

ORIGINAL ARTICLES

**High and Low Risk Dengue Haemorrhagic Fever Areas
Affecting Key Breeding Place of *Aedes aegypti* (L.)
and *Ae. albopictus* (Skuse) in Nakhon Si Thammarat,
Southern Thailand**

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ABSTRACT

This study investigated key breeding sites of *Aedes aegypti* (L.) and *Ae. albopictus* (Skuse) in high and low risk dengue haemorrhagic fever (DHF) areas. *Ae. aegypti* and *Ae. albopictus* larvae were found in 11 out of 29 types of water containers in both high and low risk DHF areas. *Ae. aegypti* larvae were found most in outdoor area containers in high risk DHF areas and in metal boxes in low risk DHF areas. On the other hand, *Ae. albopictus* larvae were found most in indoor earthen jars in low risk DHF areas and in areca containers in high risk DHF areas. The number of *Ae. albopictus* larvae found in the earthen jars and metal or plastic boxes in low risk areas were higher than in high risk DHF areas. Larval indices (i.e. HI and BI) in both high and low risk DHF areas were greater than 10 % and 50 %, respectively, which indicated high risks of DHF transmission.

Keywords: Dengue haemorrhagic fever, high and low risk DHF areas, water storage containers, *Aedes aegypti*, *Aedes albopictus*

INTRODUCTION

Dengue haemorrhagic fever (DHF), is an infectious disease caused by the dengue virus, and is a serious cause of morbidity and mortality in most countries in the tropical and subtropical areas of the world [1,2]. It is the most common mosquito-borne viral infection in the world, causing up to 100 million cases each year. DHF had been reported in Thailand since late 1950 and the incidences of DHF, an acute and severe form of the dengue virus infection have increased. Since the first DHF epidemic outbreak in 1958, epidemics have been reported from almost all parts of the country [3].

The mosquitoes that adversely affect people in southern Thailand are primarily *Aedes aegypti* (L.) and *Ae. albopictus* (Skuse) [4-7]. An epidemic of DHF occurred in southern Thailand (e.g. Samui Island in 1966 and 1967 [8]) where *Ae. aegypti* and *Ae. albopictus* were abundant, and widespread [9-11]. *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 metres as well as in a variety of other habitats in rural and suburban areas [4,5,9,12]. Ubiquitous breeding sites, such as tree holes, coconut shells, fruit peels, water jars, unused and discarded tyres, and boats holding water have been found to contain *Ae. albopictus* larvae [11].

The key breeding place of *Aedes* larvae are affected by various factors such as topographical areas, faith-based communities [13] and high/low risk DHF areas [14]. A previous study of *Aedes* larvae in high/low risk DHF areas in the northeastern region of Thailand found that earthen jars and cement tanks were the key breeding sites in both high/low risk DHF areas, however, the number of earthen jars and cement tanks were not significantly different between high/low risk DHF areas [14]. It is also found that the number of cement tanks and ant guards in low risk DHF areas were higher than in high risk DHF areas. However, study by Chansang *et al* did not separate *Aedes* larvae into *Ae. aegypti* and *Ae. Albopictus* [14]. As previous studies [13,15] show that *Ae. aegypti* prefers to lay eggs in different types of water storage containers than *Ae. albopictus*, a more detailed study separating *Aedes* larvae into *Ae. aegypti* and *Ae. albopictus* would be helpful.

Nakhon Si Thammarat is one of the highest risk DHF provinces in Thailand. There have been several DHF outbreaks in this province since 1984 at two to four year intervals (i.e. 1987, 1990 and 1995) [16]. In 2002, the cases of death due to DHF in Nakhon Si Thammarat were the highest in Thailand [16]. In 2005, eight districts in Nakhon Si Thammarat were ranked in the top ten districts with the highest incidence of DHF in Thailand [16]. In order to have an effective DHF prevention and control system in Nakhon Si

Thammarat, key-breeding sites of *Ae. aegypti* and *Ae. albopictus* larvae in high/low risk DHF areas should be investigated. This study aims to identify key breeding sites of *Ae. aegypti* and *Ae. albopictus* using larval indices, and compare types of water containers used in high and low risk DHF areas. We predicted that (1) key breeding sites of *Ae. aegypti* should differ from *Ae. albopictus* and (2) key breeding sites of *Ae. aegypti* and *Ae. albopictus* larvae in high risk DHF areas should differ from low risk DHF areas.

