SPRAY APPLICATION OF *BACILLUS THURINGIENSIS ISRAELENSIS* (BTI STRAIN AM65-52) AGAINST *AEDES AEGYPTI* (L.) AND *AE. ALBOPICTUS* SKUSE POPULATIONS AND IMPACT ON DENGUE TRANSMISSION IN A DENGUE ENDEMIC RESIDENTIAL SITE IN MALAYSIA

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Abstract. A one year study was conducted to evaluate the impact of spray application of *Bacillus thuringiensis israelensis* (Bti), strain AM65-52 on vector populations and dengue transmission in a dengue endemic state in Malaysia. Residential sites with similar populations of *Aedes aegypti* (L.) and *Aedes albopictus* Skuse were studied. One site was treated with spray application of Bti into all outdoor target vector habitats, which consisted of natural and artificial containers. The other site was not treated. The impact of spray application was measured with an indoor and outdoor ovitrap index (OI) and epidemiologic data. Significant reductions in both *Ae. aegypti* and *Ae. albopictus*, OI were observed both indoors and outdoors, in treated sites compared to untreated sites ($p<0.05$). OI reduction was achieved over time in the treated area. The OI was suppressed to below 10%. This was maintained for 4 weeks into the post-treatment phase. The outdoor OI at the untreated site remained at more than 40% for 38 weeks during the evaluation period. One dengue case occurred at the Bti treatment site at the beginning of the treatment phase, but no further cases were detected during the remainder of the treatment phase. However, there was an ongoing dengue outbreak in the untreated area with 15 serologically confirmed cases during weeks 37-54. Intensive fogging operations with pyrethroids at the untreated (Bti) site had a positive impact on *Ae. albopictus*, but not on *Ae. aegypti*.

Keywords: *Aedes aegypti*, *Aedes albopictus*, *Bacillus thuringiensis israelensis*, dengue vectors, interruption of dengue transmission, larviciding, ovitrap surveillance

INTRODUCTION

The dengue vector control program has long been used to battle *Aedes aegypti* (L.) and *Aedes albopictus* Skuse, since an effective vaccine and specific treatment are

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still unavailable in Malaysia. The current vector control program depends heavily on adulticiding as the first line of control during outbreaks. Adulticiding is conducted with the chemical insecticide(s), pyrethroids and organoposphates, within a 200 meter radius of the suspected / confirmed dengue case homes, followed by repeat fogging 7 to 10 days later. The fogging radius has been increased to 400 m in recent years (MOH, 2002). The public is also provided with temephos (Abate SG®) to use as a larvicide in water holding receptacles in their residential vicinity and to conduct source reduction as a long-term preventive measure (MOH, 2010). In 2001, the WHO applied the COMBI approach (Communication of Behavioral Impact) in Johor State based on health education and communication in a behaviorally-focused, people-centerd strategy (WHO, 2004a). Malaysia was the first country to try this approach for dengue prevention and control. A three-month COMBI program resulted in 85% of households in sampled areas carrying out the desired behavioral task over a 12 week period; 3 months later 70% were still maintaining it.

Despite these measures, dengue remains the most prominent public health disease in Malaysia since the first nationwide dengue outbreak in 1973. It threatens the health and wealth of the country, causing a significant economic and social impact every year. A total of 41,486 dengue cases with 88 deaths were reported in 2009. In 2010, by week 46 there were 42,914 cases with 125 deaths (MOH, 2010). Fatality rate increased by 60.3% compared to 2009 during the same time period. During that period, Selangor State had the highest recorded number of dengue cases (15,366 cases with 43 deaths). Globally, dengue incidence and mortalities also increased in 2009 - 2010 compared to previous years, with a reemergence of dengue cases in Florida, USA after 75 years (CDC, 2010). The increase in cases has been attributed to global warming, heavy rainfall or drought and rapid urbanization with a proliferation of man-made containers which serve as mosquito-breeding sites (CDC, 2010).

Dengue prevention and control relies on effective, applicable and sustainable vector control measures. Effective vector control prevents the emergence of adult mosquitoes, which can be achieved with larviciding. Temephos has been used in this country since the 1970's. The granular formulation is hand sown into containers, while compression sprayers are used to spread the liquid formulation. However, a limitation is the difficulty in covering all the water receptacles, both natural and artificial (WHO, 2009). However, *Aedes* spp larval resistance to temephos is present in Malaysia and in other countries (Chen *et al*, 2005; Rodríguez *et al*, 2007; Loke *et al*, 2010). Braga and Valle (2007) studied the impact of the larvicide *Bacillus thuringiensis israelensis* (Bti) against *Ae. aegypti* in temephos resistant cities in Brazil. Bti is target specific, safe and specific (Lacey, 2007). Bti toxins have virtually no threat to non-target organisms, including fish, birds and mammals. Some fish mortality has been reported at high concentrations of Bti, but mortality was attributed to the formulation components and not the Bti toxins (Lacey, 2007).

VectoBac WG is a water dispersible granule formulation of Bti Strain AM65-52. Efficacy against many vector species has been reviewed by the WHO (2004b) and its specifications are described by the WHO (2007). The formulation is free from bacterial contaminants and exotoxins, it is safe to be used in drinking water, it presents no significant hazards to users or the environment, and can be used in direct or spray applications. Setha *et al* (2007), Chandramogan (2006), Lee *et al* (2008) and Lam *et al* (2010) have confirmed efficacy of VectoBac WG in controling dengue vectors in villages, urban areas, suburban areas, residential areas and forested areas. However, those previous studies did not measure the impact of Bti on dengue cases.

This study was conducted in a dengue endemic area of Malaysia where the impact of larviciding with Bti was evaluated using vector densities and epidemiological data.

MATERIALS AND METHODS

Study design

This study was conducted in accordance with the WHO (2005) protocol for phase III: Large-Scale Field Trials.

