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High resurgence of dengue vector populations after space spraying in an endemic urban area of Thailand: A cluster randomized controlled trial

Napadol Sudsom[1], Kuaanan Techato[1], Suwich Thammapalo[2], Virasakdi Chongsuvivatwong[3], Theerakamol Pengsakul[4*]

[1]*Faculty of Environmental Management, Prince of Songkla University, Hatyai, Songkhla 90110, Thailand*

[2]*Office of Disease Prevention and Control, 12 Songkhla, Thailand*

[3]*Epidemiology Unit, Faculty of Medicine, Prince of Songkla University, Hatyai, Songkhla 90110, Thailand*

[4]*Faculty of Medical Technology, Prince of Songkla University, Hatyai, Songkhla 90110, Thailand*

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ABSTRACT

Objective: To examine the resurgence rate, house density index (HDI) and parous rate of the *Aedes aegypti* vector after space spraying carried out by the routine spraying team, and compare with the rates after standard indoor ultra low volume (SID-ULV) spraying carried out by the trained research spraying team.

Methods: Between March and September 2014, a cluster randomized controlled trial including 12 clusters (6 regular ULV, 6 SID-ULV) with totally 4 341 households was conducted, and around 20–31 houses in each cluster were selected for assessment. The parous rate and HDI of collected mosquitoes 2 days before and 1, 2 and 6 days after spraying were obtained and compared.

Results: The HDI dropped significantly from the baseline 1 and 2 days after spraying to a non-zero value in the SID-ULV treated locations but not in the regular ULV group locations. However, by 6 days after spraying, the HDI of both groups had returned to the base value measured 2 days before spraying. There were no statistically significant differences in the parous rate between groups.

Conclusions: SID-ULV is more effective in reducing *Aedes aegypti* populations. However, rapid resurgence of dengue vector after spraying in urban areas was observed in both groups.

1. Introduction

Dengue infection is a well-known rapidly spreading mosquito-borne disease, which causes significant public health problems in Thailand. The most effective way to prevent dengue virus transmission during an outbreak is exterminating the disease-carrier *Aedes aegypti* (*Ae. aegypti*) [1,2]. In emergency conditions, space spraying is the only effective means of suppressing an acute dengue virus outbreak [3,4]. However, it has been demonstrated that routine space spraying does not completely prevent secondary dengue cases [5,6]. Therefore, more field research is needed on the effect of various kinds of space spraying [7,8].

In Thailand, local administrative organizations (LAO) are the main organizations responsible for conducting space spraying. Two forms of space spraying have been implemented since 2002, ultra low volume (ULV) and thermal fog [6]. The effect of ULV spraying is more sustained for vector suppression when applied as an indoor space spraying [9], but the effectiveness is dependent on the droplet size and the application method because there is a low probability of contact between adult mosquitoes and the insecticide droplets [8]. During dengue outbreaks, LAO space spraying has been shown to be ineffective in preventing dengue transmission and evidence of the effectiveness of standard indoor ultra low volume (SID-ULV) spraying is still lacking [6,8,10,11].

The infected vector density in outbreak clusters is linked to the parity rate (PR), the proportion of female mosquitoes that

*Corresponding author: Theerakamol Pengsakul, Faculty of Medical Technology, Prince of Songkla University, Hatyai, Songkhla 90110, Thailand.
E-mail: theerakamol.p@psu.ac.th

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have laid eggs at least once [12]. To effectively interrupt transmission, the PR should be kept below 10% which results in a reduction in the adult dengue vector population of more than 97% [2,13]. While it is difficult to assess the effectiveness of measures taken to prevent secondary dengue cases, the number of female *Ae. aegypti* per house, (a good proxy for PR [2]) and the house density index (HDI), the number of adult female *Ae. aegypti* mosquitoes collected in each house for 15 min, can be employed as an indication of potential success.

The objective of this study was to verify the resurgence rate of the *Ae. aegypti* vector, by comparing the rates after regular ULV treatment carried out by the municipality and SID-ULV spraying, which was carried out by the research team.

