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

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ABSTRACT. The sixth Spanish language symposium presented by the American Mosquito Control Association (AMCA) was held as part of the 62nd Annual Meeting in Norfolk, VA, in March 1996. The principal objective, as for the previous 5 symposia, was to promote the participation in the AMCA meeting by vector control specialists, public health workers, and academicians from Latin America. This publication includes summaries of 25 presentations that were given in Spanish by participants from 6 countries in Latin America and the USA. The symposium included the following topics: ecological and genetic studies of anopheline vectors of malaria, laboratory and field evaluations of chemical control methods for several mosquito species, ecological studies and community control of *Aedes aegypti*, and reports of dengue/dengue hemorrhagic fever and Venezuelan equine encephalitis epidemics that occurred in Latin America in 1995.

INTRODUCTION

The American Mosquito Control Association (AMCA) is dedicated to the study and control of mosquitoes and other vectors and promotes the cooperation and interaction with professionals and students in this field. Although comprised mostly (*ca.* 80%) of members from the United States, there is an increasing number of AMCA members who are not from the USA. To meet the needs of this group, many of whom are from countries where vector-borne diseases (e.g., malaria, dengue, Chagas' disease, and others) are much greater public health problems than are faced in the USA, the AMCA held a Spanish language symposium in 1996 at the 62nd Annual Meeting in Norfolk, VA. This was the 6th consecutive year in which this session was held and it included 25 presentations given in Spanish by participants from 6 countries and the USA. The symposium included presentations on the following topics: ecological and genetic studies of anopheline vectors of malaria, laboratory and field evaluations of chemical control methods for several mosquito species, ecological studies and community control of *Aedes aegypti*, and reports of dengue/dengue hemorrhagic fever and Venezuelan equine encephalitis epidemics that occurred in Latin America in 1995. Summaries of 4 previous symposia have been published (Clark 1995; Clark and Suárez 1991, 1992, 1993).

Support for this unique session at the Annual Meeting continues to be good. Recognition for financial support for the 1996 symposium goes to the following sponsors and individuals: Clarke Mosquito Control Products (John L. Clarke, Jr.), ZENECA (Eduardo Moreira), ADAPCO (Allen Wooldridge), Beecomist Systems (Karl Kutzner), H. D. Hudson (Manuel Lluberas), London Fog (Bob Bonnett), Summit Chemical (Larry Kase), the Pan American Health Organization, and the Virginia Mosquito Control Association. Funds from these sponsors were used to defray travel and meeting expenses of symposium participants. With the continued interest and support from participants, sponsors, and the AMCA membership, future symposia will continue to be held.

SUMMARIES

Temporal and spatial dynamics of *Anopheles* vectors in Chiapas, México

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Ecological studies of *Anopheles* mosquito vectors were undertaken in 13 villages in the state of Chiapas, México, between February 1992 and May 1994. Our aims were to determine the biting behavior of anopheline species present (trends in their abundance by place and time and biting rhythm) and their entomological inoculation rates in relation to malaria transmission. Seven species were found associated with man. In order of abundance they were: *An. vestitipennis* (11,725 individuals collected), *An. albimanus* (4,827), *An. pseudopunctipennis* (410), *An. apicimacula* (492), *An. darlingi* (385), *An. punctimacula* (240), and *An.*

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eiseni (found only resting). All species showed outdoor: indoor biting ratios between 1.2 and 1.7 to one. Marked differences in abundance were observed among species according to village and time of year. Peaks of abundance of *An. vestitipennis, An. albimanus, An. darlingi,* and *An. punctimacula* were observed during rainy months, whereas *An. apicimacula* and *An. pseudopunctipennis* were more abundant during the dry season. Twelve-hour biting cycles were a variable feature in all species. Two to 4 types were seen, either unimodal with early or late night peaks, bimodal, or erratic. Finally, the average number of infective bites per year in *An. vestitipennis* with *Plasmodium falciparum* and *P. vivax VK247* were 1.35, whereas *An. albimanus* had 2.8 infective *P. falciparum* and 0.94 infective *P. vivax VK247* bites/year. We conclude that the 2 confirmed vectors of malaria, *An. vestitipennis* and *An. albimanus,* are not as competent as other malaria vectors and that the risk of getting infections is low compared to other regions in the world.

Observations on the biting activity of different populations of the malaria vector *Anopheles darlingi* in southern Venezuela

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Anopheles darlingi is the most efficient vector of *Plasmodium falciparum* and *P. vivax* in the Americas, nevertheless little is known about its ecology and behavior. In order to study the biting activity of this species, all-night human landing catches were carried out in Las Majadas (7°37′N, 64°50′W) during 3 consecutive nights in January, May, and October 1986; in Aripao (7°20′N, 65°10′W) during one night in July 1993; in Caicara (7°49′N, 66°12′W) during one night in October 1993; in Corobal (7°48′N, 65°42′W) during one night in October 1993 and one night in October 1994; and in Ocamo (02°50′N, 65°14′W) during 5 consecutive nights in August 1995. Mosquitoes collected outdoors in Las Majadas in January (beginning of the dry season) showed a trimodal pattern: 1990–2200 h, 0100–0200 h major peak, 0400–0500 h; whereas those collected during October (rainy season) showed a unimodal pattern with the peak of activity between 2300 and 2400 hours. During May (end of the dry season) no mosquitoes were collected. In Aripao, a trimodal pattern was observed: 1800–2000 h, 2300–2400 h major peak, 0400–0500 h during July (rainy season). In Corobal during October 1993 (rainy season) *An. darlingi* showed a bimodal activity pattern with biting peaks between 1800–1900 h (major peak) and 0200–0500 h, whereas 1 year later the pattern was entirely different with a single biting peak between 2400 and 0100 h. In Caicara, located some 120 km west of Corobal, the biting activity of *An. darlingi* had a trimodal pattern: 1900–2100 h (major peak), 2300–2400 h, 0300–0400 h. In Ocamo, located on the upper Orinoco River, indoor human landing catches showed an entirely different unimodal pattern with an extended peak between 2400–0500 hours.

