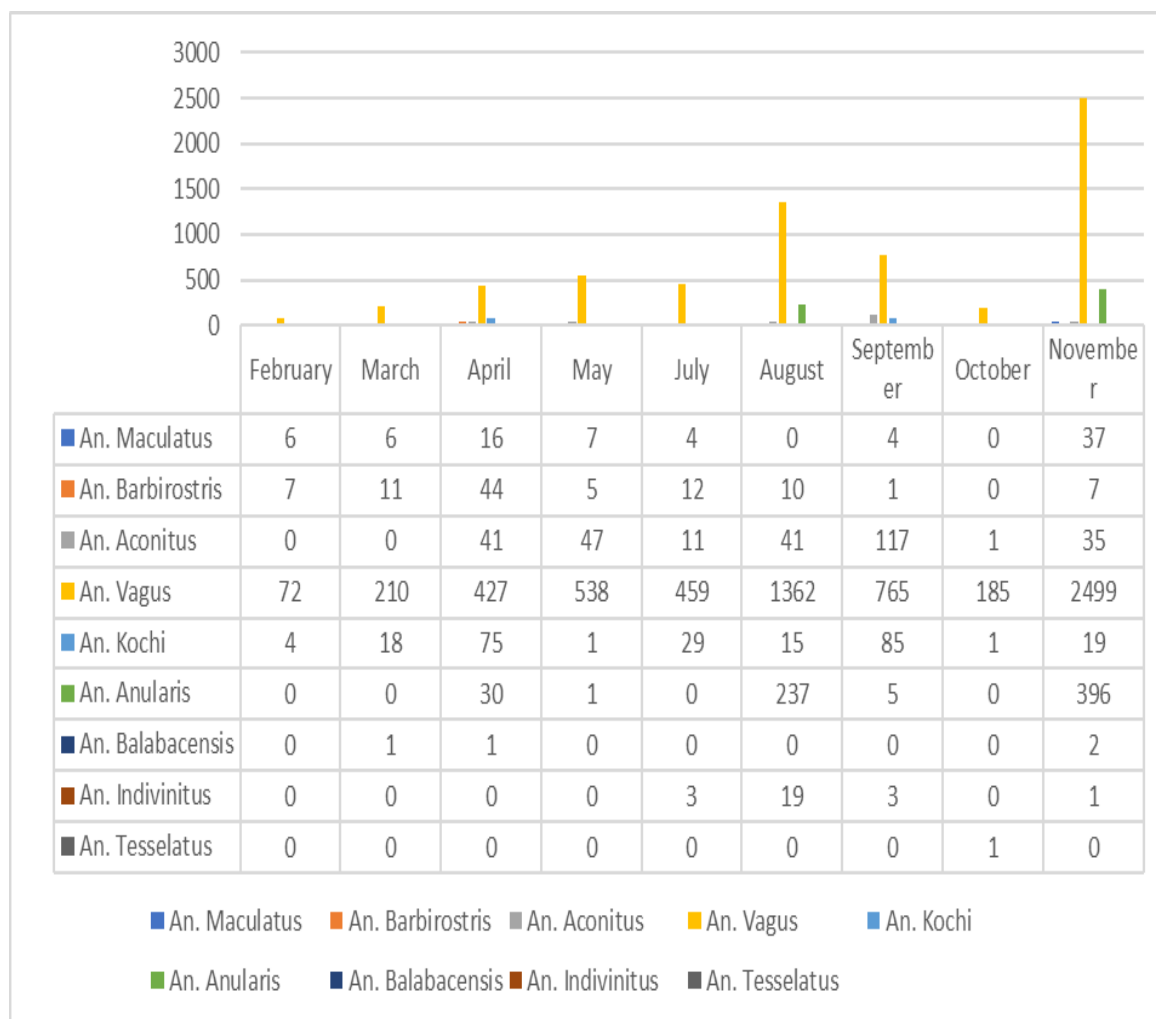


Figure 2. Monthly density of *Anopheles* species in Kulon Progo Regency, 2024.