Study site

A dengue endemic area in Shah Alam Malaysia, located 50 km from Kuala Lumpur, was selected based on reported dengue case data one year prior to the study, provided by the State Vector Control Unit, Selangor and also by the Local Authority Shah Alam (MBSA). The study area, Section 17 (N 30º2′ 43.21″; E 101º30′ 14.30″) is a highly populated residential area which covers an area of 150 hectares. However, the study focused on 2 sites in Section 17 1.3 km apart (Fig 1). Site A is 10 hectares comprised of approximately 300 houses and Site B is 8 hectares with approximately the same number of houses. The sites were separated by a dual-carriage road, which served as a buffer zone to avoid inter-movement of target vectors. Bti treatment was conducted in site B and designated as the Bti Treated Site, and site A was designated as the untreated control site (UTC). Buildings at both sites were similar. Site A is comprised of double-storey semi-detached houses and cluster houses; Site B is comprised of double-storey terrace houses. Dense vegetation was a common feature of both sites, in front of the houses and in back alleys.

Ovitrap surveillance

Twenty-nine biweekly surveillances were conducted over a 58 week period to monitor the dengue vector population. The operation was divided into a pre-treatment phase during August-November 2007 (weeks 1-14), a Bti-treatment phase from December 2007-June 2008 (weeks 15-44) and a post-Bti treatment phase from June-September 2008 (weeks 45-58).

Modified ovitraps were used for the survey as described by Lee (1992a). Ovitraps were placed in 10% of the total residential houses at each site. A total of 120 ovitraps were placed at 60 designated houses (30 houses per site per surveillance). Houses were randomly selected during the study site to avoid choosing 2 houses next to each another. The 30 houses were spread over 8 blocks at Site A and 7 blocks at Site B. From time to time, some study homes had to be changed because of objections from house owners to continue ovitrap surveillance in their homes.

Paired indoor and outdoor ovitraps were placed in the same house. Indoor ovitraps were placed in the living room in obscure and isolated spots. Outdoor traps were placed in shaded spots with abundant vegetation in the immediate vicinity of the house.

The traps were each left out for 5 days, then collected and transported back to the laboratory. The contents with the oviposition paddles were transferred individually into 500 ml plastic containers. Sufficiently seasoned tap water was

The balloons indicate number of dengue cases during study period, August 2007 - September 2008.

White lines indicate streets where ovitraps were placed.

Fig 1-Section 17, Shah Alam, Selangor, Malaysia, highlighting study Site A untreated control site and Site B Bti treated site.

added to the contents to ensure complete submersion of the paddles if the water from the ovitrap was insufficient. Ovitraps with lost paddles were still processed by adding seasoned tap water and the inner wall of the ovitrap was scraped to dislodge any mosquito eggs possibly adherent to the inner wall of the ovitrap. Beef liver (small chunks or powder) was added as larval food and to stimulate larval hatching. The containers were covered to prevent colonization by lab mosquitoes or predators. Ovitrap contents were kept in the laboratory for 7 days under ambient temperature to ensure complete hatching.

Larval identification and enumeration

Third stage (L3) larvae were identified and *Ae. aegypti* and *Ae. albopictus* larvae numbers were recorded.

Larviciding with *Bacillus thuringiensis israelensis* (Bti)

VectoBac WG (Valent BioSciences Corporation, Lot 155-563-PG) at a concentration of 3,000 ITU/mg was used in this study. Larval habitats at Site B were mapped and the total treatable area included vegetation and artificial habitats. The 8 ha site had an estimated 75% treatable area (6 ha). The boundary of the 8 ha site was also treated since natural containers were found along the boundary (1 ha). Hence, the total Bti treated area was 7 ha.

Bti application was conducted with back pack mist blowers (Stihl™ SR 420) to achieve full coverage of the site. The blowers were equipped with adjustable nozzles with 6 different orifices for varying spray volume. Generally, orifice 2 with a discharge rate of 500 ml/min was used to treat 8 m from the nozzle. Higher numbered orifices (3 and 4) with discharge rates ≥ 1 liter/minute were used to reach habitats > 8 m (vertical/horizontal) from the spray nozzle.

Bti treatment was initiated with 7 treatments on biweekly schedule, followed by 7 treatments on a weekly schedule, and ending with 4 biweekly treatments. A preliminary study was conducted to determine the required volume of water to provide an adequate coverage of the 7 ha site. The maximum labeled dosage (500 g/ha) was used due to the high population of both *Ae. aegypti* and *Ae. albopictus*, as seen with the ovitrap index (OI) during the pre-treatment phase. A 3.5 kg VectoBac® WG was mixed onsite in 160 liters of water just prior to application. Spraying was conducted between 07:30-09:30 AM. All mapped potential and actual outdoor larval habitats were sprayed, including concrete perimeter drains, roof gutters and vegetation. Four spray teams, each consisting of 1 applicator and 1 supervisor sprayed an assigned area. The same team members performed this task throughout the study. The supervisor also treated any water receptacles more than 50 liters by direct application of Bti at 8g/1,000 liters. This included all types of containers residents kept outdoors, such as aquariums or ponds with fish.

Epidemiological data

Epidemiological data and activities of the local vector-borne disease control program during the study period were collected from Shah Alam City Council (MBSA).

Data analysis

All data were subjected to statistical analysis using SPSS version 11.5 (SPSS, Chicago, IL).

The total number of ovitraps recovered with positive *Aedes* spp larvae and the total number of *Aedes* spp in each ovitrap were recorded. The ovitrap index (OI) and larval density (LD) were calculated using the following formulae:

OI = total number of positive ovitraps / total number of recovered ovitraps x 100 %; LD = total number of larvae / total number of recovered ovitraps.

Ovitrap data were analyzed with the chi-square test; larval density was analyzed with the *t*-test; significance was set at $p = 0.05$.

We attempted to determine percent reduction using Mulla's formula (Mulla *et al*, 1971), but it was not possible for weeks 37-54 because of active mosquito control activities carried out by the local city council in the untreated control site to control a dengue outbreak.

RESULTS

The impact of larviciding with Bti was evaluated with the ovitrap index (OI) and larval density (LD): overall indoor and outdoor OI (Fig 2, 3), the indoor and outdoor OI for *Ae. aegypti* (Fig 4, 5), the indoor and outdoor OI for *Ae. albopictus* (Fig 6, 7), and the LD for both species (Tables 1-3) were determined. The OI and LD were compared between untreated and Bti treated sites during the same time period.