The present study, based on a limited number of collections, showed that there are not only geographic and seasonal variations in the biting pattern of *An. darlingi* but also temporal and spatial (indoor/outdoor) variations. There is an urgent need to carry out detailed longitudinal studies to determine the biting behavior of this malaria vector in order to design and implement effective control programs. This research was funded by CONICIT-MPS-RPIV-130032-9 and PAHO.

Classic morphology of fourth instar larvae, pupae and adults of *Anopheles vestitipennis*

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We conducted a morphologic study of 4th-instar larvae, pupae, and adults of *Anopheles vestitipennis* to assess the degree of intrapopulational variability in the coastal plain of Chiapas, Mexico (14°33′54″N, 92°15′02″W). Adult female mosquitoes were collected, bloodfed in a cattle corral, transported to the laboratory, and allowed to oviposit. Resulting F_1 larvae were reared individually to obtain exuviae from 4th-instar larvae and pupae and adults were mounted upon emergence together with immature skins. Females showed variable scale patterns on the wings, but were consistent with the original pattern. Chaetotaxy of 4th-instar larvae and pupae showed several left to right asymmetries in the presence of certain setae. Pupae were characterized by the presence of setae 1-X (76%, n = 55), simple and bifurcated. This seta was also present in all males (n = 14) and was trifurcated in one male. Not previously described were setae 10-I; 8, 10, 11, 13-II; 12, 13-III; 12, 13-IV; 13-V; 12-13-VI; 12-13-VII; 10, 13-VIII; and 1-X. Certain variations in previously reported setae were also observed, namely, 0-III-VIII, 9-VIII, 1-X, 1-P and 2-P. Fourth-instar larvae

showed variations in setae 3-C, 4-C, 8-C, 9-C, 1-A, 9-P, 2-IV, V, and 6-IV, V. Finally, setae 0-II-VIII and 10, 12-P were variable compared to seta 9-P. In summary, *An. vestitipennis* manifested high phenotypic variability even though all specimens that were examined came from the same locality.

Aquatic insects associated with *Anopheles albimanus* breeding sites

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We investigated the aquatic Coleoptera, Hemiptera, and Odonata associated with *Anopheles albimanus* larval habitats and their relationship with *An. albimanus* abundance in a coastal plain of Chiapas in southern Mexico. Collections were made in 11 larval habitats defined by specific vegetation in 6 hydrologic locations. From 579 *An. albimanus* larval samples, mosquitoes were most abundant in *Ceratophyllum* habitats, averaging 9.01 larvae/dip, in *Crinum* (6.41 larvae/dip), and in *Fimristilys* (6.25 larvae/dip). Poorest larval habitats were *Eichhornia* (0.01 larvae/dip), *Pistia* (0.06 larvae/dip), and *Oryza* (0.06 larvae/dip). Similarly, 17,425 aquatic insects from 22 families and 50 genera were collected. There were 18 genera in the order Coleoptera (mainly *Tropisternus, Hydrocantus, Derallus,* and *Suphis*), 16 in the order Hemiptera (mainly *Pelocoris, Renatra, Tenagobia, Belastoma, Lethocerus,* and *Buenoa*), and 16 in the order Odonata (mainly *Telebasis, Zoniagrion,* and *Enallagma*). Aquatic insects were most abundant in the *Hymenachne* larval habitat, with 72.5, 46.6, and 33.2 individual/m^2 for Coleoptera, Hemiptera, and Odonata, respectively. A linear relation was found between the abundance of mosquito larvae and the presence of aquatic insects. The linear term suggested a significant increase in the number of larvae with increasing numbers of insects. However, this linear increase appears to change at certain insect densities, suggesting that aquatic insects may reduce the abundance of larvae. These results remained unchanged even after adjustments were made for other factors that were determinants of larval abundance.

Resistance of *Anopheles aquasalis* to the synthetic pyrethroids, deltamethrin and lambdacyhalothrin, in Sucre State, Venezuela

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A very successful malaria control program was developed in Sucre State, Venezuela, during 1992 and 1993. However, malaria has increased dramatically since 1994. The program has been supported mainly by the use of pyrethroids and organophosphate insecticides.

Samples of 20 *Anopheles aquasalis* adults were exposed at the diagnostic dosage for each insecticide on impregnated papers prepared in our laboratory by using WHO test kits and the methodology recommended by that organization. For each insecticide treatment, 5 replicates were done with the corresponding control treatment.

From the insecticide surveillance program developed in Venezuela in 1994, we found the following results: in the region of Rio de Agua, we found 3% survivors to deltamethrin in 1994, and during 2 consecutive months in 1995, 19 and 16% survivors to deltamethrin and 18 and 15% survivors to lamdacyhalothrin. In another locality, Putucual, we found 17% survivors to deltamethrin and 49% survivors to lamdacyhalothrin in 1994 and in 4 consecutive months in 1995 the percentage of survivors to deltamethrin were 47, 42, 35, and 36% and to lamdacyhalothrin were 26, 42, 15, and 29%, respectively. The high level of resistance to DDT in the same populations where resistance to pyrethroids was highest could be due to the presence of the Kdr gene. This phenomenon should be studied. No resistance was detected to the organophosphate insecticides malathion, fenitrothion, and methyl pirimiphos in all locations.

