MOSQUITO VECTOR CONTROL AND BIOLOGY IN LATIN AMERICA—A SYMPOSIUM[1]

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ABSTRACT. The first Spanish language symposium ever presented by the American Mosquito Control Association was held as part of the 57th Annual Meeting in New Orleans, LA, in March 1991. A major objective was to increase and stimulate participation in the Annual Meeting by vector control specialists and public health workers from Latin America. This publication summarizes the 25 individual presentations that encompassed the following areas: mosquito biology and ecology, evaluation of chemical and biological control measures for mosquitoes, epidemiology of dengue, and current and developing dengue prevention programs.

INTRODUCTION

The American Mosquito Control Association (AMCA) is recognized as the premier organization of its type in the world. To better serve the international vector control and public health community, a symposium with presentations in Spanish was planned, organized and held at the 57th Annual Meeting of the AMCA in New Orleans, LA. The objectives were to encourage colleagues from Latin America to attend the AMCA meeting, discuss their experiences in vector control, present results of recent studies, promote greater interaction with AMCA members and stimulate future collaboration in the resolution of vector control and vector-borne disease problems.

In planning for this symposium, all AMCA members and about 60 other governmental or university vector control personnel in Latin America were invited to submit abstracts. As a result of the enthusiastic response, 25 presentations covering a wide variety of topics were included in this session. The quality of the presentations, enthusiasm of the speakers and attendance at the session contributed to its success. The symposium's objectives were clearly met and provided a forum that has led to greater interaction between AMCA members and their neighbors in Latin America where vector-borne diseases, like malaria and dengue, are problems that must be dealt with on a daily basis.

Special recognition for support of the symposium goes to the Vector Biology and Control (VBC) Project (Andrew A. Arata), managed for the Agency for International Development by Medical Service Corporation International of Arlington, VA; the New Orleans Mosquito Control Board (Edgar S. Bordes, Jr.), American Cyanamid (William Jany), Vectec (Isaac S. Dyals) and Sumitomo Chemical America (S. Ohutsuki) for providing financial support for many of the international visitors. The VBC Project also provided copies of the abstracts (in English) for non-Spanish speakers attending the session and provided funds for publication of symposium abstracts in the *Journal of the AMCA*. As a result of the response from participants and the AMCA leadership, it is expected that this unique forum will be included in future meetings of the organization.

SYMPOSIUM ABSTRACTS

Resting sites for *Aedes aegypti* in Panamá (Sitios de reposo de *Aedes aegypti* en Panamá)

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The ground application of ultra-low volume (ULV) insecticidal fog by truck is the most common emergency measure to control *Aedes aegypti* during a dengue epidemic. Studies indicate that these mosquito populations recover within 3–7 days and that it is not a very effective control measure. These questions, and the possibility of imported cases of dengue entering Panama from nearby countries and because larval infestations (house indices) have reached 10% and above in certain metropolitan and outlying districts during 1988–89, stimulated us to investigate the resting behavior of *Ae. aegypti* in respect to the effectiveness of ULV spraying.

This project received support from the Panama Health Ministry and SNEM mosquito control, who assigned 4 technicians to work on the

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[2] Reprints may be requested from Gary G. Clark.

project. We measured each mosquito's resting place in relation to height, location light intensity and relative humidity.

Data were gathered over 30 days during October and November 1990. Collections were made during a 6-h period between 0800 and 1600 hours. Seventy-six houses, 20 mechanics' shops and 30 manholes were inspected. We collected an average of 5 mosquitoes per house. The highest number of *Ae. aegypti* collected in any single dwelling was 42 females and 23 males.

Our study compared distribution of the mosquitoes resting inside the house vs. those collected outside. We recorded the nature of the resting site, the number collected in each room and the number collected on other surfaces. Females were dissected to determine the parity state, and if possible, their physiological age (number of ovarian dilatations) and degree of feeding vs. ovarian development (Christophers' stages).

Seventy-seven percent of adult *Ae. aegypti* mosquitoes were collected inside the dwelling and 23% were found resting outside. Fifteen percent were males and 85% were females. The lesser number of males may be related to the scarcity of larval habitats near the houses or because females may have dispersed some distance since emergence in search of a bloodmeal. Males are known to disperse over shorter distances during their much shorter life span.

The largest number of female mosquitoes were found in bedrooms, but more males occurred in bathrooms. Seventy-one percent of the *Ae. aegypti* females collected were resting in protected areas under sinks or within rustic china cabinets. Fifty-seven percent of the *Ae. aegypti* were collected less than 1 m above the floor and 94% less than 2 m above the floor.

Dissections showed that 55% of the parous females had one dilatation, 25% had 2 dilatations and 20% had 3 dilatations, indicating that at least 45% had fed and laid eggs 2–3 times and that they were more likely to have come into contact with an infectious agent. Only one mosquito was found with 6 dilatations.

Gravid and semigravid females were found resting on unexposed surfaces such as clothing, under and behind furniture and other articles in the house. They were also taken from resting sites such as hanging garments, pictures, porcelain vases and ornaments on the walls where they would be more protected from ULV insecticide droplets. The remaining (42%) ones were found on exposed surfaces. Most nulliparous females appear to rest on sites that would be more exposed to ULV sprays, and we found that 66% of males rest in similar areas that would also be exposed to the spray.

Several insights were gained from our study, but we consider the following the most important:

1. A higher percentage of parous females, which represent a greater epidemiological risk regarding dengue transmission, rest in more protected areas and on surfaces where ULV spray droplets are less likely to drift.
2. Higher percentages of males and nulliparous females were found resting in the more exposed sites and would therefore be killed by routine ULV spraying.

Relationship between larval indices and adult densities of *Aedes aegypti* in El Progreso, Honduras, 1989–90 (Relación entre índices larvarios y densidad de adultos de *Aedes aegypti* en El Progreso, Honduras, 1989–90)

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To determine if there was a direct relationship between larval indices and adult densities, we performed 4 entomological surveys in 100% of the households in 8 "barrios" in El Progreso. They were conducted in June 1989 when the rainy season begins; in October 1989 when there is more rainfall; in February 1990 when the rainy season ended; and in July 1990 when the next rainy season began. We also captured adult *Aedes aegypti* mosquitoes with the CDC backpack aspirator in 30 houses in 6 "barrios" located in 8 that were surveyed for larvae. Adults were captured once per week in each "barrio."