Dengue outbreaks happened during the study period (Table 4). There was one

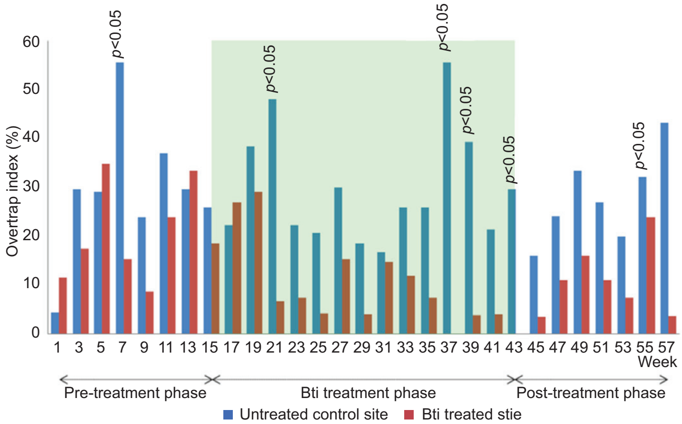

Fig 2-Overall Indoor Ovitrap Index (OI) for *Aedes* spp in Bti treated and untreated control sites.

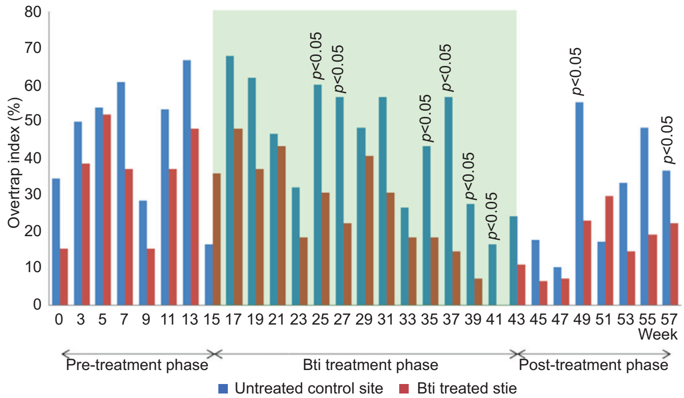

Fig 3-Overall Outdoor Ovitrap Index (OI) for *Aedes* spp in Bti treated and untreated control sites.

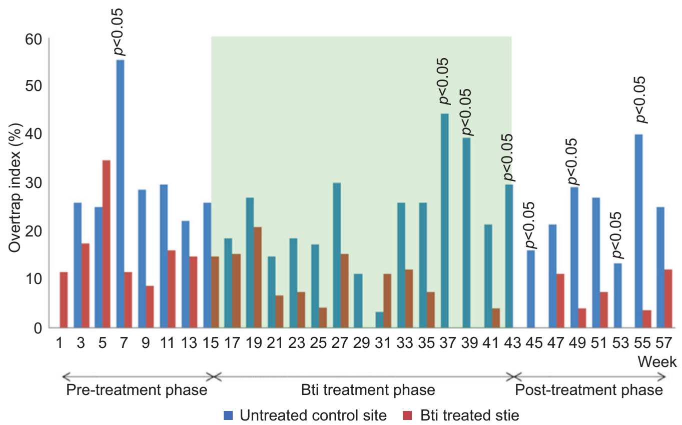

Fig 4-Indoor Ovitrap Index (OI) for *Aedes aegypti* in Bti treated and untreated control sites.

serologically confirmed case in the Bti treated site reported in January 2008 and 15 serologically confirmed cases in the untreated Bti site reported during May to early September 2008 (Fig 1). The local city council conducted adulticiding operations with pyrethroids, using thermal foggers and vehicle mounted ULV cold foggers in the affected sites with a coverage of 400 m radius. A total of 21 fogging operations were conducted, with 20 intense fogging operations during weeks 37-54 in the untreated Bti site. The effects of fogging were considered in the ovitrap surveillance analysis.

Pre-treatment phase

During the pre-treatment phase, the overall *Aedes* spp populations at both sites were of similar in density over the 14 weeks of surveillance, except for week 7 at site A, the *Ae. aegypti* indoor population (OI = 55.56 % and LD 15.85 ± 4.66) significantly contributed to a higher overall vector density at site A ($p < 0.05$). The outdoor *Ae. aegypti* population was significantly more at site A for 2 of the 7 surveillances, but it did not contribute significantly to the overall outdoor populations at the two sites ($p > 0.05$).

Pre-Bti treatment, *Ae. aegypti* was the predominant indoor species at Sites A and B with higher indoor OIs of 26.70 ± 6.14% and 16.39 ± 3.27%, respectively, in comparison

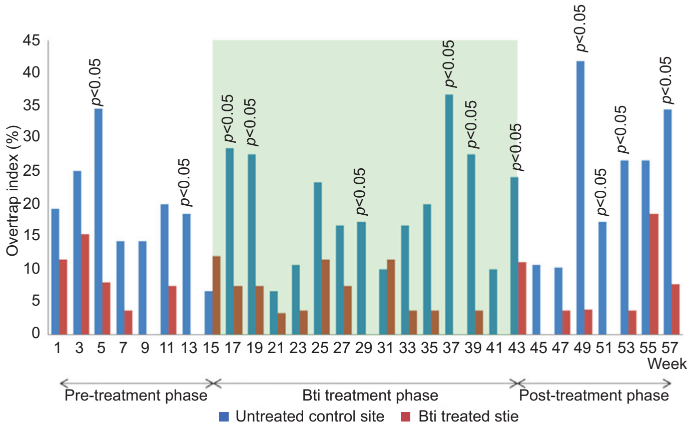

Fig 5-Outdoor Ovitrap Index (OI) for *Aedes aegypti* in Bti treated and untreated control sites.