Esterase polymorphism in insecticide susceptible/resistant populations of *Anopheles albimanus*

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Organophosphate and pyrethroid insecticides play an important role in malaria vector control. In Guatemala, natural populations of the primary

vector *Anopheles albimanus* vary widely in their degree of susceptibility to both types of compounds. Previously published field data showed a strong correlation between the activity levels of nonspecific esterases and the survival rates to fenitrothion and deltamethrin exposure.

We investigated esterase polymorphism and its relation to insecticide susceptibility in *An. albimanus.* Mosquito populations of the malaria vector from the different endemic regions in the country were sampled. Insecticide susceptibility was measured by the WHO standardized susceptibility test for adult mosquitoes and microplate assays for insensitive acetylcholinesterase and nonspecific esterases. Esterase variability was determined by means of native polyacrylamide gel electrophoresis and analyzed using BIOSYS1. Allele frequencies, proportion of polymorphic loci, means heterozygosity per locus, similarity, genetic distance, and gene flow among populations were estimated by geographic and insecticide susceptibility grouping.

Two esterase loci were selected as diagnostic: EST-1 and EST-2. Relative mobility (R_f) of bands within each locus ranged from 0.50 to 0.60 and 0.65 to 0.70, respectively. The analysis of data was based on the assumption that bands with different R_f within each region (locus), represented different alleles. Nine (EST-1/A-I) and 8 (EST-2/A-H) alleles were detected among all mosquitoes (n = 771) for loci EST-1 and EST-2, respectively.

Sampled populations were divided in 3 groups according to their level of susceptibility/resistance to fenitrothion and deltamethrin, as measured in the WHO susceptibility test. Groups and their respective percent survivorship to fenitrothion and deltamethrin standardized exposures were the following: S-FEN&DEL, ≦30% for both insecticides; R-FEN&S-DEL, >30% and ≦30%, respectively; and R-FEN&DEL, >30% for both insecticides. The EST-1 and EST-2 loci were 100% polymorphic and the higher allele frequencies were for EST-1/E and EST-2/C, ranging from 0.667 to 0.750 and 0.549 to 0.908, respectively, among all groups. Mean heterozygosity (He) for EST-1 and EST-2 loci, in groups S-FEN&DEL, R-FEN&S-DEL, and R-FEN&DEL was 0.492, 0.391, 0.409, and 0.580, 0.174, 0.191, respectively. Values of genetic distances (Nei's distance coefficients) indicate that the major difference among susceptible/resistant populations resides on locus EST-2, in association with increasing resistance to fenitrothion. Further genetic distance analysis with additional subgrouping based on geographic localization (north, east-central, and south ecological regions) reveals that geographic relationships prevail over the level of insecticide susceptibility/resistance in the determination of the population genetic structure.

Structure of *Anopheles albimanus* populations using genetic markers

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Anopheles albimanus is the principal vector of malaria in Central America and parts of South America and the Caribbean. There have been several studies of the extreme variability that this species exhibits throughout its wide geographic distribution. Furthermore, traditional methods provide little understanding of this species' population genetics. Knowledge of patterns of gene flow and dispersal rates have practical implications on control strategies for vector species. Variation in the length and copy number of intergenic spacers (IGS) of nuclear ribosomal DNA were examined to test for genetic differences among *An. albimanus* populations. Extensive collections were made in Guatemala but populations were sampled over a large range of its distribution in Central and South America. Discriminant analysis of IGS patterns in individual mosquitoes estimated a misclassification rate of only 12.4%, indicating that populations generally had unique sets of IGS length variants. The populations on the Pacific side of Central America were distinct from those on the Atlantic side of South America. Cluster analysis indicated a similar trend. The IGS diversity in Central America was 50% greater than in South America. These results suggest that the mountain ranges running through northern Central America form barriers to gene flow between *An. albimanus* populations. No evidence of gene flow was detected in South America.

It is important to test findings of this study using discrete markers such as isoenzymes, RAPD-PCR markers, or sequence analysis from selected regions of rDNA or mitochondrial DNA. Discrete nuclear markers will not be confounded in their inheritance and distribution by the effects of molecular drive. If the patterns of genetic differentiation presented in this paper are substantiated, other differences may also occur between the Pacific and Atlantic populations, including those affecting vector competence. Genetic polymorphisms generated by RAPD-PCR are useful in investigating the genetic relationships among populations in a species, overcoming limitations of the traditional methodologies.

The principle is that alleles are shared among populations because of migration, and the similarity in allele frequencies can be used to estimate the rate of migration and gene flow among populations. We propose the use of RAPD-PCR polymorphisms to examine the breeding structure of *An. albimanus* populations throughout Guatemala. Several questions will be answered in a long-term study; however, in this presentation we compare only 8 populations from 2 regions of the country and will try to answer the question about geographic barriers to gene flow among these 8 populations. Mosquitoes were collected in 2 different regions where malaria is endemic. These 2 regions are separated by mountains, which could be barriers to gene flow. Collections were made in the rainy season and the mosquitoes were brought to the laboratory alive. The DNA was extracted by the method of Livak using alcohol precipitation and was amplified randomly by 3 10-base oligomers obtained from Macromolecular Resources: Bam, C01, and C04. The products of RAPD-PCR were loaded in 1.2% agarose gels and run for 16 h at 22 V. Bands were visualized on a 302-nm ultraviolet transluminator. Analysis was done with the RAPDPLOT computer programs for analysis of RAPD-PCR developed by W. C. Black.