We observed that the larval indices increased at the beginning of the rainy season, with the highest peak (100%) for the month of greatest rainfall and decreased at the end of the rainy period. In contrast, the adult density increased at the beginning of the rainy season and later decreased, remaining constant during the months of more rainfall with a small increase at the end of the season. This variation in adult densities was directly related to the incidence of dengue cases. We observed that there was an increase in adult densities followed by the appearance of dengue cases 1–2 months later. When the adult density decreased, the dengue cases also decreased almost immediately.

We conclude that there was no direct relationship between the larval indices in households and the adult population, most likely because the breeding containers that become positive during the rainy season are not good production sites for adult mosquitoes. The increase in adult densities at the beginning and end of the rainy season occurred because of an increase in water levels, but in a certain way, it had to do with the stabilization of the breeding containers. Therefore, we must seek other risk indicators aside from those we have been dealing with until recently (e.g., presence of larvae), because the real success of a good risk indicator is that it indicates when we should act to prevent a problem (e.g., a dengue epidemic). Therefore, dengue control programs should include investigation of the number of pupae, type of breeding containers, seasonal variation, as well as abundance of adult mosquitoes, using the CDC aspirator which offers an operational advantage.

Biology and vectorial capacity of *Anopheles albimanus* on the southern coast of Guatemala (Estudio de biología y capacidad vectorial de *Anopheles albimanus* en la costa sur de Guatemala)

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From October 1987 to March 1990, we studied *Anopheles albimanus* in 2 areas on the southern coast of Guatemala. They were the village of La Blanca in Ocos San Marcos and the village of El Aceituno in Escuintla.

We collected mosquitoes between 0500 and 1200 h daily and dissected 91% of the *Anopheles* females. Indoor collections yielded 1,783 *Anopheles*, of which 340 were nulliparous and 1,279 were parous. Outdoor collections yielded 2,917 of which 515 were nulliparous and 2,114 were parous.

We marked 220 *Anopheles* using fluorescent dusts and recaptured 40. The marked mosquitoes were observed resting 369 times on sprayable surfaces. The average time at rest was 14 min and 40 seconds. Of the females recaptured, 12% died within the 24-h observation period.

At La Blanca, we dissected 5,681 *Anopheles*. Of these only 3 (0.05%) were positive for *Plasmodium vivax* sporozoites. None was positive for *P. falciparum* sporozoites.

We found that 3% of the *Anopheles* sampled had taken blood from humans and domestic animals with the highest percentage from humans. Comparing the source of the animal blood, we demonstrated by the Forage Index that the highest rate was from bovines in La Blanca and from equines in El Aceituno.

According to results from both localities, the blood meal analysis from human bait and animal shelter collections demonstrated that the female *An. albimanus* are very long-lived. The biting activity is more peridomiciliary; the resting place inside the house is less than 1 m above the floor; and with predominant peridomiciliary refuges in stalls and woodpiles.

Ecology and biology of *Anopheles albimanus* in a locality of the Pacific Coast of Nicaragua (Ecología y biología de *Anopheles albimanus* en una localidad del Pacífico de Nicaragua)

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To obtain information on the biology and ecology of *Anopheles albimanus* on the Pacific Coast of Nicaragua, we selected a locality with a high mosquito density and continuous malaria transmission for study.

Mosquitoes were collected indoors and outdoors between 0600 and 1200 h with human bait in 2 different houses, every month. A total of 41,013 specimens were captured (6,199 indoors and 34,814 outdoors). Fluorescent dust (lumogen) was used to mark 267 female *An. albimanus* after feeding on human bait in order to observe their postfeeding behavior. The number of flights, as well as height and resting time, were recorded. The physiological age was determined by ovarian dissection.

In addition, mortality and survival by gonotrophic cycle were estimated. The collections revealed a bimodal activity pattern for *An. albimanus*, with a peak between 2000 and 2200 h and another (less sharp) between 0500 and 0600 hours.

The maximum density was observed in September and October, and the lowest density was seen between the months of November and April. These differences in densities corresponded to the months of highest and lowest rainfall, respectively. The mean physiological age of mosquitoes was calculated at 1.44 days (36.2% of female mosquitoes were parous and 63.8% were nulliparous). A small percentage of female mosquitoes had 4 dilations, thus indicating that transmission may be due to a high frequency of mosquito-man contact and not to the abundance of old female mosquitoes. Most mosquitoes preferred resting on walls at 0—1 m height and on nonsprayable surfaces.

The majority of mosquitoes left the houses within 30 min after feeding, and very few remained indoors after 60 minutes. Lastly, the mortality by cycles among old female mosquitoes was 83.9%.

Current strategy for the successful control of malaria in El Salvador (Estrategia actual de control de malaria y logros alcanzados en el Salvador)

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Since 1979, the malaria control program of the Ministry of Public Health has epidemiologically stratified the problem. At the same time, it decided to change its policy toward a more realistic control strategy that was based upon an epidemiological approach. This replaced the old method for vector control using pesticides with an integrated control strategy which included the following measures:

1. Massive drug administration in high-risk areas.
2. Intradomiciliary spraying with residual insecticide in 30,000 houses, three cycles per year (bendiocarb).
3. Larvicide application, in 10-day cycles, using 44% Abate® (temephos).
4. Ultra-low volume spraying as a response to high vector density.
5. Distribution of combined drugs (chloroquine-primaquine) through the voluntary collaborators network and the general health services.
6. Source reduction by construction of small and large drainage works with community participation.

In addition to the above measures, some adaptations were introduced. These included decentralization of diagnosis and timely treatment of cases, by incorporating the 2,600 member voluntary collaborator network in the provision of a 5-day curative treatment and decentralization of planning in decision-making at operational level. Finally, vector source reduction activities were emphasized and supported by significant community participation. All control measures and new adaptations were prioritized among the different locations, according to the epidemiological importance of the problem.

The significant improvement reached in malaria control in El Salvador is evidenced by a continuous decline in morbidity, reaching the level of 9,000 reported cases per year for the last 3 years. There was a 90% reduction in the number of reported malaria cases between 1980 and 1990, with very few cases of *P. falciparum*. These achievements are the result of the application of the new control strategy.

Interagency vector control during floods in Honduras (Manejo interagencial en el control de vectores en Honduras en casos de inundaciones)

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The Ministry of Health of Honduras lacks the resources to face the health problems resulting from natural disasters alone. As a consequence, there was a need to seek assistance from other governmental agencies during October and November 1990 when extensive flooding occurred in northern Honduras.