2. Materials and methods

2.1. Study site

The study site was Songkhla City which is located at 7°12′ N, 100°36′ E on a peninsular of the east coast of Southern Thailand. The municipality covers 9.3 km^2, and is divided into 32 communities with 26000 households containing a population of 71000 people, a population density of 7400 persons/km^2 in 2013. The study was conducted in both the dry (February–July) and wet (August–January) seasons during which periods there was an average annual rainfall of 1434 mm, an average temperature of 28.4 °C and an average relative humidity of 73% (South Eastern Meteorological Center, Songkhla, 2014). The study site was selected because it is urban area prone to endemic transmission, with an average annual incidence rate of 500 per 100000 population [6,14].

2.2. Study design

A clustered randomized control trial was used with, for practical reasons, only one type of spray being used in each community. The design also covered externality effects, *i.e.*, the spray affecting nearby unsprayed houses. The trial was designed comparing 6 randomly selected SID-ULV clusters with 6 regular ULV clusters. A cluster in this study consisted of households located in a circle of 120 m in radius. In each cluster, around 20–31 houses were randomly selected for entomological assessment.

The inclusion criteria for eligible clusters were that they were all communities in dengue endemic areas with high population density; at least 100 houses; and a minimum area per cluster of at least 120 m × 120 m. The minimum distance between each SID-ULV cluster and regular ULV cluster with which it was compared was at least 1000 m. Maps of all the clusters were generated and the clusters were geo-located using high resolution satellite images (Quick Bird, USA) and Geographic Information System software (ArcGIS 9.3) from Southern Regional Geo-Informatics and Space Technology Center, Faculty of Environmental Management, Prince of Songkla University.

2.3. Spraying operations

SID-ULV spraying was conducted by well-trained officers from the Office of Disease Prevention and Control 12 (DPC-12). The application strictly followed the World Health Organization (WHO) guidelines. Regular ULV treatment was based on routine space spraying conducted by LAO. This application did not follow the WHO guidelines [15]. Both the SID-ULV and regular ULV treatments were carried out with portable ULV equipment (Fontan Portastar S, Germany). Both the SID-ULV and regular ULV treatments were conducted based on the same spraying conditions representing controlled variables within the study. These included time, wind, rain and temperature. The meteorological conditions were monitored using the same daily time slot data from the Songkhla Weather Observation Station located in the center of Songkhla City. The dates of spraying and the locations sprayed were informed to the local health department and there was regular surveillance and response to dengue cases in cooperation with the local health officers.

2.4. Calibration of chemicals and spray generators

The equipment and the insecticides used were calibrated before the field spraying operations were conducted. The measurement of the volume median diameter of the droplets produced by the ULV generators was conducted according to the slide wave technique [15]. Then 2% deltamethrin (w/v) (Type II pyrethroids insecticides) was applied for field spraying operations. A droplet bio-assay test was used to evaluate the efficacy of deltamethrin by semi-field evaluation in experimental rooms [16]. Both tests were performed at DPC-12.

2.5. Entomological surveys

The pre- and post-space spraying parameters of the *Ae. aegypti* populations were monitored. The parameters included PR and HDI assessed 2 days before, and 1, 2 and 6 days after spraying in the same houses. Adult *Ae. aegypti* mosquitoes were collected using hand-held nets by the trained collecting team following WHO guidelines [2,15]. The adult mosquitoes were collected from the living areas of around 20–31 houses in each cluster over a period of 15 min. The identification of the collected mosquitoes and the dissection of the ovaries from the female *Ae. aegypti* to establish their parity status were conducted at Faculty of Medical Technology, Prince of Songkla University.

2.6. Statistical analysis

The data were analyzed using the R statistical program (R Development Core Team) and RStudio software (RStudio, Inc., USA). The PR and HDI of the SID-ULV and regular ULV clusters were compared using line graphs and paired sample *t*-tests at a 95% confidence interval ($P < 0.05$).

