Evaluation of the present dengue situation and control strategies against *Aedes aegypti* in Cebu City, Philippines

Milagros M. Mahilum [1,2], Mario Ludwig [1], Minoo B. Madon[3], and Norbert Becker[1,2]✉

[1]*German Mosquito Control Association (KABS/GFS), Ludwigstrasse 99, 67165 Waldsee, Germany*
[2]*ICYBAC GmbH, Georg-Peter-Süß-Str.1, 67346 Speyer, Germany*
[3]*Greater Los Angeles County Vector Control District, 12545 Florence Avenue, Santa Fe Springs, CA 90670-3919, U.S.A.*

Received 11 November 2004; Accepted 22 July 2005

ABSTRACT: The present dengue situation and methods to control *Aedes aegypti* larvae in Cebu City, Philippines, were evaluated for the development of an integrated community-based dengue control program. The study included the detection of dengue infection among Filipino patients, surveying mosquito breeding sites to determine larval population density of *Aedes aegypti*, an evaluation of public knowledge, attitude, and personal protection practices against dengue, and an evaluation of the efficacy of VectoBac® DT/Culinex Tab tablets based on *Bacillus thuringiensis israelensis* against *Ae. aegypti* larvae. Of the 173 human sera samples that were assayed for dengue viruses, 94.9% were positive, 2.2% negative and 2.8% equivocal. Thirty households were randomly chosen per Barangay "Villages" (lowest level of formal local administration). Of the 489 breeding sites surveyed, 29.4% were infested with *Ae. aegypti* larvae, with discarded tires having the highest infestation rate (69.4%). A survey of people's knowledge, attitude, and practices for integrated community-based dengue control showed that 68.7% of the interviewees were aware that dengue is transmitted by mosquitoes, but only 4.3% knew that a virus was the cause of the disease. The efficacy of one and two tablets of VectoBac® DT/Culinex® Tab, based on *Bacillus thuringiensis israelensis*, was assessed against the larvae of *Ae. aegypti* exposed to sunshine and shaded water containers in semi-field and field tests. In semi-field tests, 100% mortality was achieved until the 18th and 30th day after the application of one and two tablets, respectively, in sun-exposed containers. In shaded containers, 100% mortality was observed until the 30th and 36th day after the application of one and two tablets, respectively. In field tests, the tablets were effective for approximately 3 weeks. ***Journal of Vector Ecology* 30 (2): 277-283. 2005.**

Keyword Index: Aedes aegypti, Bacillus thuringiensis israelensis, dengue viruses, Philippines.

INTRODUCTION

Dengue fever (DF) and dengue haemorrhagic fever (DHF) are major public health problems. Since the first global appearance of DHF in Manila, Philippines in 1953, five years later in Bangkok, Thailand, and 1968 in Surabaya, Indonesia, it is presently considered one of the leading viral diseases causing hospitalization and death among children and adults in 100 tropical and subtropical countries throughout America, South-East Asia, the Western Pacific islands, and Africa. About 500,000 people are hospitalized (95% of those affected are children) and about 24,000 fatalities are reported annually (Halstead 1980, 1982, WHO 1997).

Dengue fever outbreaks are rising in South America and Asia. Among the affected countries in Asia, the Philippines is considered as one of the "high risk" zones. The resurgence of dengue can be traced to rapid urbanization, poor sewage systems, and improper disposal of garbage. The persistence and numerical increase of *Ae. aegypti* (Linnaeus) as a primary vector and *Aedes albopictus* (Skuse) as a secondary vector is partly attributed to the sanitary and hygienic practices. The urbanization process has left many households with inadequate water supplies and has hastened the spread of the disease. Unfortunately, the majority of the people do not realize the seriousness of the situation until they become infected. Many people dispose of their garbage and waste just outside their door step, onto the streets, and in vacant lots. Eventually, the improperly disposed waste accumulates water during rainfall, becoming potential breeding sites for *Ae. aegypti.*

The rapid increase in the human population in the Philippines has contributed to the rising dengue problem. Between 1991-2003, the highest numbers of dengue cases were recorded in 1998, with 35,648 cases and 514 deaths. The majority of the cases were children between 1-9 years (DOH 2003).