The main purpose for developing a cooperative plan was to prevent the spread of malaria epidemics, before the flooding occurred. This was done through the execution of control activities against larval and adult anopheline populations:

1. With the cooperation of the road district of the Ministry of Communications and Public Works, draining activities were carried out to eliminate 19 breeding sites for *Anopheles albimanus* and *An. crucians*.
2. The army provided human resources and made ULV applications of insecticide in intradomiciliary sites and temporary shelters. We used fenitrotion L-100 and made 5 applications at 17 day intervals.
3. With the financial assistance of the U.S. Agency for International Development (USAID) project, *Bacillus thuringensis* var. *israelensis* was applied every 7 days for one month to 32 anopheline breeding sites (flooded fields) in the urban and rural sectors in the most malarious area of Honduras.

Although we did not quantity the number of malaria cases, we felt that this control strategy helped prevent a malaria outbreak.

Indoor low-volume insecticide spray as a new method for the control of *Anopheles albimanus* in southern Mexico. Village-scale trials of bendiocarb, deltamethrin and cyfluthrin (Aplicación de insecticida a bajo volúmen dentro de las casas como nuevo método para el control de *Anopheles albimanus* en el sur de México. Pruebas de bendiocarb, deltametrin y cyflutrin a nivel de pueblos)

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The increasing costs of DDT-alternative insecticides and their application in malaria vector control programs in developing countries have resulted in the need to optimize the use of insecticides in terms of cost-effectiveness.

With this aim, a comparative study was carried out during 1988–89 to determine the efficacy of low-volume (LV) indoor spray of bendiocarb, deltamethrin and cyfluthrin for the control of *Anopheles albimanus* in a coastal plain of southern Mexico. Three villages were used for LV treatments (one per insecticide), a fourth village was used to spray wettable powder (WP) bendiocarb, and a fifth village remained untreated and served as the control.

Low volume spraying was conducted with knapsack mistblowers Fontan R-12®, with No.

1.0 nozzles, giving an average discharge rate of 215 ml/min, which deposited droplets of 50–100 (MVD). With this technique, it was possible for each sprayman to treat 25 houses per day (as compared with a maximum of 8 houses/day/sprayman using the conventional Hudson X-Pert® compression pumps). Insecticide efficacy was measured by mortality indicators, feeding success, and indoor activity patterns of mosquitoes.

Low volume spray mortality from wall-bioassays, indoor resting and human bait collections and house curtains showed a residual effect for 10 wk, compared with the 13 wk with the WP. Repellency was lower on surfaces treated with the LV, but no change in feeding success was observed.

With this technique the spraying costs decreased by 37% with bendiocarb and 76% with deltamethrin. The spraying time was reduced by two-thirds and an effectiveness similar to WP was achieved.

Evaluation of the effectiveness of Reldan 40 WP chlorpyrifos-methyl insecticide in the laboratory for the control of malaria vectors in Colombia (Evaluación de la efectividad del insecticida Reldan 40 WP clorpyrifos-metil, a nivel de laboratorio en el control de los vectores de malaria en Colombia)

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Beyond our principal objective of reducing malaria incidence, an additional aim of our malaria control program is to evaluate insecticides that might be used as an alternative to DDT. Susceptibility tests for adults and bioassays tests, following WHO methods, were used by establishing the $LT_{99.9}$ (lethal time) for the principal vector populations. We estimated the residual activity on 3 different materials sprayed with 1 or 2 g/m^2. Additionally, tests of irritation caused by contact with the insecticide and human bait catches, inside and outside of the houses, were conducted.

Populations of *Anopheles darlingi* (Villavicencio, Choco), *An. nuneztovari* (Cúcuta, Uraba) and *An. albimanus* (Uraba) were highly susceptible, having a $LT_{99.9}$ of 3–21 min, at a concentration of 5%. Residual activity on cement with water paint and on baked brick was 6–10 wk and on wood was 10–14 weeks. There was significant residual difference between the doses used. *Anopheles darlingi* from Villavicencio was irritated when it contacted the insecticide and increased its exophagic behavior in sprayed houses.

The recommended Reldan dose is 1 g/m^2, with an average residual life between 2 and 3 months. Understanding that exophagic and irritable behavior reduce the effectiveness of insecticide, Reldan is a good alternative to organophosphates of low toxicity especially in areas where the main construction material is wood.

Entomological aspects of the Arenal-Tempisque irrigation project, Guanacaste, Costa Rica. Preliminary observations (Aspectos entomológicos del Proyecto de Irrigación de Arenal-Tempisque, Guanacaste, Costa Rica. Observaciones preliminares)

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The northern part of Costa Rica, considered as the dry Pacific zone, has a great agricultural potential, but due to its annual drought, productivity is limited to the wet season.

Servicio Nacional de Aguas Subterraneas, Riego y Avenamiento is a national institution in charge of a vast irrigation project of about 66,675 hectares. This funding agency requires a complete study of the environmental impact of the project.

Centro de Investigación y Diagnóstico en Parasitología has initiated a study of the entomological aspects under a vector monitoring plan to detect possible changes, as a result of continuous irrigation of population dynamics and diversity of mosquitoes of medical and veterinary importance. The field strategy will include use of ovitraps, larval collections, CDC, Fay, Solid State Army Miniature light trap and Weinberg adult traps; and human and animal bait collections. In the laboratory, Belkin's methodology (1967. Contrib. Am. Entomol. Inst.) will be followed for specimen preparation.

Preliminary observations from January-December 1990 consisted of 8 field collections of culicid and simuliid larvae in the area irrigated in the region of Cañas, in which the dam "Arenal-Corobici" is located.

The species so far identified include Culicidae: *Culex* (*Cux.*) *coronator*, *Cx.* (*Cux.*) *pipiens*, *Cx.* (*Cux.*) *nigripalpus*, *Cx.* (*Cux.*) *declarator*, *Cx.*(*Cux.*) *interrogator*, *Cx.* (*Cux.*) *ousqua/coronator*, *Cx.* (*Cux.*) *corniger*, *Cx. Mel.*) *erraticus*; *Psorophora* (*Gra.*) *confinnis*, *Ps.* (*Gra.*) *cingulata*, *Ps.*(*Gra.*) *howardii*; *Aedes* (*Och.*) spp.; *Haemagogus* spp.; *Anopheles* (*Nys.*) *albimanus*, *An.* (*Nys.*) *argyritarsis*, *An.* (*Ano.*) *pseudopunctipennis*; and Simuliidae: *Simulium* (*Ps.*) *haematopotum*, *S.* (*H.*) *pulverulentum*, *S.* (*S.*) *metallicum*.