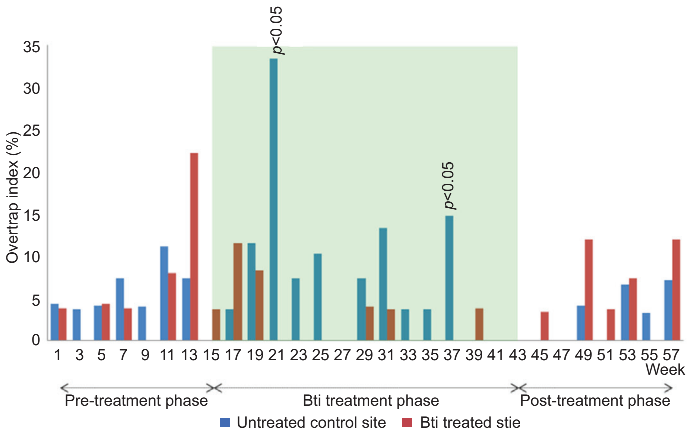

Fig 6-Indoor Ovitrap Index (OI) for *Aedes albopictus* in Bti treated and untreated control sites.

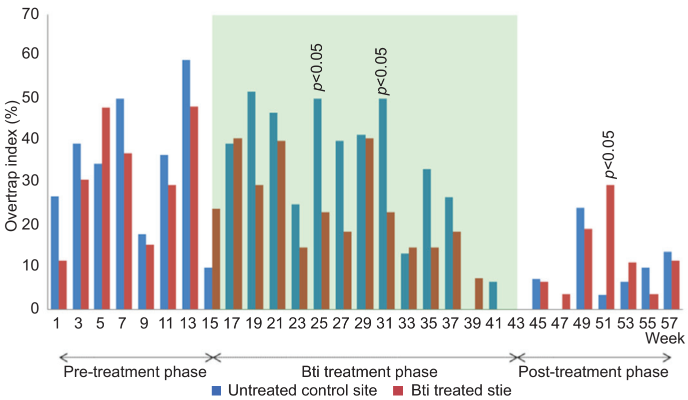

Fig 7-Outdoor Ovitrap Index (OI) for *Aedes albopictus* in Bti treated and untreated control sites.

to outdoor OIs ($p < 0.05$). *Ae. albopictus* was the predominant outdoor species at both sites with higher outdoor OIs of 37.80 ± 5.21% and 31.50 ± 5.44% at Sites A and B, respectively, in comparison to indoor OIs ($p < 0.05$). At Site A pretreatment both vector species had similar densities (44-48%), but at Site B, *Ae. albopictus* had a 15% higher density than *Ae. aegypti*.

Bti treatment and post-treatment phases

Bti treatment was initiated at Site B the week after the pre-treatment surveillance ended in week 14. Bti treatments were carried out biweekly during weeks 15-26, then weekly during weeks 27-34.

The OI for both sites is shown in Figs 2 and 3. A significant reduction in OI was observed in the Bti treated site from week 21 onward ($p < 0.05$). A similar result was observed for LD (Tables 1 and 3). This reduction was contributed by a decrease in *Ae. albopictus* indoor population.

Vector density reduction was initially observed indoors and then outdoors 10 weeks into treatment. The vector population decreased over time to an OI of 0 for both indoor and outdoor vector populations during Bti treatment.

During the biweekly intervention, a significant reduction was only observed in the outdoor *Ae. aegypti* population from week 17. A single dengue case

Table 1
Overall indoor and outdoor Larval Density (LD) in Bti treated and untreated control sites.

	Overall indoor					Overall outdoor				
Week	Bti treated	*n*	Untreated control	*n*	*p*	Bti treated	*n*	Untreated control	*n*	*p*
1	0.46 ± 0.30	26	0.43 ± 0.44	23	$p > 0.05$	3.35 ± 2.18	26	8.88 ± 5.42	25	$p > 0.05$
3	2.22 ± 1.17	23	3.11 ± 1.33	27	$p > 0.05$	5.12 ± 2.74	26	8.39 ± 2.38	28	$p > 0.05$
5	10.09 ± 3.92	23	2.96 ± 1.23	24	$p > 0.05$	14.56 ± 4.10	25	16.12 ± 5.46	26	$p > 0.05$
7	4.00 ± 2.18	24	17.22 ± 4.90	27	$p < 0.05$	7.12 ± 2.34	25	21.86 ± 4.82	28	$p < 0.05$
