Original research

North-South Gradients in Dengue Vector Ecology, Insecticide Resistance, and Community KAP in Laos: Implications for Site-Specific Vector Management

*Vilayphone Keomany [a], Malivanh Bouapha [b], Phonesavanh Silivong [a], Nanthavong Khampasong [b]**

[a] *Dali University, No. 2, Hongsheng Road, Dali City, Dali 671003, Yunnan Province, China*

[b] *National University of Laos, Dongdok Campus, Xaythany District, PO Box 7322, Vientiane 01080, Lao People's Democratic Republic*

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ABSTRACT

Objective: To clarify the species composition, biting rhythm, seasonal fluctuation, and insecticide resistance of dengue vectors in residential areas of northern (Luang Prabang), central (Vientiane), and southern (Attapeu) Laos, and to analyze residents' knowledge, attitudes, and practices (KAP) regarding dengue prevention and their association with infection. **Methods:** From 2024 to 2025, human landing catches (HLC) with full personal protection and larval container indices were conducted in three sites. WHO adult contact tube bioassays were performed in Luang Prabang and Vientiane to determine resistance to five insecticides. Structured questionnaires were administered, and laboratory-confirmed dengue infection history was analyzed using multivariable logistic regression. **Results:** In Luang Prabang, 1,847 adult mosquitoes (2 subfamilies, 5 genera, 17 species) were collected; Aedes aegypti and Ae. albopictus accounted for 1.85% (34) and 2.38% (44), respectively. Biting peaks occurred at 09:00 and 18:00. BI/HI/CI peaked in August–September, with case peaks in October. In Vientiane, 2,156 adults (2 subfamilies, 6 genera, 18 species) were collected; Ae. aegypti and Ae. albopictus accounted for 2.89% (62) and 1.56% (34). Biting peaks were at 08:30–09:30 and 17:30–18:30; BI/HI/CI peaked in May–July, with case peaks in July–August. In Attapeu, 2,341 adults (2 subfamilies, 6 genera, 19 species) were collected; Ae. aegypti and Ae. albopictus accounted for 4.12% (96) and 2.86% (67). Biting peaks were at 08:00–09:00 and 17:00–18:00; BI/HI/CI peaked in April–May, with case peaks in June. In Luang Prabang, 24 h corrected mortality of Ae. aegypti to permethrin, lambda-cyhalothrin, and deltamethrin was 67.53%, 25.47%, and 72.63% (resistant); to fenitrothion and propoxur was 100% and 98.82% (susceptible). In Vientiane, mortality to permethrin and deltamethrin was 58.24% and 65.38% (resistant), with resistance more severe than in Luang Prabang. Multivariable analysis showed that children/adolescents (<18 years) had higher infection risk across all three sites (OR=2.15–2.78). Active larval source reduction significantly lowered infection risk (OR=0.35–0.48). Significant north-south gradients were observed in vector density, seasonal peaks, and pyrethroid resistance. **Conclusion:** Significant north-south gradients exist in vector ecology, seasonal peaks, and insecticide resistance in Laos. Larval source management remains key to effective prevention, and differentiated, site-specific integrated vector management is urgently needed.

1. Introduction

Laos, situated in the Greater Mekong Subregion, faces a severe dengue endemic with marked north-south climatic gradients. Aedes aegypti and Aedes albopictus are the principal vectors [1]. Regional differences in latitude, elevation, urbanization, and monsoon timing create distinct ecological and control challenges [2]. Luang Prabang (north) is a high-elevation tourist city; Vientiane (central) is the densely populated capital with rapid peri-urban expansion; Attapeu (south) has a tropical monsoon climate with an early, prolonged rainy season. Previous studies have confirmed widespread insecticide resistance among dengue vectors in Laos

* *Corresponding author: Nanthavong Khampason*

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[3], while local residents' knowledge, attitudes and practices (KAP) related to dengue prevention remain uneven across regions [4]. Landscape variation and climatic fluctuation further shape dengue transmission patterns nationwide [5]. This study was conducted simultaneously at these three sites to reveal vector characteristics and KAP differences across Laos, providing evidence for graded, precision prevention strategies and reference for larval control alternative insecticides application [6].

