Locations and Religious Factors Affecting Dengue Vectors in Nakhon Si Thammarat, Thailand

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ABSTRACT

This study investigated the numbers of *Aedes* larvae found in relation to the religion of the people, the location of their houses, and the number of water containers in and around the house. We collected our questionnaire survey during April-May 2004 covering two different topographical areas (i.e. seaside and mountainous areas) and two religious factors (i.e. Buddhist and Muslims). We collected samples by using the stratified simple random sampling with a total of 400 households from all communities in 31 sub-districts. The results showed that there were a higher number of *Ae. aegypti* larvae in water containers in bathrooms, concrete tanks and large water jars than the number of *Ae. albopictus* larvae in both areas. *Ae. albopictus* larvae were found in higher numbers at the seaside area than in the mountainous area. On the other hand, the number of small water jars had a higher number of *Aedes* larvae in the mountainous area than in the seaside area. Considering only large water jars, covers apparently reduced the number of *Ae. aegypti* and other mosquito larvae. Regardless of the kind of cover, uncovered containers had more mosquito larvae than covered containers. More *Ae. aegypti* larvae were found in larger jars than in smaller jars and we also found more *Ae. aegypti* larvae in smaller jars which were <¼ full than at other water levels. There were three larval indices: Container Index (CI), House Index (HI) and Breteau Index (BI). All larval indices from our study indicated a high risk of Dengue Haemorrhagic Fever (DHF) transmission in both locations and religions. We found that HI in Muslim households was higher than in Buddhist households.

Key words: Dengue vectors - *Aedes aegypti* - *Aedes albopictus* - Location - Religion

INTRODUCTION

The mosquitoes that adversely affect people in southern Thailand are primarily *Aedes aegypti* L. and *Ae. albopictus* Skuse (1,2,3,4). An epidemic of DHF occurred in southern Thailand [e.g. Samui Island in 1966 and 1967 (5)] where *Ae.*

aegypti and *Ae. albopictus* were abundant, and widespread (6,7,8,9). *Ae. albopictus* is capable of breeding in a wide range of container types and water-holding habitats. In Thailand, *Ae. albopictus* has been found in forested habitats ranging in elevation from 450 to 1,800 m as well as in a variety of other habitats in rural and suburban areas (1, 9,10,11). Ubiquitous breeding sites, such as tree holds, coconut shells, fruit peels, water jars, unused and discarded tyres, and boats holding water have been found to contain *Ae. albopictus* larvae (9).

There are several factors affecting DHF incidence including water storage, climatic and vector factors. Container factors comprise of shape, type, the size of water surface, purpose for which the water used, type of materials, lids and also water consumption characteristics (4,12,13,14,15). Climatic factors comprise of monthly average rainfall, vapour pressure, and maximum, minimum, and mean temperature (16). Vector factors comprise of the strain of the virus, mosquito density, mosquito behaviour and mosquito competence, food level, duration of development, size at emergence, flight range, survival and biting activity (2,17,18,19,20,21,22). Because preventative care is an increasingly important part of the strategy, social factors that influence its use must be more closely investigated (23).

Nakhon Si Thammarat province is located in southern Thailand near Samui Island. Several epidemic manifestations have been observed at two to four year intervals (i.e. 1987, 1990, 1995 and 1998). Since 1984, there have been several DHF outbreaks in this area, especially in 1990 when the most outbreaks occurred. After that, dengue epidemics decreased and reappeared again in 1998 (24). The number of DHF cases in Nakhon Si Thammarat was the highest in Thailand in the year 2002 (i.e. 6,603 DHF cases reported or 631.40 cases per 100,000 populations) (25).

In recent decades, epidemiologists have increasingly investigated the relationships between religion and health. Although not aimed directly at religion in itself, these efforts are instructive for how religion might be insightfully studied (23, 26). Behaving in accordance with religious tenets may have impacts on health and disease transmission (27). The objectives of this study were to identify major sources of larval breeding site using several larval indices, compare the number of *Aedes* larvae between seaside and mountainous areas and compare the number of *Aedes* larvae between Buddhist and Muslim households.