This study evaluates the present dengue situation and control strategies against *Ae. aegypti* for the development of an integrated community-based dengue control program in Cebu City, and includes the detection of dengue viruses among Filipino patients, a survey of mosquito breeding sites and infestation rates of *Ae. aegypti* larvae, an evaluation of people's knowledge, attitude, and practices towards dengue infection, and an evaluation of the efficacy of *Bacillus thuringiensis israelensis (B.t.i.)* tablets against *Ae. aegypti* larvae.

MATERIALS AND METHODS

Detection of dengue viruses among the patients

Blood samples from patients were collected from January to May 1999 at the Cebu City Medical Hospital. One hundred seventy-three sera samples, consisting of 108 female and 65

male patients suspected of having dengue infection were assayed using a commercially available enzyme-linked immunosorbent assay (ELISA) test (PanBio, Australia, Cat. No. DEG-100). Sera samples were defrosted one night prior to the test according to the standard procedure. Five microwells in triplicate were used, each for positive and negative control as well as the cut-off calibrator. With 10 µL of patient's serum, 1000 µL of serum diluent (provided in the kit) was added and mixed thoroughly. Alternatively, to 10 µL of patient's serum, 90 µL of serum diluent was added and from this mixture, 20 µL added to 180µL of serum diluent and mixed again thoroughly. One hundred µL of the mixture was pipetted into each microwell of the positive, negative, and cut-off calibrator. The microwells were covered and incubated for 30 min at 37° C and were washed manually six times.

One hundred µL of horseradish peroxidase (HRP) conjugated anti-human IgG were added to each microwell, covered and incubated for 10 min at 37° C. The microwells were washed again manually and the bound complexes were visualized through the addition of 100 µL tetramethylbenzidine (TMB) in each microwell. After 30 min, the absorbance of each microwell (presence of dengue viruses) was read by using a microtiter plate reader with 450 nm wavelength. PanBio units were computed according to the standard procedure. Positive samples for dengue viruses were defined as having PanBio units of >11, negative with <9, and equivocal (either + or -) with 9-11.

Survey of mosquito breeding sites and infestation rates of *Aedes aegypti* larvae

The study was conducted in Cebu City which is located at the center of the archipelago (9°25' N and 11°30' N and between 123°25' E) about 400 miles south of Manila. The topography of Cebu is characterized by narrow coastlines, limestone plateaus, and coastal plains but with predominant rolling hills and rugged mountain ranges traversing the northern and southern lengths of the island. The climate is relatively moderate, having no distinct wet and dry season with a temperature range of between 23-33° C (73-91° F). Coolest temperatures can be felt in January and warmest in May. Cebu has a population of about 3.5 million with the city proper accounting for 718,821.

Five Barangays "Villages" in Cebu City were selected for the study because of their high incidence of dengue fever during the past five years: Lahug, Labangon, Guadalupe, San Nicolas, and Pardo (CCHR 2003).

The survey was conducted over a one month period. Thirty households were randomly chosen per Barangay. In each household, the specific breeding sites and their numbers were recorded as follows: 20 L and 50 L water containers, tin barrels, discarded tires, flower pots, tin cans, discarded plastics, and other breeding sites such as leaf axils and coconut husks. The larvae were collected randomly from major breeding sites and species determination was conducted in the laboratory according to Schoenig taxonomic key (1971, 1977). Pupae were not included for the evaluation. The number of larvae/breeding sites was estimated using the following ranges: 0, 1-10, 11-25, 26-50, and above 50 larvae/breeding site. To quantify the infestation rates, standard indices as House Index (HI), Container Index (CI), and Breteau Index (BI) were determined.

