

MOSQUITO VECTOR CONTROL AND BIOLOGY IN LATIN AMERICA—A SECOND SYMPOSIUM¹

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ABSTRACT. The second Spanish language symposium presented by the American Mosquito Control Association (AMCA) was held as part of the 58th Annual Meeting in Corpus Christi, TX in March 1992. The principal objective, as it was for the 1991 symposium, was to increase and stimulate greater participation in the AMCA by vector control specialists and public health workers from Latin America. This publication includes summaries of 25 individual presentations that were given in Spanish. The symposium included the following topics: biology and chemical control of *Aedes aegypti* and anopheline vectors of malaria; field and laboratory studies of biological control agents for *Aedes aegypti*; community participation in the prevention of dengue; and other various aspects of the biology of other medically important arthropods (e.g., *Simulium ochraceum*, *Lutzomyia* and *Culicoides*).

In 1991, the American Mosquito Control Association (AMCA), the internationally recognized leader in the field of mosquito control and research, conducted its first Spanish language symposium during its Annual Meeting in New Orleans, LA. As a result of the participation and success resulting from that session, a second symposium was organized and held at the 58th Annual Meeting in Corpus Christi, TX. As during the preceding year, the objectives were to encourage colleagues from Latin America to attend the AMCA meeting, present results of recent studies and projects, promote greater interaction with AMCA members, and stimulate future collaboration in the resolution of vector control and vector-borne disease problems in the Americas.

As in 1991, there was an enthusiastic response to participate in the symposium by our colleagues from Latin America. Had additional time been available on the program, several other presentations could have been given. As it was, 25 presentations covering a wide variety of topics were included in the symposium. The quality of the presentations, enthusiasm of the speakers, spirited discussion, and attendance at the session contributed to its success. The symposium's objectives were clearly met.

It was gratifying to see the substantial interaction between AMCA members, whether they were commercial exhibitors, scientists, or academicians, and their young counterparts from outside the US, where vector-borne diseases are more of a problem than within the continental US. It is apparent to many AMCA members

that our association has an important role and responsibility to work closely with our Latin neighbors. By holding this symposium, the AMCA is addressing this obligation. It is also clear that the participants in the Spanish symposia, which have brought added vitality to these meetings, are likely to become tomorrow's leaders in our field.

Special recognition for their generous financial support of this symposium goes to the following agencies and exhibitors and individuals: Vectec, Inc. (Isaac S. Dyals); Vector Biology and Control Project (VBC)/USAID (Andrew A. Arata); American Cyanamid Company (William Jany); Adapco, Inc. (Alan W. Wooldridge); LECO, Inc. (Kern Walcher); ICI Public Health (Julian Entwistle); Zoecon Corporation (Miguel E. Escobar); Clarke Outdoor Spraying Company (John L. Clarke Jr.); Sumitomo Chemical America, Inc. (S. Ohtsuki); London Fog, Inc. (Bob Bonnett); Summit Chemical Company (Larry Kase); Valent USA Corporation (J. Clark Hudson); Curtis Dyna-Fog, Ltd. (Donald Grayson); and Beecomist Systems, Inc. (Ed Kutzner). This support was used for registration fees, travel grants to some participants, and the simultaneous translation of the symposium. Arrangements for the translation were made by Jeff Flosi of the Local Arrangements Committee which also provided copies of the summaries (in English) for non-Spanish speakers attending the session. The VBC project has also provided funds for publication of the symposium summaries in *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 AMCA meetings.

SYMPOSIUM SUMMARIES

Distribution and biting behavior of *Anopheles* species in Casanare, Colombia (Distribución y

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comportamiento de picadura de las especies de *Anopheles* en Casanare, Colombia)

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Sixteen species of *Anopheles* were collected in the state of Casanare in Colombia from 1986 to 1991. Transmission of *Plasmodium vivax* predominates in this state with an average Annual Parasite Index of 4.0. From 11,820 specimens, 63% were *An. albimanus*, the most frequently collected and widely distributed species. The period of the day with highest human biting activity was between 1800 and 2000 h, with similar activity inside and outside the houses. In susceptibility tests to 4% DDT (1 h of exposure), this species showed an average of 27% mortality, which is compatible with resistance. This is the only species with DDT resistance in this state. *Anopheles albimanus* is not considered an important vector of malaria in this area but its role as a secondary vector is unknown. Species considered as vectors of malaria in this area are *An. darlingi* and *An. nuneztovari*. *Anopheles darlingi* was collected only in the municipalities located in the southwestern part of the state, comprising 27% of the 6,021 specimens collected in these municipalities. This species had more human biting activity at 2200 h. *Anopheles nuneztovari* was collected in the 7 municipalities studied but the density in the south was very low. Nunchia was the municipality with the highest density of this species. The human biting activity of *An. nuneztovari* began to increase at 1900 h; the maximum hour of activity was 2100 h, after which there was a slight reduction in biting activity. The other species collected as they bit humans, in relatively high numbers, were *An. brasiliensis*, *An. evansae* and *An. rangeli*. Sporadic collections were made of *An. neomaculipalpus*, *An. oswaldoi*, *An. peryassui*, *An. strodei*, *An. triannulatus*, *An. pseudopunctipennis*, *An. mediopunctatus*, *An. argyritarsis*, *An. benarrochi* and *An. neivai*.

The use of rDNA probes to differentiate *Anopheles albimanus* populations in Guatemala (El uso de sondas de rADN para diferenciar poblaciones de *Anopheles albimanus* en Guatemala)

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Anopheles albimanus is the principal vector of malaria in Central America, the Caribbean and coastal regions of South America. The taxon is behaviorally and ecologically polymorphic; how-

ever, genetic variability has not been reported as in other species of malaria vectors. Its extensive range and variable larval ecology are characteristic of a polymorphic species.

In this study we used DNA probes to compare intergenic spacer (IGS) length variation in ribosomal DNA (rDNA) among populations of *An. albimanus* from different geographic regions of Guatemala. Predominant IGS lengths may vary from one population to another, and this variation may be used to differentiate distinct populations.

Initially, the study included 3 populations (populations 2-4). Techniques were slightly modified and a second group of populations (5-9) was included. The sample size was 80 mosquitoes per population in the first group and 60 in the second group. DNA was extracted by the method of Livak (1984) and stored at -20°C until used. DNA was digested with restriction enzymes Hind III and Xho I at 5 units per mg of DNA at 37°C for 6 h. Cleaved DNA was fractionated for 18 h on 0.7% (set 7) and 0.9% (set 9) agarose gels and blotted to Genescreen filters. Filters were probed with pAal 1.4 Hind III-Xho I spacer probe radio-labeled with P³². Filters were hybridized overnight, filters were then washed and exposed to Kodak X-OMATAR film in cassettes containing 1 Dupont Lighting Plus intensifying screen.

