Tropical Biomedicine 41(4): 518-525 (2024)
https://doi.org/10.47665/tb.41.4.013

RESEARCH ARTICLE

Blood meal analysis: unveiling the feeding preferences of *Aedes aegypti* and *Aedes albopictus* in a dengue-endemic area

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ARTICLE HISTORY

Received: 3 October 2024
Revised: 1 November 2024
Accepted: 1 November 2024
Published: 31 December 2024

ABSTRACT

Studies have suggested animals as possible reservoir hosts for flaviviruses transmitted by *Aedes* mosquitoes; however, there is limited evidence for the dengue virus in Malaysia. One of the possible ways to determine the zoonotic potential for any pathogen transmission is through blood meal analysis which can provide valuable insights into the feeding preferences of the mosquitoes. Unfortunately, limited information is available on the feeding preferences of *Aedes* mosquitoes in Malaysia. Thus, this study aimed to identify the blood-feeding preferences of *Aedes aegypti* and *Aedes albopictus* from different ecotypes in Selangor, Malaysia. The field mosquitoes were collected using a modified backpack aspirator and CDC light trap. The collected mosquitoes were initially classified based on degrees of blood digestion according to the Sella scale before extracting the DNA. The presence of vertebrate DNA was detected using nested PCR, and samples positive for vertebrate DNA were further subjected to species-specific PCR targeting the common animals found at the study locations. In general, 51 of 187 field caught *Aedes* mosquitoes were positive for the presence of vertebrate DNA in their blood meal. The most frequent blood meal source was human (38.2%), followed by monkey (12.7%), bovine (10.9%), chicken (7.3%) and dog (3.6%). The human blood index (HBI) of *Ae. albopictus* collected across the four different ecotypes revealed that, *Ae. albopictus* collected near human dwellings showed 100% anthropophilic tendency. Interestingly, there were two *Aedes* mosquitoes, *Ae. aegypti* (n=1) and *Ae. albopictus* (n=1) positive for both human and monkey blood. Since sylvatic dengue continues to flourish in Southeast Asia, this finding from blood meal analysis shows the potential for zoonotic transmission by *Aedes* mosquitoes in these locations. However, further research must be carried out to understand the role of animals as potential reservoir hosts for the dengue virus, especially through the detection of the virus in the blood meal.

Keywords: *Aedes* mosquitoes; blood meal analysis; dengue virus; anthropophagic; zoophilic.

INTRODUCTION

Aedes mosquitoes are widely distributed throughout the world, with high prevalence in tropical and subtropical regions (Kraemer *et al*., 2015). It is commonly found breeding in small, water-filled containers such as tree holes, plant axils, discarded tyres, and water storage containers. They prefer clean, stagnant water and are often found in urban and suburban environments where such habitats are abundant (Nyamah *et al*., 2010). Both the *Aedes* species are vectors for several diseases, including dengue fever, Zika virus and chikungunya, making it an important public health concern in areas where it is present. Despite efforts to control vectors, variables related to climate change, urbanization and pesticide resistance have been linked to a rise in the geographic dispersion of *Aedes* species in recent years (Gómez *et al*., 2022).

In a variety of anthropogenic, climatic, and environmental circumstances, both vector species exhibit considerable ecological adaptability, which contributes to their opportunistic feeding patterns on a variety of hosts during a gonotrophic cycle (Juliano & Philip Lounibos, 2005). Typically, the dengue virus is transmitted to humans through the bite of a single infected *Aedes* mosquito; however, due to feeding disruptions, *Aedes* mosquitoes often require several blood meals to complete their gonotrophic cycle, potentially leading to clustered dengue infections (Mcclelland & Conway, 1971). After contracting an infection, the mosquitoes remain infectious throughout their lifespan, which typically ranges from a few weeks to a couple of months under favourable conditions (WHO, 2024).

Since the blood-feeding behaviour of mosquitoes plays a critical role in pathogen transmission to humans and wildlife, understanding the proportion of their diet derived from humans or their level of anthropophilic is essential for assessing the risk of disease outbreaks (Gonçalves *et al*., 2023; Xiang *et al*., 2022). For instance, *Ae. albopictus* is known for its exophagic (outdoor feeding) behaviour, with studies highlighting the importance of its blood-

Published by Malaysian Society of Parasitology and Tropical Medicine.

feeding patterns in influencing disease transmission dynamics and potentially triggering epidemics in new regions (Delatte *et al*., 2010; Richards *et al*., 2006). In the context of the dengue virus, which is primarily transmitted to humans through the bites of infected *Aedes* mosquitoes, blood meal analysis can reveal if the mosquitoes are feeding on other animals besides humans. If they are found to be feeding on animals that can also harbour the dengue virus, such as non-human primates, this indicates a potential zoonotic transmission cycle, further complicating efforts to control the disease spread (Vasilakis *et al*., 2011).

