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Entomological Assessment of Mosquito Diversity and Density in Negara District, Bali: Implications for Vector-Borne Disease Surveillance

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ABSTRACT

Indonesia has one of the highest prevalences of malaria in Asia, with annual case numbers on the rise. Despite being a major international tourist destination, the Negara Sub-district in Jembrana Regency, Bali, continues to experience notable incidences of both diseases. Entomological surveys are crucial for evaluating mosquito diversity and density, which supports effective vector control strategies. This study, conducted in five villages from September to October 2024, employed entomological survey methods to calculate the House Index (HI), Container Index (CI), Breteau Index (BI), and Density Figure (DF). Mosquito species diversity was assessed using the Shannon-Wiener diversity index and evenness (E) and dominance (C) metrics. Results indicated that HI, CI, and BI values were within the low transmission risk category, with a DF of 4 and a larva-free rate (ABJ) of ≤95%. Six mosquito species were identified: *Aedes aegypti*, *Aedes albopictus*, *Anopheles dirus*, *Armigeres subalbatus*, *Culex quinquefasciatus*, and *Mansonia uniformis*. The species diversity was moderate ($1 < H' \leq 3$), even species distribution ($E > 0.6$), and low dominance ($C \leq 0.5$). These findings suggest a relatively low risk of vector-borne disease transmission. However, given the presence of multiple disease-vector species and the region's dynamic human activity, continuous entomological surveillance remains essential to anticipate population surges and strengthen disease prevention efforts.

1. Introduction

Mosquitoes (*Culicidae*), belonging to the order Diptera, are particularly notable due to their role as vectors of significant human diseases. According to WHO data, Indonesia bears the highest burden of mosquito-borne diseases in Southeast Asia, including dengue fever, malaria, and filariasis, with cases rising annually due to factors such as poor sanitation, high population density, and inadequate vector control measures (Faraizka Amalia & Astutik 2019; Tsheten *et al*. 2021). Despite concerted efforts, these diseases remain prevalent, exacerbated by increased human mobility and inadequate public awareness of environmental hygiene (Teo *et al*. 2017; Sedda *et al*. 2018).

Bali, a prominent global tourist destination, faces a significant risk of mosquito-borne diseases like *Japanese encephalitis* (JE), dengue, malaria, and filariasis, primarily due to its international tourist traffic, expansive agricultural systems including the "subak" irrigation, and widespread pig farming, all of which create ideal mosquito breeding grounds

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(Widjajanti *et al*. 2019; Wahono *et al*. 2022). The Indonesian Ministry of Tourism emphasizes the importance of maintaining a healthy, mosquito-free environment for both residents and visitors in Bali (Kementerian Pariwisata Republik Indonesia 2016). Jembrana regency in western Bali is particularly affected, recording 1,317 dengue cases between 2019 and 2023, with Negara subdistrict experiencing the second-highest incidence of dengue and malaria in the past five years (BPS Jembrana 2024a, 2024b). Recent studies also suggest a link between international travel and the presence of JE virus strains found in mosquitoes and pigs across Bali (Tajima *et al*. 2023).

Targeted vector management strategies grounded in comprehensive data on mosquito diversity, density, and habitat preferences are urgently needed. Understanding the characteristics of mosquito larval habitats, their behavior, potential diseases they may transmit, species diversity, and population density within a specific area can serve as crucial parameters for designing more effective and targeted mosquito vector control programs, particularly in the Negara district, Jembrana, Bali.

There is a paucity of localized entomological data from high-risk subdistricts such as Negara, where ecological and demographic conditions may support unique mosquito population dynamics. This study addresses this gap by assessing mosquito diversity and vector indices to inform evidence-based, site-specific vector control. The findings are expected to contribute to more effective surveillance and disease prevention strategies in Bali and similar endemic areas.