The autoradiographs were first evaluated for the presence of IGS length variants (which appear as bands) resulting from hybridization with pAal 1.4HX probe. The 4 most intensely hybridized IGS length variants in each lane were selected and used in a discriminant analysis. The statistical analysis considered 2 data sets; first, populations 2-4 (set 7) and then populations 5-9 (set 9). A high degree of similarity was observed between the groups from the southern and eastern zones of Guatemala, based on values of the first canonical variables. Populations from these 2 zones were thus combined into 1 group, southeast, and a discriminant function was sought for the 2 groups: north and southeast. The model correctly classified 93% of the cases for set 7 and 90% of the cases for set 9, an excellent percentage. The frequency distribution of IGS length variants clearly show the different arrays of spacer length between populations.

Multigene families are influenced by a process called "concerted evolution" acting upon the turnover of DNA and which makes identity between member genes of a family (within a species) higher than between species.

These results suggest that a process of concerted evolution is taking place within populations of *An. albimanus*, and give an indication of population structure. Further studies have

been undertaken to study the IGS lengths of these populations and their genetic relationships.

Vectorial capacity of anophelines in western Venezuela (Capacidad vectorial de los anofelinos del occidente de Venezuela)

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In order to assess the entomological risk factors for malaria in western Venezuela, a longitudinal study was carried out between August 1988 and October 1989 in 3 selected villages located on the southern slope of the Andes Mountains near the Colombian border.

Mosquitoes attracted to human bait inside and outside experimental huts were collected monthly between 1900 and 0700 h, 2 nights per week per village. Each morning, the anopheline mosquitoes were identified to species and dissected for parity. Resting mosquitoes were collected monthly between 0600 and 0800 h, 4 days per week in each village. Temperature in resting places was recorded. After identification, mosquitoes were kept dry over silica gel until assayed by ELISA for host blood meal identification.

The vectorial capacity of *Anopheles nuneztovari*, *An. albitarsis* and *An. triannulatus* was estimated using the equations of Macdonald and Garrett-Jones. The estimated duration of the gonotrophic cycle of these species was 5 days and the duration of *Plasmodium vivax* sporogony was 12 days. *Anopheles nuneztovari*, the most abundant species in the 3 villages, showed the highest vectorial capacity during wet and dry seasons. During the rainy season, the estimated vectorial capacities were 5.5 for *An. nuneztovari*, 3.1 for *An. triannulatus* and 0.6 for *An. albitarsis*. During the dry season there was a significant reduction of the vectorial capacities of *An. nuneztovari* (91.8) and *An. triannulatus* (0.3), while *An. albitarsis* showed a slight increase (0.75).

The man-biting rate was the factor that showed the most significant change among species and seasons. *Anopheles nuneztovari* always had the highest man-biting rate. This factor reflects the importance of *An. nuneztovari* as the principal malaria vector in western Venezuela, especially during the rainy season when a person may receive up to 286 bites per night. These results indicate that the man-biting rate is the most important factor of vectorial capacity, and it is suggested that only this factor could be used as an indicator of vectorial capacity to evaluate control programs in western Venezuela.

Bionomics of adult populations of *Anopheles pseudopunctipennis* in the Tapachula foothills area of southern Mexico (Bionomía de la población de adultos de *Anopheles pseudopunctipennis* en el área de las colinas de Tapachula al sur de México)

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Studies on the bionomics of *Anopheles pseudopunctipennis* adults were conducted in the foothills near Tapachula, Mexico. Seasonal fluctuations in population densities, host-seeking behavior, population age-structure, weather effects on host-seeking activity, vector-parasite relationships (as defined by ELISA for malaria sporozoites), and comparisons of trapping methods were studied. Studies were conducted in 4 malaria-endemic areas along the Coatan River, from January 1989 to June 1991. Peak seasonal abundance of *An. pseudopunctipennis* occurred during the dry season which correlated with the peak malaria transmission. Vector densities were greatest in villages closer to the vector breeding sites in the Coatan River. A bimodal cycle of indoor host-seeking activity was documented. A peak of host-seeking activity occurred at 2000 h followed by a larger peak at 0100 h. Temperatures lower than 15°C resulted in reduced host-seeking activity. Moon phases also influenced nighttime activity patterns. Parous females represented a larger percentage of specimens in late-night collections. The horse-baited trap was more efficient at trapping *An. pseudopunctipennis* females than was human bait or the UV up-draft light trap. Positive specimens for both Pv210 and Pv247 *Plasmodium vivax* circumsporozoite (CS) protein polymorphs were detected in a sample of 9,386 *An. pseudopunctipennis* head-thoraces tested. These results further document the wide geographical distribution of the Pv247 CS protein polymorph in the New World.

Potential malaria vectors in northern Guatemala (Vectores potenciales de malaria en la región norte de Guatemala)*

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Malaria sporozoite and inoculation rates were measured over a 1 year period in anopheline

species from 2 endemic communities of northern Guatemala. A total of 9,337 *Anopheles albimanus*, 12,656 *An. vestitipennis*, 354 *An. neo-maculipalpus*, 14 *An. punctimacula* and 5 *An. apicimacula* were caught in all-night human bait collections and were analyzed using monoclonal antibody based ELISAs to detect sporozoites of *Plasmodium vivax* and *P. falciparum*. *Plasmodium vivax* and *P. falciparum* sporozoite antigens were only detected in *An. albimanus* and *An. vestitipennis*, the latter not previously related with *P. falciparum* transmission. Within the same locality, significant differences were observed in the sporozoite rate of *P. vivax* between *An. albimanus* (0.025%) and *An. vestitipennis* (0.041%). The sporozoite load estimated as the amount of circumsporozoite protein was significantly higher in *An. vestitipennis* (1.81 pg/well for *P. vivax* and 52.13 pg/well for *P. falciparum*) than in *An. albimanus* (0.95 pg/well for *P. vivax* and 22.21 pg/well for *P. falciparum*). The higher sporozoite rates and possibly higher parasite loads found in *An. vestitipennis* may account for differences in transmission. Seasonal variations in entomological inoculation rates were found among species and within a village with higher malaria transmission during the rainy season. The combined number of bites per man per night infected with *P. vivax* and *P. falciparum* ranged from 0.0 to 77.6 for *An. vestitipennis* and 4.4 to 17 for *An. albimanus*. These results indicate that *An. vestitipennis*, in addition to *An. albimanus*, could play an important role in the transmission of human malaria in the northern region of Guatemala.