9	0.35 ± 0.25	23	5.80 ± 2.92	25	$p > 0.05$	3.96 ± 2.03	26	5.18 ± 2.18	28	$p > 0.05$
11	4.72 ± 2.62	25	14.33 ± 5.05	27	$p > 0.05$	9.70 ± 3.14	27	17.70 ± 4.57	30	$p > 0.05$
13	17.26 ± 6.81	27	10.67 ± 3.92	27	$p > 0.05$	15.70 ± 4.11	27	27.68 ± 5.90	28	$p > 0.05$
15	6.89 ± 3.55	27	17.41 ± 6.57	27	$p > 0.05$	8.92 ± 3.21	25	3.27 ± 1.80	30	$p > 0.05$
17	12.77 ± 4.72	26	9.04 ± 4.35	27	$p > 0.05$	15.22 ± 3.79	27	21.07 ± 4.35	28	$p > 0.05$
19	6.38 ± 3.32	24	20.92 ± 6.79	26	$p > 0.05$	14.81 ± 4.79	27	21.14 ± 4.28	29	$p > 0.05$
21	3.33 ± 2.34	27	19.74 ± 6.56	27	$p < 0.05$	9.07 ± 2.68	27	14.23 ± 3.70	30	$p > 0.05$
23	0.67 ± 0.49	27	5.85 ± 3.03	27	$p > 0.05$	1.85 ± 1.04	27	3.39 ± 1.75	28	$p > 0.05$
25	2.92 ± 2.92	24	8.00 ± 3.73	29	$p > 0.05$	19.58 ± 6.54	26	21.03 ± 5.67	30	$p > 0.05$
27	6.96 ± 5.55	26	5.60 ± 1.83	30	$p > 0.05$	8.70 ± 5.84	27	14.37 ± 3.36	30	$p > 0.05$
29	2.60 ± 2.60	25	6.19 ± 3.40	27	$p > 0.05$	12.26 ± 4.04	27	15.17 ± 4.25	29	$p > 0.05$
31	6.67 ± 3.33	27	3.67 ± 2.11	30	$p > 0.05$	4.85 ± 1.84	26	10.57 ± 2.77	30	$p > 0.05$
33	3.40 ± 2.47	25	5.19 ± 2.15	27	$p > 0.05$	5.63 ± 2.86	27	5.80 ± 2.36	30	$p > 0.05$
35	4.22 ± 2.96	27	10.59 ± 5.97	27	$p > 0.05$	2.63 ± 1.23	27	11.07 ± 3.70	30	$p < 0.05$
37	0.00 ± 0.00	27	21.41 ± 6.14	27	$p < 0.05$	4.96 ± 2.66	27	18.43 ± 5.85	30	$p < 0.05$
39	0.19 ± 0.19	26	13.68 ± 4.32	28	$p < 0.05$	3.00 ± 2.58	27	5.14 ± 2.35	29	$p > 0.05$
41	0.72 ± 0.72	25	10.07 ± 4.95	28	$p > 0.05$	0.00 ± 0.00	27	4.77 ± 2.16	30	$p < 0.05$
43	0.00 ± 0.00	27	5.59 ± 1.97	27	$p < 0.05$	2.48 ± 1.65	27	11.17 ± 4.58	29	$p > 0.05$
45	0.54 ± 0.54	26	3.20 ± 1.96	25	$p > 0.05$	0.26 ± 0.18	27	2.68 ± 1.37	28	$p > 0.05$
47	4.48 ± 2.64	27	6.00 ± 2.96	28	$p > 0.05$	1.63 ± 1.15	27	1.24 ± 0.70	29	$p > 0.05$
49	2.00 ± 0.99	25	17.96 ± 6.67	24	$p < 0.05$	7.88 ± 4.67	26	14.41 ± 3.73	29	$p > 0.05$
51	1.04 ± 0.61	27	10.38 ± 4.59	26	$p > 0.05$	4.67 ± 1.77	27	7.28 ± 3.85	29	$p > 0.05$
53	0.44 ± 0.33	27	5.63 ± 2.97	30	$p > 0.05$	2.04 ± 1.26	27	5.40 ± 2.15	30	$p > 0.05$
55	2.63 ± 2.63	27	23.57 ± 6.10	30	$p < 0.05$	8.00 ± 3.42	27	9.03 ± 3.10	30	$p > 0.05$
57	4.48 ± 1.94	25	12.79 ± 4.24	28	$p > 0.05$	7.62 ± 3.63	26	9.41 ± 2.83	29	$p > 0.05$