Population genetics analysis of *Anopheles aquasalis* in Venezuela

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Anopheles aquasalis is primarily a coastal species but its geographic range extends from Nicaragua to Brazil and the Lesser Antilles. It is collected from a wide variety of habitats in both fresh and brackish water. It has a variable behavior and is a malaria vector throughout its range, which makes it an interesting subject for population analysis.

Our research was carried out in 5 sites located along the coast of Venezuela: Sinamaica, Zulia State; Cano Rico, Aragua State; Santa Fe and Yaguaraparo, Sucre State; and Capure, Delta Amacuro State. Adults were analyzed using 18 electrophoretic loci. Estimates of mean heterozygosity were between 0.104 and 0.158, and many alleles were shared in all populations. Allelic frequencies were significantly different for each comparison between any paired populations. The Nei's distance was from 0.007 to 0.042.

We did not see any correlation between the genetic structure and behavior of this vector but physiologic conditioning and environmental factors may have caused differences among populations. Our data support the hypothesis that *An. aquasalis* is a unique species. This research was supported by CONICIT RPIV-130032, PAHO, and the Ministry of Health of Venezuela.

Selective and conventional house-spray of DDT against *Anopheles pseudopunctipennis* in southern Mexico

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We evaluated the comparative effect of DDT when applied conventionally or selectively to the preferred pre- and postfeeding resting sites of *Anopheles pseudopunctipennis*. Conventional full and selective spray applications contained standard dosages of DDT (2 g/m^2). The selective spray consisted of 2 0.8-m-wide swaths of insecticide, the first starting at 0.85 m from the floor on the walls and the second starting from the base of inner roofs. Mortality on wood (wall) and tin (roof) remained at ≥90% in the fully treated house (FTH) 1 month after spray, whereas in the selectively treated house (STH), mortality on both surfaces ranged from 62.5 to 72.5%. Man-biting rates decreased 3 times indoors (5 bites/man/night [bmn]) but increased more than 4-fold outdoors (61.8 bmn) in the DDT FTH, as compared to an untreated house (UH). In the DDT STH, a 3-fold reduction in indoor biting was also observed (5.3 bmn), but no such reduction was observed in the outdoor biting mosquitoes. Mortality of indoor resting mosquitoes in the FTH and STH remained at 39 and 45%, respectively, following treatments, significantly higher than in the UH. Three hours after release in the curtained house, 97 and 99% of mosquitoes had exited the FTH and STH, respectively, compared to 77% in the UH. Feeding by mosquitoes in the curtained house was reduced to 0 and 0.3% in the FTH and STH, compared to 8.3% in the UH. Resting heights on walls varied following treatments from 0.8 to 1.2 m in the UH to 0.5 to 1.75 in the FTH and <0.6 m in the STH. In summary, although DDT residues provoked behavioral avoidance, the same control was achieved by either full or selective treatment. Expected 3% savings of insecticide offer an alternative to traditional control tactics.

Larval production and potential food sources of *Aedes aegypti* in various containers

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We evaluated 4 types of artificial water containers (2-m^3 tanks, tires, 200-liter drums, and cemetery flowerpots) that are potential breeding sites of *Aedes aegypti* in Chiapas, southern Mexico. Over a period of 10 wk, 10 containers of each type were sampled weekly. Physical–chemical characteristics in all containers were determined, and the relative importance of each container type in overall *Ae. aegypti* production was estimated by recording total larval abundance per container type and the size of emerging adults. In addition, potential food sources for larvae in each container were identified by gut content analysis. No physical–chemical parameter was correlated with mosquito larval abundance, except nitrates in flowerpots ($r = 0.36$, $P < 0.05$). Greatest larval abundance was found in tires ($\bar{x}$ = 1,312 larvae/tire), followed by tanks ($\bar{x}$ = 535), drums ($\bar{x}$ = 345), and flowerpots ($\bar{x}$ = 71). Other mosquitoes commonly found in these containers were *Culex coronator* and *Cx. quinquefasciatus*. The largest *Ae. aegypti* were collected in tanks, whereas the smallest were found in flowerpots. Gut content analysis showed that algae were more abundant in the digestive tract than protozoans. Of all bacteria isolated from water, 63, 83, 33, and 37% of bacteria species were also found inside the larval gut, respectively, in tanks, tires, drums, and flowerpots. *Aeromonas* was the most commonly found bacteria in the water, whereas *Tatunella* was the most common bacteria found in the digestive tract of *Ae. aegypti* larvae.

Aedes aegypti breeding site characterization by pupal density and associated bacteria in Panama

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The main objective of this research was to study the variation of *Aedes aegypti* behavior and the production of adults. The specific objectives proposed were to identify the most abundant breeding site in the metropolitan area, to estimate the index of pupal density for each breeding site, and to estimate and identify the bacterial flora associated.

From January 1994 to May 1995, 303 positive breeding foci of *Ae. aegypti* were found in a district of the metropolitan area in Panama. Each breeding site was identified based on larval instars found. We studied the bacterial flora of 100 of these sites. Once in the laboratory, each labeled sample was diluted 1:10, inoculated in petrifilm slides, and incubated at 37°C for a total colony count and a coliform count. The colonies counted as *Escherichia coli* or coliform were transferred to Eosine Methyl Blue agar and nutrient agar for proper identification.

Of the 303 reported sites, the total immatures recorded were 35 1st-instar larvae; 318 2nd-instar larvae; 665 3rd-instar larvae; 1,418 4th-instar larvae; and 497 pupae. The most abundant breeding containers were plastic, supporting mostly larvae and pupae. Greater pupal density was observed in plastic, rubber, plants, concrete, and car junk.