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Evaluation of malathion and Paris green for the control of *Anopheles albimanus* in a tourist area in the Dominican Republic (Evaluación de la aplicación de malation y verde de Paris para el control de *Anopheles albimanus* en una área turística de la República Dominicana)

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In order to reduce *Anopheles albimanus* populations, experimental treatments with malathion, as an adulticide, and Paris green, as a larvicide, were conducted in "El Cortecito," an important tourist area in the Dominican Republic. Ultra-low volume (ULV) applications of malathion were made with vehicle-mounted London thermal-fog generators. The thermal-fog application rate was 584 ml/ha in each of 4 field

trials. Paris Green was applied to *An. albimanus* larval habitats with a Nuvola® knapsack powder duster at the rate of 5 g/m².

Treatment efficacy was measured by conducting *An. albimanus* human bait landing counts indoors and outdoors before and after insecticide applications. These treatments produced landing rate reductions of 74 and 85%, indoors and outdoors, respectively. Larvicidal action after 2 wk showed a reduction of 91%.

Impact of partial deltamethrin resistance in *Anopheles albimanus* on the efficacy of intradomiciliary spraying for malaria control (Impacto de la resistencia parcial de *Anopheles albimanus* a la deltametrina sobre la eficacia del rociamiento intradomiciliar para el control de malaria)*

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We evaluated the efficacy of intradomiciliary spraying with deltamethrin (0.025 g/m²) for malaria control in the presence of partially resistant vectors. Six villages in the southern region of Guatemala were assigned to 1 of 2 study groups: low ($\leq 30\%$) and high ($\geq 60\%$) mosquito survivorship in the WHO "tube" bioassay. Measurements before and after indoor house-spraying estimated parameters of behavioral reactions and vectorial capacity indices of *Anopheles albimanus* in all communities, and monthly prevalence of human malaria in 2 representative villages. After 18 months of research, the mean mosquito survivorship (WHO bioassay) in the low and high categories were 49.6 and 60.8%, respectively ($\chi^2 = 88.51, P < 0.01$). Some of the entomologic data showed differences between the groups: 1) number of mosquitoes/hour/man (low, 0.9; high, 6.4); 2) number of resting mosquitoes/sprayed house (low, 0.6; high, 3.7); and 3) parity rates of mosquitoes exiting from sprayed houses (low, 46.9%; high, 22.6%). Results, however, were similar for mosquitoes exiting sprayed houses: 1) blood-feeding success (low, 43.4%; high 38.1%); and 2) survivorship rate (low, 94.3%; high, 88.3%). In addition, the monthly prevalence of human malaria did not decrease in either category. Results suggested that intradomiciliary spraying with deltamethrin has not effectively interrupted malaria transmission under the study conditions. Therefore, we propose guidelines for a more rational use of this vector control measure to: 1) consider alternative insecticides when mosquito survivorship to deltamethrin in the WHO "tube" bioassay is \geq

50%; 2) avoid the application of insecticides which are used for agricultural practices in the area, as they rapidly select for resistant mosquitoes; and 3) time the intradomiciliary spraying, not only with the malaria transmission peaks, but also with the periods of greatest susceptibility to the insecticide since it varies throughout the year.

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Variation of head chaetotaxy in *Aedes aegypti* larvae from Mexico with implications for entomological surveillance of *Ae. albopictus*
(Variaciones chaetotáxicas en la cabeza de las larvas de *Aedes aegypti* de México con implicaciones en la vigilancia de *Ae. albopictus*)

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Chaetotaxic variations in the larva of some mosquito species is well-documented. When these variations occur in certain taxonomic characters used in separating closely allied species, considerable confusion and uncertainty usually occurs. As a product of the *Aedes albopictus* surveillance program in Mexico, nearly 50,000 mosquito larvae were examined in Mexico City at the National Laboratory of Entomology. *Aedes albopictus* was not found but *Ae. aegypti* was present in nearly all localities studied. We received large numbers of mosquito larvae from 11 states, especially those near the border with the United States. Collections came from 34 types of artificial containers, of which tires, flower vases, tanks and small domestic containers yielded the most entomological material. Variation in the utility of head setae for taxonomic determination was so frequent that 5 classes of combinations were established. These variations were detected in about 7.2% of the 3,383 *Ae. aegypti* larvae studied. This variation was most common in the border states of Tamaulipas and Nuevo León.

Counts were also made of the number of cases of combinations present in Mexico. We found that the best larvae having paired setae on the head in 4 classes of the 5 mentioned were from Nuevo León state with a total of 12 combinations and Tamaulipas state with a total of 10 combinations. However, the number of larvae with double setae ranged from 6 to 28, appeared in the rest of the country. These observations indicated that wide variation may occur in different specimens of the same species. No doubt

such variations result in uncertainties in identification, particularly if there are few specimens and these happen to be "off type." Very often taxonomic keys use head setae for discrimination of closely related species (Carpenter and La Casse 1955, Darsie and Ward 1981). On the other hand, many descriptions are based on the examination of a limited number of specimens before the extent of the differences in anatomical structure has been fully determined. This fact is important and must be considered, in addition to other taxonomic characteristics, to avoid incorrect determinations. From our surveillance of dengue vectors, only *Ae. aegypti* is in Mexico although *Ae. albopictus* may appear there in the future.

Time required for peritrophic membrane formation in pupae and pharate adults (El tiempo que toma la formación de la membrana peritrofica en la pupa y adultos faratos)

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The peritrophic membrane (PM) in Diptera is of interest to vector biologists due to its role in the physiology of the vector and its potential influence on disease transmission. In the larval stage of mosquitoes, the PM is secreted by the cardial epithelium at the anterior end of the midgut and is referred to as a type I PM. In adult mosquitoes, the PM is formed by delamination of all or most of the midgut epithelium. This is referred to as type II PM. The formation of both types of PM is associated with the passage of food along the gut, organic debris from the water in larvae and blood in adults. Distension of the adult midgut by blood serves as a stimulus for PM formation.

In the pupal stage, however, another dimension is observed. The pupa/pharate adult does not feed although 2 PMs form around the meconium within the developing midgut. The first PM (PM1) forms sometime during the pupal/pharate adult period. The second PM (PM2) forms just after pupal-adult ecdysis.

The objective of this study has been to determine histologically, in several mosquito species, the nature and timing of PM1 formation in both sexes during the pupal/pharate adult period and the relation of this event to the initiation of molting.

Serial paraffin sections were made of mosquito pupae of known age post-larval-pupal ecdysis. The species studied were *Aedes aegypti*, *Ae. triseriatus*, *Culex nigripalpus* and *Psorophora columbiae*. The appearance of molting fluid was used as an indication that molting had been initiated. The pattern of degeneration of meco-

nium and the extent of PM1 formation was observed at hourly intervals throughout the pupal/pharate adult period.