3. Results

3.1. Droplet size and bio-assays

The volume median diameter of the five ULV generators (four belonging to DPC-12 and one from LAO) used in the study was measured. The equipment delivered droplets of 23, 25, 25, 26 and 26 μm respectively, which were within the acceptable ranges (5–27 μm) recommended by WHO [2].

The vector susceptibility to deltamethrin (2%, w/v) established at the DPC-12 laboratory was 99.8% knockdown and 99.9% mortality.

3.2. Results of mosquito collection

Field trials were carried out between March and September in 2014 at a total of 292 randomly selected houses in 12 clusters, of which 138 houses in 6 clusters received regular ULV treatment and 154 houses in 6 clusters received SID-ULV treatment (Figure 1). A total of 3 924 adult mosquitoes, comprising 3 161 (80.56%) *Ae. aegypti*, 13 (0.33%) *Aedes albopitus* and 750 (19.11%) other species, were collected before and after space spraying (Table 1).

As Table 1 shows, the mean number of *Ae. aegypti* per house was not significantly different between the two groups at baseline [2 days before space spraying (D_{-2}) and 6 days after space spraying (D_6)], however, there were significant differences between the groups at 1 and 2 days after spraying (D_1, D_2).

3.3. Difference in HDI after spraying

After spraying, the HDI at the sites treated with SID-ULV became significantly lower than that at baseline (D_{-2}) throughout the follow up period (Figure 2). In contrast, the HDI of the sites treated with regular ULV spraying unexpectedly increased significantly from the baseline.