The total aerobic bacterial count varied from 0 to one million per milliliter. The study of bacterial flora associated with 100 breeding sites revealed the presence of *E. coli* in 28%, *Klebsiella* sp. in 49.3%, *Enterobacter* sp. in 24%, *Citrobacter* sp. in 14.6%, *Pseudomonas* sp. in 5.8%, and 4% *Proteus* sp.

From the present study, we drew the following conclusions: In the metropolitan area of Panama, the most abundant breeding sites were plastic containers, which also presented the highest density index of pupae. In Panama, *Ae. aegypti* is found mainly in "relatively clean" water with an associated bacterial flora including coliforms and *E. coli*. The total count of aerobic bacteria present is variable with extreme limits to one million bacteria per milliliter.

Importance of nectar in the dispersal of *Aedes aegypti*

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The influence of plant abundance (nectar availability) in the presence and dispersal of female *Aedes aegypti* mosquitoes was evaluated in Huixtla, Chiapas, México. Two sets of 16 blocks within the city were selected for study, one in the outskirts (OS) and one in downtown (DS). Within these, all plants inside houses and in the streets were counted twice (in the dry and wet seasons). Four mark–recapture experiments were carried out, 2 per each set of blocks and

season. Mosquitoes were recaptured during the next morning and for 8 days, searching in and out houses over the 16-block perimeter (unmarked mosquitoes were also collected). All captured mosquitoes were classified according to their trophic status and kept at −70°C until analyzed. Sugar feeding was detected by the cold-anthrone method. During the dry season, mean abundance of plants was 15 and 6.5 per house in OS and DS, respectively, whereas in the wet season a mean of 17 and 18 plants per house was observed in each set of blocks. Mosquito recapture rates in OS were 9.8% (n = 1,500) and 8.6% (n = 3,000), respectively, in the dry and wet seasons, whereas in DS 4.2% (n = 3,000) and 7.5% (n = 3,000) of marked mosquitoes were recaptured. Of these, 8.2% (n = 147) and 8.6% (n = 257) were OS-marked fructose-positive females, in the dry and wet seasons, respectively. In DS, 4.8% (n = 126) and 8.3% (n = 225) of females were positive for sugars, in the dry and wet seasons, respectively. Trophic status was not correlated with presence of nectar in marked females. The greatest dispersal from the release point recorded was 200 m in DS. Gravid females were among those that dispersed the most (56%, n = 9). These results indicate that the local population of *Ae. aegypti* takes plant nectar 3 to 5 times more than is reported for populations in Thailand and Puerto Rico.

Clustering patterns of dengue cases during an outbreak in Puerto Rico (1991–1992) and their relationship to *Aedes aegypti* dispersal and blood-feeding behavior

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Control strategies for dengue need to target an appropriate geographic area. We studied the distribution of dengue cases in Florida, Puerto Rico (population 8,689) using a Geographic Information System (GIS). We identified 377 cases from a laboratory-based dengue surveillance system and referenced their residential addresses by digital zoning maps and a geopositioning system (GPS). We then used GIS software (ARC/INFO™) to map the cases and to determine the distance between cases with onset dates separated by 0–30 days. Weekly plots showed clustering within housing developments; these results are supported by analysis using the Gi* statistic. Forty-six percent of the nearest case pairs were separated by 11–30 days, a period roughly compatible with the sum of the intrinsic and extrinsic incubation periods of dengue. More than 90% of the neighboring case pairs were within 500 m of each other. Dengue cases were reported in 254 houses; of these 51 had multiple cases (n = 123 cases). Of the 2nd to 6th cases within a house (n = 72), 34 (47%) occurred within 5 days of each other. The observed spatial and temporal distribution of dengue cases is consistent with both dispersal and multiple feeding behavior by *Aedes aegypti*. The small size of the study area (26 km^2) may have restricted virus dispersal. Nevertheless, our results indicate that control programs that react to individual dengue cases are unlikely to be effective unless they account for the rapid temporal and spatial dissemination of the virus.

The "untadita" as an *Aedes aegypti* control method

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Since 1991, the Integrated Dengue Control Project in El Progreso, Honduras, has had an active program to promote good surveillance of permanent water containers as well as those transient breeding sites that are located in patios. Entomological evaluations have shown that most positive houses have water storage containers (water tanks and drums) that receive little and no maintenance.

Control measures that have been promoted include cleaning containers with domestic materials or biological control. This approach has been effective in a number of households; however, a significant number of positive households remain. Facing this situation and considering that chlorine and detergents are commonly used household cleaning materials, laboratory assays were carried out to measure separately the effectiveness of diluted chlorine and detergent on *Aedes aegypti* eggs, larvae, and pupae. Experimental results showed that chlorine and detergents have the same efficacy against eggs and larvae (100%) but are different for pupae (68–100% for chlorine and 98–100% for detergent). However, a large amount of these materials will be required to treat large water containers.

During the laboratory assays, it was observed that pure chlorine (undiluted) takes 10–12 min to decolor the *Ae. aegypti* egg shell and to kill the embryo. After this observation, a mixture was prepared with chlorine and commercial de-

tergents in a proportion 5:1; the mixture retained the ability to kill embryos and the detergent added some consistency when applied to the interior walls of the containers. The procedure included the application of the mixture ("untadita"), brushing the walls, and rinsing them. The effectiveness of the method was 99%. The use of "la untadita" opens the possibility of better control efforts not just for *Ae. aegypti* larvae but also of the eggs, which have not been treated in the past.