Nakhon Si Thammarat is a suitable area for this study for several reasons. First, dengue is transmitted in a variety of habitats (e.g. seaside and mountainous areas) and a mixed group of religions including Buddhists and Muslims. Second, cultural traditions and household circumstances result in extensive intentional storage of water (28). Third, dengue is often a disease of children in Thailand (29). As a result, the populations that have more children per family might have a higher dengue incidence rate. In the past few decades, there has been some development (e.g. industrial, agricultural) that may have had an impact on the abundance of *Aedes* mosquitoes by providing more habitats for these mosquitoes and thus leading to an increase in the abundance of dengue vectors.

MATERIALS AND METHODS

Data Collection

A questionnaire survey was conducted in Nakhon Si Thammarat province located 8° 32′ 16.5″ N latitude and 99° 56′ 50.7″ E longitude. We collected our questionnaire survey during April-May 2004 covering two different topographical areas (i.e. seaside and mountainous areas) and two religions (i.e. Buddhist and Muslims). We collected samples by using the stratified simple random sampling, and collected from households from all communities in 31 sub-districts. Topography and religion were assigned as stratums. One person in the collected household was identified as a sample unit. There were 100 Buddhist and 100 Muslim households in the seaside area, and 100 Buddhist and 100 Muslim households in the mountainous area. A structured questionnaire composed variables influencing the occurrence of dengue fever was designed to obtain the information through interviews and discussions with the person from the sample household. The questionnaire survey comprised of 50 questions and separated into three parts:

(1) General information (e.g. sex, age, education, occupation, income, the number of family members and clothing)

(2) Factors affecting the number of mosquito larvae (e.g. housing materials, indoor water containers : water containers in bathrooms, flower vases, and outdoor water containers : pot plants, coconut shells, used cans, water jars and concrete tanks)

(3) Cultural factors (e.g. use of tap water, the frequency of garbage collection and the frequency of chemical usage to eradicate mosquitoes).

Entomological Studies

Larval surveys were conducted by examining all water containers in selected houses from two locations and two religions. Water containers were sampled with a fishnet. Very small containers were emptied through the fishnet. Larger containers were sampled by dipping the net in the water, starting at the top of the container and continuing to the bottom in a swirling motion that sampled all edges of the container (Similar to (30,31)). We placed the mosquito larvae in plastic bags and brought them to be identified into species level in the laboratory by using a stereomicroscope. Using Rattanarithikul and Panthusiri (32) description and keys, mosquito larvae were identified with emphasis on the structure of comp scales and the number of ventral brush.

We calculated three larval indices: House Index (HI), Container Index (CI), and Breteau Index (BI). HI was defined as the percentage of houses positive for larvae. CI was defined as the percentage of water-filled containers positive for larvae. BI was defined as the number of positive containers per 100 houses (2,4,31,33,34,35). We collected data from mosquito breeding places both indoor and outdoor within 15 m of the houses (2,35). For the water jar, we classified water jars into two categories: small water jars (< 500 L) and large water jars (≥500 L).

Statistical Analysis

All variables were tested for normality using the Komogorov-Smirnov test and transformed when necessary. The equality of variances was evaluated using

Levene's test. Descriptive statistics of the data were analysed. The number of mosquito larvae, the number of *Aedes* larvae and the number of positive containers in the two locations, the two religions, and their interactions were analysed using a Two-way ANOVA. The numbers of mosquito larvae in the different water containers were compared using Mann-Whitney U tests. The numbers of mosquito larvae in different water levels were compared using Kruskal Wallis test. The larval indices were compared using Chi-square tests. The independent sampled t-test was used to compare the number of containers positive for *Aedes* larvae between seaside and mountainous areas. All significant tests were two-tailed.

RESULTS

We collected a total of 4,001 mosquito larvae from all containers. 33.6% of containers were infested with *Ae. aegypti*, 13.6% infested with *Ae. albopictus*, and 32.8% infested with both species. *Aedes* larvae cohabited with other mosquito species, such as *Culex quinquefasciatus* Say or *Toxorhynchites splendens* Wiedemann. There were a higher number of *Ae. aegypti* larvae in water containers in bathrooms, concrete tanks and large water jars than the number of *Ae. albopictus* larvae **(Table 1)**. There were no differences in the number of *Ae. albopictus* and *Ae. aegypti* larvae in pot plants, animals pans, tyres and small water jars **(Table 1)**. *Ae. albopictus* larvae were found in higher numbers at the seaside area ($\bar{x}$ ±S.D. = 1.39±5.17) than in the mountainous area ($\bar{x}$ ±S.D. = 0.84±3.38) (Mann-Whitney U test, U = 17618.50, $n_1 = n_2 = 197$, $P<0.001$).