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) in March-April 2004 covering high and low risk DHF areas. Three high risk DHF districts (i.e. Chawang, Phipun, and Na Bon districts) and three low risk DHF districts (i.e. Chula Phorn, Chian Yai, and Nop Phitham districts) were selected by using the averaged rate (DHF cases rate per 100,000 of 23 districts in Nakhon Si Thammarat were ranked from high to low cases rate. The three highest districts of DHF cases rate were classified as high DHF risk area and the three lowest districts of DHF cases rate were classified as low DHF risk) of DHF cases per year per 100,000 people in three consecutive years (i.e. 2000, 2001, 2002) to calculate the three highest/lowest DHF risk districts in Nakhon Si Thammarat (**Figure 1**).

Four hundred households in these six districts were sampled by a systematic stratified random sampling technique. By a proportional allocation method, 232 sample units were assigned to high risk DHF areas and 168 sample units were assigned to low risk DHF areas. Communities were assigned as stratum. One person in the collected household was identified as a sample unit. The structured questionnaire was composed of 29 types of water containers used to collect data.

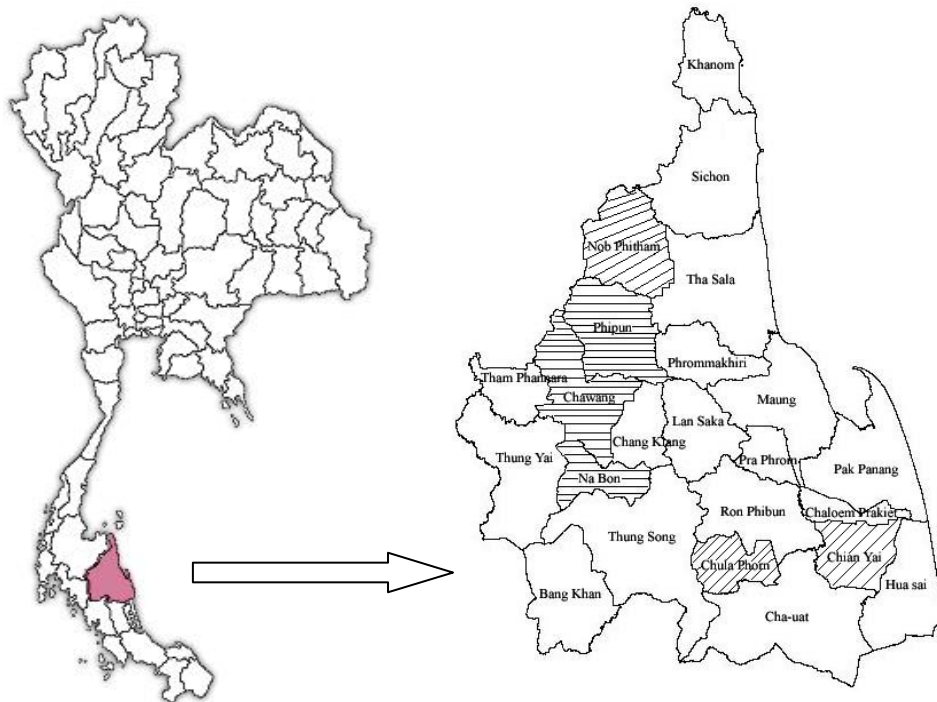


Figure 1 Three high risk DHF and low risk DHF study areas in Nakhon Si Thammarat province, southern Thailand. ▨ Low DHF area, ▨ High DHF area

Entomological studies

All water containers were sampled both indoors and outdoors. Larval surveys were conducted in the study area using fishnets. 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 [13,17]. All live mosquito larvae were collected in plastic bags, taken to the laboratory, preserved and identified up to species level using Rattanarithikul and Panthusiri's keys [18]. In this study, the 1st, 2nd instars and pupae were discarded because mosquitoes at these stages could not be identified.