Indeed, past research indicates that the feeding patterns of *Aedes* mosquitoes can significantly influence the epidemiology of dengue, especially in regions where sylvatic strains of the virus may spill over into human populations through these vectors (Gwee *et al*., 2021). In Southeast Asian and West African forests, the sylvatic-DENV transmission cycle is still present and sustained in non-human primates and *Aedes* mosquitoes, indicating possibilities for the virus to spill over into human populations, depending on the feeding preference of the suitable vector in the location (Vasilakis *et al*., 2011). Interestingly, a previous study in Malaysia successfully demonstrated the circulation of the dengue virus among forest monkeys, indicating the existence of a sylvatic transmission cycle in this country (Rudnick, 1978). The research successfully isolated the DENV-4 strain from jungle mosquitoes as well as from sentinel monkeys used in the study. A case study in 2008 reported a 20-year-old male in Malaysia developed dengue hemorrhagic fever, and the isolated dengue virus serotype 2 was found to belong to a sylvatic lineage (Cardosa *et al*., 2009).

These findings clearly underscore the crucial role of blood meal analysis in understanding the transmission dynamics of dengue and other mosquito-borne diseases, particularly in assessing the potential for zoonotic transmission (Vasilakis *et al*., 2011). Besides, blood meal analysis has been crucial in risk assessments of potential zoonotic outbreaks. Studies have highlighted how changes in mosquito feeding patterns due to environmental or human encroachment can increase human exposure to sylvatic dengue viruses, emphasizing the need for continuous monitoring (Guzman *et al*., 2010). By understanding which hosts mosquitoes have fed on, valuable information about potential reservoirs of infection can be gained, and the risk of transmission in each population can be assessed (Fikrig & Harrington, 2021). Indeed, by deciphering the host-vector interactions, more effective targeted vector control strategies can be instituted to reduce dengue transmission (Ogunlade *et al*., 2023). Hence, this study aimed to document the blood-feeding patterns of *Ae. aegypti* and *Ae. albopictus* in one of the states in Malaysia, which has recorded the highest number of reported dengue cases in the past decades (Tay *et al*., 2022).

MATERIALS AND METHODS

Study locations

Adult female *Aedes* mosquitoes were caught from four different locations within Selangor, Malaysia. The study locations consist of various ecotypes which include forest: Bukit Gasing Hiking Trail, (3°05’44.9”N 101°39’27.2”E), forest edges: Kota Damansara Community Forest (3°10’08.1”N 101°34’50.9”E), animal farm: Farm Fresh UPM (2°59’30.4”N 101°43’59.3”E) and near human dwelling: Nadayu28 Residence (3.0702° N, 101.6039° E) (Figure 1). All locations were selected within or near the Petaling district in Selangor which had reported the highest number of dengue fever cases in Malaysia for many years (Tay *et al*., 2022).

Each study location varies topographically. Bukit Gasing Hiking Trail is a 100-hectare forest reserve that splits the Federal Territory of Kuala Lumpur from the city of Petaling Jaya. Mosquito samplings were carried out along the hiking trail. On the other hand, Kota Damansara Community Forest is a recreational area with low-land forest. Mosquito samplings were carried out at the forest fringes. Interestingly, numerous long-tailed macaques (*Macaca fasicularis*) were sighted at both locations, along with other common animals such as dogs, birds, and reptiles. On the contrary, Farm Fresh UPM is a dairy farm that breeds farm animals such as dairy cattle, chickens, and ducks, whereas Nadayu28 Residence is a modern condominium residency area for students and working adults in Petaling district, Selangor. There are a lot of domestic animals, such as dogs and cats, sighted around this high-rise apartment complex.

Collection of field *Aedes* mosquitoes

Mosquito collections were carried out from September 2023 to December 2023 using a modified backpack aspirator (Jeyaprakasam *et al*., 2022) and CDC light-trap (Jeyaprakasam *et al*., 2021). Weekly collections were conducted from 0600 to 2200 hours on a rotation basis between the four locations, with 2 nights of sampling carried out each week. The modified backpack aspirator was used mainly to collect the resting *Aedes* mosquitoes. Sweeping technique was used to capture resting mosquitoes in all potential places such as under the vegetation, on the wall, in piles of debris, in tree holes, under eaves and in shaded corners of outdoor structures. On the other hand, the CDC light traps were set outdoors to trap mosquitoes at a designated place in each study location for the same duration of time. The CDC light-traps were set 1.5m above the ground in their respective locations. Light from incandescent bulbs and carbon dioxide from dry ice were used as attractants to catch the mosquitoes.

Identification and classification of field *Aedes* mosquitoes based on the Sella scale

The collected female *Aedes* mosquitoes were morphologically identified to the species level by observing the distinct characteristics at the thorax of the mosquitoes using an illustrated taxonomical key (Jeffery *et al*., 2012). The *Aedes* mosquitoes were further observed in the abdominal region using a dissecting microscope to determine their blood engorgement status. The mosquitoes were grouped into seven stages based on the Sella scale (Detinova, 1962).

