

Effect of Thermal Fogging on *Aedes* Mosquito Control, *Wolbachia* Prevalence, and Dengue Surveillance in Malaysia

Chen Zhe Tang^{1,2}, Zhen Yun Siew^{1,2,*}, Isaac Seow¹, Kenny Voon^{1,*}

¹School of Pharmacy, University of Nottingham Malaysia, Jalan Broga, 43500 Semenyih, Selangor, Malaysia.

²Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan.

*These authors contributed equally to this work as co-corresponding authors: zhenyunsiew@as.edu.tw; kenny.voon@nottingham.edu.my

ARTICLE HISTORY

Received: 27 Jun 2026
Revised: 28 Jul 2026
Accepted: 29 Jul 2026
Published: 30 Sep 2026

ABSTRACT

Dengue virus (DENV) is widespread in Malaysia, causing significant morbidity. Regular thermal fogging is conducted to prevent DENV transmission by vectors, primarily *Aedes aegypti* and *Aedes albopictus*. *Aedes* mosquitoes and larvae were collected weekly and screened for DENV and *Wolbachia*. Fogging effectiveness was assessed by comparing the mean number of larvae before and after fogging using Mann-Whitney U tests. A total of 3,844 mosquito larvae were collected, with the most identified as *Ae. albopictus* (n = 3,828, 99.58%), some as *Culex* spp. (n = 16, 0.42%), and none as *Ae. aegypti*. DENV screening yielded no positive results throughout the study, while *Wolbachia* was prevalent in the *Ae. albopictus* population. However, fogging did not significantly reduce *Aedes* larvae numbers, likely due to insecticide resistance. Specific areas with increased human activity and dense vegetation showed relatively high mosquito densities. This study investigated the presence of DENV in the *Aedes* population at a tertiary educational institution in Semenyih, Malaysia, and evaluated the effectiveness of fogging. Additionally, it seeks to explore the potential role of *Wolbachia* in reducing DENV transmission in the mosquito population and suggest alternative animal reservoirs of DENV, such as tree shrews.

Keywords: *Aedes albopictus*, *Wolbachia*, Surveillance, Vector, Fogging, One Health.

Introduction

Dengue fever is one of the most important mosquito-borne diseases affecting public health, particularly in tropical and subtropical regions worldwide (Siew et al., 2025a). The disease is caused by dengue virus (DENV), a member of the Orthoflavivirus genus, which comprises four distinct serotypes: DENV-1, DENV-2, DENV-3, and DENV-4. Transmission occurs predominantly through the bites of infected female *Aedes aegypti* and *Aedes albopictus* mosquitoes (Postler et al., 2023). Although primates are the primary hosts, DENV infection has also been reported in various animal species that frequently inhabit human-associated environments, including dogs, rodents, tree shrews, birds, and bats (Gwee et al., 2021; Siew et al., 2026a). Due to their close association with human populations, these animals have raised concerns regarding their potential involvement in

zoonotic or sylvatic (enzootic) transmission cycles, as their role as possible reservoirs of DENV remains incompletely understood (Gwee et al., 2021).

DENV has become a significant public health concern in Malaysia. Up to the 13th epidemiological week in 2024, a total of 44,144 dengue cases have been reported in Malaysia, reflecting a notable 54.42% increase compared to the same period in the previous year (Ministry of Health, 2024a). Despite the availability of dengue vaccines like CYD-TDV (Dengvaxia®) and TAK-003 (Qdenga®), they remain largely inaccessible in many countries. CYD-TDV faced controversy when the Philippines suspended its use due to concerns about enhanced severe dengue risk in individuals without prior dengue exposure (Sridhar et al., 2018). In contrast, TAK-003

demonstrated 80.2% overall vaccine efficacy, 90.4% efficacy against hospitalised dengue, and 85.9% against severe dengue haemorrhagic fever, with no significant safety risks (Biswal et al., 2020; Tricou et al., 2024; Petri et al., 2024). Although Malaysia approved TAK-003 in 2024 for individuals aged ≥ 4 years (Ministry of Health, 2024b), it is not yet part of the National Immunisation Programme, leaving the majority still at risk (perhaps). Due to the lack of treatments, dengue prevention primarily relies on monitoring and controlling mosquito populations. Vector populations are managed through the timely implementation of various control measures, such as chemical interventions (e.g., fogging), environmental management (e.g., eliminating breeding sites), and biological controls (Dorigatti et al., 2018; Siew et al., 2025a). Insecticide-based interventions are routinely employed in residential areas, schools, and high-traffic locations by local communities, private pest control operators, and the Ministry of Health to control vector populations (Akhir et al., 2022). Thermal fogging was widely accepted to be efficient in eradicating *Aedes* mosquitoes within outbreak zones, thereby stopping potential epidemics or rapidly managing an ongoing one (Chua et al., 2005). Nevertheless, despite concerted efforts to control *Aedes* mosquito populations, dengue cases in Malaysia continue to rise. Instead, the extensive use of insecticide-based interventions raises environmental and ecological concerns, including the potential development and spread of insecticide resistance, exacerbating the challenges in DENV control efforts (Dorigatti et al., 2018). Additionally, the use of insecticides like organophosphates and pyrethroids has been linked to human neurobehavioral deficits, affecting short-term auditory memory, perceptual-motor speed, coordination, and attention (Yusof et al., 2022). Furthermore, routine fogging also adversely affects non-target organisms in the environment, impacting biodiversity and regional ecology (Lee et al., 2020).

A university campus in the Selangor state of Malaysia has consistently employed fogging to manage mosquito populations on campus, aiming to reduce the risk of mosquito-associated infections. However, the necessity for ongoing fogging becomes questionable, considering the environmental, human health, and financial costs involved. Thus, the objectives of this study were

to detect the presence of DENV and *Wolbachia* in *Aedes* mosquitoes, to determine the effectiveness of fogging, as well as to identify high mosquito density areas around the campus.

Materials and Methods

Larvae collection

The study was conducted within a tertiary education institution located in Semenyih, Selangor, Malaysia, covering approximately 125 acres (50.6 hectares; 0.51 km²). Mosquito larvae were collected from 12 localities across the campus compound (Supplementary Table S1; Supplementary Figures S1 and S2). These localities were selected based on their geographical proximity and environmental characteristics.