The timing of PM1 formation in relation to molting fluid secretion was similar in all 4 species studied. Distinct membranous material was first seen shortly before the appearance of molting fluid in *Ae. triseriatus*, shortly after in *Ae. aegypti* and *Ps. columbiae*, and shortly before in *Cx. nigripalpus*. In all 4 species, granules that were probably PM1 precursor material appeared shortly before distinct membranous material was observed.

Our results indicate a temporal proximity between the timing of PM1 formation and the initiation of molting. The secretion of ecdysone or molting hormone, which initiates molting, may, therefore, be the initial stimulus for the secretion of the peritrophic membrane in mosquito pupae.

Degeneration of the meconium was apparent as an observable loss of cellular detail leading to the granular appearance of the meconial cells. Degeneration occurred in a similar pattern in all 4 species studied, beginning in the posterior portion of the meconium, followed by the anterior, and finally the middle portions.

The formation of PM1 around the meconium begins with the appearance of granules in the peritrophic space, which appear to be precursor material for PM1. As PM1 forms and the meconium degenerates and shrinks, "filaments" of PM1 become evident at the anterior and posterior ends of the meconium. The majority of the specimens we studied were females, but PM1 was also seen in males.

Age structure of *Simulium ochraceum* and transmission of *Onchocerca volvulus* in México (Estructura de la edad de *Simulium ochraceum* y la transmisión de *Onchocerca volvulus* en México)

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The aim of this work was to relate the age-structure of black flies with the daily and seasonal captures and infectivity/infection rates. Monthly catches of black flies were performed on human volunteers in a village of the southeastern onchocerciasis endemic area in the state of Chiapas, Mexico. Monthly host-seeking density of female black flies was calculated as a modified geometric mean (M_w) from 192 sampling hours (2 habitats \times 8 days \times 12 h) for each month of a year. Fly captures were made during 30 min every hour for 12 months (September 1990–August 1991) by the same collectors who

were rotated daily from one collection site to the other. Flies were dissected and examined following the Detinova (1962) technique for age determination. *Simulium ochraceum*, the main anthropophilic species, *S. metallicum* and *S. callidum* showed host-seeking patterns related with seasonal changes. *Simulium ochraceum* and *S. metallicum* populations were higher from December through February (the first part of the dry season). A slight variation in the density of *S. callidum*, greatest from October to December (the last part of the rainy season), was observed. Seasonal variations in the average infective biting rate ($\bar{x}IBD = \text{frequency of the third stage } Onchocerca \text{ volvulus larvae} \times \text{mean biting rate}$) in host-seeking parous flies were observed. The $\bar{x}IBD$ showed a unimodal pattern with a peak in the January to March period. Additionally, the $\bar{x}IBD$ pattern was similar to the "Mw" of parous biting rate. However, $\bar{x}IBD$ in host-seeking density of parous *S. ochraceum* showed a bimodal pattern with the higher peak in March and the lower peak in August. The first peak value was 2.1 infected females/day, and there were 1.0 infected females/day for the second peak. The diurnal host-seeking activity pattern for *S. ochraceum* peaked between 0800 and 1000 h. The proportion of parous biting density was higher than the nulliparous biting density between 1200 and 1700 h. Eighty-three percent of the parous *S. ochraceum* collected during 1000 h and 1600 h had large dilations in the tunica of the ovarioles. Thus, it appears that *S. ochraceum* oviposits in the morning and immediately seeks a human host. The highest percentages of infective biting *S. ochraceum* were collected at 1000, 1400 and 1600 h. The diurnal infective biting pattern closely followed the parous biting density pattern. Host-seeking density of nulliparous *S. ochraceum* showed a distinct peak between 0900 and 1000 h, higher than observed in parous flies and probably related to the daily adult emergence pattern of this species.

***Culicoides* spp. attacking cattle in Costa Rica, Honduras, Panama and Puerto Rico as potential vectors of bluetongue virus (*Culicoides* spp. atacando ganado en Costa Rica, Honduras, Panamá y Puerto Rico como vectores potenciales del virus de la lengua azul)**

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In 1991, as part of an epidemiological study of bluetongue viruses (BTV) in Central America and Caribbean Basin, 8 farms located in Costa Rica, Honduras, Panama and Puerto Rico were sampled for *Culicoides* spp. attacking cattle.

Using bovine bait, 3,884 biting midges were collected with an electric aspirator around sunrise and sunset. The predominant species captured was *Culicoides insignis* Lutz (95%), followed by *C. furens* (Poey) (3.4%), *C. filarifer* Hoffman/*C. ocumarensis* Ortiz (0.9%), *C. lahillei* (Iches) (0.7%), *C. arubae* Fox and Hoffman (<0.1%), and *C. gorgasi* Wirth and Blanton (<0.1%). Many blood-engorged specimens were collected from these species: 18% of *C. insignis*, 36% *C. furens*, 37% *C. filarifer*/*C. ocumarensis*, and 25% *C. lahillei*. No engorged *C. arubae* nor *C. gorgasi* were caught. These results confirm earlier findings that suggested that *C. insignis*, *C. furens* and *C. filarifer*/*C. ocumarensis* are potentially important vectors of BTV in the region.

Colonization of *Lutzomyia spinicrassa*, a potential vector of *Leishmania braziliensis* in Colombia (Colonización de *Lutzomyia spinicrassa*, un vector potencial de *Leishmania braziliensis* en Colombia)

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Lutzomyia spinicrassa Morales, Osorno, Osorno and Hoyos (Diptera: Psychodidae), originally described from Colombia, occurs in various parts of Colombia and Venezuela. Its importance results from its highly anthropophilic behavior and its role as a host for *Leishmania braziliensis*. In the Arboledas region in the State of Norte de Santander, Colombia, this parasite was also isolated from human beings, thus incriminating it as a potential vector of *L. braziliensis*.

On November 3, 1990, 600 *Lu. spinicrassa* females were collected with a CDC light trap and human bait. In the laboratory, the females were fed on hamster blood and males and females were fed on a 30% sucrose solution. Larvae were fed a mixture of dried bovine feces, concentrated mouse food and liver powder. The colony was kept at 23°C and 70% relative humidity. Currently, this colony is in its 6th generation with increased insect production in each generation.

These results have produced valuable information on the development of the different stages of *Lu. spinicrassa*, their requirements for food, and the influence of environmental conditions, such as temperature and relative humidity. The establishment of this colony has allowed us to study the life cycle of *Lu. spinicrassa* in our laboratory and will be very helpful for future studies of the development of *L. braziliensis* in this potential vector.

