Suppression of Dengue Transmission by Application of Integrated Vector Control Strategies at Sero-Positive GIS-Based Foci

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Abstract. A serological survey of primary school children from six schools in Chachoengsao Province, Thailand, was performed at the end of the peak of dengue transmission. GIS analysis of sero-positive cases was carried out to determine transmission foci. Vector control implementation was conducted in the foci and also within 100 meters around the foci in the treated areas by community participation in collaboration with the local government. Vector control strategies included source reduction together with the use of screen covers, a combination of Bacillus thuringiensis subsp. israelensis and Mesocyclops thermocyclopoides, and lethal ovitraps. Implementation of vector control strategies in the foci was continued until the end of the rainy season. Vector control effectiveness was monitored using entomological, serological, and clinical parameters. Results showed a significant reduction of dengue vectors as well as a decrease in sero-positive children and clinical cases in treated areas when compared with untreated areas.

INTRODUCTION

Dengue fever, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) are considered important diseases caused by vector-borne pathogens. The global prevalence of these diseases has grown dramatically in recent decades. The disease occurs in over 100 countries and territories and threatens the health of more than 2.5 billion people in urban, peri-urban, and rural areas of the tropics and subtropics. The major disease burden is in Southeast Asia and the Western Pacific. Globally, the annual number of infections is much higher than is indicated by the number of reported cases. Based on statistical modeling methods, there are an estimated 51 million infections each year. Rapid expansion of urbanization, inadequate piped water supplies, increased movement of human populations within and between countries, and further development and spread of insecticide resistance in the mosquito vector populations are some of the reasons for the increase of dengue transmission in recent years.

Thailand is located in the heart of the endemic areas for dengue and DHF. Therefore, this disease was considered one of the most important public health problems and had an impact on the economics of Thailand. The Ministry of Public Health must spend an enormous part of the net budget every year to purchase chemical insecticides for dengue vector control operation; however, this effort seems to have failed because the reported number of DHF cases in Thailand remains high. Furthermore, a large amount of the national health budget has been spent on the treatment of dengue patients. Therefore, there is an urgent need to review the strategies for preventing and controlling dengue as well as to conduct research to improve vector control methodologies.

Excellent reviews of dengue vector control have stressed several relevant aspects of successful programs (i.e., technical, social, and organizational parameters). So far, there have been quite a few examples for successful vector control in a few countries (i.e., Taiwan and Vietnam). However, in Thailand, past efforts to stop dengue transmission through vector control have failed. In this article, we report a strategy for integrated, community-based dengue control intervention suitable for semi-rural and rural Thailand that could successfully suppress dengue transmission in a targeted community. Our ultimate goal is to develop a cost-effective and practical vector control model for preventing and controlling dengue and DHF in Thailand.

MATERIALS AND METHODS

Study areas and serological survey for dengue infection. The study area was located in Hua Sam Rong and Wang Yen Subdistricts, Plaeng Yao District, Chachoengsao Province, approximately 120 km east of Bangkok, Thailand. The areas were composed of many semi-rural and rural villages clustered among rice fields and orchards. Water supply in the study area came from both tap water and rainwater but people still stored water in various types of containers. Based on recent dengue incidence records, several dengue foci and non-dengue foci in Village No. 10 of Hua Sam Rong Subdistrict and Village No. 7 of Wang Yen Subdistrict were selected as the pilot treatment and control areas, respectively.

Serological survey for dengue infection was carried out in a total of six local schools with a population of approximately 1,800 students ranging from kindergarten to grade 12. The elementary schools in Hua Sam Rong Subdistrict were Hua Sam Rong School, Ao Chang Lai School, and Sawan Nimith School, whereas those in Wang Yen Subdistrict were Tung Sadao Prachasan School, Wang Kaja School, and Wang Yen School. These schools included most of the students from the study area both in Hua Sam Rong and Wang Yen Subdistricts.