Aedes larvae were collected and identified as previously described (Teo et al., 2017). Briefly, five black opaque plastic ovitraps were deployed at each locality and filled with clean tap water to approximately two-thirds of their capacity. Ovitrap were maintained for five months, from November 2023 to March 2024, and larvae were collected at two-week intervals. Data from December 2023 and January 2024 were excluded from subsequent analyses due to sampling disruptions.

DENV and Wolbachia detection and bioinformatics analysis

Aedes mosquitoes and larvae collected from the same locality during each sampling month were pooled and homogenised using a sterile micropestle. Nucleic acid extraction was subsequently performed using the PrimeWay Viral DNA/RNA Extraction Kit (1st BASE, Malaysia) according to the manufacturer's instructions. Complementary DNA (cDNA) synthesis, polymerase chain reaction (PCR) amplification, and agarose gel electrophoresis were conducted as previously described (Siew et al., 2026a).

Reverse transcription was performed using the ReverTra Ace qPCR RT Master Mix (Toyobo, Japan). Then, PCR amplification was performed under the following thermal cycling conditions: an initial denaturation at 95°C for 2.5 min, followed by 35 cycles of denaturation at 95°C for 40 s, annealing at 55°C for 45 s, and extension at 72°C for 1 min, with a final extension at 72°C for

4 min. AmpliPLUS HiFi PCR Master Mix (2×), DNA stain, DNA ladder, and loading dye were obtained from 1st BASE (Malaysia).

The primers and positive controls used in this study were identical to those reported previously (Siew *et al.*, 2023; Siew *et al.*, 2026a) (Supplementary Tables S2 and S3, respectively). Phylogenetic analyses and pairwise alignment-based sequence identity calculation were performed as previously described (Siew *et al.*, 2023; Siew *et al.*, 2025b). The optimal nucleotide substitution model was determined prior to maximum-likelihood tree construction. *Wolbachia* supergrouping was performed using sequences as demonstrated previously (Puerta-Guardo *et al.*, 2025). Besides, reference sequences were selected from the top BLAST hits representing closely related *Wolbachia* strains, together with a divergent *Wolbachia* strain included as an outgroup.

All sequences were deposited in GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>):

Wolbachia endosymbiont of *Ae. albopictus* detected in November 2023 (GenBank: PP848270.1), February (GenBank: PP848271.1) and March 2024 (GenBank: PP848272.1).

Determination of high mosquito density areas and fogging effectiveness

The insecticide used for the thermal fogging is Resigen® (<https://www.my.envu.com/mosquito-management/products/resigen>), containing S-bioallethrin (0.8% w/w), permethrin (18.7% w/w), and piperonyl butoxide (16.8% w/w) as active ingredients.

A histogram illustrating the relative distribution of *Aedes* larvae per ovitrap was generated using data from all study localities and sampling periods. A 70th percentile threshold was applied to classify localities according to mosquito density. Localities with mean larval counts below the threshold were classified as low-density areas, whereas those exceeding the threshold were classified as high-density areas.

To evaluate fogging effectiveness, the mean number of larvae per ovitrap collected 10 days before and 10 days after fogging was compared across all study localities. Differences in larval abundance before and after fogging were visualised using box plots.

Minimum infection rate (MIR) calculation

Since testing was performed on pooled samples rather than individual larvae, the Minimum Infection Rate (MIR) is the most conservative estimate of infection prevalence. The MIR assumes that only one individual in each positive pool is infected, and was determined using the following formula:

$$MIR = \left(\frac{\text{Number of positive pools}}{\text{Total number of individuals tested}} \right) \times 1000$$

Data and statistical analysis

All statistical analyses and graphical visualisations were performed using GraphPad Prism version 9.2.0 (GraphPad Software, USA). Differences in larval abundance before and after fogging were assessed using the Mann–Whitney U test. Statistical significance was defined as $p < 0.05$.

Results

Mosquito identification and determination of high mosquito density areas

A total of 3,844 mosquito larvae were collected. Most collected mosquito larvae were morphologically identified as *Ae. albopictus* ($n = 3,828$, 99.58%), with no *Ae. aegypti* identified. Interestingly, larvae with distinct morphological features, resembling *Culex* species ($n = 16$, 0.42%), particularly *Culex tritaeniorhynchus*, a known vector of the Japanese encephalitis virus (JEV), were occasionally found (Faizah *et al.*, 2020). Figure 1 shows the distinct features of *C. tritaeniorhynchus*.

According to Figure 2, a mean larval number per ovitrap ≥ 10 over the entire study period would be categorised as a high mosquito density area, as they are situated within the top 30% in the distribution of relative frequency of *Aedes* larvae per ovitrap collected across all study sites. As such, localities 4 (Greenhouse) and 8 (Childcare Centre & Islamic Centre) were identified as high mosquito density areas, while the remaining localities had lower densities as shown in Table

1.

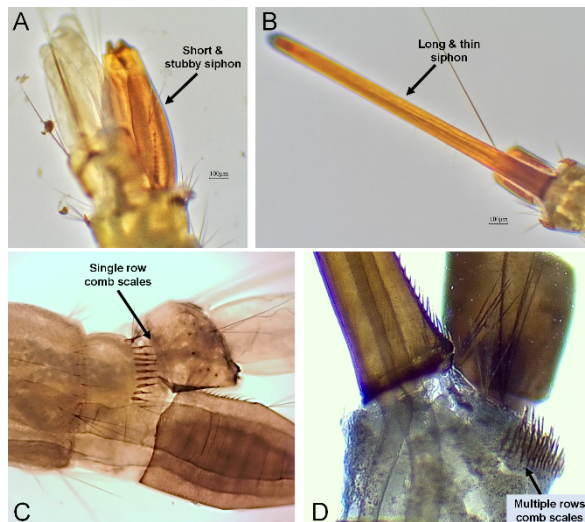


Figure 1. Morphological differences between *Ae. albopictus* and *Culex* larvae. This figure highlights the distinct features of *Ae. albopictus* (A & C) and suspected *Culex* larvae (B & D). All micrographs were captured at 40× magnification.