Sugar feeding habits of *Aedes aegypti* females from San Juan, Puerto Rico (Alimentación de nectar por *Aedes aegypti* de San Juan, Puerto Rico)

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We investigated the sugar-feeding habits of *Ae. aegypti* in San Juan, Puerto Rico, where dengue is endemic, and this species is the vector. In addition, we followed the utilization and re-synthesis of glycogen and lipids. In October 1991 and January 1992, adults were collected by power aspirator inside and outside inhabited houses in urban San Juan. Females were separated according to gonotrophic development. In addition, pupae were collected and adults analyzed at emergence. All specimens were immediately dried at 100°C and maintained at room temperature.

Only 2% of the females tested contained a trace of fructose. All females that contained blood or had mature eggs were fructose-negative. By contrast, more than half the males were fructose-positive. This confirms that the absence of fructose in females was real and not due to post-mortem losses between the time of capture and analysis. The presence of fructose-positive males indicated that although sugar sources were amply available in the environment, they were ignored by females in the presence of a blood source. Thus, it appears that females used blood both for the maintenance of energy balance and for reproduction.

Empty females, presumably young and nulliparous, had used a considerable amount of the glycogen and lipids present at emergence. Apparently, they had lived for some days without feeding. After blood feeding they accumulated new lipids and glycogen. Blood-containing and gravid females in the January collection had accumulated much more lipid than in the October collection and, therefore, must have taken larger quantities of blood. Similarly, the males took much more sugar in January. The total dependence of females on blood suggests that virus may be transmitted faster and more effectively than in sugar feeding populations.

In contrast, both female and male *Ae. aegypti* collected at a tire dump, distant from human populations, near Vero Beach, FL, were very efficient sugar feeders. Apparently, only females living in close association with a major blood source fed exclusively on blood.

Estimation of the survival rate and oviposition cycle in *Aedes aegypti* populations in Monterrey,

México (Estimación de la tasa de sobrevivencia y ciclo de oviposición en poblaciones de *Aedes aegypti* de Monterrey, México)

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The survival rate and oviposition cycle of *Aedes aegypti* in urban areas of Monterrey, Mexico, were determined. Birley and Boorman (1972), Birley (1984) and Mutero and Birley (1987) proposed using the least squares linear regression to relate the number of mosquitoes biting on any day to parous individuals collected after one oviposition cycle to estimate the average survival rate and oviposition cycle. To apply this method, a continuous time series of daily samples from a vector population is required and must separate nulliparous and parous females. Likewise, data must be transformed (filtered time series) using the function $[Z_t = X_t - \beta(X_t - 1)]$, where "X_t" is the number of parous females collected on day "t" and "X_t - 1" is the total females (parous and nulliparous) collected one day before "t," and "β" is the slope value of the linear regression applied to the data. Then a cross-correlation (CC) analysis is made. The highest correlation coefficient obtained after day 0, will determine the number of days in the oviposition cycle and the slope value in the linear regression will be the average survivorship rate per oviposition cycle.

In this study, female *Ae. aegypti* were studied daily between 1600 and 1900 h from October 14 to 29, 1991. Mosquitoes were collected outdoors

by 2 persons with exposed legs and arms. Females were separated into parous and nulliparous groups (Detinova 1962). Data were filtered to eliminate spurious CC values using an autoregressive process with a 1-day lag for total and parous females. The correlation coefficients were: 0.75 for day 0, 0.20 for day 1, -0.39 for day 2, -0.47 for day 3, 0.31 for day 4, 0.55 for day 5, -0.09 for day 6, and -0.46 for day 7. Using this method, the oviposition cycle was estimated to be 5 days, because on the fifth day the first significant correlation coefficient appeared. Also, on the fifth day, the linear regression was $Y = 5.056 + 0.498X$. From this equation the slope value is an estimator of the average survivorship rate. Therefore, according to the formula of Macdonald (1952), the daily survivorship rate was 0.87.

Laboratory and field evaluation of commercial and locally prepared formulations of *Bacillus thuringiensis* var. *israelensis* and *B. sphaericus* on Culicidae of northeastern México

(Evaluaciones de laboratorio y de campo de formulados comerciales y preparados locales de *Bacillus thuringiensis* var. *israelensis* y *B. sphaericus* con culicideos del noreste de México)

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As part of an institutional program of mosquito control research with biological and microbial control methods, we conducted laboratory

Table 1. Evaluation of *Bacillus thuringiensis* var. *israelensis* and *B. sphaericus* for control of Culicidae from northeastern Mexico.

Formulation	Species	Source	LC ₅₀ (mg/liter)
<i>B. thuringiensis</i> var. <i>israelensis</i>			
Bactimos (4500 UTI/mg)	<i>Ae. aegypti</i>	Lab.	0.04
	<i>Cx. quinquefasciatus</i>	Lab.	0.15
	<i>Cx. quinquefasciatus</i>	Field	0.01
	<i>Cx. coronator</i>	Field	0.05
ABG 61-68 (1058 UTI/mg)	<i>Ae. aegypti</i>	Lab.	0.17
	<i>Cx. quinquefasciatus</i>	Lab.	0.47
	<i>Cx. coronator</i>	Field	0.12
<i>B.t.i.</i> —FDL-90 (3600 UTI/mg)	<i>Ae. aegypti</i>	Lab.	0.05
	<i>Cx. coronator</i>	Field	0.02
	<i>Cx. quinquefasciatus</i>	Field	0.02
<i>B.t.i.</i> —FDL-91 (2571 UTI/mg)	<i>Ae. aegypti</i>	Lab.	0.07
	<i>Cx. quinquefasciatus</i>	Lab.	0.16
	<i>Cx. quinquefasciatus</i>	Field	0.03
<i>B. sphaericus</i> ABG-6185	<i>Cx. coronator</i>	Field	0.04
	<i>Cx. quinquefasciatus</i>	Lab.	0.16
	<i>Cx. quinquefasciatus</i>	Field	0.39
	<i>Cx. quinquefasciatus</i>	Lab.	0.48
FCB-SP-2	<i>Cx. quinquefasciatus</i>	Lab.	0.48
	<i>Cx. quinquefasciatus</i>	Field	0.31

and field evaluations of commercial and local design formulations (FDL) of *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*) and *B. sphaericus* on culicid species in Nuevo León state of Mexico.

Results of evaluations on culicid larvae laboratory reared and field collected larvae are shown below (Table 1).

Commercial formulations of *B.t.i.* (Bactimos and ABG 61-68) and a local design formulation (*B.t.i.*—FDL 91) was more active against laboratory-reared *Ae. aegypti* larvae than laboratory-reared *Culex quinquefasciatus*. Field-collected *Cx. quinquefasciatus* were more susceptible to the *B.t.i.* formulation (FDL-91) than those reared in the laboratory.