Peritrophic membrane and pupal-adult ecdysis in mosquitoes (Membrana peritrófica y écdisis de pupa-adulto en mosquitos)

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The peritrophic membrane (PM) in Diptera is of special interest in the field of vector biology not only because of its physiologically important role in insects, but also because of its potential influence on disease transmission. The PM is a chito-proteinous, non-cellular sheet enclosing the food in the midgut of most insects that have been studied.

Several functions have been attributed to the PM. Among these are: 1) protecting the midgut cells from abrasion by food particles; 2) serving as a barrier to parasites; and 3) acting as a selectively permeable membrane, allowing some molecules to pass while excluding others.

Although PMs in mosquitoes have been known for a long time to occur in larvae and in adult females following a blood meal, only recently has the PM been described in association with pupal stage. Romoser (1973) described 2 PMs (PM1 and PM2) which appeared to form around the meconium, i.e., the sloughed larval midgut epithelium which persists throughout the pupal period and into the adult as a mass in the developing adult midgut. In our study, preliminary observations suggested that PM1 forms early in the pupal stage while PM2 forms in recently emerged adults.

On the basis of dissections of *Aedes aegypti* pupae of known ages post-pupation and recently emerged adults of known ages post-emergence, we have determined: 1) PM1 forms between 15 and 24 h following pupation and its formation occurs after apolysis; 2) PM2 does not form during the pupal stage, but rather very soon after pupal adult ecdysis. It apparently does not form in every individual; 3) since PM2 forms at some point after the distension of the midgut by gas during ecdysis, we suggest that PM2 is analogous to the PM that forms around a blood meal, its formation being induced by the distention of the midgut epithelium; 4) the meconium and PM1 are voided from the midgut as early as 3–4 h following pupal-adult ecdysis, but there is much variation in this; and 5) PM2 appears to form only in female mosquitoes.

Functional response of some aquatic predators on *Culex pipiens* (Diptera: Culicidae) larvae (Respuesta funcional de algunos depredadores acuáticos sobre larvas de *Culex pipiens*)

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The functional response (as a statistical tool in predation) was used to evaluate the potential of aquatic insects to regulate larval mosquito populations. The tests were carried out in glass containers with 750 ml of dechlorinated water and fourth instar *Culex pipiens* larvae exposed to 9 insect genera commonly found as predators in the mosquito breeding habitat. After 24 h, we recorded the number of prey consumed. The results were analyzed according to Holling's (1959) and Rogers' (1972) functional response models.

The 9 predators were: dragonfly naiads (*Pantala* sp.; Odonata: Libellulidae), creeping water bug (*Ambrysus* sp.; Hemiptera: Naucoridae), the backswimmers (*Buenoa* sp. and *Notonecta* sp.; Hemiptera: Notonectidae), waterscorpion (*Ranatra* sp.; Hemiptera: Nepidae), predaceous diving beetles (*Laccophilus* sp. and *Thermonectes* sp.; Coleoptera: Dytiscidae), larvae of the water scavenger beetles (*Tropisternus* sp. and *Hydrophilus* sp.; Coleoptera: Hydrophilidae).

We estimated the searching capacity and "handling time" as probable indicators to choose the most effective biological control agent. The searching capacity is the ability to move in an area or volume of water to find prey. The "handling time" is the time that the entomophagous predator spends quelling, killing and eating the prey. With Holling's model, the backswimmer (*Notonecta* sp.) had the highest value of searching capacity ($a' = 0.043$) with the handling time $T_h = 0.172$; in second place came the predaceous diving beetle (*Thermonectes* sp.) with $a' = 0.042$ and $T_h = 0.0357$.

With Rogers' model, the backswimmer (*Notonecta* sp.) had the highest values of searching capacity ($a' = 0.414$) and the handling time was $T_h = 0.900$. In second place came the predaceous diving beetle (*Thermonectes* sp.) with $a' = 0.381$ and $T_h = 0.503$. We considered only the values for 24 h exposure tests.

Evaluation and persistence of two *Bacillus sphaericus* 2362 formulations on *Culex quinquefasciatus* under laboratory conditions (Evaluación y persistencia de dos formulaciones de *Bacillus sphaericus* 2362 en *Culex quinquefasciatus* bajo condiciones de laboratorio)

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We evaluated the toxicity and residual activity of 2 commercial formulations of *Bacillus sphaericus* (strain 2362) against field and laboratory strains of *Culex quinquefasciatus* larvae. The larvicidal activity of these formulations was determined by the methods of Lacey (1984) and De Barjac (1988). Six doses were prepared from a 1% stock of each formulation; one granular (ABG-6185 from Abbott Laboratories, Chicago, IL) and the other a concentrated solution (F.C., BCP- 2 Solvay, Brussels, Belgium). At the same time, we conducted a bioassay with the International Standard RST 88 of *B. sphaericus* strain 2362. Mortality was recorded at 48 and 72 h to calculate the LC_{50} and LC_{90} for each formulation. The residual effect was determined by larvicidal activity and counting of spores, following the WHO (1986) protocol. The basic dose with the LC_{50} and LC_{90} for each formulation was used in 4 repetitions with 20 larvae each. Mortality was recorded at 48, 72, 96 and 168 hours. Later, we eliminated the surviving larvae and repeated this for every 7 days through 28 days. Spores were counted, using the method of Karch (1990) before treatment and on days 7, 14, 21, and 28 posttreatment. The residual effect was only made with field-collected larvae.

The larvicidal activity at 72 h for laboratory-reared larvae for the granular formulation was: LC_{50} 0.04 mg/liter and LC_{90} 0.22 mg/liter and for the concentrated solution the values were LC_{50} 0.30 mg/liter and LC_{90} 0.24 mg/liter. For field-collected larvae, the granular formulation produced an LC_{50} of 0.19 mg/liter and an LC_{90} of 0.66 mg/liter while the concentrated solution yielded an LC_{50} of 0.24 mg/liter and an LC_{90} of 0.83 mg/liter.