Three larval indices: House Index (HI), Container Index (CI), and Breteau Index (BI) were worked out as per standard WHO guidelines. Breeding places were sampled both indoors and outdoors within 15 m of the houses [5,19]. Species and numbers of mosquito larvae in water containers at each household were classified. Mosquito larvae were identified as *Ae. aegypti*, *Ae. albopictus* or others from the biological laboratory.

Statistical analysis

All variables were tested for normality using the Kolmogorov-Smirnov test. 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 *Ae. aegypti* and *Ae. albopictus* larvae and the number of positive containers in high and low risk DHF areas were compared using independent sampled *t*-tests. The larval indices were compared using Chi-square tests. All significant tests were two-tailed.

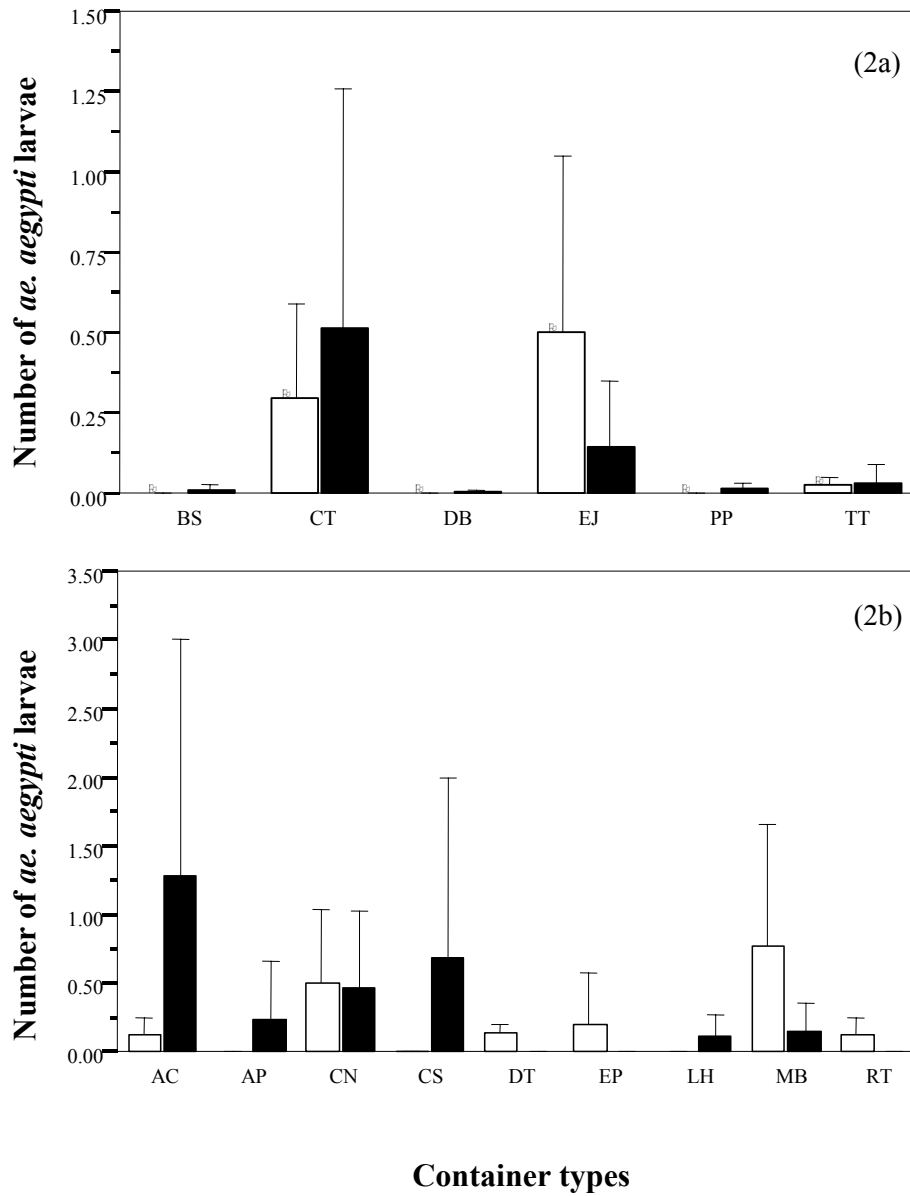
RESULTS

For indoor containers, there were a higher number of toilet tanks and cement tanks but a lower number of coolers, drained boxes, water plant pots, ant-guards, water jars and earthen jars in high risk DHF areas than in low risk areas (**Table 1**). For outdoor containers, there were a higher number of tree holes, areca husks, and banana trees but a lower number of lotus pots, discarded tyres, roof gutters, swamps, plant pots, coconut shells, vehicle repair shops and rainwater tanks in high risk DHF areas than in low risk DHF areas (**Table 1**).