Evaluation of people's knowledge, attitude, and practices towards dengue infection

The evaluation was conducted from June to November 1998 in Barangay T. Padilla, Cebu City, Philippines. This Barangay has a total surface area of 14 hectares with a population density of 857 people/ha. Interviews at 371 households were conducted randomly. The questionnaires contained information on household composition including socio-economic aspects of the individuals, experiences of dengue fever and related symptoms, and people's knowledge of the symptoms, causes, transmission, and prevention of the disease as well as vector control measures at the household.

Evaluation of the efficacy of *B.t.i.* tablets against *Aedes aegypti* larvae

VectoBac® DT/Culinex Tab tablets based on *B.t.i.* were used in the tests. The tablets (weight 384 mg) had a potency of 2,700 ITU/mg. A semi-field test was conducted from December 17, 2003 to January 28, 2004 in Cebu City. Thirty plastic water containers (each with a 50 L capacity) were used. Fifteen plastic water containers were exposed to sunshine: five of them were treated with one tablet each, another five with two tablets each and the remaining five served as a control. The water containers were covered with fine nets to prevent egg-laying by adult mosquitoes. Another 15 plastic containers were placed in a shaded area and treated in the same way as described above. Twenty larvae of *Ae. aegypti* (late 2nd and early 3rd instars) were released in each container before the start of the experiment and every three days during the study. Before the release of the new set of larvae, the remaining living larvae in each container were removed. In the control groups, all the larvae were replaced every three days. Mortality rate of the larvae was evaluated every three days starting from the application of the tablets until a mortality rate of less than 50% was observed. For statistical analyses, data were subjected to Duncan's multiple range test and/or Student's *t*-test (Köhler et al. 1984).

Eleven major breeding sites of *Ae. aegypti* that were exposed to sunshine and in shaded areas were chosen from various houses. The breeding sites included: drums of 200 L water capacity, pails of 25 L water capacity, cement catch basins of 10 L water capacity, old tires, and water jars (Table 1). Breeding sites that contained *Ae. aegypti* larvae with approximately 1 to 50 L of water were treated with one tablet, while breeding sites with more than 50 L of water were treated with two tablets. For the control group, three different breeding sites were chosen: drums (each with a 200 L capacity), plastic containers (each with a 200 L capacity) and cans (each with a 25 L capacity). Mortality rates of *Ae. aegypti* larvae were evaluated every three days starting from the application of the tablets until the breeding sites were re-infested with 4th larval instars of *Ae. aegypti*.

Table 1. Description of breeding sites in the field test.

Breeding site no.	Water Capacity (L)	Quantity Of Water Present (L)	Location (Exposed Or Shaded)	No. of *Ae. aegypti* larvae present	No. of Tablets Applied
1) Drum	200	approx. 20	shaded	40-50 larvae (2^{nd} & 3^{rd} instars)	1
2) Plastic pail	25	approx. 2	exposed	5 larvae (2^{nd} instars)	1
3) Drum	200r	approx. 5	shaded	100-150 larvae (all instars)	1
4) Drum	200	approx. 50	exposed	15-20 larvae (2^{nd}, 3^{rd} & 4^{th} instars)	2
5) Drum	200	approx. 20	exposed	150-200 larvae (all instars)	1
6) cement water catch basin	5	approx. 3	exposed	100-120 larvae (2^{nd} & 3^{rd} instars	1
7) Drum	200	approx. 2	exposed	40-50 larvae (2^{nd}& 3^{rd} instars)	1
8) Old tire	10	approx. 2	exposed	400-500 larvae (2^{nd}, 3^{rd}, & 4^{th} instars)	1
9) cement water catch basin	2,000	approx. 1,250-1,500	shaded	3,000-4,000 larvae (all instars)	4
10) Drum	200	approx. 100	shaded	150-200 larvae (3^{rd} & 4^{th} instars)	1
11) water jar	25	approx. 10	exposed	200-250 larvae (3^{rd} & 4^{th} instars)	1
12) Drum	200	approx. 50	exposed	15-20 larvae (2^{nd} & 3^{rd} instars)	Control
13) Can	25	approx. 1	exposed	10-15 larvae (2^{nd} & 3^{rd} instars)	Control
14)Plastic container	200	approx. 150	shaded	100-150 larvae (all instars)	Control