Table 1. Mean number of *Aedes* larvae collected per ovitrap in each of the 12 study localities throughout the entire study duration.

Study locality	Mean larvae numbers	High/Low mosquito density?
1	4.71429	Low
2	9.20000	Low
3	6.91935	Low
4	10.0159	High
5	7.76471	Low
6	7.62500	Low
7	6.91429	Low
8	10.1905	High
9	9.19231	Low
10	8.82857	Low
11	7.00000	Low
12	7.08824	Low

A 70th percentile threshold was applied to classify localities according to mosquito density. Localities with mean larval counts below the threshold were classified as low-density areas, whereas those exceeding the threshold were classified as high-density areas.

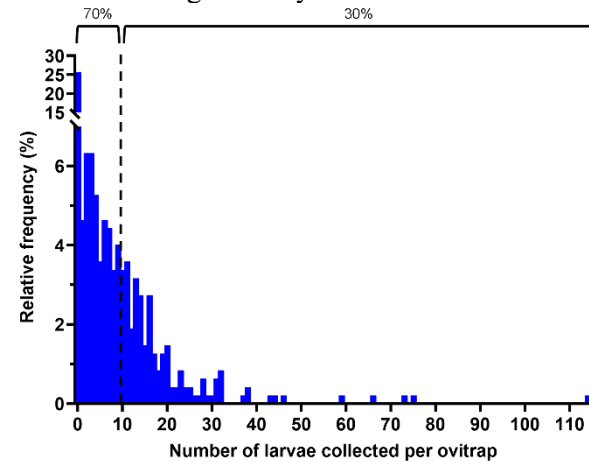


Figure 2. Distribution of the relative frequency of *Aedes* larvae per ovitrap collected across all study localities throughout the study period. The dotted line shows the cut-off value whereby ~70% of ovitraps were found to have <10 *Aedes* larvae per ovitrap, while ~30% of ovitraps collected had ≥10 larvae per ovitrap. Any locality with a mean larvae number per ovitrap that falls within that 30% range was considered a locality with high mosquito density.

Seasonal changes in mosquito density

A consistent decline in the number of *Aedes* larvae collected per ovitrap across most localities throughout the study (Table 2, Figure 3). The decline in mosquito density was associated with changes in weather patterns, particularly reduced rainfall during the latter stages of the Northeast

Table 2. The number of *Ae. albopictus* larvae collected per month across 12 study locations in the institute, Selangor.

Study locality	Mean larvae number/locality by study month			Total larvae collected
	November 2023	February 2024	March 2024	
1	5.50	6.29	3.14	231
2	24.83	8.50	2.08	276
3	7.45	6.64	6.71	429
4	18.57	5.88	5.53	631
5	10.71	4.17	5.18	254
6	12.85	5.17	5.07	305
7	10.23	6.33	4.00	242
8	17.67	7.00	5.14	428
9	13.75	8.00	1.67	239
10	12.79	10.27	1.70	309
11	9.09	4.75	7.29	259
12	8.10	8.56	5.53	241
Mean larvae/month	151.54 ^a	81.56	53.04 ^b	

^a Highest mean larvae count among the study duration is found in November 2023.

^b Lowest mean larvae count among the study duration is found in March 2024.

Monsoon and increased temperatures caused by the El Niño phenomenon. In November 2023,

heavy rainfall provided ideal breeding conditions, but as rainfall decreased in February and March 2024, fewer breeding sites were available, leading to a decrease in mosquito populations.

Impact of thermal fogging on Aedes larvae density

The effectiveness of fogging on the *Aedes* population was evaluated by comparing the mean number of larvae per ovitrap across all study localities 10 days before and after fogging (Figure 4). A slight increase in mean larvae number was noted following fogging in November 2023. The mean larvae count increased from approximately 8 to 14. Subsequent collections in February 2024 showed a slight decrease in larvae numbers from around 8 to 5 larvae. In March 2024, the larvae numbers decreased slightly from about 5 to 4.5 larvae. The increase in mean larvae in November 2023 is likely attributed to the early northeast monsoon. However, the slight increase and decreases observed in November 2023, February and March 2024 were not statistically significant ($p > 0.05$) according to the Mann-Whitney U test. Overall, no statistically significant increase or reduction in larvae counts was observed before and after fogging in any of the months studied.

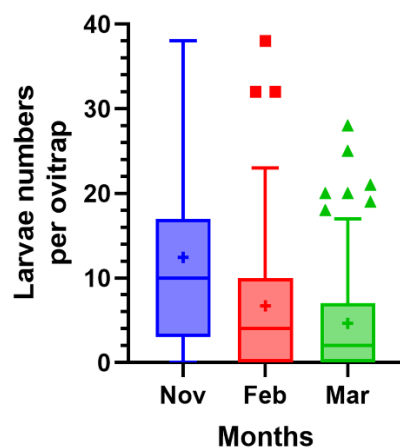


Figure 3. The reduction in *Aedes* larvae number collected per ovitrap across all study localities in the institute, Selangor. The mean number of *Aedes* larvae collected per ovitrap of each study month is indicated by the '+' symbol. The squares and triangles denote the data outliers for February and March 2024, respectively.

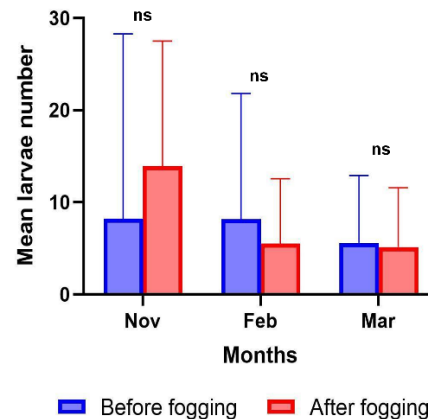


Figure 4. Mean larvae numbers before and after fogging. The graph shows mean larvae counts for November 2023, February 2024, and March 2024, with blue bars representing pre-fogging and red bars post-fogging. Error bars indicate standard deviation (SD). No statistically significant differences were found between pre- and post-fogging counts (ns, $p > 0.05$).