For indoor containers, there were a higher number of households that had a higher number of toilet tanks and cement tanks but a lower number of drained boxes, water plant pots, ant-guards, water jars and earthen jars in high risk DHF areas than in low risk areas (**Table 2**). For outdoor containers, there were a higher number of households that had a higher number of tree holes, areca husks, banana trees and plant pots but a lower number of plastic/metal boxes, cans, earthenwares, lotus pots, discarded tyres, roof gutters, swamps, coconut shells, and rainwater tanks in high risk DHF areas than in low risk DHF areas (**Table 2**).

Ae. aegypti and *Ae. albopictus* were found in 11 out of 29 types of water containers in both high and low risk DHF areas (**Figures 2a-d**). *Ae. aegypti* larvae were found most in the outdoor areca containers in high risk DHF areas and in metal boxes in low risk DHF areas (**Figure 2b**). In contrast, *Ae. albopictus* larvae were found most in the indoor earthen jars in low risk DHF areas (**Figure 2c**) and in areca containers in high risk DHF areas (**Figure 2d**). The number of *Ae. albopictus* larvae found in the earthen jars and metal or plastic boxes in low risk areas were higher than in high risk DHF areas (independent sample *t*-test, earthen jars: $t_{398} = 2.455$, $P = 0.015$; metal or plastic box: $t_{398} = 2.285$, $P = 0.023$).

The House Index (HI) and Breteau Index (BI) in both high and low risk DHF areas were greater than 10 % and 50 %, respectively, which indicated high risks of DHF transmission (**Table 3**).