A commercial formulation of *B. sphaericus* gave satisfactory results against laboratory-reared *Cx. quinquefasciatus* while the local formulation was slightly more effective in the field-collected samples. *Bacillus sphaericus* also has the ability to be recycled in dead mosquito larvae, a practical advantage over *B.t.i.*

Introduction of *Pantala* sp. and *Abedus* sp. for the biological control of immature mosquito populations (Introducción de *Pantala* sp. y *Abedus* sp. como control biológico de poblaciones de mosquitos inmaduros)

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The manipulation and release of aquatic predators as a biological control measure for mosquito larvae is an alternative for the integrated management of mosquitoes.

In "La Ciudadela" Creek of Juarez in Nuevo León state, Mexico, 3 natural pools with an area of 2 m² were studied. In one, *Pantala* sp. (Odonata: Libellulidae) naiads were released and in the second adult *Abedus* sp. (Hemiptera: Belostomidae) were released. The third pool was an untreated control. Ten predators of each species were released every 2 weeks. Each week, 10 samples were collected from all pools and all fish were removed.

The organisms in the collection were separated into: immature mosquitoes, predators, herbivores, copepods and others (i.e., small crustaceans and scavenger beetles). The results were analyzed by analysis of variance for the immature mosquito population. *Anopheles pseudo-punctipennis* and *Culex pipiens* were the mosquito species collected. No difference in imma-

ture mosquito abundance was found after releases of *Pantala* sp., *Abedus* sp. and the untreated control ($F = 0.26$, $P > 0.05$). These introductions showed the same efficacy as natural predators on mosquitoes.

Prey selection by three aquatic predators (Selectividad de presas de 3 depredadores acuáticos)

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The prey selectivity of 3 aquatic predators was evaluated in systems with *Culex pipiens* and *Chironomus*.

Adult *Buenoa* sp. and *Notonecta* sp. backswimmers (Hemiptera: Notonectidae) and third instar larvae of the water scavenger beetle (*Tropisternus* sp.) (Coleoptera: Hydrophilidae) were the predators studied. Fourth instar *Culex pipiens* and *Chironomus* sp. larvae were used as prey. All insects were collected from the Psequeria River in Escobedo, Nuevo Leon. The tests were conducted in glass containers with 750 ml of dechlorinated water. One predator was exposed for 24 h to several densities of fourth instar larvae of *Cx. pipiens* and *Chironomus* sp. at a 1:1 rate (1 *Cx. pipiens*: 1 *Chironomus* sp.). The preference of prey was analyzed by functional response models of Holling (1959) and Rogers (1972), and the selectivity coefficients of Ivlev (1961) and Jacob (1974).

In terms of functional response, the prey preference was expressed as the highest value of searching capacity (a') and lowest value of handling time (Th). According to Holling's model, the backswimmers showed a preference for *Cx. pipiens* larvae where *Buenoa* sp. had an $a' = 0.035$ and an $a' = 0.021$ for *Chironomus* sp. The respective "Th" values were 1.458 and 2.255. *Notonecta* sp. had an $a' = 0.032$ to mosquito larvae and an $a' = 0.029$ to midge larvae. The respective "Th" values were 0.252 and 0.371. *Tropisternus* sp. larvae showed selectivity for midge larvae with an $a' = 0.021$ versus an $a' = 0.001$ for mosquito larvae. The respective "Th" values were 14.340 and 62.629.

According to Rogers' model, *Buenoa* sp. showed a greater preference for *Cx. pipiens* larvae ($a' = 0.042$; Th = 0.103), versus *Chironomus* sp. ($a' = 0.035$; Th = 2.999). Similarly, *Notonecta* sp. preferred mosquito larvae ($a' = 0.279$; Th = 0.825) over midge larvae ($a' = 0.214$; Th = 0.963). As in the Holling model, *Tropisternus*

sp. also selected midge larvae ($a' = 0.734$; $Th = 2.714$) in lieu of mosquito larvae ($a' = 0.050$; $Th = 66.136$).

Field collections of copepods from Nuevo León and Coahuila, Mexico (Colecciones de campo de copépodos alrededor de Nuevo León y Coahuila, México)

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Copepods have been identified as a promising form of biological control for *Aedes aegypti* mosquitoes. With the objective of identifying copepod candidates for biocontrol, field collections were made in Nuevo León and Coahuila, Mexico. Copepods which are common in a variety of aquatic and semiaquatic habitats were collected with a standard plankton net. The following species were identified: *Acanthocyclops robustus* (Sars), *Acanthocyclops vernalis* (Fischer), *Ectocyclops phaleratus* (Koch), *Eucyclops agilis* (Koch), *Eucyclops speratus* Herrick, *Mesocyclops longisetus* Dussart, *Macrocyclus fuscus* (Jurine), *Macrocyclus albidus* (Jurine), *Microcyclus rubellus* (Lilljeborg), and *Megacyclus viridis* (Jurine). From these collections, the larger species (i.e., *Me. longisetus*, *Ac. robustus*, *Ma. albidus* and *Ma. fuscus*) will be evaluated as candidates for *Ae. aegypti* control.

Predatory capacity in the laboratory of two copepod species from Nuevo León, México, with potential as biocontrol agents for *Aedes aegypti* (Capacidad de depredación en el laboratorio de dos especies de copépodos con potencial como agentes de biocontrol de *Aedes aegypti* en el estado de Nuevo León, México)

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In the functional response models of Holling (1959) and Rogers (1972), the searching capacity or attack coefficient can be determined. "Good" entomophagy is mathematically expressed as a' and is defined as the predatory capacity to move in a system test (area or volume), with a maximum theoretical value of 1. The other quality is handling time, expressed as Th , and it is the

time that the entomophagous predator spends to locate, catch and kill its prey, with ideal value of 0 or near it.

The predatory capacities of 2 copepods (*Macrocyclus albidus* and *Mesocyclops longisetus*) on *Aedes aegypti* larvae were evaluated. Both copepod species were reared in the laboratory and the tests were carried out in containers with 7.6 ml (V1), 700 ml (V2) and 1,500 ml (V3). One copepod was introduced into the container and exposed to 8 densities (varying from 1 to 128) of first instar *Ae. aegypti* larvae and replicated 30 times. After 24 h the numbers of prey killed was recorded. The results were analyzed by functional response models of Holling (1959) and Rogers (1972).