2. Materials and methods

2.1. Study Sites and Period

Site 1 (North): Urban residential area of Luang Prabang (19° 53′ N, 102° 08′ E, ~300 m above sea level), monitored continuously from January 2024 to December 2025.
Site 2 (Central): Peri-urban community in Vientiane (17° 58′ N, 102° 36′ E, ~170 m above sea level), monitored continuously from January 2024 to December 2025.
Site 3 (South): Urban residential area in Attapeu Province (14° 48′ N, 106° 50′ E, ~100 m above sea level), monitored continuously from January 2024 to December 2025.
These sites represent three ecological types: northern montane monsoon, central plain transition, and southern tropical humid.

2.2. Adult and Larval Surveys

Adult survey: Human landing catches (HLC) and CDC miniature light traps (John W. Hock) were used. For HLC, two fixed points per site (center and edge) were selected, with two trained collectors per point. Collections were made for 15 minutes per time slot, with hourly rotation, recording Ae. aegypti and Ae. albopictus landings from 06:00 to 18:00. All HLC personnel wore long-sleeved protective clothing, used repellents (DEET ≥20%), and slept under bed nets; daily health monitoring was conducted, with no dengue or malaria infections reported during the study. Light traps were hung indoors and outdoors at 1.5 m height, operating from 18:00 to 06:00, for 3 consecutive nights per month. Captured mosquitoes were frozen, identified using Chinese Mosquito Taxonomic Keys and Southeast Asian Mosquito Identification Guide, and recorded by species, sex, and number.
Larval survey: Visual inspection of indoor and outdoor water-holding containers. Systematic sampling of 100 households (Luang Prabang), 120 (Vientiane), or 100 (Attapeu) was conducted monthly. Positive containers, total containers, and positive houses were recorded to calculate Breteau Index (BI), House Index (HI), and Container Index (CI). Seasonal dynamics were analyzed in relation to monthly dengue case reports from local health departments.

2.3. Insecticide Resistance Bioassays

Conducted in Luang Prabang and Vientiane. Field-collected Ae. aegypti females (F0) were reared to F1 in the laboratory. Three- to five-day-old non-blood-fed females were tested using the WHO adult contact tube method (WHO/CDS/GMP/2016.3). Diagnostic doses were: 3.20% permethrin, 0.08% lambda-cyhalothrin, 0.20% deltamethrin, 0.25% fenitrothion, and 0.03% propoxur. Four replicates of 25 mosquitoes each (total 100) were tested per insecticide, alongside a susceptible reference strain (Ae. aegypti New Orleans, Sudan) and an acetone-treated blank control. After 1 h exposure, mosquitoes were transferred to recovery tubes; mortality was recorded at 24 h. All mortality rates were corrected using Abbott's formula. Control mortality ranged from 3% to 5%, within acceptable limits. Resistance was classified according to WHO 2022 guidelines: corrected mortality >98% = susceptible; 80–98% = possible resistance; <80% = resistant [7].

2.4. Questionnaire Survey and Infection Confirmation

A structured face-to-face questionnaire collected demographic data, symptom recognition (fever, hemorrhage), information sources, protective behaviors, and active larval source reduction practices (container covering, no water storage, vegetation clearing). Sample size was calculated using $n = Z^2 p(1-p)/d^2$ (expected infection rate p=12%, margin of error d=0.05), yielding a minimum of 163; with 20% attrition, ≥200 participants were surveyed per site. Stratified random sampling was used by age group (<18, 18 – 35, 36 – 59, ≥60 years) and sex. The questionnaire was pre-tested (n=30) and validated (Cronbach's α=0.84; content validity index=0.86).
Infection history was based on laboratory-confirmed diagnoses from local health facilities: NS1 antigen positivity, RT-PCR detection of dengue virus nucleic acid, or IgM seroconversion in convalescent sera. Data were analyzed using SPSS 26.0. Categorical variables were compared using χ^2 or Fisher's exact tests; inter-site comparisons used Kruskal–Wallis H or Mann–Whitney U tests. Multivariable logistic regression was used to identify independent risk factors; variables with P<<0.20 in univariate analysis were entered into the multivariable model, reporting adjusted odds ratios (OR) with 95% confidence intervals (CI). Significance was set at α=0.05.