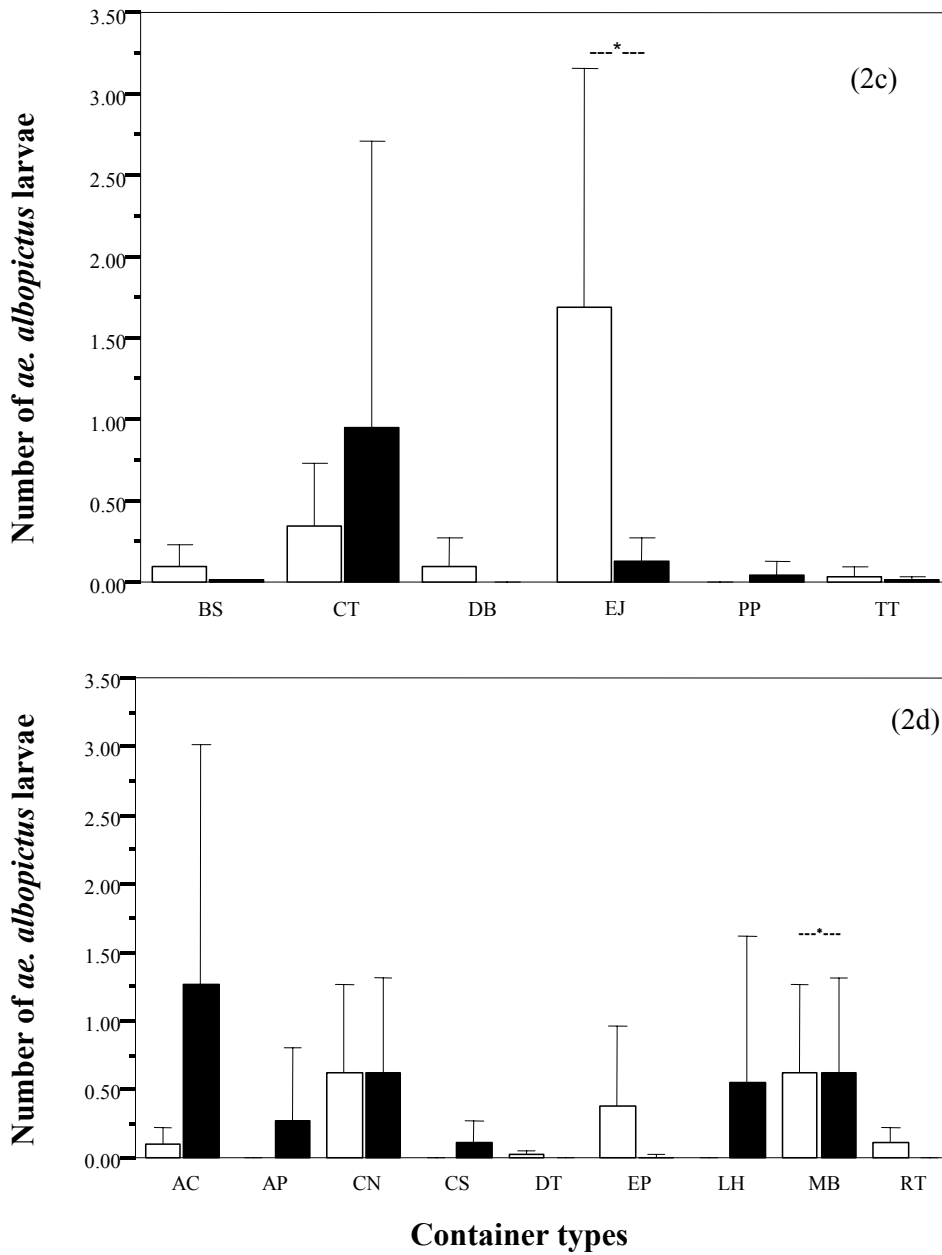


Figure 2 The number of *Aedes* larvae in containers (mean \pm S.E.). (a) indoor, and (b) outdoor *Ae. aegypti* positive containers, (c) indoor, and (d) outdoor *Ae. albopictus* positive. * $P < 0.05$, ** $P < 0.01$, \square low and \blacksquare high DHF risk areas, BS = basin (water jar), CT = cement tank, DB = drained box, EJ = earthen jar, PP = plant pot, TT = toilet tank, AC = areca container, AP = animal pan, CN = can, CS = coconut shell, DT = discarded tyre, EP = earthen pot (earthenware), LH = leaf or husk, MB = metal box and RT = rainwater tank.

Table 1 The number of water containers ($\bar{x} \pm SD$) in high and low risk DHF areas, * $P < 0.05$, ** $P < 0.01$.

Containers types	The number of water containers		
	High risk	Low risk	t-test
Indoor			
Toilet tanks	4.45 ± 3.03	2.44 ± 5.73	$t_{397} = 04.53^{**}$
Cement tanks	3.18 ± 4.21	1.52 ± 5.61	$t_{398} = 03.39^{**}$
Cooler with plates	0.03 ± 0.19	0.04 ± 0.19	$t_{358} = -00.55$
Vases	0.17 ± 0.77	0.21 ± 0.68	$t_{384} = -00.55$
Coolers	0.04 ± 0.19	0.12 ± 0.49	$t_{398} = -02.40^*$
Drained Boxes	0.45 ± 0.65	0.55 ± 0.48	$t_{398} = -03.51^{**}$
Water plant pots	0.16 ± 0.86	0.99 ± 3.19	$t_{398} = -03.78^{**}$
Ant-guards	1.09 ± 1.79	1.99 ± 2.04	$t_{398} = -04.71^{**}$
Water jars	0.03 ± 0.18	0.29 ± 0.64	$t_{398} = -05.89^{**}$
Earthen jars	1.17 ± 0.91	4.08 ± 4.04	$t_{398} = -10.60^*$
Outdoor			
Tree holes	1.76 ± 02.24	0.40 ± 01.05	$t_{398} = 7.29^{**}$
Leaf and areca husks	3.05 ± 03.60	1.44 ± 02.79	$t_{398} = 4.83^{**}$
Banana trees	8.67 ± 26.31	3.63 ± 06.34	$t_{398} = 2.44^*$
Areca containers	1.11 ± 01.49	0.94 ± 01.06	$t_{397} = 1.29$
Water plant pots	0.12 ± 00.55	0.06 ± 00.55	$t_{398} = 1.03$
Plastic/metal boxes	1.79 ± 06.76	1.53 ± 01.72	$t_{269} = 0.58$
Cans	1.77 ± 06.99	2.24 ± 03.94	$t_{376} = -0.86$
Animal pans	0.89 ± 01.17	1.06 ± 01.51	$t_{398} = -1.26$
Earthenwares	0.02 ± 00.16	1.63 ± 06.61	$t_{398} = -1.47$
Lotus pots	0.15 ± 00.57	0.39 ± 01.60	$t_{397} = -2.09^*$
Discarded tyres	0.29 ± 00.89	0.54 ± 01.33	$t_{397} = -2.31^*$
Roof gutters	0.00 ± 00.00	0.53 ± 00.33	$t_{398} = -2.44^*$
Swamps	0.17 ± 00.40	0.29 ± 00.48	$t_{398} = -2.77^{**}$
Plant pots	0.02 ± 00.20	0.26 ± 01.27	$t_{398} = -2.82^{**}$
Coconut shells	2.81 ± 03.67	5.59 ± 10.66	$t_{398} = -3.74^{**}$
Vehicle repair shops	0.39 ± 00.66	0.79 ± 00.89	$t_{398} = -5.18^{**}$
Rainwater tanks	0.65 ± 01.12	1.91 ± 02.55	$t_{398} = -6.69^{**}$