Stage 1 features mosquito with empty stomach without blood and ovaries undeveloped. Stage 2 shows a freshly blood-fed female, still with undeveloped ovaries. In Stage 3, the blood meal darkens, with blood-free areas extends to the 2–2.5 sternites and 4–5 tergites. By Stage 4, blood becomes very dark, and free areas extend to the 2.5–3 sternites and 5–6 tergites. Stage 5 presents blackened blood

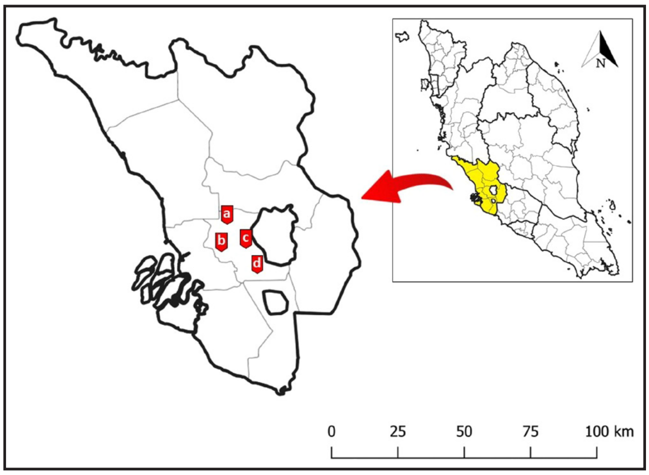

Figure 1. Map of Selangor state in Malaysia showing the sampling locations for the study: **(a)** Kota Damansara Community Forest (Forest edges), **(b)** Nadayu28 Residence (Human dwellings), **(c)** Bukit Gasing Hiking Trail (Forest) and **(d)** Farm Fresh UPM (Animal farm).

Table 1. Prevalence of *Aedes* mosquitoes collected based on different study locations

Mosquito species	Bukit Gasing (Forest)	Kota Damansara Community Forest (Forest fringes)	Farm Fresh, UPM (Farm)	Nadayu28 Residences (Near human dwellings)
Ae. aegypti	5 (12.2%)	16 (40.0%)	8 (13.8%)	45 (93.8%)
Ae. albopictus	36 (87.8%)	24 (60.0%)	50 (86.2%)	3 (6.2%)
Total	**41**	**40**	**58**	**48**

with blood-free areas reaching the 2.5–3.5 sternites and 6.5–7.5 tergites. Stage 6 sees remaining black blood confined ventrally, as developing ovaries fill the abdomen. Finally, Stage 7 shows no visible blood and the eggs are fully developed (Detinova, 1962).

Sample preparation and blood meal analysis

The collected female *Aedes* mosquitoes were dissected with the aid of a dissecting microscope. The abdomen was separated from the head and thorax and stored in 95% ethanol for genomic DNA extraction. The abdomens of the mosquitoes were individually homogenized, and the DNA was extracted using the DNeasy tissue kit (Qiagen, Germany) according to the manufacturer's protocol. The extracted DNA was subjected to a nested PCR assay that targets the vertebrate mitochondrial Cytochrome *c* Oxidase Subunit I (*COI*) gene to detect the presence of DNA from the vertebrate host using primers and protocol described by Alcaide *et al*. (2009) with slight modifications, which were previously described by Jeyaprakasam *et al*. (2022).

Samples which were positive for vertebrate DNA were further subjected to species-specific PCR using the primers developed by Gunathilaka *et al*. (2016). Individual tubes were used for PCR amplifications to detect seven common vertebrates found at the study locations: cat, dog, chicken, bovine, human, wild boar and monkey. A final volume of 30 µL was used for the PCR amplification, which contained 1.25 U of GoTaq DNA polymerase (Promega), 1× Green GoTaq reaction buffer (Promega), 3.0 mM $MgCl_2$ (Promega), 0.2 mM of dNTPs mixture (Promega), 0.25 µM of forward and reverse primers, and 3 µL of DNA template. The cycling parameters include a 4-min initial denaturation at 94°C, followed by 35 cycles of 94°C for 30 s, 62°C for 60 s, 72°C for 60 s, and a final extension step lasting 10 min at 72°C (Jeyaprakasam *et al*., 2022).

Statistical analysis

The data were analysed using statistical software SPSS version 25 (IBM, New York, USA). A Pearson's chi-square test of independence, with a significance level set at α=0.05, was employed to determine whether the species distribution of *Aedes* mosquitoes collected was significantly associated with the type of collection method used. On the other hand, to identify the anthropophilic nature of the *Aedes* species, human blood index (HBI) was calculated using the formula below (Pappa *et al*., 2011):

$$\text{HBI} = \frac{\text{Number of mosquitoes which have fed on humans}}{\text{Total number of mosquitoes whose blood meals have been identified}}$$

RESULTS

Composition of *Aedes* mosquitoes based on different ecotypes

A total of 187 *Aedes* female mosquitoes were collected throughout the study period with 74 *Ae. aegypti* and 113 *Ae. albopictus*. The highest number of *Aedes* mosquitoes were caught at Farm Fresh (n=58), followed by Nadayu28 Residences (n=48), Bukit Gasing (n=41) and Kota Damansara Community Forest (n=40) (Table 1). *Aedes aegypti* was mostly collected near human dwellings at Nadayu28 Residences while *Ae. albopictus* was collected mostly in forests, forest fringes and farm areas.