Enzyme-linked immunosorbent assay (ELISA) tests for serology were carried out according to the MAC-ELISA test routinely used at the Center for Vaccine Development (CVD), Mahidol University at Salaya. Sera were first tested for IgM isotypes by using a mixture of tetravalent dengue virus antigens and...
alternatively for IgG using Dengue-2 antigens prepared by sonication in standard borate saline, pH 9.0, with 1% Triton X-100.

All antigen preparations were clarified by centrifugation and frozen in aliquots at −80°C until used. Antigens for both assays were optimized using reference antisera. Sample sera were screened at an initial dilution of 1:100 and titrated 4-fold. Sera were considered IgM-positive if the adjusted optical density (OD) were equal or greater than 0.5 at the 1:100 dilution (as compared with reference sera). In this particular study, we also observed elevated levels of IgG-positive sera in the second year upon completion of dengue control intervention. Sera that exceeded 1.00 were considered positive. The ELISA data are interpreted according to a method modified from Innis and others12 and Johnson and others.13

GIS mapping of dengue foci and Aedes-positive containers. Locations of all houses in both treatment and control areas were measured by a Leica GS5 + global positioning system (GPS) unit (Leica Geosystems Inc., Torrance, CA), which had an error of ± 3 meters. The GIS software to manipulate the field data was ArcPad (ESRI, Redlands, CA) of the Pocket PC iPAQH 3850 (Compaq Information Technologies Group, Palo Alto, CA) that was linked to the GPS unit. The field-collected data were transferred to a personal computer at the Center of Excellence for Vectors and Vector-Borne Diseases (CVVD), Faculty of Science, Mahidol University at Salaya, Nakhonpathom. The GIS map of the study area was then produced from the digital-based map of the Royal Thai Survey Department of Thailand (scale of 1:50,000) using the ArcView program (ESRI, Redlands, CA).

The treatment areas for a pilot dengue vector control intervention program were based on high anti-dengue immunoglobulin (IgM and IgG) in local school children, as measured, conducted after the peak transmission of dengue. The areas of dengue foci were defined as a group of houses within a 100-m radius of the houses that had IgM- and IgG-positive students. The areas outside of dengue foci were defined as groups of houses within a 100-m radius of the houses that had no IgM- and IgG-positive students. The survey of water containers positive for Aedes immatures, both inside and outside of dengue foci of treated and control areas, was conducted according to the methodologies described in Strickman and Kittayapong14 and the data were used to generate the GIS map for targeting vector control intervention.

Integrated vector control strategies applied at dengue foci of treated areas. Implementation of vector control strategies were based on four simple methodologies as follows: 1) source reduction/clean-up campaign followed by weekly garbage pick-up; 2) screen covers for water jars; 3) a combination of M. thermocyclopoideis (copepod) and B. thuringiensis subsp. israelensis (Bti) for various permanent containers other than water jars; and 4) permethrin-treated lethal ovitraps for controlling adult mosquitoes. These vector control methodologies were implemented in the transmission foci of the treated areas. Control activities were also conducted in the schools where the children from the treated areas attended.

Before the control activity was carried out, a clean-up campaign was organized in the treated areas just before the rainy season by the Local Administrative Authorities of Hua Sam Rong Subdistrict together with the participation of households, school children, public health volunteers, and public health officers to discard all garbage and disused containers in these areas. The clean-up campaign did not affect the untreated areas located in another subdistrict. After the campaign, weekly garbage pickup was organized by the Local Administrative Authority of Hua Sam Rong Subdistrict and was continued throughout the rainy season. During the control intervention, three types of screen covers designed to interfere as little as possible with water use habit in the community were used to prevent development of Aedes immatures in water jars, which are the most common and productive breeding containers.15 A combination of local predaceous copepods and locally produced Bti in liquid formulation was inoculated into water containers that could not be covered by any screen cover types. These containers could be categorized as cement bath basins, cement foot-bath basins, hygiene jars in the bathroom, ant traps, flower vases, metal or plastic drums, animal-watering vessels, and miscellaneous containers (e.g., discarded tires, pots, bowls, and buckets). The numbers of copepods and the volume of Bti inoculated into these containers depended on sizes or capacity of containers. The volume of Bti used in different types of breeding containers followed Chansang and others.16 Five to ten chemical-based lethal ovitraps, which were composed of a black plastic cup lined with permethrin-treated filter paper were produced locally and distributed in each house of the treated areas to kill the adult Ae. aegypti. These lethal ovitraps were a modification of those developed by Zeichner and Perich.17