Table 3. The BLAST percentage identity of *Wolbachia* strains obtained in this study.

<i>Wolbachia</i> strain	Percentage identity (%)	Top 10 reference strains
PP848270	97.36	CP031221, CP041923, CP080546, CP041924, OZ034794, MN307069, CP102671
	97.19	OZ034710, CP003883, OZ034788
	99.83	PP848272
	96.04	CP031221, CP041923, CP080546, CP041924, MN307069, CP102671
PP848271	96.00	MW718008
	95.83	MW718098, MW718027
	99.83	PP848271
	96.04	CP031221, CP041923, CP080546, CP041924, MN307069, CP102671
PP848272	96.00	MW718008
	95.83	MW718098, MW718027

DENV and Wolbachia detection in Ae. albopictus

In total, 36 pools were screened for DENV and *Wolbachia*. All samples collected were found negative for DENV, but *Wolbachia* was detected in all pools (Figure 5) with an estimated MIR of 0.937% (9.37 infected larvae per 1,000 larvae). Phylogenetic analysis (Figure 6A) and the pairwise alignment-based sequence identity calculation (Figure 6B) were performed to characterise *Wolbachia* obtained in this study.

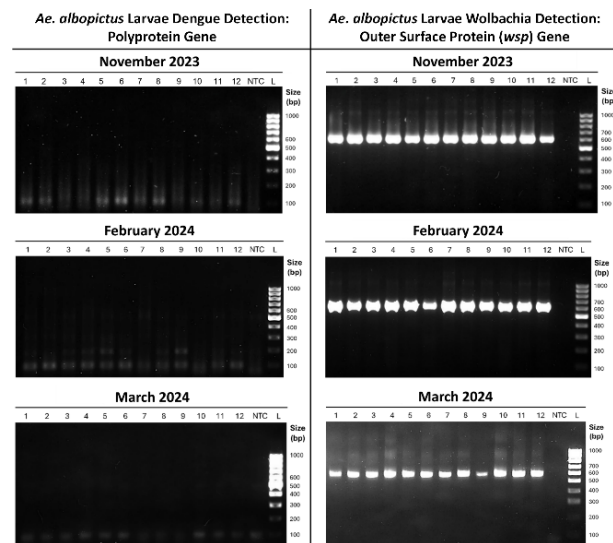


Figure 5. Agarose gel images of DENV and *Wolbachia* detections in all localities. Lanes labelled '1' to '12' correspond to the localities 1 to 12, respectively. The lane labelled 'NTC' depicts the no template control, whereas the lane labelled 'L' contains the DNA ladder. The successfully amplified band at 600 bp corresponds to the PCR product of the *Wolbachia* *wsp* gene.

According to **Figure 6A**, all *Wolbachia* identified in this study were grouped within the supergroup B. However, the *Wolbachia* endosymbiont of *Ae. albopictus* detected in November 2023 (GenBank: PP848270) has slight differences in its surface protein gene when compared to the *Wolbachia* detected in February (GenBank: PP848271.1) and March 2024 (GenBank: PP848272.1). *Wolbachia* PP848270 is clustered closely with the strain wPip, while PP848271 and PP848272 are with strain wAlbB. The percentage identity of each *Wolbachia* sequence was assessed using NCBI BLAST (Table 3). For each strain, the top 10 BLAST hits demonstrated high sequence similarity, with nucleotide identities greater than 95%. Thus, the *Wolbachia* isolates obtained in this study were assigned to the mosquito-associated *Wolbachia* lineage and were most closely related to members of Supergroup B (wAlbB lineage). Owing to the use of a partial *wsp* gene, definitive strain identification was not possible.

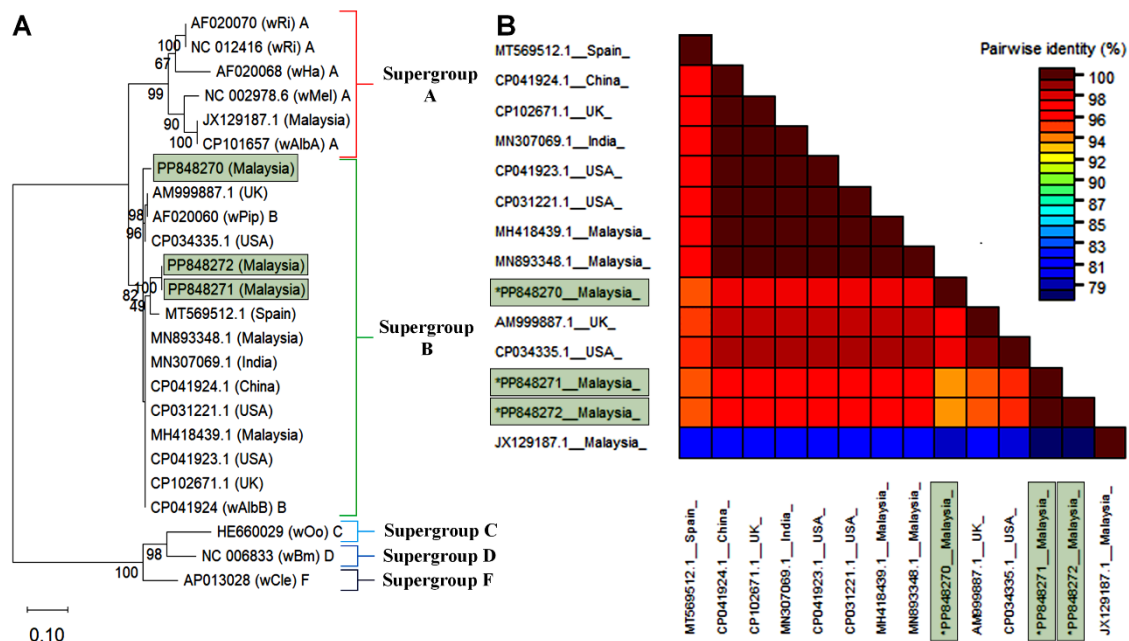


Figure 6. (A) Phylogenetic tree displaying the evolutionary relationships between various *Wolbachia* bacterial strains from different countries, including sequences from India, the USA, the UK, China, and Malaysia. The sequences highlighted in green represent newly identified strains from Malaysia. Bootstrap values are provided at the relevant nodes, indicating the statistical support for each branching point. The scale bar represents 0.10 substitutions per site. (B) The pairwise alignment-based sequence identity calculation of *Wolbachia* strains obtained in this study against reference strains.