Table 2 The number of households ($\bar{x} \pm SD$) that had indoor/outdoor water containers in high and low risk DHF areas, * $P < 0.05$, ** $P < 0.01$.

Containers types	The number of households		
	High risk	Low risk	t-test
Indoor			
Toilet tanks	0.80 ± 0.40	0.62 ± 0.48	$t_{398} = 4.07^{**}$
Cement tanks	0.80 ± 0.40	0.64 ± 0.48	$t_{398} = 3.66^{**}$
Cooler with plates	0.03 ± 0.16	0.04 ± 0.20	$t_{311} = 0.83$
Vases	0.06 ± 0.25	0.12 ± 0.32	$t_{398} = 1.87$
Coolers	0.03 ± 0.18	0.08 ± 0.27	$t_{398} = -1.88$
Drained Boxes	0.44 ± 0.49	0.65 ± 0.47	$t_{398} = -4.25^{**}$
Water plant pots	0.06 ± 0.24	0.27 ± 0.59	$t_{398} = -4.96^{**}$
Ant-guards	0.30 ± 0.46	0.51 ± 0.50	$t_{398} = -4.35^{**}$
Water jars	0.03 ± 0.19	0.21 ± 0.41	$t_{398} = -6.08^{**}$
Earthen jars	0.80 ± 0.40	0.84 ± 0.37	$t_{398} = -1.11^*$
Outdoor			
Tree holes	0.49 ± 0.50	0.20 ± 0.40	$t_{398} = 6.25^{**}$
Leaf and areca husks	0.56 ± 0.49	0.40 ± 0.67	$t_{398} = 4.83^{**}$
Banana trees	0.59 ± 0.49	0.46 ± 0.50	$t_{358} = 2.62^*$
Plant pots	0.07 ± 0.25	0.02 ± 0.15	$t_{398} = 2.07^*$
Vehicle repair shops	0.06 ± 0.26	0.04 ± 0.20	$t_{398} = 0.97$
Animal pans	0.52 ± 0.51	0.50 ± 0.50	$t_{365} = 0.41$
Areca containers	0.43 ± 0.49	0.55 ± 0.51	$t_{355} = -2.38$
Tree containers	0.01 ± 0.11	0.12 ± 0.71	$t_{398} = -2.21$
Plastic/metal boxes	0.42 ± 0.49	0.59 ± 0.49	$t_{363} = -3.35^{**}$
Cans	0.42 ± 0.49	0.64 ± 0.48	$t_{398} = -4.64^{**}$
Earthenwares	0.01 ± 0.11	0.22 ± 0.61	$t_{398} = -5.00^{**}$
Lotus pots	0.12 ± 0.41	0.26 ± 0.44	$t_{398} = -3.37^*$
Discarded tyres	0.13 ± 0.33	0.22 ± 0.42	$t_{398} = -2.48^*$
Roof gutters	0.00 ± 0.00	0.05 ± 0.31	$t_{398} = -2.36^*$
Swamps	0.16 ± 0.37	0.28 ± 0.45	$t_{398} = -2.89^{**}$
Coconut shells	0.50 ± 0.50	0.66 ± 0.64	$t_{308} = -2.69^*$
Rainwater tanks	0.36 ± 0.48	0.62 ± 0.49	$t_{360} = -5.25^{**}$