Evaluation of vector control implementation. Before the source reduction/clean-up campaign, a survey of immature mosquitoes was conducted in the treated areas in Hua Sam Rong Subdistrict to determine the key breeding containers for targeting vector control implementation. After the control activities were carried out in the treated areas, the efficacy of combined vector control agents was evaluated weekly by the observation of Ae. aegypti immature reappearance. Immature surveys were conducted and recorded as the number of containers that were either positive or negative for Aedes larvae. Replication of copepods and Bti would be conducted in any water containers that had mosquito larvae and pupae. In addition, weekly mosquito landing collections were performed by two persons to evaluate the overall control intervention. For the untreated areas in Wang Yen Subdistrict, which served as the control community, no mosquito control was performed during this study. However, monthly immature surveys and weekly adult landing collections by two persons were conducted to compare the results between treated and untreated areas due to the vector control intervention. The total numbers of dengue patients from both treatment and control areas admitted to local hospitals and reported to the Local Public Health Office before and after the control intervention were compared. In addition, evaluation of IgG–IgM positive cases12,13 based on serological surveys in schools before and after the control intervention were intended to be clear determinants of the success of this integrated vector control intervention program. Evaluation of the vector control program by mosquito landing collection and serological survey of school children had been approved by the Ethical Advisory Committee of the Ministry of Public Health of Thailand.
RESULTS

GIS mapping of dengue foci and *Aedes*-positive containers. Serological surveys of primary school children from six local schools in Plaeng Yao District, Chachoengsao Province, eastern Thailand were analyzed to locate the dengue foci for targeting vector control implementation as well as to determine the success of vector control intervention in the areas of concentrated transmission. When compared between the first-year and the second-year serological results, the overall IgG–IgM positive rates were 1.85% (30 of 1,625) and 6.72% (118 of 1,755), respectively. The houses of IgG–IgM positive students and the number of immature positive containers in the treated and untreated areas were mapped by GIS and are shown in Figure 1. The entomological parameters (i.e., the average number of positive containers per house and the average number of pupae per house with 95% CI) in treated and untreated areas were reported in Table 1. For the treated

![GIS map](image_url)

**Figure 1.** The GIS map showing the location of houses of IgG-IgM positive students and the number of containers positive for *Aedes* mosquitoes in the (A) treated and (B) untreated areas.
community, the average number of positive containers per house with 95% CI in the dengue foci was $4.45 \pm 0.33$ (3.79–5.10) and out of dengue foci was $2.51 \pm 0.27$ (1.97–3.04), which was significantly different ($t = -3.493, P = 0.001, df = 150$). Based on these entomological survey data, the major breeding containers were identified to be various sizes of water jars and cement bath basins.