3. Results

3.1. Mosquito Species Composition and Vector Proportion

Luang Prabang: 1,847 adult mosquitoes collected, belonging to 2 subfamilies, 5 genera, and 17 species (Table 1). Dominant species were Culex quinquefasciatus (28.37%) and Cx. tritaeniorhynchus (24.31%). Ae. aegypti and Ae. albopictus accounted for 1.85% (34/1,847) and 2.38% (44/1,847), totaling 4.23%.
Vientiane: 2,156 adults collected, belonging to 2 subfamilies, 6 genera, and 18 species. Dominant species were Cx. tritaeniorhynchus (31.45%, 678/2,156), Cx. quinquefasciatus (27.18%, 586/2,156), and Cx. gelidus (15.82%, 341/2,156). Ae. aegypti and Ae. albopictus accounted for 2.89% (62/2,156) and 1.56% (34/2,156), totaling 4.45%.
Attapeu: 2,341 adults collected, belonging to 2 subfamilies, 6 genera, and 19 species. Dominant species were Cx. tritaeniorhynchus (35.07%, 821/2,341), Cx. quinquefasciatus (26.91%, 630/2,341), and Cx. gelidus (16.19%, 379/2,341). Ae. aegypti and Ae. albopictus accounted for 4.12% (96/2,341) and 2.86% (67/2,341), totaling 6.98%.
The combined proportion of Aedes mosquitoes differed significantly among the three sites (χ^2=14.83, P=0.001); Attapeu was significantly higher than Luang Prabang (χ^2=13.62, P<<0.001) and Vientiane (χ^2=6.45, P=0.011).

Table 1. Comparison of adult mosquito species composition across three sites.

Species	Luang Prabang (*n*=1,847)		Vientiane (*n*=2,156)		Attapeu (*n*=2,341)	
	Quantity	Percentage	Quantity	Percentage	Quantity	Percentage
Ae. aegypti	34	1.85	62	2.89	96	4.12
Ae. albopictus	44	2.38	34	1.56	67	2.86
Cx. tritaeniorhynchus	449	24.31	678	31.45	821	35.07
Cx. quinquefasciatus	524	28.37	586	27.18	630	26.91
Cx. gelidus	187	10.12	341	15.82	379	16.19
Others	609	32.97	455	21.10	348	14.85

Note: "Others" includes mainly Anopheles spp., Mansonia spp., Armigeres spp., and Uranotaenia spp.

3.2. Biting Rhythm

Ae. aegypti showed a bimodal biting pattern at all three sites, with morning peaks shifting earlier from north to south. Luang Prabang: peaks at 09:00 (28.4% of diurnal landings) and 18:00 (31.2%); Ae. albopictus peaked at 18:00 (42.6%). Vientiane: Ae. aegypti peaks at 08:30–09:30 (27.8%) and 17:30–18:30 (30.5%); Ae. albopictus peaks at 08:30 (18.4%) and 17:30–18:30 (35.2%). Attapeu: Ae. aegypti peaks at 08:00–09:00 (29.2%) and 17:00–18:00 (32.1%); Ae. albopictus activity was relatively uniform during daytime (8–14% per time slot), with a slight increase at 17:00–18:00 (28.5%).

Morning peak times differed significantly among sites (Kruskal–Wallis H=8.76, P=0.013), with Attapeu and Vientiane significantly earlier than Luang Prabang..

3.3. Larval Seasonality and Case Peaks

Luang Prabang: Year-round larval presence. BI, HI, and CI peaked in August–September (BI=19.2, HI=23.0, CI=11.5), with dengue case peaks in October (48 cases/month) (Table 2).