Prevalence of *Aedes* mosquitoes by collection methods

The modified backpack aspirator yields a higher number of mosquitoes (n=157) compared to the CDC light trap (n=30) for both the *Aedes* species (Table 2). A Pearson's chi-square test of contingencies (with α=0.05) was used to evaluate whether the species *of Aedes* mosquitoes collected was influenced by the method of collection. The chi-square test was not statistically significant, χ^2 (1, *N*=187) = 0.126, p> 0.05, indicating the collection methods did not influence the species of *Aedes* mosquitoes collected from the study locations. This suggests that the distribution of mosquito species is independent of the collection method.

Sella score of blood meal digestion for the collected *Aedes* mosquitoes

Mosquitoes caught were further observed for their abdominal engorgement using a dissecting microscope and classified based on the Sella Scale (Table 3). A total of 187 mosquitoes were evaluated, comprising *Ae. aegypti* 39.57% (n=74) and *Ae. albopictus* 60.43% (n=113). Most mosquitoes were classified as Sella Stage 1, indicating mosquitoes which were unfed and undeveloped ovaries. As the Sella scores increase, indicating a more advanced stage of blood digestion, the number of mosquitoes in each category declines, with only a small percentage of *Aedes* mosquitoes classified at Stage 5 (3.74%) and Stage 6 (3.21%). No mosquitoes were observed at Stage 7, which denotes fully developed eggs without any trace of blood.

Table 2. Prevalence of *Aedes* mosquitoes caught using different collection methods

Mosquito species	Modified backpack aspirator	CDC light trap
Ae. aegypti	63 (40.1%)	11 (36.7%)
Ae. albopictus	94 (59.9%)	19 (63.3%)
Total	**157**	**30**

Table 3. The number of *Aedes* mosquitoes grouped based on different degrees of blood digestion according to the Sella scale

Sella Scale	*Ae. aegypti*	*Ae. albopictus*	Total
1	42	80	122
2	20	10	30
3	2	9	11
4	3	8	11
5	4	3	7
6	3	3	6
7	0	0	0
Total	**74**	**113**	**187**

Table 4. The source of blood meal for *Aedes* mosquitoes

Blood sources	Human	Chicken	Dog	Cat	Bovine	Monkey	Wild boar	Unidentified
Ae. aegypti	15	1	0	0	0	3	0	7
Ae. albopictus	6	3	2	0	6	4	0	8
Total (%)	21 (38.2)	4 (7.3)	2 (3.6)	0 (0.0)	6 (10.9)	7 (12.7)	0 (0.0)	15 (27.3)

Table 5. Number of *Aedes* mosquitoes with single and multiple blood meals

Aedes species	n	Single (n = 1)	%	Mixed (n = 2)	%	Unknown	%
Ae. aegypti	25	17	68.0	1	4.0	7	28.0
Ae. albopictus	26	15	57.7	3	11.5	8	30.8
Total	51	32	62.7	4	7.9	15	29.4

Blood meal analysis of the field caught *Aedes* mosquitoes

Overall, 187 *Aedes* mosquitoes were caught from 4 sampling locations, in which 51 mosquitoes were detected positive for vertebrate DNA (27.3%). The *Aedes* mosquitoes which were positive for blood meal were further subjected to PCR to identify the blood meal source, using primers targeting the common animals sighted in the area or expected to be present in the sampling locations. In general, the most frequent blood meal source for *Aedes* mosquitoes was human (38.2%), followed by monkey (12.7%), bovine (10.9%), chicken (7.3%) and dog (3.6%) (Table 4). None of the *Aedes* mosquitoes were positive for cat or wild boar's DNA. However, 15 vertebrate-positive samples (27.3%) from *Aedes* mosquitoes could not be identified for the source of the blood meal, suggesting the presence of DNA from vertebrates other than the seven species screened in this study.

Blood meal analysis for *Ae. aegypti* revealed that humans are the most preferred source of blood meal (57.7%), followed by monkeys (11.5%) and chickens (3.8%). For *Ae. albopictus*, both humans and bovines are equally preferred as blood meal sources, with each having a 20.7% preference. This is followed by monkeys (13.8%), chickens (10.3%) and dogs (6.9%). However, both *Ae. agypti* and *Ae. albopictus* have 26.9% and 27.6% of unidentified blood sources, respectively. Blood meal analysis showed that most *Aedes* species fed from a single source (62.7%) (Table 5).