RESULTS

Detection of dengue viruses infection among patients

94.9% (164 out of 173) of the sera samples were positive for dengue viruses, comprising 101 females and 63 males. Only 2.2% (4 out of 173) were negative, comprising 3 females and 1 male and 2.8% (5 out of 173) comprising 4 females and 1 male were equivocal.

Survey of mosquito-breeding sites and infestation rates of *Aedes aegypti* larvae

Out of 873 mosquito larvae sampled, 799 were *Aedes aegypti* (90%), 74 *Ae. albopictus* (9%), and *Ae. scutellaris* (1%). Of the 489 breeding sites, 29.4% (142) were found to be infested with mosquito larvae. In terms of percentages of the infested breeding sites, discarded tires had the highest rate with 69.4% (50 out of 72). Discarded plastic containers were 68.4% (13 out of 19), and other breeding sites such as leaf axils, coconut husks, and flower pots were next with >50%. Among the breeding sites with the least number of larvae were 20 L and 50 L capacity containers with 3.9% (7 out of 195) and 5% (1 out of 20), respectively (Table 2). According to the indices, Barangay Lahug ranks first with a house index of 93.3% followed by Guadalupe with 56.7%, San Nicolas with 43.3%, Labangon with 36.7%, and Pardo with 33.3%. For the container and Breteau indices, similar ranking was recorded as Barangay Lahug showed the highest rate of 55.7% and 230, respectively, and Barangay Pardo the lowest with 14.4% and 50, respectively (Table 3).

Evaluation of people's knowledge, attitude, and practices towards dengue

The average age of the respondents was 38 years, 79.8% females and 20.2% males. The average number of persons in a household was six and the average number of children was three. Sixty-three percent of the population consisted of children below 15 years of age and the average age of residence was 22. Twenty-one percent of the respondents were housewives and 40% of them attended high school. Forty-nine percent of the respondents' homes were located near open canals and 57% were constructed with cement and wood. Furthermore, 64% of the respondents had a direct source of tap water.

One hundred sixty-nine of 371 (45.5%) respondents had experienced general febrile conditions during the last six months before the interview. Many of the interviewees (65%) mentioned fever, however, only 9.4% reported the typical symptom of body pains and 17.8% reported headache. While 68.7% of the interviewees were aware that the disease is transmitted by mosquitoes, only 4.3% of the respondents knew that a virus was the cause of the disease.

About half (52.3%) of the respondents were aware of the potential danger of mosquito breeding sites and were also aware that adulticiding (fogging) was being done in their community. 48.5% of the respondents commented negatively on fogging and 67.7% agreed that fogging should be done in their Barangays. Vector control measures at the household level reported by the respondents included: cleaning up the surroundings, using mosquito nets, repellents (mosquito coils), and screening their homes. Almost half of the respondents were aware of the Barangay-initiated anti-dengue drive

Table 2. Specific types of mosquito-breeding sites and larval infestation in five selected Barangays.

Breeding sites	Number of larvae				Total breeding sites		Total sites surveyed	% of sites w/ larvae
	1-10	11-25	26-50	50 and above	w/ larvae	w/out larvae		
Water container (up to 20 L)	4	3	0	0	7	188	195	3.59
Water container (up to 50 L)	0	0	1	0	1	19	20	5.0
Barrels	12	4	4	2	22	56	78	28.2
Discarded tires	22	11	9	8	50	22	72	69.44
Flower pots	6	1	0	1	8	8	16	50
Tin cans	8	1	0	0	9	17	26	38.46
Discarded plastics	6	1	2	4	13	6	19	68.42
Other breeding sites (e.g. leaf axils, etc.)	16	5	8	3	32	31	63	50.79
Total					142	347	489	
Percentage (%)					70.96	29.04	100	

programs and 48% were willing to attend them.