Vientiane: BI, HI, and CI peaked in May–July (BI max=26.5, HI=29.0, CI=15.2), with case peaks in July–August (55 cases).

Attapeu: BI, HI, and CI peaked in April–May (BI max=28.0, HI=31.5, CI=16.8), with case peaks in June (62 cases).

BI peak timing differed significantly among sites (Kruskal – Wallis H=15.34, P=0.001), showing a clear north-south gradient: Attapeu (April – May) → Vientiane (May – July) → Luang Prabang (August – September), consistent with local rainy season onset.

Table 2. Monthly Breteau Index (BI) and dengue case counts across three sites (2024–2025)

Month	Luang Prabang		Vientiane		Attapeu	
	BI	Cases	BI	Cases	BI	Cases
Jan	2.0	0	4.0	3	6.5	5
Feb	3.5	1	6.0	5	9.0	8
Mar	5.0	2	10.5	10	15.0	15
Apr	8.0	5	18.0	22	28.0	28
May	12.5	12	26.5	38	25.5	45
Jun	15.0	25	24.0	55	20.0	62
Jul	18.0	35	22.0	52	16.0	48
Aug	19.2	42	18.0	35	12.0	30
Sep	17.0	48	14.0	28	9.0	18
Oct	12.0	48	10.0	15	6.0	10
Nov	6.0	15	6.5	8	4.0	5
Dec	3.0	3	4.0	3	3.0	2

Note: Case counts are monthly averages across 2024–2025, rounded to the nearest integer.