Besides single blood meal, there was also a small proportion of *Aedes* mosquitoes with mixed blood meal (Table 5). Among 25 *Ae. aegypti*, 68.0% (n=17) had single blood meals, 4.0% (n=1) had mixed blood meals, and 28.0% (n=7) had unknown sources. On the other hand, for *Ae. albopictus* (n=26), 57.7% (n=15) had single blood meals, 11.5% (n=3) had mixed blood meals, and 30.8% (n=8) had unknown blood sources. Overall, out of 51 mosquitoes, 62.7% (n=32) had single blood meals, 7.9% (n=4) had mixed blood meals.

For *Ae. aegypti*, humans were the most common source of single blood meals (n=14), followed by monkeys (n=2) and chickens (n=1). Additionally, one *Ae. aegypti* mosquito tested positive for both human and monkey DNA in its blood meal. Besides these three vertebrates, no other animal DNA was detected in the blood meals of *Ae. aegypti*. In contrast, *Ae. albopictus* fed on a broader range of animals, including humans (n=5), bovine (n=5), monkeys (n=2), chickens (n=2), and dogs (n=1). However, no DNA from cats or wild boars was found in either mosquito species (Table 6).

Table 6. Blood sources of *Aedes* mosquitoes with single and multiple blood meals

Number of vertebrates' DNA found in the blood meal	Vertebrates	*Ae. aegypti*	*Ae. albopictus*
1	Cat	–	–
	Chicken	1	2
	Bovine	–	5
	Dog	–	1
	Human	14	5
	Monkey	2	2
	Wild boar	–	–
2	Bovine + Chicken	–	1
	Dog + Monkey	–	1
	Human + Monkey	1	1
	Unidentified	7	8
Total		**25**	**26**

In general, the type of blood meal taken by the *Aedes* mosquitoes was highly dependent on the location of the mosquito collection (Figure 2). For example, *Ae. albopictus* from Farm Fresh exhibited preferences primarily for farm animals found at the location, including bovines (n=6) and chickens (n=3). In both Bukit Gasing and Kota Damansara Community forests, *Ae. albopictus* was found positive for human, monkey, and dog DNA. On the other hand, *Ae. aegypti* found at Bukit Gasing, showed a strong preference for humans (n=5) and monkeys (n=3). At Nadayu28 Residence, a residential area, both *Ae. albopictus* and *Ae. aegypti* fed exclusively on humans. In summary, *Ae. albopictus* demonstrated a broader host range across the locations, while *Ae. aegypti* predominantly fed on humans, with occasional feeding on monkeys.

Comparison of Human Blood Index (HBI) of *Aedes mosquitoes* from different outdoor locations

Generally, *Ae. aegypti* shows a high preference for human blood near human dwellings (HBI of 1.00 at Nadayu28 Residences), while this preference decreases in more natural environments such as farms (HBI of 0.20 at Farm Fresh, UPM), forest fringes (HBI of 0.50 at Kota Damansara Community Forest) and forested area (HBI of 0.56 at Bukit Gasing) (Table 7). On the other hand, *Ae. albopictus* exhibits a lower overall HBI compared to *Ae. aegypti*, with the highest index observed near human dwellings (HBI of 0.75 at Nadayu28 Residences). Its HBI is significantly lower in forested and farm environments, with a complete absence of human blood