Figure 1-2, show the monthly changing of *Anopheles* mosquito density in all Kulon Progo. The results demonstrated a consistent pattern of higher density during the transitional rainy seasons, more pronounced at the beginning and end of each year, highlighting an opportunity to boost surveillance efforts in entomological monitoring.

This longitudinal surveillance indicates that *Anopheles* density in Kulon Progo is spatially and temporally heterogeneous. The consistently elevated MHD in Nanggulan, together with the emergence of measurable vector activity in Kokap and Sentolo in 2024, suggests that district-level risk maps should be updated regularly rather than relying solely on historical classifications. These findings are operationally important because, in elimination and post-elimination settings, the continued presence of vectors in ecologically suitable areas can sustain the risk of transmission re-establishment even when local case numbers are low or absent (Hewavitharane et al., 2025; Walshe et al., 2024; Zhang et al., 2018).

The seasonal fluctuation of mosquito density was observed on a monthly basis, having higher density is noted in months with transition from dry to rainy and vice versa periods which are consistent with previous studies that highlighted the influence of

humidity, rainfall, and development sites abundance (Hewavitharane et al., 2025; Mwalimu et al., 2024; Noeth et al., 2025). Similar seasonal patterns have been reported in Sri Lanka, where vector abundance in the post-elimination era varied significantly with climatic conditions and collection site, and in Tanzania, where longitudinal surveillance demonstrated recurring monthly peaks associated with rainy seasons across different ecological strata (Hewavitharane et al., 2025; Mwalimu et al., 2024; Rozo-López & Mengual, 2015). Surveillance at the China–Myanmar border also showed that vector densities may rise seasonally despite marked progress toward elimination, emphasizing the need for continued entomological vigilance in receptive settings (Zhang et al., 2018). In Kulon Progo, the recurrent density peak in Nanggulan may be related to local habitat characteristics, including riverine environments and irrigated agricultural areas, which have previously been identified as receptive landscapes for malaria vectors (Elia et al., 2024). The larval survey showed that *Anopheles* breeding habitats were generally associated with the presence of vegetation, shaded conditions, water temperature, and pH. These environmental characteristics indicate that larval productivity in the study area was influenced by microhabitat conditions that provide suitable protection, temperature stability, and water quality for larval development (Radl et al., 2024). Although environmental variables were not formally analyzed in the present study, the repeated temporal pattern suggests that local ecological drivers are likely contributing to vector persistence.

The predominance of *An. vagus* and the repeated detection of *An. aconitus*, *An. barbirostris*, *An. kochi*, and *An. anularis* are consistent with the broader Indonesian context, where malaria receptivity is shaped by diverse *Anopheles* fauna across ecotopes (Nurwidayati et al., 2024). However, high abundance alone does not establish vector competence or confirm ongoing transmission. Studies from Ethiopia and western Kenya show that suspected or secondary vectors may contribute to residual transmission, but incrimination requires sporozoite testing, blood-meal analysis, and behavioral assessment (Mustapha et al., 2021; Tarekegn et al., 2024),.

From a public health perspective, these results support the value of routine, localized entomological surveillance as an early warning component of malaria elimination strategy. The persistent high density in Nanggulan and the emergence of Kokap and Sentolo as areas of vector activity indicate that surveillance maps and control priorities should be updated regularly rather than assuming that historical risk patterns remain unchanged. Similar conclusions have been drawn in malaria elimination settings in Thailand and Côte d'Ivoire, where entomological monitoring has been used to guide local decision-making, prioritize receptive areas, and support timely vector control responses (Kouassi et al., 2023; Walshe et al., 2024). In this context, monthly surveillance can help district health authorities identify when and where vector density begins to increase, enabling more targeted environmental management, larval source reduction, and other locally appropriate vector control measures. This approach is also consistent with integrated vector management principles, which emphasize the use of site-specific entomological evidence to tailor interventions to local ecology and operational needs (Okara et al., 2010; Otolorin et al., 2025; Vu et al., 2021).