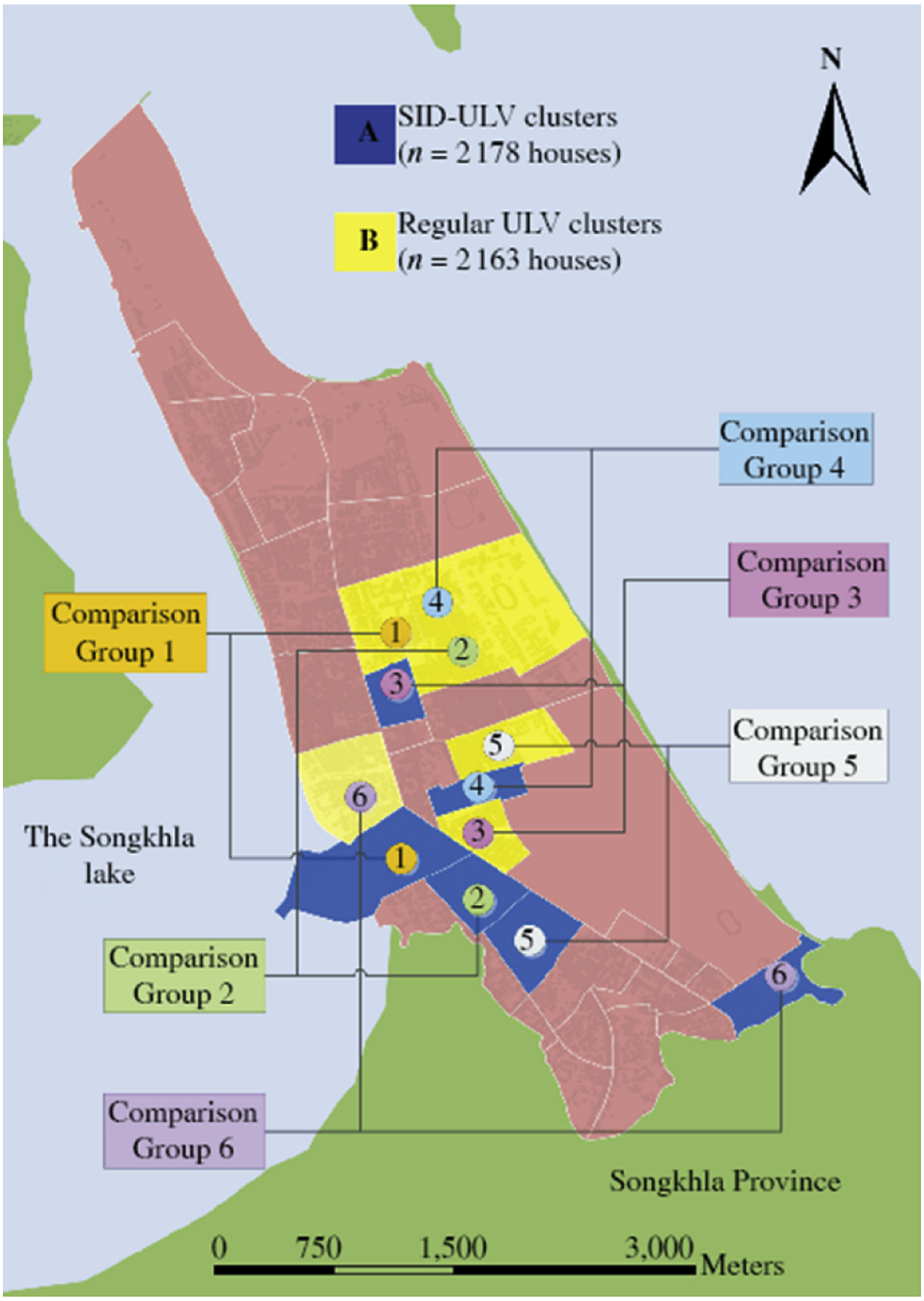

Figure 1. A map of six comparison groups studied.
Comparison Group 1: Thasaan (A, n = 444) and Bonwuakao (B, n = 360); Comparison Group 2: Watthasalahuayang (A, n = 185) and Watchaimongkon (B, n = 413); Comparison Group 3: Ruamjaiphatthana (A, n = 396) and Wangkhiaowangkao (B, n = 320); Comparison Group 4: Noksuan (A, n = 344) and Watsraket (B, n = 391); Comparison Group 5: Kubo (A, n = 445) and Wachirasoiku (B, n = 365); Comparison Group 6: Kaoseng (A, n = 364) and Banbon (B, n = 314). A: SID-ULV clusters; B: Regular ULV clusters; n: Number of houses.

Table 1

Comparison of number of adult mosquitoes collected by days before and after regular ULV versus SID-ULV spraying.

Day of collection	Group sprayed (6 clusters)	*Ae. aegypti*		*Aedes albopitus*		Other species		Total by days	
		n (%)	Mean per house (SD)	*n* (%)	Mean per house (SD)	*n* (%)	Mean per house (SD)	*n* (%)	Mean per house (SD)
D_{-2}	Regular ULV	404 (79.68)	2.93 (3.59)	0	0	103 (20.32)	0.75 (1.92)	507 (100)	3.67 (4.36)
	SID-ULV	564 (81.86)	3.66 (4.66)	0	0	125 (18.14)	0.81 (1.90)	689 (100)	4.47 (5.07)
	P-value	–	0.14	–	–	–	0.77	–	0.15
D_1	Regular ULV	486 (81.00)	3.52 (4.08)	10 (1.67)	0.08 (0.92)	104 (17.33)	0.75 (2.00)	600 (100)	4.35 (4.97)
	SID-ULV	286 (80.34)	1.86 (2.81)	2 (0.56)	0.02 (0.13)	68 (19.10)	0.44 (1.00)	356 (100)	2.31 (3.07)
	P-value	–	0.00[a]	–	0.40	–	0.10	–	0.00[a]
D_2	Regular ULV	371 (82.26)	2.69 (3.43)	0	0	80 (17.74)	0.58 (1.73)	451 (100)	3.27 (4.11)
	SID-ULV	301 (81.57)	1.95 (2.95)	1 (0.27)	0.01 (0.09)	67 (18.16)	0.44 (1.10)	369 (100)	2.40 (3.24)
	P-value	–	0.05[a]	–	0.34	–	0.39	–	0.05[a]
D_6	Regular ULV	348 (77.68)	2.52 (3.90)	0	0	100 (22.32)	0.72 (1.91)	448 (100)	3.25 (4.80)
	SID-ULV	401 (79.56)	2.60 (5.01)	0	0	103 (20.44)	0.67 (1.50)	504 (100)	3.27 (5.54)
	P-value	–	0.88	–	–	–	0.78	–	0.97
Total (% of all species)		3 161 (80.56)	–	13 (0.33)	–	750 (19.11)	–	3924 (100)	–