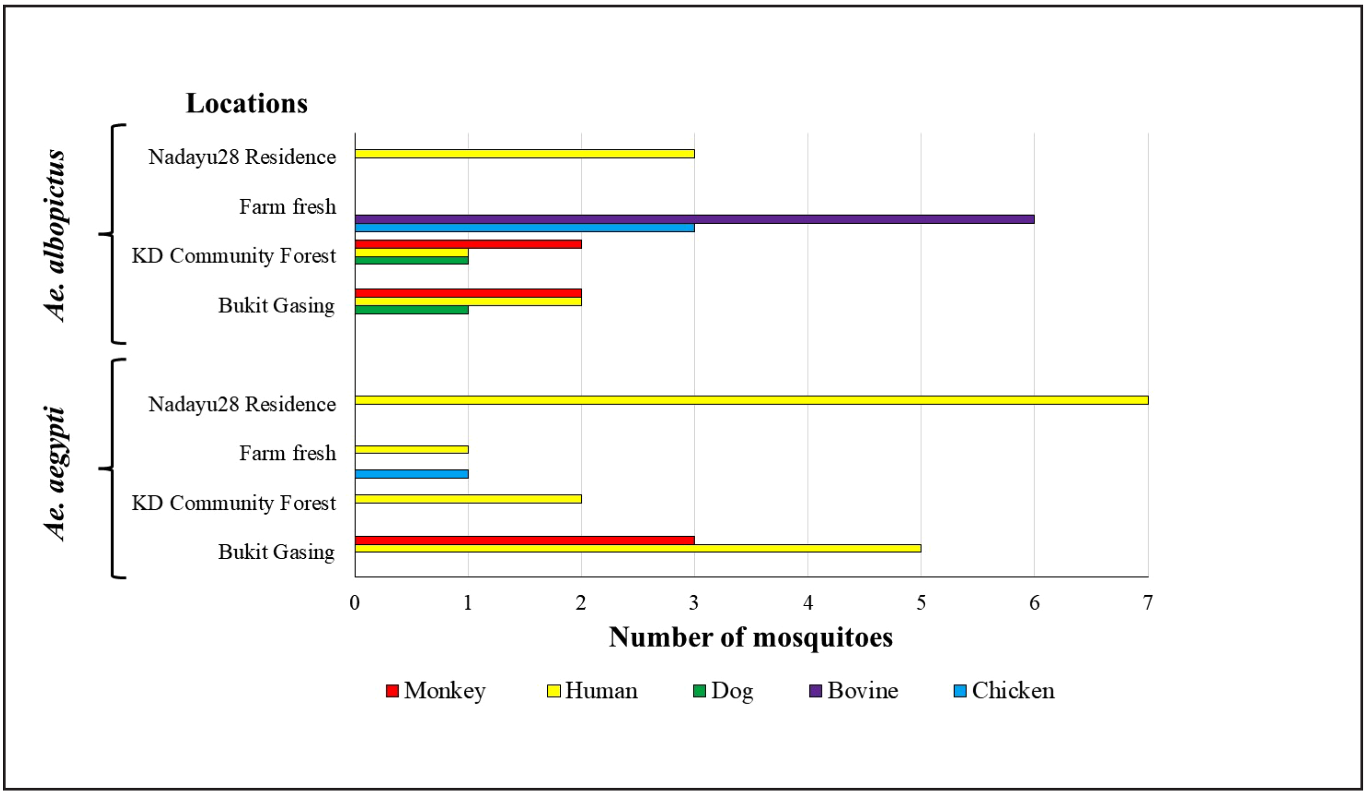

Figure 2. Blood meal preference of *Ae. albopictus* and *Ae. aegypti* based on geographical locations.

Table 7. The human blood index (HBI) of both *Ae. aegypti* and *Ae. albopictus* collected from different ecotypes

Mosquito species	Bukit Gasing (Forest)	Kota Damansara Community Forest (Forest fringes)	Farm Fresh, UPM (Farm)	Nadayu28 Residences (Near human dwellings)
Ae. aegypti	0.56	0.50	0.20	1.00
Ae. albopictus	0.29	0.20	0.00	0.75
Average	**0.44**	**0.33**	**0.07**	**0.91**

preference on farms (HBI of 0.00 at Farm Fresh, UPM). The average HBI across both species follows a similar pattern, being highest near human dwellings (0.91 at Nadayu28 Residences) and lowest in farm environments (0.07 at Farm Fresh, UPM), indicating a stronger association of both mosquito species with human presence in urban areas compared to rural or natural settings.

DISCUSSION

Dengue fever remains a major public health problem, especially in Asia, where 70% of the world's cases are reported (Bhatt *et al*., 2013). Malaysia experienced a significant surge in dengue outbreaks, totalling 67,775 cases as of June 2024, compared to 51,331 cases reported in 2023 during the same period, marking a notable escalation of dengue cases from the previous year (Shiow Chin & Gimino, 2024). Mortality due to dengue-related comorbidities also increased 29.7% during the same timeframe from 2023 to 2024 (Shiow Chin & Gimino, 2024). The study locations, which are in the state of Selangor, have consistently recorded the highest dengue cases in Malaysia for the past decades (Tay *et al*., 2022).

Both *Ae. aegypti* and *Ae. albopictus* are the primary and secondary vectors, respectively, for the dengue virus in Malaysia, which is involved in both sylvatic and human transmission cycles (Johari *et al*., 2019). In this study, more *Ae. albopictus* were collected (60.4%) compared to *Ae. aegypti* (39.6%). This outcome was anticipated, as all collections were conducted outdoors and more in forested areas compared to residential areas. *Aedes albopictus* is well known for its exophagic behavior and a strong preference for mammals, with a significant portion of its blood meals derived from humans in certain regions (Cui *et al*., 2021; Kamgang *et al*., 2012). However, it also feeds on other mammals, though less frequently (Ponlawat & Harrington, 2005). This feeding pattern, involving both humans and other animals, may contribute to the zoonotic transmission of pathogens from wildlife or domestic animals to humans (Gwee *et al*., 2021). In contrast, *Ae. aegypti* are typically endophagic mosquitoes that have adapted well to urban environments, often seeking hosts within human dwellings (Facchinelli *et al*., 2023). Interestingly, a low number of *Ae. aegypti* were also found in the outdoor collections near forests, forest fringes and farm areas. The study locations are not densely forested areas. Although located within or at the edges of forests, these locations experience heavy human traffic due to recreational activities. For example, Bukit Gasing Forest is a popular hiking spot, while Kota Damansara Community Forest is a recreational area with residential housing bordering its perimeter. This may explain the collection of *Ae. aegypti* in these areas, albeit in very low numbers. Indeed, *Ae. aegypti* are frequently found in environments where humans are present, mainly due to their strong attraction to human hosts (Kamau *et al*., 2023).