2. Materials and Methods

This study employed a descriptive-exploratory design with a qualitative approach, conducted from September to October 2024, in five villages within the Negara Sub-district, Jembrana Regency, Bali: Kaliakah, Banyubiru, Baluk, Cupel, and Pengambengan. These villages were selected purposively based on data from the Jembrana District Health Office, which reported a high incidence of dengue hemorrhagic fever (DHF) and malaria in these areas over the past five years. The study population included all residential buildings within the selected villages. Mosquito larvae sampling was conducted randomly at potential breeding sites, including bathtubs, buckets, used cans, and other water-holding containers, following the national entomological survey guidelines by Garjito *et al* (2017).

Larvae collected from the field were stored in labeled containers and transported to the Optical Laboratory, Biology Study Program, UIN Maulana Malik Ibrahim Malang, for further analysis. In November 2024, species identification was carried out using visual (macroscopic) and microscopic methods based on the morphological characteristics of the larvae and adult mosquitoes.

2.1. Field Observation

Field observations were carried out regularly to obtain data on the presence of mosquito larvae in residential areas. Buildings were randomly selected, prioritizing those with occupants available and willing to allow inspection. Within each selected building, all types of water-holding containers-such as buckets, bathtubs, jars, and used cans-were thoroughly examined for larvae. When a container was found positive for mosquito larvae, larvae collection was performed using the single larvae dipping method, following standard entomological procedures. Additionally, brief interviews with homeowners were conducted to gather supporting data on container maintenance, specifically the frequency of cleaning practices. The detection of larvae relies on visual inspection, where the presence of larvae is identified through direct observation of water surfaces and container interiors.

2.2. Sample Collection

This study employed a cluster sampling method, wherein the overall population was divided into clusters and a subset of clusters was selected randomly for sampling. All residential buildings within the selected clusters had an equal probability of inclusion. The sampling area focused on Negara Sub-district in Jembrana Regency, which was identified as a high-risk area due to the elevated incidence of dengue fever and malaria over the past five years. Negara comprises 12 villages, of which three—Kaliakah, Banyubiru, and Pengambengan—were purposively selected based on disease prevalence data from BPS Jembrana (2023). To achieve broader geographic representation and logistical feasibility, two additional villages, Baluk and Cupel, were selected randomly, resulting in five sampling locations.

In each village, 100 residential buildings were selected for the entomological survey based on the following inclusion criteria: location within one of the five selected villages, willingness of the residents to participate, and presence of water-holding containers. Buildings with water containers for keeping fish (e.g., aquariums or fish ponds) were excluded, as these inhibit mosquito larval development. Larval sampling followed the mosquito vector surveillance guidelines (Garjito *et al*. 2017). The single larvae dipping method was employed, using a 100 ml beaker to scoop water 2-3 times from each container. Water was filtered using a fine mesh net if larvae were not visible through scooping. Environmental parameters such as temperature, pH, humidity, and GPS coordinates were also measured at each sampling site and documented using standardized data sheets. All collected larvae were stored in labeled plastic containers, indicating sample location, container type, and collection time to ensure traceability and facilitate species identification. This structured sampling and data collection process ensured systematic, representative, and scientifically valid findings regarding the presence and distribution of mosquito larvae across the study area.

Three types of containers: controllable sites, disposable sites, and under-controllable sites. Controllable sites refer to water storage containers that humans can regulate in terms of water volume and cleanliness, such as bathtubs, buckets, and similar containers. Disposable sites are water-holding containers that are often neglected and considered waste, making them difficult to maintain in a clean condition. For example, discarded cans, used tires, or coconut shells filled with rainwater are often found in these areas. Under controllable sites are water-holding places that remain under continuous human supervision, such as fishponds and aquariums. This classification was adapted from the mosquito entomological survey guidelines and the journal Aspirator Vol. 11(1), Kinansi *et al*. 2019.

2.3. Mosquito Rearing and Species Identification

Collected mosquito larvae were reared to adulthood in a controlled environment to facilitate accurate species identification. The larvae were placed in small, specially designed plastic containers that prevent mosquito escape and minimize any potential risk to the surrounding environment. Mosquito light traps were also installed around the rearing area to enhance monitoring and ensure containment. The larvae were observed daily using visual inspection for 7–12 days, depending on species-specific development rates. Once the mosquitoes reached the adult stage, they were carefully collected, sorted by container and collection site, and transferred into labelled Petri dishes. Each label included information on the village of origin and the type of breeding container. The number of adult mosquitoes from each sample was recorded on structured data collection sheets. Adult mosquitoes were selected for identification because they exhibit more distinct morphological features, enabling clearer differentiation between species.