Table 1. The mean (± S.D.) number of mosquito larvae in different water containers. Natural containers comprise of coconut shells, tree holes, bamboo clump, ditches and small ponds. Mann-Whitney U test *$P<0.05$.

Type of water containers	*Ae. aegypti*	*Ae. albopictus*	$n_1 = n_2$	Statistical test
Water containers in bathrooms	0.25±1.79	0.01±0.11	318	U = 49286.0*
Pot plants	0.21±0.82	0.38±2.04	29	U = 407.0
Animal pans	0.10±0.50	0.06±0.42	52	U = 1326.5
Concrete tanks	0.88±3.00	0.04±0.19	56	U = 1394.0*
Tyres	0.33±0.58	0.00±0.00	3	U = 3.0
Small water jars	0.45±2.42	0.69±5.82	195	U = 19003.5
Large water jars	1.25±5.57	0.35±3.70	149	U = 10338.5*
Natural containers	0.01±0.09	0.01±0.09	121	U = 7320.50

The number of mosquito larvae, the number of *Aedes* larvae and the number of positive containers were not different between seaside and mountainous areas, or between Buddhist and Muslim households **(Table 2)**. However, there was some interaction in the number of *Aedes* larvae between location and religion **(Table 2)**. A number of small water jars had a higher number of *Aedes* larvae in the mountainous area than in the seaside area (t_{29} = -2.373, $P<0.05$, **Figure 1**).

When we compared the number of mosquito larvae in small and large water jars between water jars without lids and with different types of lids, we found a higher number of *Ae. albopictus* in small water jars with metal lids than without lids (Mann-Whitney *U* test, $U_{267,79}$ = 9917.50, P<0.05, **Table 3**) and a higher number of other mosquito larvae in small water jars without lids than metal lids ($U_{267,79}$ = 9486.50, P<0.01, **Table 3**). There was a higher number of *Ae. aegypti* and other mosquito larvae in large water jars without lids than metal lids and any lids (for *Ae. aegypti*: without lids versus metal lid: $U_{269,39}$ = 4790.50, P<0.01; without lids versus any lids: $U_{269,92}$ = 11617.50, P<0.01; other mosquito larvae: without lids versus metal lids: $U_{269,39}$ = 481