Figure 1. Geographic and Climatic Gradient Across Three Survey Sites in Laos

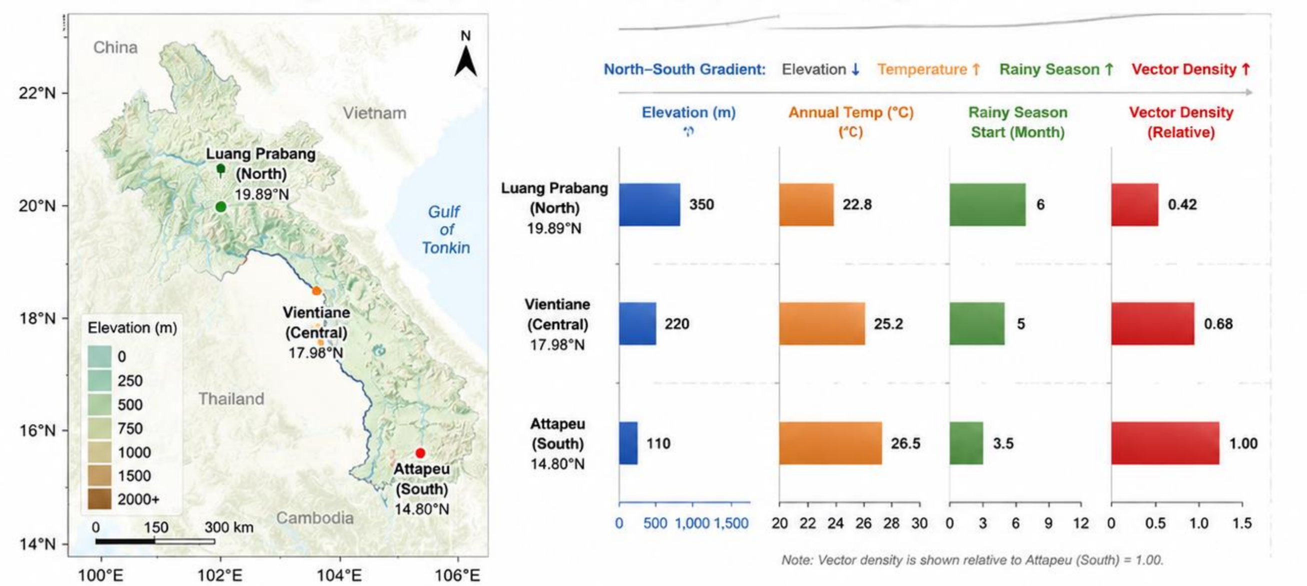


Figure 2. Diurnal Biting Pattern of *Aedes aegypti* Across Three Sites

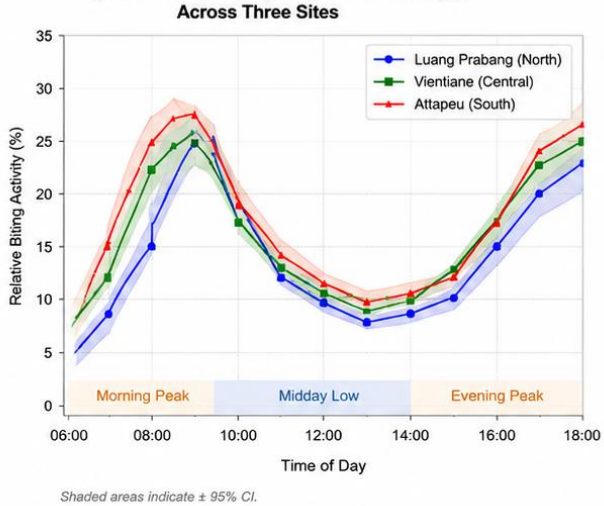
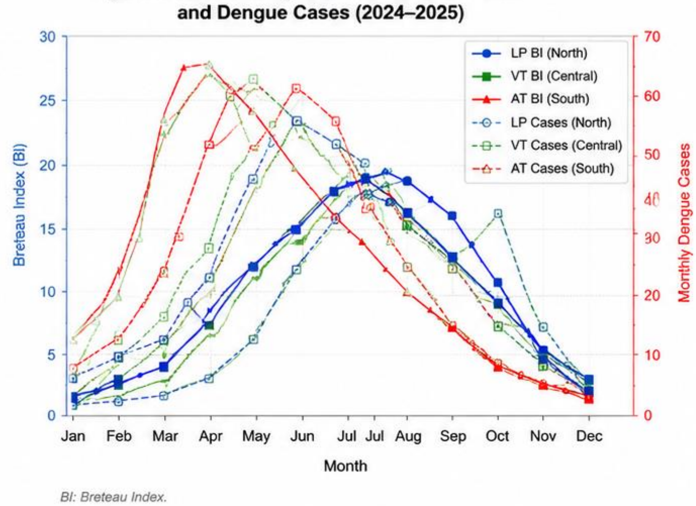


Figure 3. Seasonal Dynamics of Breteau Index and Dengue Cases (2024–2025)



3.4. Insecticide Resistance

Luang Prabang: F1 Ae. aegypti showed corrected 24 h mortality of 67.53%, 25.47%, and 72.63% to permethrin, lambda-cyhalothrin, and deltamethrin, respectively (all <80%, resistant); mortality to fenitrothion and propoxur was 100% and 98.82% (susceptible) (Table 3).

Vientiane: F1 Ae. aegypti showed corrected mortality of 58.24%, 18.65%, and 65.38% to permethrin, lambda-cyhalothrin, and deltamethrin, respectively (all <80%, resistant). Mortality to permethrin and deltamethrin was significantly lower than in Luang Prabang (χ^2=4.32, P=0.038; χ^2=5.18, P=0.023), indicating more severe resistance. Mortality to fenitrothion and propoxur was 99.02% and 97.56% (susceptible / possible resistance).

3.5. KAP and Infection Association

Luang Prabang: 198 residents surveyed; 46 (23.2%) had laboratory-confirmed dengue infection history. Using the ≥60-year group as reference, multivariable analysis showed minors (<18 years) had 2.34-fold higher risk (*P*=0.015), and the 36–59-year group had higher risk (OR=1.89, *P*=0.018). Television as an information source was protective (OR=0.52, *P*=0.014). Active container covering (OR=0.41, *P*=0.001), no water storage (OR=0.38, *P*=0.001), and vegetation clearing (OR=0.45, *P*=0.004) significantly reduced infection risk.