[a]: *P*-values reported are the results of independent sample *t*-tests weighted by number of houses per cluster (138 houses for regular ULV and 154 houses for SID-ULV).

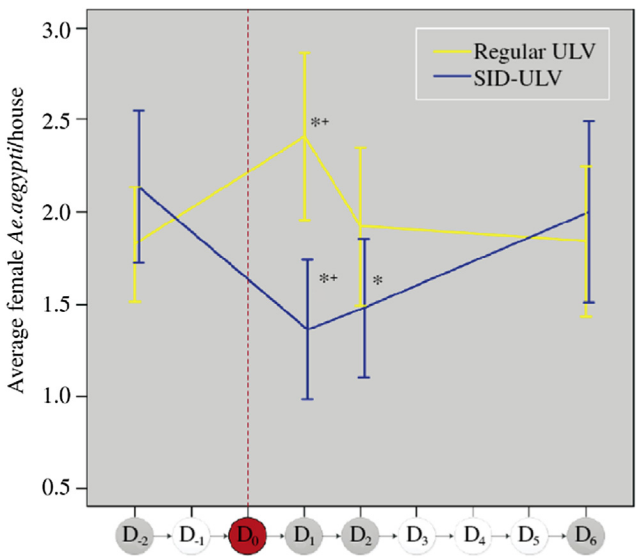

Figure 2. Variability of average number of female *Ae. aegypti* per house after SID-ULV versus regular ULV spraying.

*: Significant difference in vector density compared with the base values of the same group (D_{-2}) (*t*-test, $P < 0.05$); +: Significant difference between groups (*t*-test, $P < 0.05$).

In a comparison between the two groups (ULV and SID-ULV) on the same days, the only statistical significance was found on D_1 where the HDI increased in the regular ULV group but decreased dramatically in the SID-ULV group.

Table 2

Comparison of parity status of female *Ae. aegypti* collected between regular ULV clusters and SID-ULV clusters.

Day of collection	Parity status	Regular ULV	SID-ULV	*P*
		n (%)	*n* (%)	
D_{-2}	Parous	68 (38.4)	86 (37.1)	0.28
	Nulliparous	8 (4.5)	9 (3.9)	0.35
	Undetermined	101 (57.1)	137 (59.0)	0.09
D_1	Parous	90 (36.6)	37 (27.8)	0.14
	Nulliparous	12 (4.9)	11 (8.3)	0.95
	Undetermined	144 (58.5)	85 (63.9)	0.37
D_2	Parous	61 (36.1)	55 (37.4)	0.51
	Nulliparous	8 (4.7)	10 (6.8)	0.18
	Undetermined	100 (59.2)	82 (55.8)	0.52
D_6	Parous	46 (34.9)	59 (36.2)	0.50
	Nulliparous	5 (3.8)	6 (3.7)	–
	Undetermined	81 (61.3)	98 (60.1)	0.91

P-values reported are the result of independent sample *t*-tests weighted by number of houses per cluster.