The findings also have implications for how malaria-free status is sustained in districts with heterogeneous ecology. Post-elimination programmes often prioritize case surveillance, yet vector surveillance remains essential for understanding receptivity and

anticipating reintroduction risk (Hewavitharane et al., 2025; Walshe et al., 2024; Zhang et al., 2018). In Kulon Progo, the coexistence of persistently high-density areas and newly emerging areas of vector activity suggests that elimination gains will be more secure if routine entomological surveillance is institutionalized alongside epidemiological surveillance. Such integration is particularly important in settings where mobility, land-use change, and seasonal environmental variation may alter vector distribution more rapidly than administrative risk maps are updated.

Several limitations should be acknowledged. First, mosquito identification was based solely on morphology, which is appropriate for routine surveillance but may not fully resolve cryptic or closely related species; therefore, some taxa may require future confirmation using molecular methods (Weeraratne et al., 2017). Second, no examination for *Plasmodium* sporozoites was performed, so infectivity-based indicators such as sporozoite rate and entomological inoculation rate could not be estimated. Third, the study did not assess blood-feeding preference, biting time, insecticide susceptibility, or environmental covariates quantitatively, which limits inference regarding vector behavior and the mechanisms underlying spatial heterogeneity. Finally, human landing catch remains highly informative for host-seeking density, but it is also sensitive to operational variation among collectors, and this should be considered when comparing densities across time and place (Acheé et al., 2015).

Despite these limitations, the study provides useful operational evidence that *Anopheles* density and species composition in Kulon Progo are dynamic and geographically uneven. The persistence of high density in Nanggulan, together with the emergence of vector activity in Kokap and Sentolo, reinforces the need for adaptive surveillance and locally responsive vector management. Future work should strengthen these findings by incorporating climatic data, molecular confirmation of selected taxa, and infection testing to better define the entomological basis of malaria receptivity in this malaria-free district.

4. CONCLUSION

This study showed that *Anopheles* density and species composition in Kulon Progo Regency varied substantially across subdistricts and between 2023 and 2024, indicating that malaria receptivity remains spatially heterogeneous in this malaria-free district. Nanggulan consistently exhibited the highest mosquito density, while Kokap and Sentolo emerged as additional areas of vector activity in 2024. The predominance of *An. vagus*, together with the recurrent detection of several other morphologically identified *Anopheles* taxa, suggests that local receptivity is shaped by a diverse and changing vector community. Operationally, these findings support the institutionalization of routine monthly entomological surveillance within district malaria prevention strategies. Local health authorities should prioritize high-density areas for intensified monitoring, update receptive-area maps using current vector data, and align vector control with seasonal and spatial density patterns. Because infectivity, vector competence, and environmental determinants were not directly measured, the findings should be interpreted as evidence of receptivity rather than confirmed local transmission. Future studies should incorporate environmental monitoring, molecular species confirmation, sporozoite detection, and insecticide susceptibility testing to strengthen early warning systems in Kulon Progo and similar malaria-free districts.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Fardhiasih Dwi Astuti and Ali Sukamto conceptualized the study, developed the research design, coordinated data collection, conducted data analysis, and drafted the initial manuscript. Supported data collection, transcription, coding, and organization of qualitative data. Fardhiasih Dwi Astuti and Shafira Putri Hermayanti contributed to data validation. All authors contributed to manuscript revision, approved the final version of the manuscript, and agreed to be accountable for all aspects of the work.

ACKNOWLEDGEMENT

The authors sincerely thank the Health Office of Kulon Progo Regency (Dinas Kesehatan Kabupaten Kulon Progo) for its essential role in supporting this study. Its contribution to field coordination, operational facilitation, and the implementation of entomological surveillance was fundamental to the conduct of this research. The authors further acknowledge the dedication of the field staff and local personnel involved in mosquito collection and data documentation.

FUNDING

This research received no external funding.

DECLARATION OF ARTIFICIAL INTELLIGENCE USE

During the preparation of this work, the authors used ChatGPT to enhance the clarity of the writing. After using ChatGPT, the authors reviewed and edited the content as needed and take full responsibility for the publication's content.

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