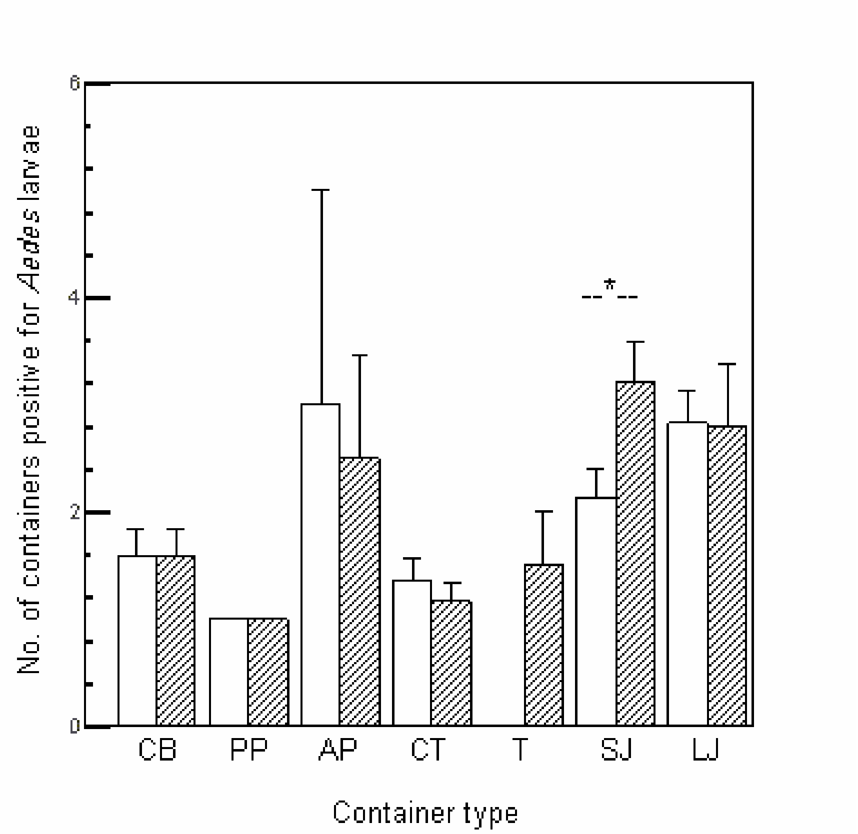

Figure 1. The mean (± S.E.) number of containers positive for *Aedes* larvae between Seaside (☐), and Mountainous (▨) areas. Container type includes water containers in bathrooms (CB), flower pot plates (PP), animal pans (AP), concrete tanks (CT), tyres (T), small water jars (SJ) and large water jars (LJ). *P<0.05.

The water level and the size of the water jars affected the number of mosquito larvae, both *Ae. aegypti* and *Ae. albopictus*, in water jars **(Table 4)**. More *Ae. aegypti* larvae were found in larger jars than in smaller jars (for smaller jar: $\bar{x}$ ±S.D.: 2.6±5.3; larger jar: 11.6±13.4; t_{17} = -2.6, P<0.05) and we also found more *Ae. aegypti* larvae in smaller jars which were <¼ full than at other water levels (Kruskal Wallis test, W_4 = 17.64, P<0.01, **Table 4**). The number of *Ae. albopictus* were not significantly different in various water levels both in small and large water jars.

Table 2. The mean $(\pm \text{S.D.})$ number of mosquito larvae, the number of *Aedes* larvae and the number of positive containers in different locations in Buddhist and Muslim households. * P<0.05.

The number of	Seaside area		Mountainous area		Statistical tests		
	Buddhist	Muslim	Buddhist	Muslim	Location	Religion	Location × Religion interaction
Mosquito larvae	11.9±56.9	10.4±33.0	10.4±27.9	7.4±25.0	$F_{1,396}$=0.4	$F_{1,396}$=0.4	$F_{1,396}$=0.4
Aedes larvae	0.5±2.2	2.5±7.0	2.8±11.2	0.9±3.4	$F_{1,396}$=0.3	$F_{1,396}$=0.0	$F_{1,396}$=7.7*
Positive containers	4.7±8.1	2.2±1.0	2.8±1.8	3.0±2.0	$F_{1,82}$=0.5	$F_{1,82}$=0.1	$F_{1,82}$=0.1

Table 3. The mean (± S.D.) number of mosquito larvae in small and large water jars with different types of lid. Any lids composed of plastic, nylon, cement or wood.

Type of lids	Small water jar			Large water jar		
	Ae. aegypti	***Ae. albopictus***	**Other mosquito larvae**	***Ae. aegypti***	***Ae. albopictus***	**Other mosquito larvae**
None	0.6±2.7	0.5±2.9	9.2±31.4	1.8±6.2	0.2±0.7	6.2±16.8
Metal	0.5±2.7	1.2±8.7	6.5±32.0	0.5±1.8	0.0±0.2	0.4±1.6
Any lids	0.0±0.1	0.0±0.1	0.4±2.1	1.2±6.1	0.5±4.7	1.8±9.1

Table 4. The mean (± S.D.) number of mosquito larvae at different water levels in small and large water jars.