The residual effect was reflected in larval mortality for a period of 28 days following a single application of the LC_{50} or LC_{90} dose of either formulation. The number of colonies formed increased exponentially over time. The initial spore count for the granular formulation was from 219 to 549 spores/ml for the LC_{50} and LC_{90}, respectively, and from 6.44×10^{10} and 2.18×10^{11} spores/ml in the final count for the LC_{50} and LC_{90}, respectively. For the concentrated solution, the initial count was from 148 to 295 spores/ml and the final count was from 2.18×10^{11} to 5.5×10^{10} spores/ml for the LC_{50} and LC_{90}, respectively.

Effect of sublethal doses of Abate® on some biological parameters of *Aedes aegypti* (Efecto de dosis subletales de Abate® sobre algunos parámetros biológicos de *Aedes aegypti*)

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The effect of Abate® applied at concentrations of 0.016, 0.020 and 0.025 mg/liter to fourth stage *Aedes aegypti* larvae and the effect on the weights of pupae and adults, quantity of blood ingested, and adult longevity were studied. We used the insecticide from the standard solutions provided with the WHO test kit and diluted it in distilled water to obtain the above concentrations. The larvae were in the F_2 generation and had been collected in an urban area of Monterrey, Mexico, and reared on an artificial diet. Females were fed with a 10% solution of honey and rabbit blood during each gonotrophic cycle while males received honey alone. For each concentration, we randomly selected 20 larvae which were then individually separated. Later, the pupae and recently emerged adults were weighed to 0.01 mg. Later, each female was confined with 3 males in a small cage ($10 \times 10 \times 10$ cm) and a 25 cm^2 piece of moist paper was provided for oviposition. The females were also weighed again after their first bloodmeal.

We observed a decrease in weight in pupae and in adults of both sexes from those individuals that survived the 3 concentrations of Abate. The lowest weights found in the pupae and adults (1.09 ± 0.07 and 0.54 ± 0.07 mg) were 31 and 33% lower than their respective controls. The lowest pupal (2.55 ± 0.27 mg) and adult (1.20 ± 0.09 mg) weights were only 10 and 6%, respectively, less than controls that were not exposed to sublethal concentrations of Abate. The average weight of blood ingested by females exposed to the maximum concentration of Abate was 0.036 ± 0.06 mg. This reduction of 10% was not statistically different from the untreated controls.

Longevity of males exposed to the insecticide was 30.96 ± 2.19 days and 20% longer than the controls. No difference in female longevity was found.

Evaluation of oviposition rates in preliminary releases of *Toxorhynchites theobaldi* in suburban areas in Mexico (Evaluación de la tasa de oviposición en liberaciones preliminares de *Toxorhynchites theobaldi* a nivel suburbano en Mexico).

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Toxorhynchites theobaldi is a mosquito predator that has been considered as a control agent for *Aedes aegypti*, the mosquito vector of dengue viruses. Previous experience with other *Toxor-*

hynchites species has demonstrated that before beginning a control program using these mosquitoes, it is necessary to know as much as possible about their biological characteristics, especially those pertaining to oviposition.

From the spring through the fall of 1990, we released 20, 20, 40 and 80 gravid females (9–11 days old) in a walnut grove at the edge of an urban area of Monterrey, Mexico. The adults that we used in these experiments were obtained from a laboratory colony. For oviposition sites, we provided 10 used automobile tires, placed vertically at the base of tree trunks, in a rectangular pattern of 20 × 40 m. Oviposition was recorded daily for 17 days.

The average daily oviposition rate varied between releases. The release of 80 females had the highest average oviposition rate (242.9) while that of 40 females was higher (97.5) than those (35.9 and 48.2) of 20 females. The oviposition pattern was adjusted to the inverse regression equation ($Y = a + b/X$), where Y equals the number of eggs deposited per day and X is the number of days post-release. We observed this tendency in the fall release of 80 females.

In terms of positive tires, the release of 80 females (69.4%) was equal to that of the release of 40 females (64.1%) and larger than that of the 2 releases of 20 females (48.2 and 45.9%). We found a negative linear correlation between percent of positive tires and days post-release. This was quite notable for the releases of 20 females, less so for the release of 40 and poor for the release of 80.

The lower fall temperatures affected flight activity of the released females and the oviposition pattern of 80 females was very irregular, but they followed the increases and decreases of temperature within the range of 13–23°C ($r^2 = 0.81$). The increased number of released females had a tendency to find a greater number of oviposition sites. Additional studies of the dispersion and oviposition in larger urban areas are necessary to determine the potential of this species as a control agent for *Ae. aegypti.*

Functional response of *Toxorhynchites theobaldi* on *Aedes aegypti* larvae (Respuesta funcional de *Toxorhynchites theobaldi* sobre larvae de *Aedes aegypti*)

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We studied the functional response of *Toxorhynchites theobaldi* larvae on *Aedes aegypti* larvae. The variation in number of larval *Ae. aegypti* consumed by each larval predator was recorded for the 4 larval predator stages and at 8 larval prey densities of 10, 20, 30, 40, 50, 60, 70 and 80. Tests were conducted in an individual Petri dish and replicated 8 times. After 48 h of exposure, we recorded the number of larvae consumed. The insectary had an ambient temperature of 28°C, 48% relative humidity and 14:10 light:dark cycle.

We plotted the number of larvae consumed by the prey species at different prey densities and tested the observed points with the chi-square test, to the linear model ($Na = a + bNo$) and the Holling II ($Na = Tta'/1 + Tha'$) where Na equals prey consumed at the density No, and a and b are the intercept and the angle of the linear equation. In Holling, Tt is the time of predator exposure (in hours), Th is handling time for the prey, and a′ is the rate of instantaneous attack.

The functional response was described by the linear model for the second and fourth predator instars. The equations were $Na = 5.6095 + 0.6149No$ and $Na = 2.0632 + 47No\ 0.8479No$, respectively. Holling II describes the best functional response with the first and third instars with equations $Na + (0.02448)\ (48)\ No/1 + (0.02448)\ (0.47385)\ No$ and $Na = (0.02455)\ (48)\ No/1 + (0.02455)\ (0.4643)\ No$, respectively.

Aedes aegypti larval control using cyclops in Puerto Rico (Control larvario de *Aedes aegypti* con ciclops en Puerto Rico)

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Cyclops are one of the commonest forms of freshwater zooplankton in the world. These tiny crustaceans have recently been recognized as exceptionally promising biological control agents in some *Aedes aegypti* production sites where they attack and kill first-instar larvae.