Vientiane: 215 residents surveyed; 58 (27.0%) had confirmed infection history. Using the ≥60-year group as reference, minors (OR=2.56, *P*=0.004) and the 18–59-year group (OR=1.92, *P*=0.026) had higher risk. Gardeners (OR=2.15, *P*=0.007) and clerical staff (OR=1.78, *P*=0.032) were at higher risk. Bed net use (OR=0.48, *P*=0.009), water removal (OR=0.35, *P*=0.001), no water storage (OR=0.42, *P*=0.005), and vegetation clearing (OR=0.39, *P*=0.003) were protective.

Attapeu: 203 residents surveyed; 61 (30.0%) had confirmed infection history. Using the ≥60-year group as reference, minors (OR=2.78, *P*=0.002) and the 36–59-year group (OR=2.15, *P*=0.008) had higher risk. Farmers (OR=2.45, *P*=0.005) and outdoor vendors (OR=2.08, *P*=0.019) were at higher risk. Container covering (OR=0.48, *P*=0.012), water removal (OR=0.38, *P*=0.002), and bed net use (OR=0.44, *P*=0.007) were protective.

Table 3. Insecticide resistance bioassay results for Ae. aegypti in Luang Prabang and Vientiane

Insecticide	Diagnostic dose (%)	Luang Prabang (*n*=100)		Vientiane (*n*=100)	
		Corrected mortality (%)	Status	Corrected mortality (%)	Status
Permethrin	3.20	67.53	Resistant	58.24	Resistant
Lambda-cyhalothrin	0.08	25.47	Resistant	18.65	Resistant
Deltamethrin	0.20	72.63	Resistant	65.38	Resistant
Fenitrothion	0.25	100.00	Susceptible	99.02	Susceptible
Propoxur	0.03	98.82	Susceptible	97.56	Possible resistance
Blank control (acetone)	—	3.2	—	4.5	—

Note: Blank control mortality is reported as raw (uncorrected) percentage.

Table 4. Multivariable logistic regression of dengue infection risk across three sites

Variable	Luang Prabang (*n*=198)		Vientiane (*n*=215)		Attapeu (*n*=203)	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Age <18 years	2.34 (1.18–4.65)	0.015	2.56 (1.34–4.89)	0.004	2.78 (1.45–5.32)	0.002
Age 36–59 years	1.89 (1.12–3.21)	0.018	1.92 (1.08–3.42)	0.026	2.15 (1.22–3.79)	0.008
High-risk occupation	—	—	Gardener 2.15 (1.23–3.76)	0.007	Farmer 2.45 (1.32–4.55)	0.005
			Clerk 1.78 (1.05–3.02)	0.032	Vendor 2.08 (1.13–3.83)	0.019
Information source (TV)	0.52 (0.31–0.88)	0.014	—	—	—	—
Larval source reduction						
Container covering	0.41 (0.24–0.71)	0.001	—	—	0.48 (0.27–0.86)	0.012
Water removal	—	—	0.35 (0.19–0.64)	0.001	0.38 (0.21–0.69)	0.002
No water storage	0.38 (0.21–0.69)	0.001	0.42 (0.23–0.77)	0.005	—	—
Vegetation clearing	0.45 (0.26–0.78)	0.004	0.39 (0.21–0.72)	0.003	—	—
Bed net use	—	—	0.48 (0.28–0.83)	0.009	0.44 (0.25–0.78)	0.007

Note: Variables with P>0.20 in univariate analysis were not entered into the multivariable logistic regression model, indicated by "—".