Species identification was conducted using a combination of macroscopic and microscopic methods, referring to the Identification Key for Mosquito Species in Indonesia published by the Indonesian Ministry of Health (2024a). This process included mosquitoes that emerged from reared larvae and adult mosquitoes captured via light traps at the collection sites. All specimens were handled and processed post-mortem following entomological laboratory safety standards.

2.4. Data Processing

Data processing in this study was carried out through four structured stages. First, data editing was conducted to ensure that all collected information was complete, accurate, and consistent. Second, a data coding process was applied, in which certain variables were assigned codes or attributes to facilitate further analysis. Third, the data entry stage involved inputting and organizing the coded data using Microsoft Excel 2016 for tabulation and QGIS 3.40.0 for spatial mapping and geographic data visualization. Finally, in the data tabulation stage, the processed data were categorized and summarized into analytical groups that were aligned with the research objectives, allowing for meaningful interpretation and further statistical examination.

Data on the density and distribution of mosquito populations are obtained through measurements of several indices, namely the House Index (HI), Container Index (CI), Breteau Index (BI), and Larvae Free Number (ABJ), all of which are included in the calculation of the entomological index. According to the Indonesian Ministry of Health (2024b), the mosquito entomological index can be calculated using the following formula:

a. House index (HI) is the number of houses positive for mosquito larvae from all the houses examined

$$HI = \frac{\text{Number of houses that are positive mosquito larvae}}{\text{Total number of places checked}} \times 100\%$$

b. Container index (CI) is the number of containers in which larvae were found from all containers examined

$$CI = \frac{\text{Number of containers that are positive mosquito larvae}}{\text{Total number of containers checked}} \times 100\%$$

c. Breteau index (BI) is the number of containers with larvae in 100 houses

$$CI = \frac{\text{Number of containers that are positive mosquito larvae}}{\text{100 places checked}} \times 100\%$$

Based on the values of the House Index (HI), Container Index (CI), and Breteau Index (BI), mosquito larval density (Density Figure, DF) can be classified as low, moderate, or high. A low category is indicated by DF = 1, a moderate category by DF = 2–5, and a high category by DF = 6–9. The following table 2.3 presents the classification of mosquito larval density into low, moderate, and high categories.

Table 2.3. Categories of larval density based on larval indices (Sorisi & Pijoh 2017)

Density figure	HI	CI	BI	Category
1	1-3	1-2	1-4	Low
2	4-7	3-5	5-9	Moderate
3	8-17	6-9	10-19	Moderate
4	18-28	10-14	20-34	Moderate
5	29-37	15-20	35-49	Moderate
6	38-49	21-27	50-74	High
7	50-59	28-31	75-99	High
8	60-76	32-40	100-199	High
9	≥ 77	≥ 41	≥ 200	High

2.5. Data Analysis

Quantitative data, including the number of mosquito larvae and adult mosquitoes, as well as environmental parameters such as temperature, pH, and humidity, were compiled and presented in tabular form. Statistical analysis was performed using IBM SPSS version 31. The Chi-square test was applied to assess differences in container types (controllable sites, disposable sites, and undercontrollable sites) and environmental parameters (temperature, pH, and humidity) in relation to ideal breeding conditions for mosquito larvae. The Geographic Information System (GIS) software, QGIS version 3.40.0, was utilized to generate maps of sampling locations based on recorded geographic coordinates, thereby visualizing the spatial distribution. Additionally, correlation analyses were conducted in SPSS to identify relationships between environmental factors and mosquito population parameters.

3. Results

3.1. Mosquito Population Density in Five Villages of Negara District, Jembrana Regency, Bali

Five hundred buildings across five villages in the Negara District, Jembrana Regency, Bali, were inspected as part of an entomological survey. Of these, 130 buildings (26%) were positive for mosquito larvae. Additionally, 160 water-holding containers were identified as breeding sites for mosquitoes. Statistical analysis revealed a strong link between container type and the presence of mosquito larvae ($p < 0.05$), with controllable sites, such as bathtubs, harboring the highest number of larvae. To quantify the extent of mosquito infestation, several entomological indices were calculated, including the House Index (HI), Container Index (CI), Breteau Index (BI), Density Figure (DF), and Free Larvae Index (ABJ) (Table 1).