Social acceptability of a control method for *Aedes aegypti*

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As part of an integrated *Aedes aegypti* control project in Honduras, a network of volunteers has been developed in 12 neighborhoods. The volunteers have been actively visiting their neighbors to encourage them to control containers such as "pilas" (laundry tanks) and drums, which have been demonstrated to be the most important breeding sites of *Ae. aegypti* in El Progreso. The need for an effective method for cleaning the pilas and drums led to the development of the method known as "la untadita" (5 parts of chlorine and one part of detergent).

The untadita method was demonstrated to be effective against *Ae. aegypti* eggs, larvae, and pupae under laboratory conditions, and both volunteers and project staff facilitated the method by promoting it at the community level. In order to observe the use of la untadita under controlled conditions, a community was selected for study. The community fulfilled the following conditions: a high house index (last entomological survey had 32.7), an unreliable water supply, an organized volunteer group, and a small neighborhood (less than 250 houses).

Twelve housewives were selected and followed for 8 wk. The *Ae. aegypti* life cycle (especially egg and larval stages) and the steps of la untadita were explained and housewives were asked to repeat the procedures and give a practical demonstration of the method. During interviews, housewives considered the method to be very useful.

After 8 wk, housewives were visited again and pilas were observed in order to determine the presence of eggs, larvae, pupae, and algae; frequency of recent cleaning; and the application of la untadita. At that time, 9 (75%) of the housewives were using the method on a weekly basis and had no *Ae. aegypti* eggs, larvae, or pupae; the remaining 3 (25%) had a positive pila and cleaned their pilas less than once a week. The advantages of the method were identified in terms of controlling the breeding sites and reducing costs but a disadvantage was mentioned: it takes longer than other ways of cleaning the pila.

Aedes aegypti resting preference on untreated and deltamethrin-treated crepe paper and plastic foam surfaces

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The present study is based on a paper presented by John Edman and colleagues at the 1995 annual meeting of the American Mosquito Control Association, which described preliminary results from research in Thailand where black resting boxes were used to collect *Aedes aegypti* indoors. Tests used boxes covered with black cloth treated with deltamethrin and showed good control of adult *Ae. aegypti* inside homes where they were placed.

Our goal was to design an inexpensive "black resting box" using dark-colored crepe paper instead of cloth. A large (1-m^3) rearing cage was modified for use in observing mosquito resting behavior. Red and black crepe paper glued to card stock was used to make cones of each color, which were mounted in a cardboard box placed inside the observation cage. The box (32 cm wide × 32 cm high × 23 cm deep) that held the 4 cones (12 cm wide × 20 cm deep) faced the observer and was taped to the bottom of the observation cage. Fifty (male or female) mosquitoes were used for the test and counts of the number of resting adults inside each cone were made every 30 min for 5 h.

A significant percentage of the *Ae. aegypti* adults chose to rest in the black crepe paper cones. Averages for the black crepe paper cones were 36% for males and 47% for females, whereas only 15% of the males and 18% of the females rested in the red crepe paper cones. The relative position of the cones did not appear to have any significant effect. Some mosquitoes appeared to prefer to rest on the narrow collar of black plastic foam around the opening of each cone, so trials were later conducted using cones made from black plastic air conditioner filter material. In those tests, an average of 42% of males and 60% females rested inside the plastic foam cones, whereas 31% of males and 37% of females rested inside the black crepe paper cones.

Tests were then conducted to determine if

black crepe paper and black plastic foam cones would remain attractive when treated with deltamethrin insecticide. We used K-Othrine (2.5% deltamethrin wettable powder) mixed according to the label (10 g/liter of water). Two applications were sprayed on the surface of each cone using a small garden sprayer. Surprisingly, many of the *Ae. aegypti* adults continued to rest inside the treated cones. Figures for resting males and females were 37 and 46%, respectively, in the treated plastic foam cones, whereas the counts for the treated black crepe paper cones were 24 and 32% for males and females, respectively. Thus, the treated surfaces remained attractive as resting sites. When 50 bloodfed females were placed into the observation cage with 2 treated plastic foam cones and 2 untreated black crepe paper cones, all died after only 4 h exposure. However, in tests using 2 treated crepe paper cones and 2 untreated plastic foam cones, only 62% mortality was observed after 4 h, but 100% mortality was seen after 24 h. In earlier tests using all untreated cones, we observed from 20 to 26% mortality in males and 10 to 12% mortality in females after 24 h.

Although the more costly plastic foam material was somewhat more attractive than the black crepe paper, it was decided that its greater expense was not justified in our initial field experiments. In January 1996, field tests were made inside open mechanical repair shops on an *Ae. aegypti*-infested island connected to the mainland by a causeway. Seventeen cardboard resting boxes with deltamethrin-treated black crepe paper inside were placed throughout the shops. No determination of the effectiveness of control was made, but in several instances dead *Ae. aegypti* adults were found inside the treated black resting boxes. Future studies in Panama should allow a better assessment of the killing effect of these low-cost resting boxes and their value in controlling *Ae. aegypti* inside homes during dengue epidemics.