Evaluation of the efficacy of *B.t.i.* tablets against *Aedes aegypti* larvae

VectoBac® DT/Culinex Tab tablets were very effective against the larvae of *Ae. aegypti*. In the semi-field test, 100% mortality of *Ae. aegypti* larvae was obtained until the 18th day and 57% mortality on the 27th day by the application of one tablet in containers exposed to sunshine. Comparison of controls with statistical means showed that after the 33rd day, there was no significant ($P<0.01$) efficacy of the tablets remaining. With the application of two tablets in exposed areas, 100% mortality of the larvae was observed until the 30th day and 55% on day 33. In shaded areas, a mortality rate of 100% was achieved until the 30th day, with 85% on the 33rd day and 58% on the 36th day with the application of one tablet. By comparison with controls ($P<0.01$), it could be demonstrated that after the 42nd day, the tablets had completely lost their efficacy. With the application of two tablets in shaded areas, 100% mortality was achieved until the 36th day, 82% on the 39th day, and 55% day 42. Using Duncan's multiple range test, it was demonstrated that in the sun exposed area, efficacy of B.t.i. dropped significantly ($P<0.01$) after the 27th day (one tablet) and 33rd day (two tablets) respectively, whereas in the shaded area, 100% efficacy was still observed. In the control group, in shaded and in sunny areas, mortality rates of 1 - 5 % were observed during the course of the study (Table 4).

In field tests, the tablets were effective for approximately 3 weeks (Table 5). In the control group, the containers were infested from the beginning until the end (3 weeks) of the experiment with increasing numbers of larvae.

Table 3. House, container, and Breteau index values in each Barangay.

Barangay	House index	Container index	Breteau index
Guadalupe	56.66	29.89	90
Lahug	93.33	55.37	230
San Nicolas	43.33	25.27	83.33
Labangon	36.66	15	56.66
Pardo	33.33	14.44	50.0

DISCUSSION

Detection of dengue viruses infection among patients

The results obtained from the ELISA tests showed a very high rate (94.9%) of patients positive for dengue viruses. These figures correlate with the results obtained by Buerano et al. (2000) who showed that 80.4% of sera samples were positive for dengue viruses. These figures are not surprising because the samples were collected from a hospital where poor patients came for treatment, having no money for proper medication, and perhaps preferring to take some herbal medications. These people usually come to the hospital only when they become severely ill.

Survey of mosquito-breeding sites and infestation rates of *Aedes aegypti* larvae

The species composition obtained in this study correlates to the results of Schoenig (1977). The abundance of mosquito-breeding sites and the rate of larval infestations are closely correlated with densely populated areas. Barangays Lahug and Guadalupe are the most densely populated among the five Barangays surveyed and also had the highest number of breeding sites and larval infestation rates, with most of the breeding sites classified as artificial. Thus, the breeding sites of mosquitoes can be diminished by the local community by

Table 4. Average mortality rate of *Ae. aegypti* larvae in the semi-field test: shaded and exposed to sunlight.