A *Ma. albidus* strain (La Chueca) showed a searching capacity of $a' = 0.033$ (V1), $a' = 0.030$ (V2) and $a' = 0.069$ (V3) according to Holling's model; and by Rogers' model $a' = 0.056$ (V1), $a' = 0.057$ (V2) and $a' = 0.043$ (V3). The *Ma. albidus* strain (Linares) showed a higher searching capacity than La Chueca ($a' = 0.034$) with Holling's model and Rogers ($a' = 0.069$), and *Ma. albidus* strain (El Yerbaniz) had the lowest values, $a' = 0.028$ and $a' = 0.048$. *Mesocyclops longisetus* strain (Acuacultura F.C.B.) showed a high searching capacity ($a' = 0.037$ and $a' = 0.068$).

Based on these laboratory tests, the best candidates for control of *Ae. aegypti* were *Ma. albidus* (Linares strain) and *Mesocyclops longisetus* (Acuacultura F.C.B. strain).

Mass cultivation of copepods used for the biocontrol of *Aedes aegypti* (Cultivo masivo de copépodos utilizados en el biocontrol de *Aedes aegypti*)

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Cyclopoid copepods (planktonic microcrustaceans) have been identified as a promising new form of biological control for container breeding mosquito species such as *Aedes aegypti* (Linn.). A simple method for indoor and outdoor cultivation of *Mesocyclops aspericornis* (Daday) and *Macrocyclus albidus* (Jurine) copepods is presented. For indoor cultivation, laboratory facilities should have at least 3×2 m of floor space, hot and cold water, a 20 liter sink, and shelves of sufficient size and capacity to support large plastic rearing pans ($45 \times 35 \times 18$ cm). Good quality water is critical for maintenance of healthy copepod cultures. Separate stock cul-

tures of commercially available *Paramecium caudatum* and *Chilomonas* sp. can easily be started with 900 ml of dechlorinated water in glass bottles. A 10 cm² piece of fresh lettuce is added to the bottle at the time these protozoa are added to the water. If too much lettuce is added, anaerobic conditions, deleterious to the culture, will develop. If the protozoan population in the culture bottle is high (more than 500 specimens per 600× field), they can be harvested for feeding the copepods. The contents of the flask are filtered through a standard tea strainer into a bucket to eliminate lettuce particles. About 50–100 ml of the stock culture should be retained to maintain the stock culture. Additional dechlorinated water and lettuce should be added to this stock to provide fresh water and new food supply to stimulate the rapid regeneration of the protozoan population. In each plastic tray, pour ca. 2.5 liters of the food culture (i.e., 3 containers with 800–850 ml). This will produce a 3 cm depth in the tray, which is sufficient for copepod cultivation. Add 20–30 copepod specimens and a piece (20 cm²) of lettuce and cover the trays with aluminum foil to minimize surface evaporation. After 3 weeks, this procedure will yield about 2,000 adult copepods per tray. The copepods can be harvested using a 200 mesh Nitex strainer.

We used inexpensive plastic wading pools (1 m wide × 8 cm deep) to increase copepod production and begin outdoor cultivation. To begin culture in this venue, we added 1 bucket (2 liters) of the stock food culture (*Paramecium caudatum* and *Chilomonas* sp.), 1 bucket of dechlorinated tap water, 30 cm² of fresh lettuce, and about 50 copepods. Some were covered with a net to minimize contamination of the outdoor cultures.

Did ULV prolong an epidemic of dengue in Florida, Puerto Rico? (¿La aplicación UBV prolonga una epidemia de dengue en Florida, Puerto Rico?)

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A model of dengue transmission presented at AMCA in 1991 by Newton and Reiter predicted that ultra-low volume (ULV) treatments during an epidemic would have little effect on the *total number of cases*, but that the *period of transmission* would be prolonged. This year we examined the impact of ULV on a real dengue epidemic in Yanes, an urbanization of 425 houses on the outskirts of the town of Florida (population 8,689).

Over more than 6 months (week 28, 1991 to week 4, 1992) we received 350 blood samples from presumptive dengue cases in Florida. Peak incidence was in week 39. The base case of the model indicated a duration of ca. 3 months in a community of 10,000. One severe case of dengue hemorrhagic fever (DHF), and one fatal case were confirmed (both from Yanes, weeks 44 and 45). The regional authorities treated the area weekly or biweekly with ULV (91% malathion, 100 ml/ha), starting in week 34 and continuing for 5 months. Source reduction teams treated the homes of suspected cases, and an energetic informational campaign was conducted through several community organizations.

We collected adult mosquitoes by power aspirator during weeks 37 and 38, after the 4th and 5th ULV treatments. Two hundred eighteen bedrooms in 96 randomly chosen houses yielded 822 female mosquitoes (3.8/bedroom) or ca. 2 mosquitoes per person, the same ratio as used in the base case of the model. One mosquito was positive for dengue-2 virus by head-squash (0.12% vs. ca. 1% predicted); 2 others are pending further examination. A premise survey in 69 randomly selected houses (week 38), gave a House Index (HI) of 61% and Breteau Index (BI) of 109. In week 47, after the widely publicized fatal case, indices were substantially lower (HI = 30%; BI = 40).

A serosurvey and a questionnaire survey were conducted (weeks 45–48) in all houses used for adult collections. Preliminary results show 276 of the 332 samples (83%) were positive for IgG, of which 20 (6%) were secondary infections (high IgG titer). Forty-nine samples were positive for IgM (18%), of which 9 (3%) were secondary infections. Our preliminary conclusions are: 1) the epidemic was substantial; 2) despite the intensive, highly visible ULV operation, a sizable population of adult mosquitoes persisted, including insects infected with dengue virus; 3) transmission continued for more than 6 months, remarkable for such a small community; 4) the epidemic may have been prolonged by the ULV treatments; and 5) larval indices remained high, even after a widely publicized fatal case and an intensive educational campaign.

Mosquito control with high school students as health educators in Bucaramanga, Colombia (Estrategias de control de mosquitos utilizando estudiantes de bachillerato como educadores de salud en la ciudad de Bucaramanga, Colombia)

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Before 1984, the "Dirección de Campañas Directas" (previously called Servicio de Erradica-

ción de la Malaria), ran the Mosquito Control Program in the city of Bucaramanga, using traditional methods without citizen participation and media publicity. In 1985, the city assumed control of this program through the Bucaramanga Health Institute.

The current program involves high school seniors who have received training on *Aedes aegypti* biology and control. Each year, these students (approximately 500) visit 50–70% of the houses in this city of 70,000 houses. The students visit the houses to educate the residents and provide brochures that explain our program objectives and how to control *Ae. aegypti* production sites. They also advise people how they can become involved in the program and get information about *Ae. aegypti* populations.

Since 1985, the *Ae. aegypti* population and the number of people with dengue fever have decreased.