A possible mechanism involved in the resistance of *Aedes aegypti* in Venezuela

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Insecticide resistance levels to organophosphates, carbamates, and pyrethroids were determined in mosquitoes from 8 states in Venezuela. A strain of *Aedes aegypti* from field collections in Miranda State and homozygous for resistance to deltamethrin (FR50 100X) was maintained in the laboratory to study the mechanism of insecticide resistance. Bioassays with the synergist piperonyl butoxide (PB), an inhibitor of multifunction oxidase enzymes, showed that these enzymes are not involved in resistance to the organophosphate insecticides (malathion and fenitrothion) and the pyrethroids (lambdacyhalothrin and cyfluthrin). *In vitro* assays on microtiter plates for elevated esterase and modified acetylcholinesterase (AChE) showed no evidence of esterase or AChE activity against the same insecticides with frequencies of 0.07 and 0.0, respectively. There was no cross-resistance between deltamethrin and DDT nor between lamdacyhalothrin, cypermethrin, and cyfluthrin, indicating that the Kdr gene was not involved in resistance to pyrethroid insecticides. These results showed evidence that the glutathion alkid transferase enzymes are involved in resistance to deltamethrin.

Dengue outbreak in Cali, Colombia

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The city of Cali is located near the equator (3°27′N, 76°32′W) and 1,100 m above sea level with about 2 million inhabitants. It experiences distinct seasons of heavy rainfall annually from April to May and November to January. Transmission of dengue viruses and dengue fever (DF) have been systematically reported since 1980, with documented circulation of dengue 1, 2, and 4 serotypes, sometimes simultaneously. From January to October 1995 there were 1,200 suspected cases of DF reported in Cali; 638 DF cases had hemorrhagic manifestations, of which 26 fulfilled WHO criteria and were confirmed by laboratory tests as dengue hemorrhagic fever (DHF). Compared with the 5 preceding years, the number of confirmed cases increased by 72%. During the 1995 outbreak, 5 virus isolations were made; 2 were DEN-1 and 3 were DEN-2. Two DHF deaths were confirmed by laboratory testing; in one of these, DEN-2 virus was isolated. The highest incidence in terms of the distribution of DF in Cali was in Comuna 2, a deprived sector of 500,000 inhabitants living in poor sanitary conditions; however, cases were widespread around the city. Forty-seven percent of DF cases were females and the age group ranging from 15 to 44 years was the most affected. Strategies to control the outbreak includ-

ed chemical insecticides, temephos for larvae and ULV malathion for adults; occasionally, fish (*Poecilia reticulata*) and *Bacillus sphericus* were also used as biological control methods.

Dengue hemorrhagic fever in Mexico, 1995

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Dengue fever is emerging as a major public health problem in Mexico due to the dramatic increase in the number of severe cases reported in 1995. Dengue hemorrhagic fever (DHF) was first detected in 1984 in the southern state of Yucatán. Since then, DHF cases were sporadically identified until the end of 1994 when 30 cases were reported. The situation in 1995 dramatically changed due to a shift from a passive to an intensive search for dengue cases in areas where dengue transmission was detected. This active surveillance produced a major increase in the identification of DHF cases throughout the country.

Data from 1995 showed that DHF started to appear in early January with the peak number of cases reported during September and a steady decline through December, a period in which 386 DHF cases were identified and 355 were laboratory-confirmed. The age group distribution showed that only 20% of the severe cases affected those under 15 years old and that DHF cases occurred in those age groups (15–44 years) where classic dengue is more frequently observed. Most of the DHF cases were reported by the social security health system (71%), followed by the public health sector (26%) and private physicians (3%). The case fatality rate of DHF was 7.8%, a situation that points out the combination of the severity of the clinical picture, the delay in seeking health services, the delay in proper diagnosis of DHF, and the lack of clinical expertise in the management of this new clinical entity in Mexico.

The geographic distribution of DHF cases suggested that the risk factors leading to the severe form of dengue are widely spread, because 19 states with different ecological conditions reported DHF cases in 1995. Sinaloa reported 34% of all cases, followed by Veracruz (22%) and Tamaulipas (10%). The number of dengue virus isolates obtained from August to December 1995 was the highest (22%) of the 616 isolates obtained since 1982. The prevalence of serotypes that were isolated in 1995 follows: DEN-1, 17%; DEN-2, 42%; DEN-3, 11%; and DEN-4, 38%.

The identification of 19 isolates of DEN-3 in Chiapas, Veracruz, Puebla, San Luis Potosi, and Tamaulipas reflects an improvement in the surveillance system activities implemented in 1994 and intensified during 1995. The role of this serotype in the causation of DHF epidemics is still to be determined because none of the DHF cases or fatal cases were associated with this serotype, which is circulating for the first time in Mexico. Nevertheless, the widespread susceptibility of the Mexican population to serotype DEN-3 must be considered a potential risk factor and detailed epidemiologic analysis is necessary to define the role of DEN-3 in the transmission dynamics of dengue in the country.

Venezuelan equine encephalitis, actual situation. Main vectors

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Venezuelan equine encephalitis (VEE) virus was isolated and characterized by Beck and Wyckoff in 1938. Since then, VEE has circulated in a silent enzootic cycle in sylvatic foci in several regions of Venezuela. Major epidemics/epizootics of VEE occurred in 1938 and 1942. In 1962, an outbreak started northwest of Lake Maracaibo (Zulia State) and spread rapidly through the coastal areas and reached the northeast region (Sucre State) in 1964. The last report of epizootic VEE virus activity occurred during the period December 1992 to January 1993 in the state of Trujillo (east of Lake Maracaibo).

The main foci of sylvatic VEE in Venezuela are located in the states of Zulia, Falcon, Yaracuy, and Miranda. In June 1995, a major epidemic/epizootic of VEE virus started on the eastern side of Lake Maracaibo (Falcon State) and eventually spread to the states of Zulia, Yaracuy, Lara, and Cojedes. In addition to the equine cases, 11,244 human cases and 15 fatalities were reported.