Moreover, the collection technique utilized also influences the number of *Aedes* mosquitoes collected in the study, where most of the collection was obtained through a modified backpack aspirator (84.0%), and only a few *Aedes* mosquitoes were collected through CDC light traps (16.0%). The inability of CDC light trap to collect a good number of mosquitoes was also reported in the previous study (Jeyaprakasam *et al*., 2021). The difference in the number of *Aedes* mosquitoes collected could be due to the principle behind the method. The CDC light traps, which primarily attract flying insects like mosquitoes, are less effective at capturing engorged adult mosquitoes that tend to rest after feeding, compared to modified backpack aspirators, which are specifically designed to target resting mosquitoes (Maia *et al*., 2011; Sriwichai *et al*., 2015). Better maneuverability in a variety of locations is another benefit of a modified backpack aspirator, which enables the collector to access challenging areas where mosquitoes are likely to rest (Maia *et al*., 2011). This method facilitates efficient sampling of resting adult mosquitoes, which is crucial for understanding their feeding behavior and potential as disease vectors.

Although it is widely believed that animals serve as reservoir hosts for flaviviruses such as the yellow fever virus and the Zika virus (Gwee *et al*., 2021), there is not enough data to support this theory for the spread of the dengue virus in Selangor, Malaysia. Indeed, the zoonotic transmission of these flaviviruses is highly dependent on the feeding behaviors of the mosquitoes (Huang *et al*., 2014). Unfortunately, there are very limited studies on the blood meal of *Aedes* mosquitoes, and to the best of our knowledge, no blood meal analysis has been conducted on *Aedes* mosquitoes in Peninsular Malaysia. There has only been one blood meal study conducted in Borneo Malaysia, which documented the blood meals of *Ae. albopictus* and mosquitoes from other genera (Young *et al*., 2020). Similarly, in Peninsular Malaysia, only one previous blood meal study was conducted which focused on the feeding preferences of *Anopheles* mosquitoes (Jeyaprakasam *et al*., 2022). Indeed, blood meal analysis is instrumental in assessing the likelihood of alternative transmission routes, such as the zoonotic potential of dengue transmission.

Thus, this current study was conducted to elucidate the blood meal preference of *Aedes* mosquitoes in Selangor, Malaysia, focusing on the blood meal analysis of *Aedes* mosquitoes collected from different ecotypes such as forest, forest edges, farm, and human dwelling outdoor locations. This is essential as different ecotypes with the presence of different hosts might influence the blood-feeding patterns of *Aedes* mosquitoes (Fikrig *et al*., 2022). Different ecotypes typically represent distinct ecological settings, each with its own unique assemblage of animal species. In this study, both the Bukit Gasing hiking trail and Kota Damansara Community Forest consist of forested areas, making animals such as monkeys, particularly *Macaca fasicularis*, a common sight. On weekends, hikers often brought along dogs for recreational activities. This is in parallel with the findings from the study where both monkey and dog DNA were identified during the blood meal analysis of *Ae. albopictus* from both locations. Interestingly, a very small number of *Ae. aegypti* (n=2) from Bukit Gasing were also detected to be positive for the monkey's DNA. This finding is parallel to a past study conducted in Thailand where a few *Ae. aegypti* (n=4) were found to be positive with the monkey's DNA (Khaklang & Kittayapong, 2014).

Notably, from the current study, there were also *Ae. aegypti* (4.0%) and *Ae. albopictus* (3.8%) positive for both human and monkey DNA in a mixed blood meal. However, the percentage seems lower compared to the previous study, which recorded 70.0% for *Ae. aegypti* while 87.50% for *Ae. albopictus* (Khaklang & Kittayapong, 2014). Close contact between humans and monkeys in these recreational areas, which are in forested areas, might potentially increase the risk of zoonotic disease transmission as monkeys are known reservoirs for viruses like CHIKV, DENV and JE (Tongthainan *et al*., 2020). In Malaysia, CHIKV was first isolated from long-tailed macaques and was linked to human outbreaks in 1998 and 2006 (Apandi *et al*., 2009). On the other hand, in the Philippines, JE, DENV, and CHIKV were reported to be highly prevalent among monkeys (Inoue *et al*., 2003). Given the genetic similarities between humans and primates, humans are likely susceptible to these infections. Mixed blood-feeding by *Ae. aegypti* and *Ae. albopictus* on both species could potentially facilitate pathogen transmission, raising public health concerns. This is especially true when the sylvatic strain of DENV-4 is found circulating in *Ae. aegypti* and *Ae. albopictus* mosquitoes in urban regions of Peninsular Malaysia which underscores these vectors' vulnerability to sylvatic DENV infection (Johari *et al*., 2019). However, further studies with larger sample sizes and the detection of these viruses are necessary to gain a better understanding of the zoonotic transmission dynamics in the study locations.