In 1990, several dengue hemorrhagic fever (DHF) cases occurred in Colombia, but in spite of its geographical location, there were no reported DHF cases in Bucaramanga. In 1991, the program was not functional because of a lack of people to work in the program and the *Ae. aegypti* population and the dengue fever cases increased.

Community organization for *Aedes aegypti* control: an integrated approach (Organización comunitaria para el control del *Aedes aegypti*: un esfuerzo integrado)

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Vector control programs have had a tradition of an independent structure and a single-disease approach in their activities. Consequently, collaboration with other health institutions and programs has rarely occurred.

Since 1991, the Integrated Dengue Control Project has been elaborating an inter-institutional cooperative effort to achieve sustainable dengue control in El Progreso, Honduras. In January 1992, a cooperative agreement with the National University was signed. Since then, the entomological and biological control research have been conducted jointly by personnel of both institutions.

Meanwhile, El Progreso's Health Center has collaborated in the development of community participation strategies for prevention and control of *Aedes aegypti* and dengue. The health communication effort also included malaria and cholera prevention, in order to transform isolated, single-disease messages into an integrated environmental health message.

The following methodology of community organization was used: In order that our message could be communicated face-to-face, as much as possible, the communities were divided in zones (40–60 households per zone). During immunization sessions carried out in each zone, an invitation to a zone meeting was delivered by project facilitators. In these meetings, discussions about community health concerns were raised. So far, problems perceived to be important, such as frequent backyard flooding, have been immediately addressed. Practical demonstrations of appropriate and feasible solutions have been followed by the formation of a five-member community group ("Grupo de Salud Integral") to lead the implementation of these solutions in its zone. It is proposed that 1–10 integrated zone groups per neighborhood be organized in this manner in 10 neighborhoods in El Progreso; one activity of these groups would then be face-to-face communication of an integrated health message from neighbor-to-neighbor, with emphasis on *Ae. aegypti* source reduction and domestic hygiene.

Prevention of dengue and dengue hemorrhagic fever through education and community participation in the Dominican Republic (La prevención del dengue y el dengue hemorrágico a través de la educación y la participación comunitaria en la República Dominicana)

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The principal objective is to educate the public in the Dominican Republic about the prevention of dengue and dengue hemorrhagic fever (DHF). It is part of a 2-year project supported by a grant from the Rotary Foundation of Rotary International through the Rotary Club of San Juan, Puerto Rico. The Rotary Club of Santo Domingo-Arroyo Hondo was the local coordinator for the project. The project has 4 technical components for which a participating agency is primarily responsible. They are:

1. Mass education through the development and distribution of posters and brochures about prevention and control of dengue/DHF (Dominican Infectious Disease Foundation).

2. Preparation of videocassettes for the education of physicians and other public health personnel involved in the clinical and epidemiological aspects of dengue/DHF (Ministry of Health).

3. Incorporation of a formal program of primary education with booklets designed to enable students to: identify the mosquito *Aedes aegypti*,

recognize sites where the mosquito is produced and how they can be eliminated, and characteristics of dengue/DHF. The booklets will be illustrated and designed for the student population to be trained (e.g., it will present drawings of different flies including the mosquito vector of dengue). In addition, it will illustrate the importance of the primary level student's participation in the program (Ministry of Education).

4. A massive education for residents of the barrios of Guachupita and Gualey in Santo Domingo about community participation in the control and elimination of the mosquito vector of dengue/DHF will be conducted. This intervention will include lectures supported by audiovisual materials. Videocassettes will be prepared including scenes from the pilot barrios with residents of the barrios appearing as actors (Autonomous University of Santo Domingo and community leaders).

In the short-term, the project will cover: 5,000 doctors, nurses and health educators; 53,000 primary schools students in the first to fifth grades; and 20,000 residents in the two pilot barrios. Results of all 4 intervention components will be evaluated. In the long-term, with the implementation of the program at the national level, the program will train 6,500 doctors, nurses and health educators and protect the 7,000,000 inhabitants of the Dominican Republic.

Community and civic organizations join for dengue prevention in Mayaguez, Puerto Rico (Movilización de la comunidad para la prevención del dengue en Mayaguez, Puerto Rico)

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In early 1991, Mayaguez on the western coast of Puerto Rico was selected as the site for implementation of a community-based, dengue/dengue hemorrhagic fever project. The project was sponsored and facilitated by the Rotary Club of San Juan (through a grant from the Rotary Foundation) and the Dengue Branch of the Centers for Disease Control, U.S. Public Health Service. Local leadership and direction of the project was by regional environmental health officials and local Rotary clubs. Other agencies/groups that actively participated in the project included: Department of Education, City of Mayaguez, Head Start program, Rural Hous-

ing Administration, Boy Scouts, and the Mayaguez campus of the University of Puerto Rico.

Three similar communities were selected: Río Hondo, La Soledad, and Tres Hermanos. Tres Hermanos served as an untreated control and received no intervention. Interventions in the 2 treatment areas emphasized the following activities: cleanup campaigns, house-to-house visits by personnel from participating agencies, an elementary school program, displays in local stores, and local agency, Head Start, and church meetings.

In March 1991, prior to the intervention, we conducted an entomological evaluation in the 3 areas. An effort was made to inspect all of the houses in these areas. That was not possible but a total of 476 premises were inspected. At that time, the inspectors said nothing about the dengue prevention project that was going to be implemented. In June 1991, the entomological evaluation was repeated in these 3 areas and 434 of the same houses inspected in March were resurveyed.

Results of the pre-intervention and post-intervention surveys are summarized below:

Area (houses)	Index					
	House (%)		Container (%)		Breteau	
	Pre-	Post-	Pre-	Post-	Pre-	Post-
Río Hondo (151)	13	7	6	2	25	9
La Soledad (173)	30	17	26	6	120	26
Tres Hermanos (110)	8	14	4	9	10	32

The percent change (i.e., reduction) in these *Aedes aegypti* indices after the intervention was substantial. In Río Hondo and La Soledad, the house index was reduced by almost 70%, the container index and Breteau index each dropped by around 90%. All 3 indices increased in the untreated area.

In both intervention areas, the greatest changes (i.e., reduction) in frequency of containers with water were seen in tires, discarded appliances, and bromeliads. No reduction was seen in any container type in Tres Hermanos. A reduction in positivity was seen in both areas in all types of containers. Another post-intervention survey will be conducted in April 1992 to determine if this impact has been sustained.

Initial results from this community-based intervention emphasizing community collaboration and sanitary education suggest that *Ae. aegypti* indices can be reduced without the use of insecticides. Further efforts are required to determine if these reductions effectively prevent cases of dengue and dengue hemorrhagic fever.