3.4. Comparison of parity status

Female *Ae. aegypti* from regular ULV group locations (724) and SID-ULV group locations (675) were dissected to examine their ovaries. The PR status of the females dissected was classified into 3 types, parous, nulliparous, and undetermined (Figure 3). Table 2 summarizes the differences in parity status between treatment groups on the day after spraying. None of

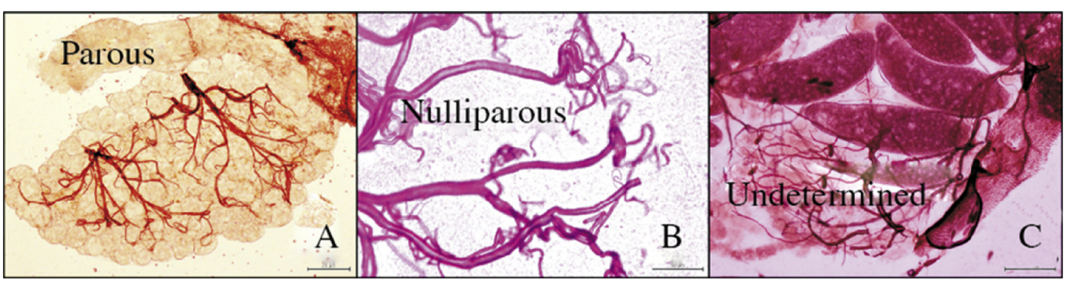

Figure 3. Types of parity status of dissected female *Ae. aegypti*.

A: Parous (ovaries that have completed at least one gonotrophic cycle); B: Nulliparous (ovaries that have not completed a full gonotrophic cycle); C: Undetermined (Christophers' stage III or IV of oocyte and gravid females).

the groups had a PR of less than 10% and no significant difference was detected between the two groups at any of the time periods.

4. Discussion

Our study demonstrated that SID-ULV spraying transiently decreased the density of, but did not eliminate, *Ae. aegypti* in the first few days after spraying, but the resurgence to baseline density followed within one week. On the other hand, regular ULV spraying did not reduce the density but was followed by a possibly transient increase of *Ae. aegypti* density. Based on mosquitoes collected at sites treated with both types of spraying, a high percentage of the mosquitoes were parous throughout the monitored period.

The finding of only a small change in mosquito density after spraying raises several questions. The bio-assay conducted in the laboratory revealed high susceptibility, and the previous studies of natural resistance to deltamethrin in Songkhla by DPC-12 reported that deltamethrin induces the specified level of susceptibility (> 98% mortality).

The rebound effect of new mosquito populations may reduce the effectiveness of space spraying, because space spraying is not effective in controlling immature mosquitoes. There is need for integrated control approaches including spraying combined with environmental management since otherwise space spraying may not be sustained over long periods [7,10]. It is notable that in this study, there were minor increases in the percentages of emerging nulliparous mosquitoes after spraying.

Thus it is likely that resurgence can be due to immigrating mosquitoes. When exposed directly to deltamethrin, the major behavioral responses of *Ae. aegypti* are contact irritancy, increased movement and flying [17], and rapid flight escaping from sprayed areas to unsprayed areas [18]. After space spraying, exposed or unexposed mosquitoes immigrate from untreated areas back to treated areas [19]. The behavioral responses of mosquitoes to pyrethroids are likely to cause a reduction in the effectiveness of space spraying, and the association between these phenomena needs further investigation [20,21].

In addition, the urban environment in which this study was conducted, with high house density, is likely to play an important role in the high resurgence rate of dengue vector populations and thus in efforts to suppress vector populations, because the externality effects of nearby unsprayed houses can reduce the effectiveness of space spraying. This suggests that intensive space spraying should be conducted in urban areas.

This study shows that the resurgence of dengue vectors after space spraying in urban areas is rapid, and within 6 days of spraying, adult *Ae. aegypti* populations returned to their base value. SID-ULV is an effective method of space spraying which has greater potential to control adult dengue vector populations than regular ULV spraying. However, SID-ULV spraying or regular ULV spraying applied as a single intervention to suppress dengue vector populations may be an ineffective measure and the externality effects on nearby unsprayed houses requires further study.

Conflict of interest statement

The authors declare that they have no competing interests.

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