n indicates number of recovered ovitraps.

was seen in January 2008; the unchanged indoor *Ae. aegypti* density and unchanged indoor and outdoor *Ae. albopictus* densities (p>0.05) during the first 2 months of Bti treatment led us to implement weekly Bti treatment from weeks 27-34 to suppress the vector population. Seven weekly Bti treatments were conducted which resulted in a significant reduction in *Ae. aegypti* (both indoors and outdoors) from week 37 (p<0.05) (Figs 4 and 5 and Table 2). Weekly Bti treatments reduced *Ae. albopictus* (indoors and outdoors), but there was not a significant difference from the untreated site. This could be due to decreased *Ae. albopictus* at the Bti untreated site because of intensive pyrethroid fogging operations conducted by the local city council during weeks 37-54 at that site (Fig 1 and Table 4). Seventeen of the pyrethroid foggings were conducted within a 400 m radius of the untreated control site.

Table 2
Indoor and outdoor Larval Density (LD) for *Aedes aegypti* in Bti treated and untreated control sites.

	Aedes aegypti indoor					*Aedes aegypti* outdoor				
Week	Bti treated	*n*	Untreated control	*n*	*p*	Bti treated	*n*	Untreated control	*n*	*p*
1	0.38 ± 0.29	26	0.00 ± 0.00	23	$p > 0.05$	0.85 ± 0.52	26	2.48 ± 1.63	26	$p > 0.05$
3	2.22 ± 1.17	23	2.85 ± 1.32	27	$p > 0.05$	2.50 ± 2.19	26	1.93 ± 1.00	28	$p > 0.05$
5	9.52 ± 3.65	23	2.17 ± 1.02	24	$p > 0.05$	1.16 ± 0.89	25	8.15 ± 3.74	26	$p > 0.05$
7	3.46 ± 2.15	24	15.85 ± 4.66	27	$p < 0.05$	0.76 ± 0.76	25	5.29 ± 2.95	28	$p > 0.05$
9	0.35 ± 0.25	23	5.48 ± 2.92	25	$p > 0.05$	0.00 ± 0.00	26	2.32 ± 1.26	28	$p > 0.05$
11	4.04 ± 2.58	25	10.63 ± 4.86	27	$p > 0.05$	1.52 ± 1.41	27	6.47 ± 2.90	30	$p > 0.05$
13	2.37 ± 1.82	27	8.96 ± 3.78	27	$p > 0.05$	0.00 ± 0.00	27	6.57 ± 3.43	28	$p > 0.05$
15	4.33 ± 2.63	27	17.41 ± 6.57	27	$p > 0.05$	3.44 ± 1.97	25	1.90 ± 1.68	30	$p > 0.05$
17	7.96 ± 3.76	26	6.04 ± 3.36	27	$p > 0.05$	3.07 ± 2.35	27	10.00 ± 4.17	28	$p > 0.05$
19	5.46 ± 3.31	24	13.85 ± 5.83	26	$p > 0.05$	4.33 ± 3.70	27	7.00 ± 2.78	29	$p > 0.05$
21	3.33 ± 2.34	27	8.11 ± 5.10	27	$p > 0.05$	0.33 ± 0.33	27	2.20 ± 1.80	30	$p > 0.05$
23	0.67 ± 0.49	27	5.70 ± 3.03	27	$p > 0.05$	0.52 ± 0.52	27	1.96 ± 1.65	28	$p > 0.05$
25	2.92 ± 2.92	24	6.41 ± 3.51	29	$p > 0.05$	8.00 ± 5.10	26	7.40 ± 4.18	30	$p > 0.05$
27	6.96 ± 5.55	26	5.60 ± 1.83	30	$p > 0.05$	5.93 ± 5.81	27	5.90 ± 2.98	30	$p > 0.05$
29	0.00 ± 0.00	25	4.41 ± 3.04	27	$p > 0.05$	0.00 ± 0.00	27	2.45 ± 1.24	29	$p > 0.05$
31	5.89 ± 3.29	27	0.40 ± 0.40	30	$p > 0.05$	2.81 ± 1.78	26	1.40 ± 1.00	30	$p > 0.05$
33	3.40 ± 2.47	25	5.15 ± 2.14	27	$p > 0.05$	2.15 ± 2.15	27	2.93 ± 1.56	30	$p > 0.05$
35	4.22 ± 2.96	27	10.33 ± 5.98	27	$p > 0.05$	0.33 ± 0.33	27	4.53 ± 2.61	30	$p > 0.05$
37	0.00 ± 0.00	27	16.26 ± 5.58	27	$p < 0.05$	0.00 ± 0.00	27	12.90 ± 5.32	30	$p < 0.05$
39	0.00 ± 0.00	26	13.68 ± 4.32	28	$p < 0.05$	0.15 ± 0.15	27	5.14 ± 2.35	29	$p < 0.05$
41	0.72 ± 0.72	25	10.07 ± 4.95	28	$p < 0.05$	0.00 ± 0.00	27	3.40 ± 2.00	30	$p > 0.05$
43	0.00 ± 0.00	27	5.59 ± 1.97	27	$p < 0.05$	0.00 ± 0.00	27	11.17 ± 4.58	29	$p < 0.05$
45	0.00 ± 0.00	26	3.20 ± 1.96	25	$p > 0.05$	0.00 ± 0.00	27	2.36 ± 1.37	28	$p > 0.05$
47	4.48 ± 2.64	27	6.00 ± 2.96	28	$p > 0.05$	0.96 ± 0.96	27	1.24 ± 0.70	29	$p > 0.05$
49	0.64 ± 0.64	25	16.33 ± 6.64	24	$p < 0.05$	0.31 ± 0.31	26	9.72 ± 3.42	29	$p < 0.05$
51	0.85 ± 0.60	27	10.38 ± 4.59	26	$p > 0.05$	0.00 ± 0.00	26	7.21 ± 3.85	29	$p > 0.05$
53	0.00 ± 0.00	27	5.03 ± 2.95	30	$p > 0.05$	0.04 ± 0.04	27	5.20 ± 2.16	30	$p < 0.05$
55	2.63 ± 2.63	27	21.57 ± 6.01	30	$p < 0.05$	6.22 ± 3.06	27	7.30 ± 2.00	30	$p > 0.05$
57	1.72 ± 1.09	25	10.86 ± 4.15	28	$p < 0.05$	4.81 ± 3.41	26	7.41 ± 2.80	29	$p > 0.05$

n indicates number of recovered ovitraps.

Biweekly treatment was carried out during weeks 35 - 44. The indoor and outdoor OI and LD for *Ae.aegypti* were significantly lower than the untreated site during this phase ($p < 0.05$). The suppression was evident for the next 14 weeks into the post-treatment phase, with significant suppression of both indoor and outdoor *Ae. aegypti* OI and LD compared to untreated site ($p < 0.05$). The effects of the pyrethroid fogging operations at the untreated control site could be the reason for not observing a significant reduction in the OI and LD in *Ae. albopictus* at the Bti treated site during the post-treatment phase.

DISCUSSION

This study at Shah Alam was the result of the Ministry of Health's desire

Table 3
Indoor and outdoor Larval Density (LD) for *Aedes albopictus* in Bti treated and untreated control sites.