We collected 18 cyclops species from their natural habitats in Puerto Rico and identified and cultured 3 good larvivorous candidates. Through laboratory bioassays, we determined that *Mesocyclops aspericornis* was the best candidate for field trial applications, followed by *Macrocyclops albidus* and *Mesocyclops* n.sp.

In April 1990, we added *Me. aspericornis* and *Ma. albidus* to 25 tires located outdoors under natural conditions. About 50 individuals were applied per tire. They have survived, multiplied and maintain a constant population, while preventing the appearance of *Ae. aegypti* larvae. In adjacent tires and during this same period, *Ae. aegypti* production continued unaffected.

In previous entomological surveys in a community in Caguas, Puerto Rico, we identified 55 gallon drums, discarded appliances and cisterns as the most common containers that would lend themselves to the application of cyclops. In addition, large tire accumulations in public dumps were identified as good candidates for cyclops applications.

Recently, we initiated field applications of *Me. aspericornis* in the above containers. After having gained the community's confidence during prior project activities, residents accepted, without resistance, this larval control procedure and some have asked for multiple applications. Along with the cyclops application, residents received a pamphlet with basic information about these predators. The principal reaction was one of general disbelief, since the residents thought that the cyclops were too small to kill *Ae. aegypti* larvae. Preliminary results indicate that the cyclops survive and continuously prey on larvae, unless the container becomes completely dry.

Effectiveness, risks and costs of spatial ULV applications with Malathion CE-96 and Sumithion L-100 against *Aedes aegypti* in Panama (Eficacia, riesgo y costo de las aplicaciones espaciales ULV con Malatión CE-96 y Sumitión L-100 contra *Aedes aegypti* en Panamá)

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This study was conducted on the university campus and 3 districts of the metropolitan area of Panamá City, Panamá to evaluate ULV applications of malathion and Sumithion against *Aedes aegypti.* For evaluation we used bioassay cages with 10 bloodfed females, plastic jars with fifteen 3rd and 4th stage larvae in 50 ml of water and silicone-coated slides. Each of these 3 evaluation methods was located inside and adjacent to each, systematically selected house and left for a 2-h exposure period. Before and after the ULV treatment, we determined the level of serum cholinesterase (CHE) in operators and residents. During the test, we recorded temperature, humidity and wind.

Malathion CE-96, applied at the rate of 127 ml/min (103 ml/ha), produced an average mortality (recorded at 24 h) of 56 and 82% in adults and 6.8 and 28.6% in larvae, respectively. Similarly, Sumithion L-100 applied at 122.5 ml/min (94.2 ml/ha) produced an average mortality of 56 and 83% in adults and 14.6 and 54.7% in larvae, inside and outside the houses, respectively. When the average bioassay mortality was compared at 2 and 24 h post-treatment, the results obtained were 34 and 68.6% for malathion and 31 and 69.6% for Sumithion.

The levels of CHE before and after treatment were not significantly different in operators or residents. The concentration of insecticide droplets was from 24–33/cm^2 for Malathion and from 26 to 50 for Sumithion. The average diameter (DMV) for 85% of the droplets was 11.5 microns for malathion and 12.0 microns for Sumithion. During the 8 field tests, weather conditions varied between 26.5–28.5°C, 60–76% relative humidity and windspeed from 2 to 10 km/hour.

The project included 8 ULV applications in an area of 100 ha, where we selected 115 houses. Personnel used in this trial included 3 technicians, 2 operators and 2 investigators to complete 160 h of work. The total cost for the valuation of the test was $968, equivalent to $9.68/ha for insecticide, personnel, CHE analysis and fuel.

Measurement of aerial and ULV spraying efficacy in Antioquia, Colombia (Medición de la eficacia de fumigaciones UBV aérea y con LECO en Antioquia, Colombia)

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To prevent a dengue epidemic in 1989, the Colombian government alerted the community through different campaigns and took safety precautions. In the state of Antioquia, there were at least 100 suspected and 3 confirmed cases of dengue hemorrhagic fever.

To reduce the adult mosquito population, applications of malathion were made with vehicle-mounted LECO units and by aircraft. By LECO, the application rate was 500 cc/ha in 4 localities in Medellín. For each outbreak case, the operating range covered was 200 meters. The same dosage was sprayed by aircraft in the municipalities of Carepa and Chigorodo.

Prior to each application, 5% of the dwellings were surveyed by collecting *Aedes aegypti* mosquitoes for 20 min in each house. After spraying was completed, the procedure was repeated to calculate the post-spray index, which was then compared with the pre-spray index. The results favored the LECO applications where the adult mosquito population was reduced by more than 50% in the 4 study areas. The indices did not show any important reduction with the aerial application.

The selective application showed good results for its significant reduction of adult population. The aerial application was highly ineffective, mainly because of the difficult weather conditions, the workers' inexperience and the type of houses. With its high costs, this operation can only be recommended for special cases.

Utility of ultra-low volume application of insecticides for the control of *Aedes aegypti* during dengue and dengue hemorrhagic fever epidemics (La utilidad de insecticidas aplicados por medio de volumen ultra-reducido (VUB) para el control de *Aedes aegypti* durante epidemias de dengue y fiebre hemorrágica de dengue).

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In recent years there has been a controversy concerning the usefulness of ultra-low volume (ULV) application of insecticides for the control of diseases transmitted by *Aedes aegypti*. Numerous trials from the 1960s until the present have shown widely different results, varying from no detectable control to virtually complete elimination of the adult vector population. Of the multitude of factors contributing to these differences, the most important are apparently dosage, insecticide susceptibility, house construction, block configuration and route of the spray vehicle.

One of the most important reasons for differing results has been differences in dosages of insecticide applied. For example, in Puerto Rico where ULV application has resulted in little or no control, less than 73 ml/hectare of malathion was applied. However, in most of Latin America where better reductions have been observed, the usual target dosage is 305 ml/hectare. In both cases, the same discharge rate (127 ml/min) was employed, but in Puerto Rico application is made only once along each street and only on those streets with the same compass orientation (i.e., only North to South and South to North, but not East to West), whereas in Latin America the vehicle circles each city block, resulting in passes in both directions along every street.