**Entomological, serological, and clinical monitoring.** Entomological, serological, and clinical parameters were used to evaluate our dengue control intervention. Results showed a
dramatic reduction in the number of the Aedes-positive containers in the treated areas after the application of these vector control strategies (Figure 2). In addition, results of the mosquito landing collections in treatment and control areas are demonstrated in Figure 3. These results confirmed that the number of Aedes mosquitoes in the treated areas, especially inside the dengue foci, was significantly reduced after implementation of the vector control intervention program. However, Ae. aegypti were still present in the community even though the number was low when compared with the untreated areas. To evaluate whether our control intervention had any effect on dengue transmission in the treated community, serological data collected before and after intervention from children living in treated and untreated areas were compared. Despite the higher dengue incidences in the second year when compared with the first year, the proportion of IgG-IgM positive students in the treated areas was reduced from 13.46% to 0% whereas those from untreated areas increased from 9.43% to 19.15% (Table 2). In addition, dengue cases reported at the local hospital were recorded and the proportion of dengue cases per 100,000 populations is shown in Table 2. There were no dengue cases in the treated areas increased in the untreated areas when compared between the years before (217.9/100,000) and after (322.2/100,000) intervention. These results confirmed that the focal vector control intervention could successfully suppress dengue transmission in the treated areas.

**DISCUSSION**

Thailand has a long, distinguished history of research on dengue prevention and control. Recently, the case fatality rate of dengue in Thailand declined from 13% in 1958 to 0.34% in 1998. This improvement resulted from the long experience of clinical diagnosis and management more than the vector control program. Appropriate control methodologies of mosquito vectors are still needed for the long-term suppression of dengue vector populations below the threshold level for dengue transmission. It has been recommended that the policy on vector control programs in Thailand should emphasize environmental management methods and community involvement. So far, there has been no distinct record of any successful dengue control program using these recommended strategies. Our pilot dengue control intervention program demonstrates the use of a combination of both governmental top-down and community-based bottom-up approaches. The combination approach is expected to have a faster effect and last longer than either one of the approaches alone. This dengue vector control program is currently expanding to other communities in the same area by the local government. However, sustainability and scale-up of this successful program at a national level are still in question and a challenge to the public health authorities.

In Thailand and in a few other countries in Southeast Asia, water jars are the main containers used for storing water. These containers are the major breeding sites for dengue vectors, especially Ae. aegypti. Aluminum lids are commercially available to cover these water jars. However, Strickman and Kittayapong demonstrated that the dengue vectors could get inside to lay eggs and that the immature vectors could still develop in the water jars that were permanently covered with the aluminum lids. Three types of simple screen covers that could prevent development of immature mosquitoes were developed to suit the water use habit of the people in the community. These screen covers were implemented in the previous intervention program and the efficiency was evaluated to be high (Kittayapong and Strickman, unpublished data). However, education was necessary to convince the community to use these screen covers to prevent the development of Aedes immatures in preference to the use of insecticide spraying to control adult mosquito vectors. Moreover, these types of screen covers could be applied to use in other Southeast Asian countries where similar types of water jars are the main

![Figure 3](image.png)

**Figure 3.** Reduction of landing Aedes aegypti female mosquitoes in the transmission foci of treated areas after dengue vector control intervention.

<table>
<thead>
<tr>
<th>Positive containers/house</th>
<th>Pupae/house</th>
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<tbody>
<tr>
<td>(Mean ± SE) (95% CI)</td>
<td>(Mean ± SE) (95% CI)</td>
</tr>
<tr>
<td>Treated area</td>
<td>3.39 ± 0.22 (2.95-3.82)</td>
</tr>
<tr>
<td>Inside dengue foci</td>
<td>4.45 ± 0.33 (3.79-5.10)</td>
</tr>
<tr>
<td>Outside dengue foci</td>
<td>2.51 ± 0.27 (1.97-3.04)</td>
</tr>
<tr>
<td>Untreated area</td>
<td>1.42 ± 0.15 (1.13-1.72)</td>
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<th>Comparison of serologically positive children and clinical cases of dengue in treated and untreated areas before and after vector control intervention</th>
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<tr>
<td><strong>Serological cases</strong></td>
</tr>
<tr>
<td>% IgG-IgM positive (n)</td>
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<tr>
<td>Year 1 (treated)</td>
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<tr>
<td>Treated area</td>
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<tr>
<td>Untreated area</td>
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sources for water storage, as the cost per screen cover is low and they usually last for 1–2 years (Kittayapong and Strickman, unpublished data).