4. Discussion

This three-site survey reveals significant north-south gradients in dengue vector ecology across Laos. Attapeu (south) had the highest Aedes proportion (6.98%), earliest vector peak (April–May), and earliest case peak (June); Vientiane (central) showed intermediate values (4.45%, May﹣July); Luang Prabang (north) had the lowest proportion (4.23%) and latest peak (August﹣September). This gradient aligns closely with climatic parameters: Attapeu has the lowest latitude (14° 48′ N), lowest elevation (~100 m), highest annual temperature (26.5°C), and earliest rainy season onset (March–April), favoring year-round early vector proliferation. Luang Prabang, at higher elevation (~300 m) and lower temperature (22.8°C), with a rainy season starting in June, shows correspondingly delayed peaks. These findings indicate that a "one-size-fits-all" national control calendar is inappropriate; instead, a differentiated "south-early, central-mid, north-late" strategy is required.

The consistent 1–2 month lag between larval peaks and case peaks at all sites provides a critical intervention window. Attapeu should initiate source reduction in March, Vientiane in April, and Luang Prabang in July, before case outbreaks occur.

Insecticide resistance testing revealed severe and regionally heterogeneous pyrethroid resistance. Both Luang Prabang and Vientiane Ae. aegypti were resistant to all three pyrethroids tested, with Vientiane showing more severe resistance (permethrin mortality 58.24% vs. 67.53%). This finding is in line with nationwide resistance investigations in Laos [8] and regional surveillance across the Southeast Asia Region [9], and reflects the global trend of widespread pyrethroid resistance in Ae. Aegypti [10].This likely reflects longer and more intensive pyrethroid use history in the capital, compounded by urban heat island effects accelerating resistance evolution. Fenitrothion and propoxur remain largely effective and should be used as rotational insecticides during outbreaks. However, propoxur in Vientiane is approaching the possible resistance threshold (97.56%), underscoring the need for a national resistance monitoring network and molecular follow-up studies (e.g., kdr mutation screening). Neighboring Cambodia has also reported similar resistance profiles in *Aedes* populations, suggesting cross-border diffusion risk [11].

KAP analysis consistently confirmed larval source reduction as the core protective strategy. Active container covering, water removal, and no water storage reduced infection risk by 55–65% (OR=0.35–0.48) across all sites, consistent with findings from Brazil and Vietnam. Television-based health education was effective in Luang Prabang, while personal protection (bed nets) showed stronger effects in Vientiane and Attapeu, suggesting northern sites should prioritize mass media coverage, while central and southern sites require intensified community mobilization and commodity distribution. Age and occupation risk profiles highlight the need for targeted interventions among students, young adults, and outdoor workers (farmers, vendors, gardeners), which is consistent with research on travel-related and occupational dengue risk in the region [12]. Spatio-temporal characteristics of dengue transmission in Laos also require differentiated population intervention strategies [13].

Limitations: Resistance bioassays were not conducted in Attapeu due to lack of local F1 rearing facilities and susceptible reference strains; this should be addressed in future studies. The cross-sectional questionnaire design precludes causal inference. Infection history relied on past laboratory confirmations, potentially missing mild or unreported cases. Future research should expand to eastern provinces (e.g., Savannakhet), incorporate molecular resistance markers, and employ prospective cohort designs. National vector control policies should be formulated based on the Global Vector Control Response framework [14].

5. Conclusion

Significant north-south gradients exist in dengue vector ecology, seasonal peaks, and insecticide resistance in Laos. Attapeu (south) has the highest vector density and earliest transmission season; Vientiane (central) has the most severe pyrethroid resistance; Luang Prabang (north) has the latest peak. Active larval source reduction by residents is the most effective protective measure. We recommend a differentiated "south-early, central-mid, north-late" control calendar: source reduction campaigns in Attapeu (March–April), Vientiane (April–May), and Luang Prabang (July–August). A national insecticide resistance monitoring network should be established, pyrethroid mono-therapy restricted, and fenitrothion/propoxur rotation promoted, with targeted protection for minors and outdoor occupational groups.

Conflict of Interest: All authors declare no conflicts of interest.

Ethics Statement: This study was approved by the National University of Laos Medical Ethics Committee. Mosquito collection permits were obtained from health departments in all three sites. All participants provided written informed consent; guardians consented on behalf of minors.

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