The House Index (HI) values across all villages ranged narrowly from 25% to 27%, indicating a relatively uniform distribution of larval-positive houses. The Container Index (CI) varied between 11.2% and 14%, reflecting the proportion of water containers acting as breeding sites. Notably, Banyubiru and Pengambengan exhibited the highest Breteau Index (BI) values (35), suggesting higher densities of breeding containers per 100 houses. In contrast, Baluk and Cupel reported lower BI values (28), indicative of moderate larval density. Despite differences in CI and BI values, the Density Figure (DF) remained constant at four across all villages, classifying the mosquito-borne disease transmission risk as moderate for 2024. The Free Larva index (ABJ) ranged from 73% to 75%, revealing persistent larval presence despite ongoing vector control measures.

Geospatial analysis using QGIS 3.40.0 showed that larval-positive sampling points were more densely distributed in villages with lower ABJ values, particularly Kaliakah and Banyubiru (Figure 1). These villages also had higher population densities and more water storage containers, which likely contributed to increased prevalence of breeding sites. In contrast, Baluk and Cupel displayed fewer concentrated points, which is consistent with their higher ABJ values and lower BI.

This study also considered environmental conditions at the time of sampling, including temperature, pH, and humidity (Table 2). The ambient temperature across the

Table 1. Entomological indices in five villages of Negara District, Jembrana Regency, Bali

Component	Results				
	Kaliakah	Banyubiru	Baluk	Cupel	Pengambengan
House Index (HI)	27	27	25	25	26
Container Index (CI)	14	14	11	11	14
Breteau Index (BI)	34	35	28	28	35
Density Figure (DF)	4	4	4	4	4
Free Larva index (ABJ)	73	73	75	75	74