Date	Average Mortality Rate Of 5 Containers					
	Shaded Area			Exposed Area		
	1 tablet	2 tablets	Control	1 tablet	2 tablets	Control
3 days	100% a	100% a	2% a	100% a	100% a	4% a
6 days	100% a	100% a	1% a	100% a	100% a	3% a
9 days	100% a	100% a	2% a	100% a	100% a	3% a
12 days	100% a	100% a	0% a	100% a	100% a	0% a
15 days	100% a	100% a	2% a	100% a	100% a	2% a
18 days	100% a	100% a	2% a	100% a	100% a	0% a
21 days	100% a	100% a	0% a	91% a	100% a	2% a
24 days	100% a	100% a	0% a	77% a	100% a	0% a
27 days	100% a	100% a	2% a	57% b	100% a	0% a
30 days	100% a	100% a	1% a	35% b	100% a	0% a
33 days	85% a	100% a	1% a	4% c	55% b	1% a
36 days	58% b	100% a	3% a			
39 days	27% c	82% a	1% a			
42 days	5% c	55% b	3% a			

Values followed by the same letter are not significantly different ($P<0.01$)

Table 5. Mortality rate of *Ae. aegypti* larvae in the field test.

Duration	Treated container number Mortality (%)											Control Number of larvae		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
3 days	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	7 (2^{nd} & 3^{rd} instars; 8 pupae)	8 (2^{nd} & 3^{rd} instars; 2 pupae)	15-20 (2^{nd} & 3^{rd} instars)
6 days	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	20-30 (3^{rd} instars)	20-30 (2^{nd} & 3^{rd} instars)	5-10 (3^{rd} instars; 5 pupae)
9 days	100%	100%	100%	100%	100%	100%	100%	100%	infested	100%	100%	18 (3rd & 4th instars; 2 pupae)	20-30 (2^{nd} & 3^{rd} instars; 5 pupae)	30-50 (1^{st} & 2^{nd} instars)
12 days	100%	100%	100%	100%	100%	100%	100%	infested (30-50 2^{nd} & 3^{rd} instars)	infested (1.00-2000 1^{st} & 2^{nd} instars)	100%	100%	100-150 (all instar larvae)	50-100 (all instar larvae; 10-20 pupae)	50-75 (2^{nd} & 3^{rd} instars)
15 days	100%	100%	100%	100%	100%	water dried up	100%			100%	water emptied	100-150 (all instar larvae)	50-100 (all instar larvae)	20-30 (3^{rd} instars; 10 pupae)
18 days	100%	100%	100%	100%	100%		water dried up			100%		100-150 (all instar larvae)	50-100 larvae (all instars)	10-20 (2^{nd} & 3^{rd} and 4^{th} instars)
21 days	infested (100-150 2^{nd} instars)	infested (5-10 1st instars)	100%	infested (10-20 1^{st} & 2^{nd} instars)	infested (50-75 3^{rd} & 4^{th} instars)					infested (50-100 1^{st} & 2^{nd} instars)		100-150 (all instar larvae)	50-100 (all instar larvae)	20-30 (3^{rd} + 4^{th} instars)

1,3,4,5,7,10, and 12 = drum (each has 200 L water capacity).
2 = plastic pail (each has 25 L water capacity).
6 and 9 = cement water catch basin.
8 = old tire.

strictly following proper sanitation and hygienic practices. In the Philippines, environmental strategies to reduce the population of mosquito vectors are also practiced. An example is a memorandum entitled "4 o'clock Habit" or "Kill the Mosquito, Knock Out Dengue" that was issued in 1996 by the President of the Philippines. At 4:00 every afternoon, bells at universities, churches, and city halls rang, requesting people to go to their respective areas to clean up and reduce mosquito-breeding sites. However, when recommending source reduction for *Ae. aegypti* larvae by emptying or destroying discarded containers, the usefulness of these practices to homeowners should be considered (Nathan and Knudsen 1991).

The Philippine government has declared June as the month of "Dengue Awareness." Little Dengue Health Brigades have also been established, consisting of students distributing basic information about dengue to schools and the general public. Aside from the program mentioned above, a Dengue Task Force has also been created, composed of trained people that are responsible for educating the public about dengue as well as monitoring the existence of dengue viruses. Press conferences and media campaigns (radio and television) are also held to provide information to the public on the disease and appropriate control strategies.