	Aedes albopictus indoor					*Aedes albopictus* outdoor				
Week	Bti treated	*n*	Untreated control	*n*	*p*	Bti treated	*n*	Untreated control	*n*	*p*
1	0.08 ± 0.08	26	0.43 ± 0.44	23	$p > 0.05$	2.50 ± 2.12	26	6.19 ± 3.95	26	$p > 0.05$
3	0.00 ± 0.00	23	0.26 ± 0.26	27	$p > 0.05$	2.62 ± 1.13	26	6.46 ± 2.16	28	$p > 0.05$
5	0.57 ± 0.57	23	0.79 ± 0.79	24	$p > 0.05$	13.40 ± 4.05	25	7.96 ± 3.66	26	$p > 0.05$
7	0.54 ± 0.54	24	1.37 ± 0.99	27	$p > 0.05$	6.36 ± 2.01	25	16.57 ± 4.55	28	$p > 0.05$
9	0.00 ± 0.00	23	0.32 ± 0.32	25	$p > 0.05$	3.96 ± 2.03	26	2.86 ± 1.90	28	$p > 0.05$
11	0.68 ± 0.64	25	3.70 ± 2.22	27	$p > 0.05$	8.19 ± 3.00	27	11.23 ± 3.65	30	$p > 0.05$
13	14.89 ± 6.69	27	1.70 ± 1.49	27	$p > 0.05$	15.70 ± 4.11	27	21.11 ± 5.50	28	$p > 0.05$
15	2.56 ± 2.56	27	0.00 ± 0.00	27	$p > 0.05$	5.48 ± 2.83	25	1.37 ± 0.78	30	$p > 0.05$
17	4.81 ± 3.35	26	3.00 ± 3.00	27	$p > 0.05$	12.15 ± 3.43	27	11.07 ± 3.12	28	$p > 0.05$
19	0.92 ± 0.70	24	7.08 ± 4.47	26	$p > 0.05$	10.48 ± 3.57	27	14.14 ± 3.64	29	$p > 0.05$
21	0.00 ± 0.00	27	11.63 ± 4.9	27	$p < 0.05$	8.74 ± 2.70	27	12.03 ± 3.48	30	$p > 0.05$
23	0.00 ± 0.00	27	0.15 ± 0.12	27	$p > 0.05$	1.33 ± 0.63	27	1.43 ± 0.69	28	$p > 0.05$
25	0.00 ± 0.00	24	1.59 ± 1.38	29	$p > 0.05$	11.58 ± 4.83	26	13.63 ± 4.61	30	$p > 0.05$
27	0.00 ± 0.00	26	0.00 ± 0.00	30	-	2.78 ± 1.26	27	8.47 ± 2.42	30	$p < 0.05$
29	2.60 ± 2.60	25	1.78 ± 1.70	27	$p > 0.05$	12.26 ± 4.04	27	12.72 ± 3.60	29	$p > 0.05$
31	0.78 ± 0.78	27	3.27 ± 2.10	30	$p > 0.05$	2.04 ± 0.82	26	9.17 ± 2.70	30	$p < 0.05$
33	0.00 ± 0.00	25	0.04 ± 0.04	27	$p > 0.05$	3.48 ± 2.03	27	2.87 ± 1.90	30	$p > 0.05$
35	0.00 ± 0.00	27	0.26 ± 0.26	27	$p > 0.05$	2.30 ± 1.21	27	6.53 ± 2.94	30	$p > 0.05$
37	0.00 ± 0.00	27	5.15 ± 3.60	27	$p > 0.05$	4.96 ± 2.66	27	5.53 ± 3.29	30	$p > 0.05$
39	0.19 ± 0.19	26	0.00 ± 0.00	28	$p > 0.05$	2.85 ± 2.43	27	0.00 ± 0.00	29	$p > 0.05$
41	0.00 ± 0.00	25	0.00 ± 0.00	28	-	0.00 ± 0.00	27	1.37 ± 0.99	30	$p > 0.05$
43	0.00 ± 0.00	27	0.00 ± 0.00	27	-	2.48 ± 1.65	27	0.00 ± 0.00	29	$p > 0.05$
45	0.54 ± 0.54	26	0.00 ± 0.00	25	$p > 0.05$	0.26 ± 0.18	27	0.32 ± 0.26	28	$p > 0.05$
47	0.00 ± 0.00	27	0.00 ± 0.00	28	-	0.67 ± 0.67	27	0.00 ± 0.00	29	$p > 0.05$
49	1.36 ± 0.80	25	1.63 ± 1.63	24	$p > 0.05$	7.58 ± 4.68	26	4.69 ± 2.31	29	$p > 0.05$
51	0.19 ± 0.19	27	0.00 ± 0.00	26	$p > 0.05$	4.85 ± 9.33	26	0.07 ± 0.07	29	$p < 0.05$
53	0.44 ± 0.33	27	0.60 ± 0.54	30	$p > 0.05$	2.00 ± 1.26	27	0.20 ± 0.15	30	$p > 0.05$
55	0.00 ± 0.00	27	2.00 ± 1.83	30	$p > 0.05$	1.78 ± 1.78	27	1.73 ± 1.27	30	$p > 0.05$
57	2.76 ± 1.73	25	1.93 ± 1.50	28	$p > 0.05$	2.81 ± 1.61	26	2.00 ± 1.03	29	$p > 0.05$

n indicates number of recovered ovitraps.

to conduct a study in a high risk dengue endemic site to determine novel, effective methods to prevent dengue outbreaks (MOH, 2006). A previous study conducted in a dengue endemic site in Selangor State in 2004-2005 showed the appropriate application of Bti, VectoBac® WG suppressed vector populations (Lee *et al*, 2008). In this 58 week study we found using the same technology suggested by Chandramogan (2006), Lee *et al* (2008) and Lam *et al* (2010), can suppress *Ae. aegypti* and *Ae. albopictus* populations at a dengue endemic site with a temephos resistant population and eventually interrupt dengue transmission in humans.

Vector population significantly decreased with time and remained suppressed even after treatment was stopped for 3 months. Similar post-treatment sup-

Table 4
Epidemiological data of serologically confirmed dengue cases and dengue vector control program by adulticiding with pyrethroids in section 17, Shah Alam, Selangor during January-September 2008. (Data Source: Shah Alam City Council).

No.	Month	No. cases		Date	Insecticide	Application method	Address	Remarks
		DF	DHF					
1.	January	1	0					
2.	February	0	0	22/02/2008	Resigen®	Thermal	17/10	T
3.	March	0	0					
4.	April	0	0					
5.	May	3	0	09/05/2008	Resigen®	Thermal	17/39	UTC[a]
				09/05/2008	Resigen®	Thermal	17/39B	UTC[a]
				16/05/2008	Resigen®	Thermal	17/51	UTC[a]
6.	June	6	0	04/06/2008	Resigen®	Thermal	17/51	UTC[a]
				04/06/2008	Resigen®	Thermal	17/39	UTC[a]
				05/06/2008	Resigen®	Thermal	17/50	UTC[a]
				10/06/2008	Resigen®	Thermal	17/51	UTC[a]
				10/06/2008	Resigen®	Thermal	17/52	UTC[a]
				17/06/2008	Resigen®	Thermal	17/45B	UTC
				24/06/2008	Resigen®	Thermal	17/44	UTC
				24/06/2008	Resigen®	Thermal	17/51	UTC[a]
				25/06/2008	Resigen®	Thermal	17/3B	UTC[b]
				30/06/2008	Resigen®	Thermal	17/54	UTC
7.	July	3	0	09/07/2008	Mospray®	Thermal	17/19	UTC
				10/07/2008	Mospray®	Thermal and ULV	17/35B	UTC
				21/07/2008	Mospray®	Thermal	17/18	UTC[c]
				25/07/2008	Mospray®	Thermal	17/52A	UTC[a]
				25/07/2008	Mospray®	ULV	17/45B	UTC
8.	August	2	0	26/08/2008	Mospray®	Thermal and ULV	17/17B	UTC[b]
9.	September	1	0	02/09/2008	Mospray®	Thermal and ULV	17/18	UTC[c]

[a]UTC, Streets which were under ovitrap surveillance for untreated control site; T, Streets which were under ovitrap surveillance for the Bti treated site; UTC, Streets which were not covered under ovitrap surveillance but these streets are within 400 m radius of the untreated control site; [b]UTC, Streets which were not covered under ovitrap surveillance but these streets are within 400 m radius from Bti treated site; [c]UTC, Streets which were not covered under ovitrap surveillance but these streets are more than 400 m radius from untreated control and Bti treated sites.

pressions have been observed in studies conducted in urban areas of Singapore (Chandramogan, 2006) and in suburban and temporary settlement areas in Malaysia (Lee *et al*, 2008).