In this study, only *Ae. albopictus* collected from Farm Fresh were positive for cattle's DNA. This clearly demonstrates that host availability plays a crucial role in determining the feeding preference of the mosquitoes (Takken & Verhulst, 2013). In fact, this study further revealed that host-seeking behaviours and blood-feeding patterns of *Aedes* mosquitoes are significantly influenced by the presence of available hosts. Interestingly, a study on the seroprevalence of JE, West Nile and DENV-2 in India showed the presence of these flaviviruses in domestic animals such as dogs, pigs and cattle at varying percentages (Mall *et al*., 1995). These animals are potential hosts that can harbor these viruses and may contribute to the transmission cycle indirectly through mosquito vectors (Gwee *et al*., 2021). This further complicates the efforts to reduce cases in areas with a high endemicity of dengue and highlights the need for further research on zoonotic transmission pathways. Understanding these dynamics is crucial for developing effective public health strategies to mitigate the risk of flavivirus infections in humans.

In this study, the HBI was calculated to determine the anthropophilic nature of *Aedes* mosquitoes caught from different outdoor locations. Based on the blood meal analysis conducted, *An. aegypti* collected from the residential areas (Nadayu28 Residences) had the highest average HBI (1.00) compared to other locations such as forests (0.56), forest fringes (0.50) or farm areas (0.20). This aligns with the well-known anthropophilic biting behavior of *Ae. aegypti* (Facchinelli *et al*., 2023). Similarly, *Ae. albopictus* also exhibited a higher degree of anthropophily in samples collected from outdoor areas of Nadayu28 Residences compared to other outdoor locations, which were primarily surrounded by forests or farm settings. This suggests that *Ae. albopictus* from human-inhabited outdoor areas is more anthropophilic in nature. Although *Ae. albopictus* is mainly known as a zoophilic species, it has excellent adaptability where it can also exhibit anthropophilic behavior, especially in areas where human activity dominates or where its natural habitats have been altered (Marques & De Castro Gomes, 1997). This dual-feeding tendency enables *Ae. albopictus* to thrive in both rural and suburban environments, increasing its potential as a vector for human disease and potentially facilitating zoonotic transmission (Gwee *et al*., 2021; Richards *et al*., 2006).

This study had several limitations, most notably the absence of screening for dengue viruses or other flaviviruses in the captured mosquitoes, which would have provided a clearer understanding of potential zoonotic transmission. Furthermore, a larger sample size is necessary to better assess the blood-feeding preferences of mosquitoes across different locations in dengue-endemic areas. Additionally, mosquitoes collected on a Sella scale of 3 to 6 might be challenging to detect the blood meal sources, as some of the DNA may have already been partially digested (Jeyaprakasam *et al*., 2022). Consequently, the success rate for amplification is lower in these mosquitoes compared to fully engorged ones. Indeed, blood meal analysis is critical for understanding the feeding patterns of *Aedes* mosquitoes in diverse ecotypes, offering valuable insights into the ecological context of disease transmission and helping to assess the risk of disease spread in varied environments (Gonçalves *et al*., 2023). The distribution of vector-borne diseases is shaped by interactions between vectors, hosts, and the environment (Kamgang *et al*., 2012). Thus, analyzing blood meals across different ecological settings contributes to a deeper understanding of disease dynamics, including the potential for zoonotic transmission. This approach also aids in mapping disease transmission networks by identifying the host associations of blood-feeding vectors and tracing the flow of pathogens between different host species and geographic locations (Al-Rashidi *et al*., 2022). As animals can act as reservoirs for dengue transmission, such findings can enhance our ability to predict and manage disease outbreaks, particularly those involving zoonotic transmission (Gwee *et al*., 2021).

CONCLUSION

Based on the feeding preference of the *Aedes* mosquitoes from this study, it can be concluded that the primary source of dengue virus transmission at the study locations might be mainly from the infected human hosts. Nevertheless, the interesting findings of mosquitoes, which were positive for both human and monkey blood, underscore the importance of further study on the potential zoonotic transmission of dengue viruses to humans in areas where there is close contact between humans and wildlife, such as monkeys. This is especially crucial since there is a risk of spillover into urban environments, given the increasing deforestation and rapid urbanization, which bring humans in close contact with wildlife. This research underscores the need for ongoing surveillance and targeted interventions to manage dengue risk in changing environments. Further studies should be carried out using a larger sample size of mosquitoes, including the detection of dengue viruses in those mosquitoes positive for both human and other vertebrate blood.

ACKNOWLEDGEMENTS

We extend our gratitude to the science officer, Ms. Azura Rosidi, as well as medical laboratory technologists, Mrs. Rafiah Mohamed Rapiai and Mrs. Balkhis Bashuri, for their invaluable assistance in both the administration of the research project and the fieldwork. Additionally, we also extend our sincere appreciation to Assoc. Prof. Dr Ismarulyusda Ishak, for her valuable guidance on the statistical analysis. This study was funded by the UKM Early Career Researcher Award (Grant number: NN-2024-014).

Conflict of Interest Statement

The author declares that they have no conflict of interest.

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