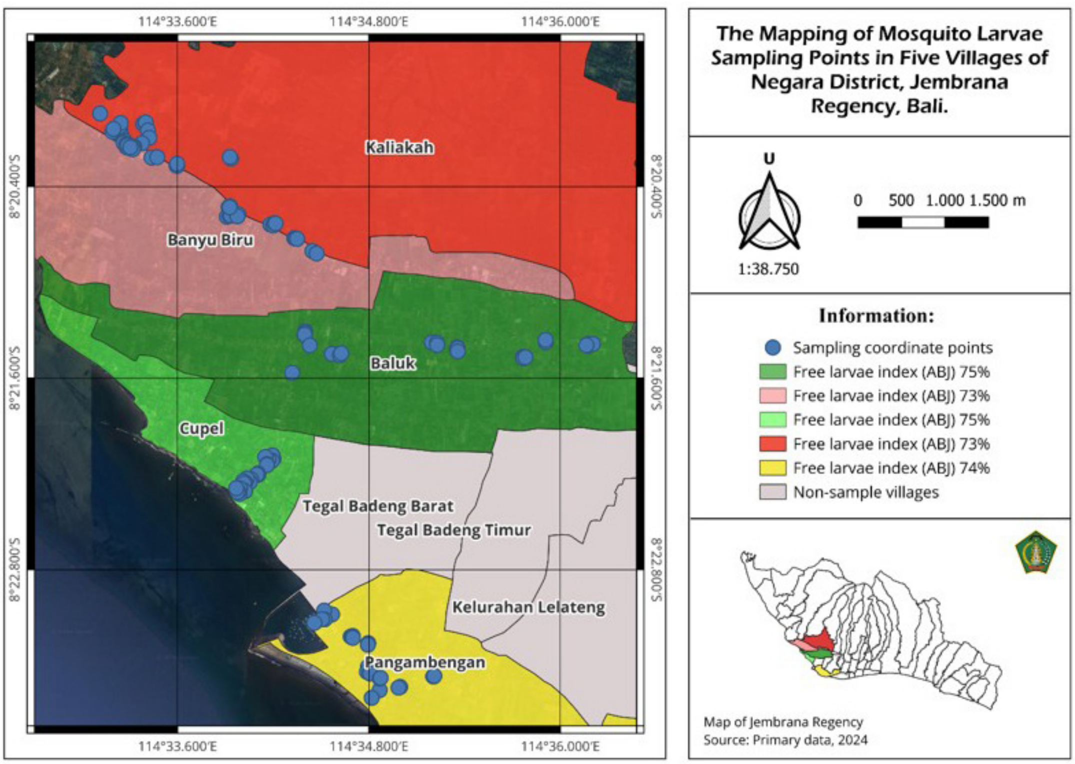

Figure 1. Geospatial analysis of mosquito larvae distribution in Five Villages of Negara District, Jembrana Regency, Bali using QGIS 3.40.0

Table 2. Environmental conditions in five villages of Negara District

Village	Temperature (°C)	pH	Humidity (%)	Height (mdpl)
Kaliakah	26-30	6-7	72-80	250-700
Banyubiru	26-32	6-8	72-82	250-700
Baluk	28-32	5-8	74-78	250-700
Cupel	29-33	7-8	72-78	250-700
Pengambengan	28-32	6-8	74-80	250-700

villages ranged from 26°C to 33°C, which falls within the optimal range for mosquito breeding. Water pH varied between 5 and 8, with a neutral pH (around 7) being most favorable for mosquito larval survival. Humidity levels ranged from 72% to 82%. Statistical analysis using the Chi-square test in IBM SPSS version 31 revealed that both temperature and humidity significantly influenced mosquito breeding site conditions ($p < 0.05$). In contrast, water pH did not show a statistically significant effect ($p > 0.05$).

3.2. Diversity of Mosquito Species in Five Villages of Negara District, Jembrana Regency, Bali

Mosquito species were collected and identified from five villages in the Negara District, Jembrana Regency, using macroscopic and microscopic techniques. Species determination was conducted based on morphological characteristics, following the identification key provided in Indonesia's Key Book of Determination and Identification of Mosquito Species (Ministry of Health 2024a). Specimens with identical diagnostic features were grouped as the same species.

A total of six mosquito species were identified: *Aedes aegypti*, *Aedes albopictus*, *Anopheles dirus*, *Culex quinquefasciatus*, *Mansonia uniformis*, and *Armigeres subalbatus*. Among these, *Aedes aegypti* was the most prevalent (271 individuals), whereas *Mansonia uniformis* was the least abundant (12 individuals) (Table 3). *Aedes aegypti* is a well-known vector of the dengue virus. At the same time, *Mansonia uniformis* plays a role in transmitting the Venezuelan equine encephalitis virus and is a key vector of lymphatic filariasis caused by filarial nematodes.

Biodiversity analysis using the Shannon-Wiener index (H') indicated that mosquito diversity in all habitat types was moderate ($1 < H' \leq 3$). The highest species diversity was observed in ditch environments (H' = 1.52), followed by gardens/rice fields (H' = 1.49) and buildings (H' = 1.47). Livestock pens had slightly lower diversity (H' = 1.37), while ovitraps recorded the lowest diversity (H' = 1.23). The species evenness index (E) across all habitat types exceeded 0.6, indicating a relatively even distribution of mosquito species without significant dominance by a single species. This finding is consistent with the calculated dominance index (C), which in all habitats remained ≤ 0.5, suggesting no species overwhelmingly dominated the mosquito population (Table 4).

4. Discussion

The present study highlights the ecological and epidemiological profile of mosquito populations in Negara District, Jembrana Regency-a region with a history of endemic diseases and growing urban tourism development. While entomological indices such as HI, CI, BI, and DF fell within the low-risk range, the consistent presence of larvae across surveyed villages reflects persistent breeding activity. This result suggests that vector control efforts may be insufficient in eliminating microhabitats that are conducive to mosquito development (Day 2016).