There were no recorded dengue cases at the Bti treated site during peak months (May - July 2008), but 15 cases occurred outside the Bti treated site. The absence of dengue cases in the Bti treated site was probably due to reduced vector populations caused by Bti spraying. During the Bti treatment and post-treatment phases, both vector densities remained lower than at the untreated site. Overall, the outdoor OI at the untreated site was consistently high at 68%. The high vector density could have led to a dengue outbreak from week 37 onwards. The threshold OI in Malaysia needed to initiate dengue vector control was determined to be 10% (Lee, 1992b) and a possible dengue outbreak happened at about 30% (Kamilan, 2001). The dengue outbreak at the untreated site, and the low vector density with no dengue cases at the Bti treated site confirms the need to routinely monitor the vector population and conduct larviciding to prevent dengue outbreaks.

Adulticiding conducted by the local city council at the outbreak site during weeks 37-50 did reduce the *Ae. albopictus* population, but did not suppress the *Ae. aegypti* population. Thus, the overall indoor and outdoor OI remained significantly higher in the site treated with adulticides compared to the site treated with Bti ($p < 0.05$) (Table 1). The lack of impact from adulticiding could be due to pyrethroid resistance among *Ae. aegypti* mosquitoes. Field mosquitoes collected from the two study sites at the time of this study were resistant to 0.75% permethrin, with a 4.4% mortality at 24 hours using WHO (1981) adult mosquito testing methods (Loke, 2012).

Larviciding can be beneficial when effective larvicides and application technology compatible to the environment are used. Temephos resistance has been detected in Malaysia and should not be the larvicide of choice in endemic areas. *Ae. aegypti* from the two study sites in Shah Alam have a decreased susceptibility to temephos at 0.02 mg/l which over time has given a mortality range of 11-100% (Loke *et al*, 2010).

Bti has been used in many localitions for more than a decade without any reports of resistance developing in the mosquito populations (Becker and Ludwig, 1993; Lacey, 2007). Bti is a stomach poison and must be ingested by the larvae. Thus, it needs to be dispersed as droplets of appropriate size into larval feeding areas; this has been successfully achieved by aerial and ground applications. In Malaysia, simulated field studies with spray application of Bti have been conducted; efficacy of sprayed Bti droplets was measured with cups of water holding larvae. Complete to decreasing range of mortality was observed with increasing distance from spray equipment (Lee *et al*, 1996; Seleena and Lee, 1998; Seleena *et al*, 1999).

Dengue vector control in urban/suburban areas requires ground application. In this study Bti was applied to all mapped larval habitats. Residential areas had few water storage containers (>50 liters) either indoors or outdoors, in treated and untreated sites, since both sites had piped water. We only treated 9 such containers with direct Bti application at 8 g/1,000 liters. Our main focus was outdoor spray application, targeting concrete perimeter drains, roof gutters and vegetation. We treated potential dry and wet habitats. Treatment of outdoor containers, in the absence of indoor container habitats, was sufficient to cause a significant improvement in indoor mosquito control, as seen by the significant reduction in indoor OI and LD.

Concrete drains are covered, but have openings at regular intervals. These were seen to hold stagnated clear water on all days. Drain covers prevented us from conducting thorough larval surveillance to detect the presence of immature *Aedes* spp in the drains. The clear drain water has been found to support higher oviposition activity with 6 fold more eggs than seasoned tap water and support full colonization to successful emergence of adults (Chen *et al*, 2007). Thus, in the absence of any other artificial containers, concrete drains were considered as the principal potential habitat for *Ae. aegypti*; hence, we applied larvicide to the drain waters. There was a significant reduction in indoor and outdoor OI in the *Ae. aegypti* population during the study period, showing treatment was effective. Similar results were observed in Taman Samudera where concrete perimeter drain waters were also treated with Bti (Lee *et al*, 2008).

We also treated all vegetation in the front and back yards of homes, since the vegetation able to hold water is known to be a habitat for *Ae. albopictus* (Hawley, 1988; Braks *et al*, 2003). There was a good reduction in indoor and outdoor OI and LD for *Ae. albopictus*. The WHO (2009) in its guidelines for dengue diagnosis, treatment, prevention and control stated larvicides may be impractical to apply in hard-to-reach natural sites, such as leaf axils and tree holes, which are common habitats of *Ae. albopictus*. However, with suitable equipment, back pack mist blowers and vehicle mounted ULV generators, wide areas of treatment with fine Bti droplets can target these micro-habitats in vegetation and significant reductions in adult *Ae. albopictus* can be achieved in dense vegetation (Lam *et al*, 2010).

Public health education emphasizes keeping residential vicinities free of *Aedes* spp larvae and source reduction. The public are encouraged to check for larvae within their homes and in the surrounding vicinity at least once a week; the maximum fine imposed on an owner for failing to do so is RM 500.00 (USD 156.00) (NST, 2010). Residents are aware of this regulation and do play their part in preventing dengue outbreaks. Despite these efforts, dengue still occurs. This could be due to ongoing habitats outside public jurisdiction, such as perimeter drains and vegetation. Concrete perimeter drains have concrete covers in front of residents' homes with openings at end of the home, the drains have stagnated rain water and most have leaf litter lining the drain. Malaysia, which has tropical weather, receives an average rainfall of 2,000 mm. Thus, a well designed and constructed drainage system is required where litter is prevented from entering into waterways, but flow of water is unobstructed and the drain can completely dry out after rainfall. Respective government agencies need to ensure the drains are built to specifications where they will not serve as vector habitats. Also the agencies must be committed to continuous maintenance of the drains, and not be dependent on residents.

Source reduction, environmental maintenance and integrated regular larviciding with Bti to provide sustainable dengue control may prevent dengue outbreaks. Training of applicators to identify and treat larval habitats is an important component in a dengue control program utilizing Bti as a larvicide.

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