A large number of publications from many countries have demonstrated the success of biological control agents such as predaceous copepods for controlling mosquito vectors. They have been used as a single application and in combination with other techniques.20–26 The combination of Bti and copepods was reported to prolong the control efficiency in the laboratory and in a semi-natural situation.16–27 Because clean-water containers was one of the routine habits of householders in rural Thailand, reaplication of these biological control products was necessary. However, our results first demonstrated the successful application of this combined method in a natural field situation in Thailand.

The use of ovitraps in the surveillance of Aedes vectors was recommended by the World Health Organization.28 However, some models of ovitraps could be modified to be lethal to both adults and immatures of Ae. aegypti.17,29–31 The advantages of lethal ovitraps for controlling Aedes vectors are related to their specificity and efficiency against only container breeders, particularly Ae. aegypti. In addition, lethal ovitraps could be integrated with other control methodologies such as chemical control and biological control. In Mexico, a newly designed sticky ovitraps was used to determine the dispersal flight range of Ae. aegypti and it was then recommended for the dengue control program.32 Integration of lethal ovitraps in our control activities was expected to help reduce the adult populations emerged from immatures that were not killed by physical or biological control approaches. Lethal ovitraps might also prevent immigration of Ae. aegypti to uninested areas.

In conclusion, integrated vector control tools (i.e., screen covers), a combination of copepods and Bti and lethal ovitraps, together with the community participation for a source reduction/clean-up campaign were targeted in the dengue foci of the treated areas where higher numbers of both sero-positive cases and Aedes-positive containers were detected. This intervention program demonstrates that an integration of simple vector control tools together with community participation could suppress dengue transmission. All vector control tools were locally produced using local strains or cheap materials, which made this program cost effective. However, our study design could not determine which vector control tools were most effective. The refinement of this vector control program could be conducted using cluster randomized trials, which is the standard method used for the evaluation of new health interventions.33 With this method, both single and combined vector control tools could be simultaneously evaluated and compared in several randomized communities.

Our vector control intervention program was conducted in areas of concentrated transmission based on the results from a serological survey of school children who live permanently in the study area. The use of serological data in this study should increase the opportunity to identify dengue foci for vector control intervention as the asymptomatic cases were also detected. However, even though such serological data are useful for research, they are not easily obtained and may be too expensive for routine monitoring of dengue control programs. In this case, the history of reported DHF cases may be a more practical tool for producing the GIS map of the risk areas to target vector control effort. Our preliminary data showed the correlation of sero-positive and reported cases of DHF. The public health system is in place in all provinces in Thailand to report dengue cases. The mapping of reported DHF cases to locate the specific areas for targeting vector control should be feasible and should help reduce the cost required for successful dengue control, especially in most developing countries.

Received February 1, 2006. Accepted for publication May 25, 2007.

Acknowledgments: We are grateful to the officers of Plaeng Yao Hospital and the Local Public Health Office for their assistance in the serological surveys; the Local Administrative Office of Hua Sam Rong Subdistrict for their help in coordinating the source reduction/clean-up campaign and organizing routine garbage pickup during implementation. The authors thank Mr. Tanong Aimmak for coordinating the overall project; Mr. Somboon Srimarit for collecting mosquitoes and GPS positioning of houses; and also people in the study area of Hua Sam Rong and Wang Yen Subdistricts, Plaeng Yao District, Chachoengsao Province for their good cooperation. The ethical approval of this study was granted by the Ethical Advisory Committee of the Ministry of Public Health of Thailand.

Financial support: This investigation received financial support from the UNICEF/UNDP/World Bank/WHO Special Programme for Tropical Diseases Research and Training (TDR/RC/R00786) and the Mahidol University Research Grant (SCBI-47-T-217).

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