Compared to findings in similar tropical rural-urban transition zones, the moderate species diversity (H' between 1.2 and 1.5) and high evenness observed here indicate a relatively balanced mosquito population, yet capable of sustaining multiple vector-borne diseases. This distribution contrasts with urban settings where *Aedes aegypti* often becomes overwhelmingly dominant. The coexistence of *Aedes*, *Anopheles*, *Culex*, and *Mansonia* in similar ecological zones is less commonly reported. It marks a unique feature of this landscape, pointing to overlapping transmission potentials for dengue, malaria, and filariasis (Day 2016; Wilke *et al*. 2019). Calculation of the mosquito diversity index remains important even though all mosquito species have the potential to transmit disease. This result is due to the fact that each species has distinct biological characteristics, behaviors, and epidemiological roles in the spread of disease. By understanding the level of species diversity and dominance, we can assess the potential risk of disease transmission and develop more specific and effective control strategies (Keesing 2006). Additionally, the diversity index can also reflect the stability of the mosquito ecosystem and serve as an indicator of the success of vector control intervention efforts in a given area.

The spatial patterns of species presence-particularly in household and livestock-related containers-suggest

Table 4. Species diversity (H'), even species distribution (E), and dominance index (C) of mosquito populations by habitat type

Place	Species diversity (H')	Even species (E)	Dominance (C)
Building/house	1.47	0.82	0.28
Ditch/drain	1.52	0.85	0.25
Garden/rice field	1.49	0.83	0.26
Livestock pen	1.37	0.77	0.28
Ovitrap	1.23	0.68	0.36

Table 3. Identified mosquito species and their distribution by habitat category in five villages of Negara District

Species	Number of species per place category					Total
	Building	Ditch	Garden	Livestock pen	Ovitrap	
Aedes aegypti	213	12	5	6	35	271
Aedes albopictus	130	12	3	0	22	167
Culex quinquefasciatus	52	12	4	7	4	79
Armigeres subalbatus	58	1	4	1	4	68
Anopheles dirus	40	4	11	6	5	66
Mansonia uniformis	9	2	0	1	0	12

behavioral adaptations of mosquito species to human and animal proximity. This result differs from prior observations in more forested areas where *Anopheles dirus* predominates near vegetative cover. Moreover, despite relatively low HI and BI, the moderate Free Larvae Index (ABJ) implies that larval habitats may be more cryptic or distributed beyond traditional breeding sites, challenging standard control protocols (Rose *et al*. 2020; Sorrels 2021).

Environmental parameters recorded in this study, such as temperature and humidity, were conducive to mosquito proliferation. However, the lack of pH influence on larval viability aligns with previous research showing species-level tolerance to a broad pH range. Still, the simultaneous influence of microclimate and human practices-such as water storage behavior and waste disposal-deserves further scrutiny in this socio-geographic context. Notably, this study integrates species ecology with entomological risk indices in a peri-coastal district renowned more for its tourism than for vector research. The coexistence of six medically important species in a small geographic range suggests the potential for overlapping transmission cycles, which may be amplified by mobility, tourism influx, and climate variation (Rose 2020; McMahon and Wimberly 2023).

While the current vector indices indicate a low immediate transmission risk, the ecological richness and adaptability of mosquito species in Negara District highlight the need for continuous entomological surveillance. The coexistence of multiple disease vectors in proximity to human populations underscores the urgency of implementing integrated vector control strategies that extend beyond reactive interventions. Future research should explore seasonal fluctuations, patterns of insecticide resistance, and spatial modeling using remote sensing technologies to enable more targeted and predictive mosquito management. Sustained monitoring efforts, coupled with active community engagement, will be essential for maintaining low disease incidence and preventing future outbreaks in this dynamic landscape (Mulyaningsih 2024).

Additionally, a limitation of this study lies in the exclusive use of the Shannon-Wiener diversity index to assess mosquito species diversity. Although widely used, this index has been critiqued for its limited interpretability in ecological contexts. Alternative metrics-such as Hill numbers, Chao estimators, and rarefaction curves-offer more robust and informative insights into species richness and community structure. However, the application of these advanced metrics was not feasible in the present study due to constraints in sample size and relatively low species richness. Future research with broader sampling coverage and higher taxonomic resolution is recommended to enable a more comprehensive and reliable ecological assessment.

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