Factor	Small water jar		Large water jar	
	Ae. aegypti	***Ae. albopictus***	***Ae. aegypti***	***Ae. albopictus***
Water level in water jar				
<1/4	11.00±10.15	1.33±2.31	0.00±0.00	0.00±0.00
1/4	0.36±1.22	0.18±0.66	2.89±7.94	5.00±15.00
1/2	0.38±1.28	0.60±3.61	1.93±7.96	0.12±0.50
3/4	0.92±4.32	0.27±0.96	0.47±2.56	0.07±0.37
Full	0.05±0.22	0.94±7.87	1.02±4.36	0.00±0.00

CI was not different between the two locations, the two religions and there was no interaction between location and religion (Chi-square: Location: χ_1^2 = 0.18, ns; Religion: χ_1^2 = 0.18, ns; Location × Religion interaction: χ_1^2 = 0.22, ns, **Table 5**). HI was different between Buddhist and Muslim households (Chi-square: Religion: χ_1^2 = 4.06, P<0.05, **Table 5**) but HI was not different between the seaside and mountainous areas and there was no interaction between location and religion (Chi-square: Location: χ_1^2 = 2.53, ns; Location × Religion interaction: χ_1^2 = 2.44, ns, **Table 5**). BI was not different between the two locations and the two religions but there was interaction between location and religion (Chi-square: Location: χ_1^2 = 0.36, ns; Religion: χ_1^2 = 0.23, ns; Location × Religion interaction: χ_1^2 = 4.68, P<0.05, **Table 5**).

Table 5. Larval abundance indices of *Aedes* mosquitoes in Nakhon Si Thammarat, Thailand by location and religion.

Laval Indices	Seaside area		Mountainous area		All areas
	Buddhist	Muslim	Buddhist	Muslim	
Container Index (CI)	6.41	4.48	6.24	5.92	5.74
House Index (HI)	11	26	24	28	22.25
Breteau Index (BI)	79	57	66	80	70.5

DISCUSSION

Our results showed that *Ae. aegypti* larvae were found more in water containers in bathrooms, concrete tanks and large water jars than *Ae. albopictus*. Our results confirmed Thavara et al's (2) finding that water storage jars and concrete tanks in bathrooms were the main breeding sites of *Aedes* larvae and flower vases, pot saucers, drinking water jar, ant guards and natural sites served as minor breeding sites. However, Thavara et al (2) did not distinguish between *Ae. aegypti* and *Ae. albopictus* larvae in their study.

This study showed that there was a greater number of *Ae. aegypti* in larger containers including water containers in bathrooms, concrete tanks and large water jars. This suggests that adult *Ae. aegypti* may have strong ovipositional preferences based on container size. This kind of preference has been shown in other mosquito species. For example, *Culex. pervigilans* preferred to lay eggs in small surface area containers rather than large surface area containers (36). Thavara et al (2) showed that water jars and concrete tanks in bathrooms were the main breeding sites of *Aedes* larvae. When we compared the percentage of positive containers, we found that the animal pans and small water jars had higher percentage of *Aedes* larvae than other containers. It is possible that these containers may contain sufficient nutrition to support larval development. Strickman and Kittayapong (31) showed that small water jars used for pickling fish or vegetables contained more larvae than either standard water jars or ant traps. They suggested that these dirty sites contained sufficient nutrition to support more larval development.

Our results showed that *Ae. albopictus* were found more in seaside areas than in the mountainous area. However, Thavara et al (9) showed that *Ae. albopictus* inhabits sites 450-1,800 m above sea level in rural and suburban areas. This difference could be due to the fact that we conducted our survey on the mainland (i.e. Nakhon Si

Thammarat province) but they conducted their study on Samui Island. The number and type of mosquito larvae were not different between Buddhist and Muslim houses. There was some interaction in the number of *Aedes* larvae between location and religion. This means that in the seaside area, we found that the number of *Aedes* larvae in Muslim households was higher than in Buddhist households. On the other hand, in the mountainous area, the number of *Aedes* larvae in Muslim households was lower than in Buddhist households. This could be because Buddhist households in the mountainous area had a higher number of containers than Muslim households.

Considering only large water jars, covers apparently reduced the number of *Ae. aegypti* and other mosquito larvae. Regardless of the kind of cover, uncovered containers had more mosquito larvae than covered containers. This result confirms Strickman and Kittayapong (31), and Luemoh et al (4) studies. They found that containers with lids had a lower number of mosquito larvae.

The water level affects the number of mosquito larvae in the water jars. We found that the number of *Ae. aegypti* larvae in the small water jar was highest at <¼ water level. It is possible that *Ae. aegypti* females prefer to lay eggs in smaller containers with low water level or mortality factors influencing the survival of eggs and larvae. This result has also been observed in other mosquito species. For example, *Culex. pervigilans* larvae were found more in small containers with low water levels (36).