Table 3 The number of households and containers, and larval indices in high and low risk DHF areas.

	High risk DHF areas			Low risk DHF areas		
	<i>Aedes</i> spp.	<i>Ae.</i> <i>aegypti</i>	<i>Ae.</i> <i>albopictus</i>	<i>Aedes</i> spp.	<i>Ae.</i> <i>aegypti</i>	<i>Ae.</i> <i>albopictus</i>
No. of households	232	232	232	168	168	168
No. of positive households	39	31	28	75	51	46
No. of containers	6518	6518	6518	4715	4715	4715
No. of positive containers	91	88	66	141	121	105
Larval Index						
HI	16.81	13.36	12.06	44.64	30.36	27.38
CI	1.39	1.35	1.01	2.99	2.56	2.22
BI	39.22	37.93	28.45	83.93	72.02	62.50

DISCUSSION

The key breeding place distribution plays a very important role in determining the most suitable and effective methods of vector control in specific areas. *Ae. aegypti* and *Ae. albopictus* may have different key breeding places from one area to another [13,15]. Pong and Nam's [15] study showed that the key breeding site of *Ae. aegypti* in the east of Hanoi were concrete tanks. The key breeding sites in the south of Hanoi were jars, while in the central coast of Vietnam, they were ant traps; and in the Thanhbinh commune of Danang province they were toilet tanks [15]. Our study supports previous studies [13,15] that the key breeding sites of *Aedes* larvae depend on the types of *Aedes* species, and high/low risk DHF areas.

Ae. aegypti prefers to lay eggs in different containers to *Ae. albopictus* [13,15]. Pong and Nam [15] studied *Aedes* larval occurrence in Vietnam and found that *Ae. aegypti* larvae were mostly found in drums, jars, concrete tanks and discarded objects. On the other hand, *Ae. albopictus* larvae were mainly found in jars, discarded objects, drums and aquariums. Wongkoon *et al* [13] studied *Aedes* larval occurrence in Nakhon Si Thammarat, Thailand and found *Ae. aegypti* and *Ae. albopictus* larvae in six water storage containers including pot plants, animal pans, tyres, small water jars, bathroom tanks, and concrete tanks. They found that from these six containers, there were a higher number of *Ae. aegypti* larvae in water containers in bathrooms and concrete tanks than *Ae. albopictus* [13]. Our results supported previous findings and showed that the key breeding place of *Ae. aegypti* and *Ae. albopictus* in high risk DHF areas were the preserved areca jars, the container that preserved some areca for long time. On the other hand, in low DHF risk areas, the key breeding

place of *Ae. aegypti* were metal boxes and the key breeding place of *Ae. albopictus* were earthen jars. This suggests that water in areca containers should be emptied out regularly in order to eliminate the key breeding places of both *Aedes* larvae.

Our results showed that there were a higher number of positive containers including ant guards, basins, earthen jars, plant pots, animal pans and rainwater tanks in low risk areas than in high risk areas. On the other hand, there were a higher number of positive cement tanks in high risk DHF areas than in low risk DHF areas. This suggests that cement tanks may be the main breeding site of *Aedes* mosquitoes. Therefore, cement tanks should be cleaned regularly, and filled larvicide every three months in order to eliminate the main breeding place of *Aedes* mosquitoes.