Discussion

Mosquito density and environmental influences

High mosquito density areas, such as the

Childcare Centre & Islamic Centre and the Greenhouse, were strongly associated with environmental factors, particularly abundant vegetation and high levels of human activity. The

surrounding vegetation likely facilitates mosquito proliferation, as *Aedes* mosquitoes preferentially utilise dense foliage for shelter from predators and adverse weather conditions (Supplementary Figure S3) (Adnan *et al.*, 2021). In addition, vegetation may serve as both a resting habitat during the day and a source of sugar meals through nectar feeding (Adnan *et al.*, 2021). Furthermore, anthropogenic activities, particularly during periods of increased rainfall, may lead to the accumulation of stagnant water in artificial containers, providing suitable breeding habitats for *Ae. albopictus*, a vector with a strong preference for container-associated oviposition sites (Adnan *et al.*, 2021; Harrison *et al.*, 2021).

High levels of human activity may also contribute to increased mosquito density by providing abundant blood meal sources. As anautogenous insects, *Aedes* mosquitoes require blood meals for egg development, and previous studies have demonstrated a positive correlation between blood intake and fecundity (Gulia-Nuss *et al.*, 2015). Accordingly, high human traffic in areas such as the Childcare Centre may facilitate mosquito feeding and subsequent reproduction (Ellwanger *et al.*, 2021).

The observed reduction in mosquito populations during the later stages of the study may be attributed to shifting weather patterns in Selangor. According to the Meteorological Department Malaysia (2024), reduced rainfall is expected during the later phase of the Northeast Monsoon. This decrease in rainfall, combined with elevated temperatures associated with the El Niño phenomenon, likely resulted in reduced humidity and fewer available breeding sites, contributing to a decline in *Aedes* populations (Meteorological Department Malaysia, 2024). These findings highlight the dynamic interplay between environmental factors and mosquito population dynamics, where seasonal variability can substantially influence mosquito abundance and underscore the need for adaptive vector control strategies.

Evaluating the efficacy of thermal fogging and implications for resistance management

A similar study reported only a slight and statistically insignificant reduction in the number of *Aedes* larvae collected during the fogging phase compared with the pre-fogging period (Chua *et al.*, 2005). Interestingly, the authors observed that

larval abundance immediately after fogging was higher than that recorded before fogging. This phenomenon may be attributed to the unintended effects of chemical insecticides on the natural predators of *Aedes* mosquitoes, including frogs, mosquitofish and spiders. The reduction of these predator populations may diminish natural biological control, thereby facilitating the persistence or rebound of *Aedes* larval populations following fogging interventions.

In this context, the limited effectiveness of thermal fogging observed in this study may be partly attributable to the unintended impact of insecticides on dragonflies and damselflies, which are important biological control agents of mosquito populations. Both adult dragonflies and damselflies, and their aquatic nymphs, are known to prey on mosquitoes at different life stages, including adults and larvae (Priyadarshana and Slade, 2023). Consequently, reductions in dragonfly populations resulting from insecticide exposure may weaken natural predation pressure on mosquitoes, thereby diminishing the long-term effectiveness of fogging interventions. These findings highlight the importance of considering the ecological consequences of insecticide-based vector control strategies, particularly their effects on non-target predator species that contribute to the natural regulation of mosquito populations.

Moreover, the limited effectiveness of fogging may also be attributed to the selection pressure imposed by repeated insecticide applications. Such pressure can promote the survival and reproduction of insecticide-resistant mosquitoes, thereby reducing the efficacy of vector control measures over time. Najdah *et al.* (2020) reported that *Ae. albopictus* populations from all surveyed localities exhibited resistance to permethrin, one of the active ingredients in the fogging insecticide formulation used at the present study site. Notably, populations from nearby localities, including Bandar Rinching and Taman Teknologi, demonstrated high and moderate levels of resistance, respectively. Given the geographical proximity of these sites, a similar resistance profile may be present among the *Aedes* populations examined in this study, potentially contributing to the observed ineffectiveness of fogging.

While knockdown resistance (*kdr*) gene mutations (S989P, F1534C, V1016G, and A1007G)

associated with pyrethroid resistance have been documented in *Ae. aegypti* in Malaysia, they have not been detected in *Ae. albopictus* (Akhir *et al.*, 2022). Nevertheless, *Ae. albopictus* can still develop pyrethroid resistance through alternative mechanisms, particularly via the overexpression of cytochrome P450 (CYP) enzymes such as CYP6N3 and CYP6P12, which have been previously identified in Malaysian mosquito populations (Ishak *et al.*, 2016; Nurul-Nastasea *et al.*, 2023). This adaptive capacity highlights the need for further investigation into the underlying resistance mechanisms and underscores the importance of implementing integrated vector management strategies to mitigate the risk of resistance development.

The present findings highlight a critical gap in the current understanding of *Aedes* mosquito population dynamics and the ecological consequences of chemical control measures. There is a need to explore alternative vector control strategies, including biological control using natural predators, habitat modification, and strengthened community engagement in mosquito management. A more holistic approach that integrates ecological considerations with sustainable practices may improve the effectiveness of vector control programmes while reducing reliance on chemical insecticides.