Evaluation of people's knowledge, attitudes, and practices towards dengue

The knowledge, attitudes, and practices towards dengue were limited. Many of the interviewees (65%) mentioned fever, however, only 9.4% reported typical symptoms of body pains and 17.8% reported headache. While 68.7% of the interviewees were aware that the disease is transmitted by mosquitoes, only 4.3% of the respondents knew that a virus was the cause of the disease. The respondents' fragmentary knowledge on the disease and transmission appears to be one of the reasons why people do not follow the instructions to reduce mosquito-breeding sites (Gubler, 1998). The results of this study agree with the results obtained by Kroeger et al. (1995) on community-based dengue control in Colombia, South America regarding knowledge and practice and the potential contribution of the biological larvicide *B.t.i.* In their study, they found that many interviewees (70%) mentioned fever, but only one-third reported the typical symptoms of muscle and rheumatic pain. To address this problem, there is a need to disseminate information, education, and communication activities systematically. Educational activities could be carried by social mobilization organized by the community, interpersonal communication such as women's groups, and mass media broadcasts, radio, newspapers, and videos.

Evaluation of the efficacy of *B.t.i.* tablets against *Aedes aegypti* larvae

B.t.i. tablets showed promising results against *Ae. aegypti* larvae. In semi-field tests, the duration of the efficacy of *B.t.i.* tablets was longer in shaded water containers than in containers exposed to sunlight. This result is correlated with that of Melo Santos et al. (2001) on the evaluation of a new tablet formulation based on *B.t.i.* for the control of *Ae. aegypti*. In their study, the authors found that *B.t.i.* was effective for 35 days in containers exposed to sunlight and for more than 50 days in shaded containers. The same results were obtained from the study done by Kroeger et al. (1995) on *B.t.i.* tablets in Colombia. The authors found that *B.t.i.* lasted for more than a month.

In water containers exposed to sunlight, the long-term effect of the tablets was reduced because *B.t.i.* was inactivated by sunlight (Becker et al. 2003). The UV-A/B of sunlight reaching the earth's surface is considered responsible for the photo-degradation and consequent loss of toxicity (Glare and O'Callaghan 1998). Experiments under semi-field conditions also showed that the duration of the efficacy can be increased by higher concentrations of *B.t.i.* (two tablets instead of one). A lower dose (one tablet/50 L water) was less effective and is not recommended, particularly because of decreasing water temperature (Becker and Margalit 1993) and with increasing numbers of mosquito larvae, the efficacy of *B.t.i.* decreases (Becker et al. 1992).

In field tests, the duration of the efficacy of *B.t.i.* tablets was shorter (about 21 days) than in semi-field conditions (up to 36 days in shaded containers and up to 30 days in sun-exposed containers). This may be because the conditions in natural breeding sites cannot be controlled. Various factors have to be considered, including rainfall and thunderstorms. Heavy rain was experienced during the field study causing the mosquito-breeding containers to overflow, thus having a negative impact on the efficacy of *B.t.i.*.

B.t.i. is a very promising microbial agent and should be incorporated in integrated mosquito control management programs to reduce the incidences of diseases such as DF and DHF in the tropics. In suitable formulations, this microbial agent is a useful supplement or replacement for conventional pesticides. It can also be assumed that with the use of *B.t.i.* in an integrated program, the onset of resistance can be prevented (Becker et al. 1991). On the other hand, *B.t.i.* offers an ecologically defensible compromise between the need of humans to protect themselves from nuisance mosquitoes and current environmental policies focusing on protection of sensitive ecosystems by the use of non-selective methods.

Acknowledgments

Heartfelt thanks go to Dr. Beate Ruch-Heeger, Ms. Agnes Tomayao, and Ms. Abigail Genelsa for their assistance during the field study. The authors especially thank Valent BioSciences, ICYBAC GmbH, German Mosquito Control Association (KABS), Gesellschaft zur Förderung der Stechmückenbekämpfung e.V. (GFS), and Lion's Club (Goldener Hut) for financial support of the study.