The National Institute of Communicable Diseases (37) defined high risk of DHF transmission when BI was ≥50, or HI was ≥10, and low risk of transmission when BI was ≥5, or HI was ≥1. All larval indices from our study indicated a high risk of DHF transmission in both locations and religions. Other studies on DHF in Thailand have shown similar trends (2,4). We found that HI in Muslim households was more than in Buddhist households. However, a study by Luemoh et al (4) reported that HI among Buddhists was 81.2 where as among Muslims, HI was 0. There was some interaction between location and religion in BI. We found that Buddhist households in seaside area had higher BI than in Muslim households. On the other hand, Muslim households in mountainous areas had higher BI than in Buddhist households. From previous studies on larval indices in Thailand, they found that BI was 20-515, HI was 0-89 and CI was 5-60 (2,4,31,33). Our results showed that BI was 57-80, HI was 11-28 and CI was 4.48-6.41. All larval indices in our studies were very low. This could be because larval field survey was done in the dry season when available oviposition sites were low. Thavara et al (2) showed that water jars, tanks and natural sites were infested with *Aedes* larvae more in the rainy season than the dry season on Samui Island.

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ผลกระทบทางด้านลักษณะภูมิประเทศและศาสนาต่อพาหะนำโรคไข้เลือดออกในจังหวัดนครศรีธรรมราช

งานวิจัยนี้เป็นการศึกษาจำนวนลูกน้ำยุงลายและจำนวนภาชนะเก็บน้ำที่พบในบ้านและบริเวณบ้านของชาวพุทธและมุสลิม ในบริเวณชายฝั่งทะเลและบริเวณเทือกเขา ผู้วิจัยเลือกกลุ่มตัวอย่างโดยใช้วิธีการสุ่มแบบอย่างง่ายในชั้นภูมิ โดยเลือกตัวอย่างมา 400 ครัวเรือน จากทั้งหมด 31 ตำบล ผลการศึกษาพบว่า มีลูกน้ำยุงลายชนิด *Ae. aegypti* ในภาชนะเก็บน้ำในห้องน้ำ บ่อซีเมนต์ และโอ่งน้ำขนาดใหญ่ มากกว่าลูกน้ำยุงลายชนิด *Ae. albopictus* นอกจากนี้ยังพบลูกน้ำยุงลายชนิด *Ae. albopictus* ในบริเวณชายฝั่งทะเลมากกว่าบริเวณเทือกเขา ในขณะที่โอ่งน้ำขนาดเล็กในบริเวณเทือกเขามีจำนวนลูกน้ำยุงลายมากกว่าบริเวณชายฝั่งทะเล เมื่อพิจารณาเฉพาะโอ่งน้ำขนาดใหญ่ การมีฝาปิดโอ่งน้ำสามารถลดจำนวนลูกน้ำยุงลายชนิด *Ae. aegypti* และลูกน้ำชนิดอื่น หากไม่สนใจชนิดของฝาปิด โอ่งน้ำที่ไม่มีฝาปิดจะมีจำนวนลูกน้ำมากกว่าโอ่งน้ำที่มีฝาปิด พบลูกน้ำยุงลายชนิด *Ae. aegypti* ในโอ่งน้ำขนาดใหญ่มากกว่าโอ่งน้ำขนาดเล็ก นอกจากนี้ยังพบลูกน้ำยุงลายชนิด *Ae. aegypti* ในโอ่งน้ำขนาดเล็กที่มีระดับน้ำน้อยกว่า ¼ ของโอ่งมากกว่าที่ระดับน้ำอื่นๆ การศึกษาดัชนีลูกน้ำทั้ง 3 ชนิด ได้แก่ Container Index (CI) House Index (HI) และ Breteau Index (BI) สรุปได้ว่าดัชนีทั้ง 3 ชนิดบ่งชี้ว่า พื้นที่ที่ศึกษาทั้งสองบริเวณและทั้งสองศาสนา เป็นพื้นที่ที่มีความเสี่ยงสูงต่อการติดเชื้อโรคไข้เลือดออก และพบดัชนีลูกน้ำชนิด HI ในครัวเรือนของมุสลิมมากกว่าครัวเรือนชาวพุทธ

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