Insights into DENV and Wolbachia Dynamics in Ae. albopictus and other reservoirs

The consistent absence of dengue virus (DENV) in *Ae. albopictus* samples, together with the detection of *Wolbachia*, suggests that the presence of these endosymbionts might play a role in DENV propagation in local mosquito populations. *Wolbachia* are intracellular bacteria known to reduce arboviral transmission either by directly inhibiting viral replication or by shortening the lifespan of the mosquito vector, thereby reducing the likelihood of successful virus transmission (Mustafa *et al.*, 2016). One study suggested that *Wolbachia* suppresses viral replication and inhibits dengue virus binding to Aag-2 *Aedes* mosquito cells (Lu *et al.*, 2020). In Malaysia, 91.6% of *Ae. albopictus* individuals were reported to be superinfected with both the wAlbA and wAlbB *Wolbachia* strains. In the present study, DENV was not detected in the *Aedes* populations sampled from Semeniyih. Although previous studies have demonstrated that certain *Wolbachia* strains can suppress DENV replication in

mosquitoes, the naturally occurring *Wolbachia* detected in our *Ae. albopictus* populations should not be interpreted as the explanation for the absence of DENV, as this relationship was not investigated in the present study. This observation only suggests the possible role of *Wolbachia* in reducing vector competence and, consequently, the transmission potential of DENV (Joanne *et al.*, 2015).

The slight phylogenetic differences observed in *Wolbachia* strains over time may reflect ongoing evolutionary dynamics or environmental factors influencing their populations. Insights from phylogenetic analyses can inform the development of more effective biological control strategies tailored to specific regions or mosquito populations. By identifying the most prevalent and effective *Wolbachia* strains in different environments, public health initiatives can implement more targeted interventions to mitigate dengue outbreaks and other mosquito-borne diseases (Ou *et al.*, 2023).

Interestingly, common tree shrews (*Tupaia glis*) from the same study site were found to harbour a range of pathogens of concern, including *mycobacteria*, dengue virus, and orthoreovirus (Siew *et al.*, 2024; Siew *et al.*, 2025b; Siew *et al.*, 2026a). The detection of dengue virus serotype 2 (DENV-2) supports the possibility that mammals may play a role in maintaining or amplifying viral circulation within multi-host transmission cycles. The close genetic relationship between the detected DENV-2 sequences and those reported from other regions further highlights the global connectivity of viral populations. This suggests potential pathways for cross-border viral movement, underscoring the importance of continued molecular surveillance and epidemiological monitoring.

Limitations

This study has several limitations. First, the relatively short sampling period may have resulted in incomplete temporal coverage and limited assessment of seasonal variation. Extending surveillance to at least one year would provide a more robust evaluation of seasonal dynamics and the effectiveness of thermal fogging. Second, the inclusion of an unfogged control site would better distinguish the effects of fogging from natural seasonal fluctuations in mosquito populations. Third, whole-genome

sequencing of both *Wolbachia* strains and *Aedes* mosquitoes was not performed, limiting genetic, evolutionary, and host–symbiont–virus interaction analyses. The partial *wsp* gene sequencing is not sufficient for definitive strain identification. In addition, *Wolbachia* screening was conducted using pooled mosquito samples, precluding estimation of individual infection prevalence. Finally, data on mosquito predator abundance were not collected, limiting assessment of the broader ecological impacts of thermal fogging.

Conclusion

This study identified the presence of *Wolbachia* in *Ae. albopictus* while no DENV was detected in the local mosquito population. Despite ongoing fogging efforts, no significant reduction in *Aedes* population density was observed, highlighting the need for more effective and sustainable vector control strategies. Notably, the detection of DENV-2 in tree shrews from the same region represents a novel finding, raising the possibility of an alternative mammalian host involved in the local transmission ecology of the virus. However, a substantial knowledge gap remains regarding the apparent discrepancy between the detection of DENV-2 in tree shrews and its absence in *Aedes* mosquitoes, warranting further investigation into the underlying ecological and virological mechanisms.

Acknowledgements

We extend our heartfelt gratitude to the dedicated Hiroto Aoyama and E-Jayn Chong for their invaluable contributions and support. The authors declare no competing interests. The authors wish to thank Apical Scientific Sdn. Bhd. for their invaluable technical support in advancing our research.

Funding

No specific funding was received.

Conflict of Interest Statement

The authors declare no conflicts of interest.

Data Availability

All data generated or analyzed during this study are included in this published article and its

Supplementary Materials. Further inquiries can be directed to the corresponding author, Z.Y. Siew (siewzy0@gmail.com).