What degree of reduction of the natural mosquito population, and for how long is it necessary to interrupt, or at least reduce transmission of dengue or yellow fever? This is very difficult to determine in the field because we are unable to predict what the transmission would have been in the *absence* of the treatment, and transmission in untreated comparison areas cannot be assumed to be comparable to the treated areas. However, we can design mathematical models based on what we know or assume about the dynamics of disease transmission. Preliminary models show that ULV application alone will have very little effect on the total number of cases of dengue because the adult mosquito populations recover very rapidly to previous densities, but it will decrease the *rate* of transmission, spreading it over a longer period of time. If elimination of breeding sites (source reduction) were done instead of ULV application, there would be a reduction in total number of cases because of the longer lasting reduction of mosquito densities, but the implementation would be so slow that the epidemic would be over before a great reduction could be achieved. If the 2 methods were used together, they would have a synergistic effect, resulting in greater reduction in transmission than the sum of the reductions when they were each used alone, ULV application delaying the peak of the epidemic thereby giving more time for the slower method of source reduction to reduce number of cases.

Architecture, ventilation, *Aedes aegypti* and ULV (Arquitectura, ventilación, *Aedes aegypti* y VUB)

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Aedes aegypti is an indoor species that favors heavily sheltered sites when it is not feeding or ovipositing. Vehicle-dispensed and aerial ULV applications for *Ae. aegypti* control are therefore essentially drift spraying operations from the street or the sky into the closet. To understand this drift, we must consider airflow into and around buildings. The mechanics of such airflow are complex, but predictable. Discussion in this paper is limited to the more important principles relevant to single story houses.

Air is deflected over and around buildings, creating calm zones of pressure differential, positive on the windward sides and negative to leeward. Air flows from inlets in positive areas to outlets in areas of lower pressure. The larger the negative calm area, the greater the potential for indoor flow. The proportions of a structure determine the form of the air movement. For example, behind a house of equal height, width and depth, the depth of the calm area is twice its height. The orientation of the house affects air movement by the same basic parameters: height, overall width to windward and depth. Because of the frictional drag of the roof, a thin structure, such as a wall, provides a larger calm area than a house of the same height and width, and the deeper the house, the less deep is the calm area. A wide house has a wider, deeper calm area than a narrow one, but roof-height still determines its height. Roof slope and pitch greatly increase the height and depth of the calm area. Buildings on stilts channel airflow under the floor as well as over the roof. Buildings in lines may protect downwind buildings from potential airflow, and tend to channel airflow along the streets.

Air has momentum—it will circulate in the interior of a house if the inflow is oblique to the alignment of the inlet/outlet, but will pass through in a straight, narrow stream if exterior and interior flow are in line. Velocity of air is determined by the inlet/outlet ratio. Maximum interior airflow occurs when the outlet is larger than inlet, because negative pressure is established inside the building. If inlets are larger than outlets, positive pressure is established indoors, so more airflow is deflected around the house. Vegetation, window forms, insect screens, overhangs, awnings and many other structures are also important.

In view of this complexity we can expect great variation in the potential efficacy of outdoor ULV applications. We certainly cannot expect comparable results against *Ae. aegypti* in the Caribbean as we do with an exophilic species such as *Ae. taeniorhynchus* in a typical mosquito abatement district in the USA. Moreover, the enormous differences in architecture and street plans, between countries and even within cities, means that the impact on *Ae. aegypti* in a village of lattice-walled wooden houses on stilts in South East Asia may be very different from that in a cinder-block and concrete urbanization in South America. For these reasons, the efficacy of ULV in any area can only be known with certainty if careful evaluations are made *in situ*.

Dengue activity in Puerto Rico in 1990 (Actividad de dengue en Puerto Rico en 1990)

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During the last 3 decades, Puerto Rico has experienced 11 epidemics of dengue and, beginning in 1986, from 6,000 to 11,000 cases of dengue-like illness have been reported annually. During the last 5 years, three serotypes (DEN-1, DEN-2 and DEN-4) have been isolated. The identification of these serotypes and serologic documentation of dengue virus transmission has been the result of a cooperative, laboratory-based surveillance program between the Puerto Rico Department of Health and the Centers for Disease Control. During 1990, 7,660 cases were reported to the Dengue Branch, of which 1,725 were confirmed by laboratory testing.

Although there is transmission throughout the year, the annual seasonal increase in dengue activity in Puerto Rico normally begins in August, usually peaking in October or November, and diminishing sharply by January or February. Increased dengue activity in 1990 began in August and was first recognized in the city of Lares in the western mountains. The outbreak was first thought to be caused by measles. From there, significant dengue activity was observed in the Ponce region on the southern coast with cases eventually confirmed from all municipalities. Highest incidence rates were seen on the southern coast, in several mountain municipalities and along the eastern end of the island.

Dengue-2 was the most frequently (72%) isolated serotype, followed by DEN-4 (21%), and was found throughout much of the island. It was isolated during every month of the year and from all municipalities where virus isolates were obtained. All 3 serotypes were isolated from 4 municipalities.

The highest percentage (36%) of laboratory confirmed cases and highest incidence (0.65/1,000 population) were in the age-group less than 15 years of age. Severe cases of dengue continue to be detected in Puerto Rico. This was reflected in 1990 by the number of cases that were hospitalized (150/1,000 confirmed cases) and the substantial number of cases with hemorrhagic manifestations (195/1,000 confirmed cases).

In 1990, we serologically identified a presumptive case of dengue with a fatal outcome. The case involved a 18 year-old female who resided in a locality west of the San Juan metropolitan area. This was the first such case since 1986 when 3 fatal cases were attributed to dengue/dengue hemorrhagic fever.

Socioeconomic impact of an outbreak of dengue in Lares, Puerto Rico (Impacto socioeconómico de un brote de dengue en Lares, Puerto Rico)

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We present preliminary findings obtained from a household survey conducted after an outbreak of dengue in Lares, Puerto Rico. Data were collected to document both the geographical distribution and the socioeconomic impact of the outbreak.

A random sample of households was selected based on the total number of occupied housing units. The number of household members ranged between 2 and 11 ($\bar{x} = 4$). A total of 417 individuals were included and only female heads of households were interviewed. Of those surveyed, 23% reported at least one dengue case within the family. Among these 22 households, there were 56 suspected cases reported (13% of the 417 persons surveyed).

Symptomatology was consistent with the clinical picture of classical dengue. Children ($n = 19$) represented 55% of the cases and 68% of those who visited a doctor during the outbreak.