REFERENCES CITED

Becker, N., S. Djakaria, A. Kaiser, O. Zulhasril, and H.W. Ludwig. 1991. Efficacy of a new tablet formulation of an asporogenous strain of *Bacillus thuringiensis israelensis* against larvae of *Aedes aegypti*. Bull. Soc.

Vector Ecol. 16: 176-182.

Becker, N., M. Zgomba, M. Ludwig, D. Petric, and F. Rettich. 1992. Factors influencing the activity of *Bacillus thruringiensis* var. *israelensis*, treatments. J. Am. Mosq. Contr. Assoc. 8: 285-289.

Becker, N. and J. Margalit. 1993. Use of *Bacillus thuringiensis israelensis* against mosquitoes and blackflies. *Bacillus thuringiensis, An Environmental Biopesticide: Theory and Practice.* John Wiley and Sons Ltd. pp. 147-170.

Becker, N., D. Petric, M. Zgomba, C. Boase, C. Dahl, J. Lane, and A. Kaiser. 2003. *Mosquitoes and their control.* Kluwer Academic/Plenum Publishers. New York. 497 pp.

Buerano, C., I. Ibraham, R. Contretras, F. Hasebe, R. Matias, F. Natividad, and A. Igarashi. 2000. IgM-capture ELISA of serum samples collected from Filipino dengue patients. Southeast Asian J. Trop. Med. Publ. Hlth. 31: 524-529.

Cebu City Health Record (CCHR). 2003. Cebu City, Philippines. 112 pp.

Department of Health (DOH). 2003. Cebu City, Philippines. 98 pp.

Ellis, R. 2001. Municipal Mosquito Control Guidelines. Health Canada, Bureau of Infectious Diseases.

Glare, T.R. and M. O' Callaghan. 1998. Environmental and health impacts of *Bacillus thuringiensis israelensis.* Report to the Ministry of Health, Wellington, NZ.

Gubler, D.J. 1998. Dengue and dengue haemorrhagic fever. Clin. Microbiol. Rev. 11: 480-496.

Halstead, S.B. 1980. Dengue haemorrhagic fever-a public health problem and a field for research. Bull. Wld. Hlth. Org. 58: 1-21.

Halstead, S.B. 1982. WHO fights dengue haemorrhagic fever. Wld. Hlth. Org. Chron. 38: 65-67.

Köhler, W., G. Schachtel, and P. Voleske. 1984. *Biometrie.* Springer Verlag, Berlin. 246 pp.

Kroeger, A., U. Dehlinger, G. Burkhardt, W. Atehortua, H. Anaya, and N. Becker. 1995. Community based dengue control in Colombia: people's knowledge and practice and the potential contribution of the biological larvicide B.t.i. (*Bacillus thuringiensis israelensis*). Trop. Med. Parasitol. 4: 241-246.

Melo Santos, A., E.G. Sanches, and F. de Jesus. 2001. Evaluation of a new tablet formulation based on *Bacillus thuringiensis* serovar. *israelensis*: Control of *Aedes aegyypti.* Mem. Instit. Oswaldo Cruz. 96: 859-860.

Nathan, M.B. and B. Knudsen. 1991. *Aedes aegypti* infestation characteristics in several Caribbean countries. J. Am. Mosq. Contr. Assoc. 7: 400-404.

Schoenig, E. 1971. Mosquitoes in Cebu City and adjacent areas: an ecological survey. Phil. Sci. 8: 21-32.

Schoenig, E. 1977. Distribution and abundance of mosquito species in Metropolitan Cebu, their relation to public health and their control. Phil. Sci. 14: 34-51.

WHO, 1997. World malaria situation in 1994. Part I. Population at risk. Weekly Epidem. Rec. 72: 269-274.