Larval surveillance during this study was important to find out the extent of prevalence of vectors in a locality. HI, CI and BI were used in this study to help stratifying DHF risk areas for further control and monitoring of the vector population in defined areas. All larval indices from our study indicated a high risk of DHF transmission in both high/low risk DHF areas. Other studies on DHF in Thailand have shown similar trends [4,5,7,13]. HI and BI in both high and low risk DHF areas were higher than the WHO standard for high risk DHF areas (i.e. 10 % HI and 50 BI). This indicates a high risk of DHF transmission in Nakhon Si Thammarat Province, Thailand. Surprisingly, all larval indices for both *Ae. aegypti* and *Ae. albopictus* in high risk DHF areas were lower than in low risk DHF areas. This may be because the Nakhon Si Thammarat Provincial Health Office had a publicity campaign for the control and prevention of DHF particularly in high risk DHF areas. Both indoor and outdoor water containers in high risk areas had been emptied out or cleaned regularly. This resulted in a low number of *Aedes* larvae in high risk areas.

CONCLUSION

Nakhon Si Thammarat province is one of the highest DHF risk provinces in Thailand. A better understanding of key-breeding sites of *Ae. aegypti* and *Ae. albopictus* larvae in this area will help us to design a more effective DHF prevention and control system. This study clearly demonstrated that key breeding sites of *Ae. aegypti* and *Ae. albopictus* differ between high and low DHF risk areas. In high DHF risk area, key breeding sites of *Ae. aegypti* and *Ae. albopictus* are preserved areca jars. On the other hand, in low DHF risk area, the main key breeding site of *Ae. aegypti* are plastic boxes but the main key breeding site of *Ae. albopictus* are earthen jars. Provincial Health Office may have to design a campaign for DHF prevention more specifically in each locality.

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บทคัดย่อ

ศุภวรรณ พรหมเพรา มัลลิกา เจริญสุธาสิณี และ กฤษณะเดช เจริญสุธาสิณี

พื้นที่เสี่ยงต่อโรคไข้เลือดออกสูงและต่ำที่มีผลกระทบจากภาชนะเก็บน้ำที่ยุ่งลาย *Aedes aegypti* และ *Ae. albopictus* เลือกวางไข่ ในจังหวัดนครศรีธรรมราช ภาคใต้ของประเทศไทย

การศึกษาค้นคว้านี้ได้ทำการศึกษาพื้นที่เสี่ยงต่อโรคไข้เลือดออกสูงและต่ำที่มีผลกระทบจากภาชนะเก็บน้ำที่ยุ่งลาย *Aedes aegypti* และ *Ae. albopictus* เลือกวางไข่ พบว่า พบลูกน้ำยุ่งลาย *Ae. aegypti* และ *Ae. albopictus* ในภาชนะ 11 ชนิดจากภาชนะที่สำรวจทั้งหมด 29 ชนิดในพื้นที่เสี่ยงต่อโรคไข้เลือดออกสูงและต่ำ ลูกน้ำยุ่งลาย *Ae. aegypti* ในพื้นที่เสี่ยงต่อโรคไข้เลือดออกสูงพบมากที่สุดใไหหมาก และในพื้นที่เสี่ยงต่อโรคไข้เลือดออกต่ำพบมากที่สุดในกล่องโลหะ ในทางกลับกัน ลูกน้ำยุ่งลาย *Ae. albopictus* ในพื้นที่เสี่ยงต่อโรคไข้เลือดออกต่ำพบมากที่สุดในโอ่งน้ำในบ้าน และในพื้นที่เสี่ยงต่อโรคไข้เลือดออกสูงพบมากที่สุดในไหหมาก โอ่งน้ำ กล่องโลหะ และกล่องพลาสติก ในพื้นที่ความเสี่ยงต่ำมีจำนวนลูกน้ำยุ่งลาย *Ae. albopictus* สูงกว่าในพื้นที่เสี่ยงต่อโรคไข้เลือดออกสูง ดัชนีจำนวนลูกน้ำ (HI and BI) ทั้งในพื้นที่เสี่ยงต่อโรคไข้เลือดออกสูงและต่ำมีค่าสูงกว่าค่ามาตรฐานขององค์การอนามัยโลก ร้อยละ 10 และ 50 ตามลำดับ กล่าวได้ว่าพื้นที่นี้มีความเสี่ยงต่อการระบาดของโรคไข้เลือดออกสูง