The urban area produced 67% of the cases. Communities characterized by a low socioeconomic status reported the highest number of cases.

Documentation on direct cost of medical care indicated that while the outbreak did not produce serious cases requiring secondary or tertiary level of care, primary care was sought at least once by the majority of households who reported cases (86%). The local health center was the main source (66%) of this care. Medicaid was the health insurance most frequently mentioned as a source of payment for medical treatment. Other direct costs for transportation, prescription and over-the-counter medicines, and laboratories were not significantly high.

Indirect costs were also documented. Surveyed households reported 300 sick-days among the cases with clinical dengue, an average of 5 sick-days per person. Students missed 9 school-days, an average of 3 days per student. Although most of the adult cases were in housewives and unemployed males, employed persons lost 38 work-days, an average of 4 days per employee. Lost income per day ranged from $29 to $84. Housework was negatively impacted by the outbreak. The vast majority (91%) of the female homemakers interviewed reported not being able to complete all their housework normally performed during the days when they or their family members were sick with dengue.

Psychological costs were also documented. The majority (86%) of interviewed female caretakers reported a high level of stress related to lack of information about the illness and logistics (e.g., transportation to the health provider) as well as emotional stress (e.g., feelings of anxiety and nervousness) while they or their family members were sick with dengue.

Experience of community control of dengue in Honduras and future perspectives (Experiencias de control comunitario de dengue en Honduras y perspectivas futuras)

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Dengue fever is endemic in Honduras where the vector, *Aedes aegypti* was reintroduced in the 1960s. In 1977, the dengue virus was present in Honduras, and during 1978, the first epidemic occurred in the northern coast when about 100,000 cases were reported nationwide. Several outbreaks have occurred in subsequent years. Vector control methods such as the ultra-low volume application of insecticides against the adult mosquitoes or biological control are not adequate to deal with the problem when they are used alone.

Since *Ae. aegypti* is a domestic mosquito and its breeding sites are in and around human dwellings, human attitude and behavior regarding real and potential breeding sites can be part of the problem. Since they can be part of the solution, community participation in vector control programs is being emphasized.

During 1989 and 1990, a baseline study was conducted in El Progreso (population 70,000), Honduras. Epidemiological, entomological and social science data were gathered and analyzed. A small-scale educational program was developed which showed that knowledge and attitude toward *Ae. aegypti* breeding sites can be positively modified to improve vector control.

During 1991, community participation activities for *Ae. aegypti* control will be expanded to 10 communities in El Progreso (previous phase included only four) and with a wider scope of work: 1) community organization/mobilization, 2) school involvement, and 3) development of educational materials and programs.

After evaluating the success of the first phase, Ministry of Health officers have been requested to extend the benefits of this experience to other urban centers in the country as well as sharing experiences with health personnel in the other sanitary regions of Honduras, seeking to adapt methodology to their local vector control programs.

Simultaneously, a biological control research component will be initiated in El Progreso to begin application of *Mesocyclops* (crustaceans) as a complementary *Aedes* larval control method at the community level. Later, the applied biological control technology will be shared with community residents to support their efforts, as well as to maximize vector reduction.

The project is a result of an agreement for cooperation between the Honduran Ministry of Health and the Rockefeller Foundation in *Aedes aegypti* control.

The dengue control program in Mexico and perspectives for the future (El programa de control de dengue en México y perspectivas para el futuro)

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The recent history of dengue transmission in Mexico started in 1978 along the southern border with Guatemala. Since then, dengue has spread in epidemic fashion and is now endemic in 29 of 32 states in the country. The total number of cases reported through 1990 was 227,229, and 86% of the cases were found in only 13 states. After the eradication of *Aedes aegypti* in 1963, efforts to keep the country free of the vector were fruitless. The first dissemination of the infection and the recent transmission of dengue in high attitude areas demonstrated how difficult the control has been.

During the 1980s, the control program was dependent on the use of chemical tools for insecticide spraying and larval control. As in many other Latin American countries, the program faced problems and limitations in budget, trained human resources and equipment. These were not new but became more severe as transmission in different regions and the risk of dengue hemorrhagic fever increased. Since the solution to vector-borne diseases was traditionally with the government, the community perceived that the solution for dengue or malaria was outside its domain. The present situation hallmarks a point where the feasibility of investing in a technically based solution where specialized personnel, spraying machines and insecticide or larvicide use is out-of-hand. This is also because the vector is a domestic mosquito that breeds in man-made containers in the domestic setting, generated by specific human behavior and patterns of consumption. There is a need to create social awareness and recognize our participation and responsibility as a community, in generating a solution to the problem.

The program in Mexico is beginning to develop educational tools where the community is actively involved in their development and content. Different approaches to control are also being tested where breeding sites are controlled by routine activities like trash recycling schemes and biological control strategies, which will be developed and implemented.

Prevention strategies for dengue epidemics in Panama (Estrategias en la prevención de epidemias de dengue en Panamá)

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Many of the dengue epidemics in several Latin American countries since the 1970s could have been controlled by integrated health activities. The specific activities to be employed in such strategies include: 1) education of health personnel, 2) community education, 3) monitoring of febrile illnesses, 4) use of sentinel centers for active surveillance, and 5) preparation for an eventual outbreak. The case of Panama will be examined to illustrate how these activities are coordinated to produce reduced larval indices, monitor dengue virus activity and prepare for an outbreak.

The absence of dengue virus in Panama is surprising, given the fact that Panama was reinfested with *Aedes aegypti* in August 1985 (the overall house index has exceeded 5% every rainy season since 1986) and Panama's population is highly susceptible to dengue, since the last outbreak ended in 1942. In an effort to combat the possibility of a dengue virus epidemic, Gorgas Memorial Laboratory, the Ministry of Health, social security system, PAHO and community leaders have joined efforts in 1990 to implement a program to prevent epidemic dengue. This program included all 5 strategies.

Larval indices have shown a downward trend without the use of chemical control. Laboratory tests on 1,780 suspected cases of dengue studied since September 1988, employing virus isolation, IFAT, IgM-ELISA, HI and NT, indicated no dengue virus activity in Panama, except for 2 imported cases detected through the established surveillance system.

This is the first example in which a dengue-free country with *Ae. aegypti* reinfestation has made a concerted effort to reduce larval indices, while simultaneously monitoring dengue virus activity and preparing for an eventual outbreak of dengue.