References

- Adnan, R. A., Ramli, M. F., Othman, H. F., Asha'ri, Z. H., Ismail, S. N. S., & Samsudin, S. (2021). The Impact of Sociological and Environmental Factors for Dengue Infection in Kuala Lumpur, Malaysia. *Acta tropica*, 216, 105834. <https://doi.org/10.1016/j.actatropica.2021.105834>
- Akhir, M. A. M., Wajidi, M. F. F., Lavoué, S., Azzam, G., Jaafar, I. S., Awang Besar, N. A. U., & Ishak, I. H. (2022). Knockdown resistance (kdr) gene of *Aedes aegypti* in Malaysia with the discovery of a novel regional specific point mutation A1007G. *Parasites & vectors*, 15(1), 122. <https://doi.org/10.1186/s13071-022-05192-z>
- Biswal, S., Borja-Tabora, C., Martinez Vargas, L., Velásquez, H., Theresa Alera, M., Sierra, V., Johana Rodriguez-Arenales, E., Yu, D., Wickramasinghe, V. P., Duarte Moreira, E., Jr, Fernando, A. D., Gunasekera, D., Kosalaraksa, P., Espinoza, F., López-Medina, E., Bravo, L., Tuboi, S., Hutagalung, Y., Garbes, P., Escudero, I., ... TIDES study group (2020). Efficacy of a tetravalent dengue vaccine in healthy children aged 4-16 years: a randomised, placebo-controlled, phase 3 trial. *Lancet (London, England)*, 395(10234), 1423–1433. [https://doi.org/10.1016/S0140-6736\(20\)30414-1](https://doi.org/10.1016/S0140-6736(20)30414-1)
- Chua, K. B., Chua, I. L., Chua, I. E., & Chua, K. H. (2005). Effect of chemical fogging on immature *Aedes* mosquitoes in natural field conditions. *Singapore medical journal*, 46(11), 639–644.
- Dorigatti, I., McCormack, C., Nedjati-Gilani, G., & Ferguson, N. M. (2018). Using *Wolbachia* for Dengue Control: Insights from Modelling. *Trends in parasitology*, 34(2), 102–113. <https://doi.org/10.1016/j.pt.2017.11.002>
- Ellwanger, J. H., Cardoso, J. D. C., & Chies, J. A. B. (2021). Variability in human attractiveness to mosquitoes. *Current research in parasitology & vector-borne diseases*, 1, 100058. <https://doi.org/10.1016/j.crpvbd.2021.100058>
- Faizah, A. N., Kobayashi, D., Amoa-Bosompem, M., Higa, Y., Tsuda, Y., Itokawa, K., Miura, K., Hirayama, K., Sawabe, K., & Isawa, H. (2020). Evaluating the competence of the primary vector, *Culex tritaeniorhynchus*, and the invasive mosquito species, *Aedes japonicus japonicus*, in transmitting three Japanese encephalitis virus genotypes. *PLoS neglected tropical diseases*, 14(12), e0008986. <https://doi.org/10.1371/journal.pntd.0008986>
- Gulia-Nuss, M., Elliot, A., Brown, M. R., & Strand, M. R. (2015). Multiple factors contribute to anautogenous reproduction by the mosquito *Aedes aegypti*. *Journal of insect physiology*, 82, 8–16. <https://doi.org/10.1016/j.jinsphys.2015.08.001>
- Gwee, S. X. W., St John, A. L., Gray, G. C., & Pang, J. (2021). Animals as potential reservoirs for dengue transmission: A systematic review. *One health (Amsterdam, Netherlands)*, 12, 100216. <https://doi.org/10.1016/j.onehlt.2021.100216>
- Harrison, R. E., Brown, M. R., & Strand, M. R. (2021). Whole blood and blood components from vertebrates differentially affect egg formation in three species of anautogenous mosquitoes. *Parasites & vectors*, 14(1), 119. <https://doi.org/10.1186/s13071-021-04594-9>
- Ishak, I. H., Riveron, J. M., Ibrahim, S. S., Stott, R., Longbottom, J., Irving, H., & Wondji, C. S. (2016). The Cytochrome P450 gene CYP6P12 confers pyrethroid resistance in kdr-free Malaysian populations of the dengue vector *Aedes albopictus*. *Scientific reports*, 6, 24707. <https://doi.org/10.1038/srep24707>
- Joanne, S., Vythilingam, I., Yugavathy, N., Leong, C. S., Wong, M. L., & AbuBakar, S. (2015). Distribution and dynamics of *Wolbachia* infection in Malaysian *Aedes albopictus*. *Acta tropica*, 148, 38–45. <https://doi.org/10.1016/j.actatropica.2015.04.003>
- Lee, N. S. M., Clements, G. R., Ting, A. S. Y., Wong, Z. H., & Yek, S. H. (2020). Persistent mosquito fogging can be detrimental to non-target invertebrates in an urban tropical forest. *PeerJ*, 8, e10033. <https://doi.org/10.7717/peerj.10033>

- Lu, P., Sun, Q., Fu, P., Li, K., Liang, X., & Xi, Z. (2020). *Wolbachia* Inhibits Binding of Dengue and Zika Viruses to Mosquito Cells. *Frontiers in microbiology*, 11, 1750. <https://doi.org/10.3389/fmicb.2020.01750>
- Meteorological Department Malaysia. (2024). METMalaysia - Monthly Weather Bulletin. Available online: <https://www.met.gov.my/en/iklim/buletin-cuaca-bulanan/>. Accessed 30 June 2024
- Ministry of Health. (2024a). Kenyataan Media Ketua Pengarah Kesihatan – Situasi Semasa Demam Denggi Di Malaysia Minggu Epidemiologi 13 (24 Mac 2024 – 30 Mac 2024). Available online: https://www.moh.gov.my/index.php/database_stores/store_view_page/17/2509. Accessed 9 March 2025.
- Ministry of Health. (2024b). Keputusan Mesyuarat Pihak Berkuasa Kawalan Dadah Kali Ke-393 Pada 8 Februari 2024. Available online: <https://www.npra.gov.my/easyarticles/images/shared/One-Stop%20Centre/Kenyataan-Media-KPK--keputusan-mesyuarat-PBKD-393---Zolgensma-dan-QDenga-v7-09022024.pdf>. Accessed 9 March 2025.
- Mustafa, M. S., Rastogi, V., Gupta, R. K., Jain, S., Singh, P. M., & Gupta, A. (2016). *Wolbachia*: The selfish Trojan Horse in dengue control. *Medical journal, Armed Forces India*, 72(4), 373–376. <https://doi.org/10.1016/j.mjafi.2015.07.002>
- Nurul-Nastasea, S., Yu, K.-X., Rohani, A., Zurainee, M. N., Tengku-Idris, T. I. N., Dianita, R., Sabrina, M. R., & Wan Najdah, W. M. A. (2023). Insecticide resistance status of *Aedes aegypti* and *Aedes albopictus* in Malaysia (2010 to 2022): A review. *Asian Pacific Journal of Tropical Medicine*, 16(10), 434–445. <https://doi.org/10.4103/1995-7645.378561>
- Ou, D., Qiu, J. H., Su, Z. Q., Wang, L., & Qiu, B. L. (2023). The phylogeny and distribution of *Wolbachia* in two pathogen vector insects, Asian citrus psyllid and Longan psyllid. *Frontiers in cellular and infection microbiology*, 13, 1121186. <https://doi.org/10.3389/fcimb.2023.1121186>
- Petri, E., Biswal, S., Lloyd, E., Tricou, V., & Folschweiller, N. (2024). Early onset of protection of the TAK-003 dengue vaccine: Data from the DEN-301 clinical trial. *Vaccine*, 42(26), 126309. <https://doi.org/10.1016/j.vaccine.2024.126309>
- Postler, T. S., Beer, M., Blitvich, B. J., Bukh, J., de Lamballerie, X., Drexler, J. F., Imrie, A., Kapoor, A., Karganova, G. G., Lemey, P., Lohmann, V., Simmonds, P., Smith, D. B., Stapleton, J. T., & Kuhn, J. H. (2023). Renaming of the genus *Flavivirus* to *Orthoflavivirus* and extension of binomial species names within the family *Flaviviridae*. *Archives of virology*, 168(9), 224. <https://doi.org/10.1007/s00705-023-05835-1>
- Priyadarshana, T. S., & Slade, E. M. (2023). A meta-analysis reveals that dragonflies and damselflies can provide effective biological control of mosquitoes. *The Journal of animal ecology*, 92(8), 1589–1600. <https://doi.org/10.1111/1365-2656.13965>
- Siew, Z. Y., Khoo, C. T., Ong, G. K., Muhamad, B., Leong, P. P., Wong, S. T., Tan, B. S., Leong, C.-O., Low, D.-E., & Voon, K. (2024). TB or not TB: emerging mycobacteriaceae detected in a human patient, tree shrews, and soil. *Discover Medicine*, 1(1), 143. <https://doi.org/10.1007/s44337-024-00175-8>
- Siew, Z. Y., Lai, Z. J., Ho, Q. Y., Ter, H. C., Ho, S. H., Wong, S. T., Gani, M., Leong, P. P., & Voon, K. (2023). Bat coronavirus was detected positive from insectivorous bats in Krau Wildlife Reserve Forest. *Tropical biomedicine*, 40(4), 462–470. <https://doi.org/10.47665/tb.40.4.012>
- Siew, Z. Y., Ngai, Z. N., Wong, S. T., Chew, X. Y., Tan, B. S., Leong, C. O., Koh, R. Y., Leong, P. P., & Voon, K. (2025b). Tupaia orthoreovirus: The first successful isolation of a novel Mammalian orthoreovirus from treeshrews in Malaysia. *Journal of virological methods*, 337, 115189. <https://doi.org/10.1016/j.jviromet.2025.115189>
- Siew, Z. Y., Seow, I., Lim, X. R., Tang, C. Z., Djamil, F. M., Ong, G. K., Leong, P. P., Wong, S. T., & Voon, K. (2025a). Arboviruses: the hidden danger of the tropics. *Archives of virology*, 170(7), 140. <https://doi.org/10.1007/s00705-025-06314-5>
- Siew, Z. Y., Tang, C. Z., Musa, S. N. A., Seow, I., Johari, N. A., Leong, P. P., Wong, S. T., & Voon, K. (2026a). Treeshrews as a potential reservoir: First detection of dengue virus serotype 2 in Malaysian treeshrew faeces. *Journal of virological methods*, 339, 115256. <https://doi.org/10.1016/j.jviromet.2025.115256>
- Siew, Z. Y., Zariman, N. F., Wan Sa'idi, W. S. M., Lui, Z. Y., Muthu Coomahesha, H. S., Seow, I., Shafie, N. J., Wong, S. T., Abdul Razak, M. F. A., Gani, M., Sindang, S. W., & Voon, K. (2026b). Surveillance of Zoonotic Pathogens and Taxonomic Identification of Non-volant Small Mammals in Peninsular Malaysia. *Tropical Life Sciences Research*, 37(1), 293–313. <https://doi.org/10.21315/tlsr2026.37.1.14>
- Sridhar, S., Luedtke, A., Langevin, E., Zhu, M., Bonaparte, M., Machabert, T., Savarino, S., Zambrano, B., Moureau, A., Khromava, A., Moodie, Z., Westling, T., Mascareñas, C., Frago, C., Cortés, M., Chansinghakul, D., Noriega, F., Bouckennooghe, A., Chen, J., Ng, S. P., ... DiazGranados, C. A. (2018). Effect of Dengue Serostatus on Dengue Vaccine Safety and Efficacy. *The New England journal of medicine*, 379(4), 327–340. <https://doi.org/10.1056/NEJMoa1800820>
- Tricou, V., Yu, D., Reynales, H., Biswal, S., Saez-Llorens, X., Sirivichayakul, C., Lopez, P., Borja-Tabora, C., Bravo, L., Kosalaraksa, P., Vargas, L. M., Alera, M. T., Rivera, L., Watanaveeradej, V., Dietze, R., Fernando, L., Wickramasinghe, V. P., Moreira, E. D., Jr, Fernando, A. D., Gunasekera, D., ... Wallace, D. (2024). Long-term efficacy and safety of a tetravalent dengue vaccine (TAK-003): 4·5-year results from a phase 3, randomised, double-blind, placebo-controlled trial. *The Lancet. Global health*, 12(2), e257–e270. [https://doi.org/10.1016/S2214-109X\(23\)00522-3](https://doi.org/10.1016/S2214-109X(23)00522-3)
- Teo, C. H. J., Lim, P. K. C., Voon, K., & Mak, J. W. (2017). Detection of dengue viruses and *Wolbachia* in *Aedes aegypti* and *Aedes albopictus* larvae from four urban localities in Kuala Lumpur, Malaysia. *Tropical biomedicine*, 34(3), 583–597.
- Wan Mohamad Ali, W. N., Ahmad, R., Nor, Z. M., & Hassan, A. F. (2020). SPATIAL DISTRIBUTION, ENZYMATIC ACTIVITY, AND INSECTICIDE RESISTANCE STATUS OF *Aedes aegypti* AND *Aedes albopictus* FROM DENGUE HOTSPOT AREAS IN KUALA LUMPUR AND SELANGOR, MALAYSIA. *Serangga*, 25(3), 65–92. <https://ejournal.ukm.my/serangga/article/view/40468>
- Yusof, M. Z., Cherrie, J. W., Samsuddin, N., & Semple, S. (2022). Mosquito Control Workers in Malaysia: Is Lifetime Occupational Pesticide Exposure Associated With Poorer Neurobehavioral Performance?. *Annals of work exposures and health*, 66(8), 1044–1055. <https://doi.org/10.1093/annweh/wxac038>
- Zhou, W., Rousset, F., & O'Neil, S. (1998). Phylogeny and PCR-based classification of *Wolbachia* strains using wsp gene sequences. *Proceedings. Biological sciences*, 265(1395), 509–515. <https://doi.org/10.1098/rspb.1998.0324>