Control measures were oriented toward the vaccination of the susceptible equine population but it only covered 30% of the horses. Vector control was based on aerial and ground spray applications of insecticides to control adult mosquitoes. Entomological studies in Zulia and Yaracuy states have shown potential vectors of the VEE virus in species from the genera *Aedes, Culex, Psorophora, Mansonia,* and *Anopheles.* The main sylvatic hosts investigated were small mammals, particularly rodents, but the hosts included *Didelphis marsupialis* (opossum), *Proechimys guyannensis guairae* (caisgua rat), *Ako-*

don venezuelensis (field mouse), and *Tupinambis nigropunctatus* (tegu lizard, matoreal).

Venezuelan equine encephalitis in La Guajira, Colombia: entomological aspects

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A Venezuelan equine encephalitis (VEE) epizootic epidemic occurred in the counties of Maicao, Uribia, Riohacha, and Manaure of La Guajira State, Colombia, during September and October 1995. According to a household survey, there were an estimated 45,000 human cases, with 26 reported fatalities. This summary reports the results of the entomological survey, as well as the vector control interventions that were implemented.

Potential breeding places for mosquitoes (e.g., saltwater pools, lakes, crab holes, "jagueyes," or local water reservoirs) were surveyed. Larvae were identified to species. Adults were collected with a Shannon trap, stored in liquid nitrogen, and then identified and sent to the virology laboratory at the Instituto Nacional de Salud for attempted virus isolation. Immature forms of the following species were identified: *Aedes taeniorhynchus, Psorophora confinnis, Culex quinquefasciatus, Culex coronator, Culex nigripalpus, Anopheles albimanus, Anopheles neomaculipalpus,* and *Uranotaenia lowii.* The main breeding places were saltwater pools, where *Ae. taeniorhynchus* had the highest density. In spite of the failure to isolate viruses, this species has been incriminated as the main vector of VEE, based on the epidemiological evidence. In addition to equine vaccinations, an integrated mosquito control program was developed using *Bacillus sphaericus* GRISELESF 2362 plus pyrethroid and organophosphate insecticides, which had a great impact on the adult mosquito population density.

Life cycle of *Clerada apicicornis* (Heteroptera: Lygaedae), a potential biological control agent of *Rhodnius prolixus,* under laboratory conditions

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Clerada apicicornis Signoret is a small heteropteran insect that feeds on the hemolymph of *Rhodnius prolixus* and other triatomid bugs. For this reason, this insect is a potential alternative for the biological control of *R. prolixus,* the most important vector of *Trypanosoma cruzi* in Colombia. In order to better understand the life cycle of this species, 40 pairs of adult *C. apicicornis,* one male and one female, were placed in individual containers. The F_1 offspring were obtained from these adults to determine developmental times. The adult and nymphal stages were kept in glass flasks and fed on 3rd- to 5th-instar nymphs of *Blaberus atropus* cockroaches. The flasks were then placed on metal shelves and maintained at 20–24°C and 71.5% relative humidity. The eggs of each adult pair were collected on milano and crepe paper every 5 days. Eggs and nymphs were observed daily until they reached adulthood. Life cycle duration, egg and nymphal stage natality and mortality, and F_1 adult survival rates were determined. In addition, population parameters such as net reproduction rate, generation time, and intrinsic rate of natural growth were obtained for 29 female *C. apicicornis.*

Detection of dog tick infections of *Borrelia burgdorferi* in Monterrery, Mexico, using polymerase chain reaction

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Lyme disease is a multisystem infection caused by the tick-borne spirochete *Borrelia burgdorferi.* The disease was first recognized in 1976 and is the most frequently reported tick-borne infection in the world today.

This report presents results of a study conducted from August 1994 to May 1995 in 4 villages around the city of Monterrey in northeastern Mexico. The objectives were to detect *B. burgdorferi* DNA in ticks collected from domestic dogs by using the polymerase chain reaction (PCR), as well as to identify the tick species vectoring the spirochete. A total of 651 ticks were collected from 70 (72.1%) of 97 dogs. Tick species collected were *Rhipicephalus sanguineus* (521, 80%), *Dermacentor variabilis* (57, 8.7%), *Amblyomma cajennse* (60, 9.2%), and *Boophilus annulatus* (13, 2.0%). Pools of ticks were examined by the PCR assay for *B. burgdorferi.* Spirochetes were detected in 4 (6.6%) of 64 pools of *D. variabilis.* The PCR assay in ticks is the first report of *B. burgdorferi* in Nuevo León, México. This study demonstrated that the amplification of *B. burgdorferi*-specific se-

quence using PCR can be applied as a diagnostic method in tissue of ticks collected from dogs.

Experimental transmission of Powassan virus (Flaviviridae) by *Ixodes scapularis* ticks (Acari: Ixodidae)

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Powassan virus (POW) causes serious and often fatal encephalitis, particularly in children. This virus was identified and characterized in 1958, when it was isolated from the brain of a 5-year-old boy who died of encephalitis in the town of Powassan, Ontario, Canada. Since then, symptomatic human cases have been reported from North America (New York State [9], Ontario [5], Quebec [4], Pennsylvania [1], and Massachusetts [1]) and southeastern Russia. Human asymptomatic cases have been reported from northern México.

In New York State, the geographic distribution of *Ixodes scapularis* and its hosts overlaps with that of POW virus in 14 of 46 counties; also, this tick aggressively bites humans. Therefore, it was relevant to determine if *I. scapularis* could efficiently transmit POW virus under experimental conditions.

Five transmission experiments were performed using hamsters and rabbits to feed immature and adult ticks, respectively. Virus isolations and titrations were performed in 2-day-old suckling white mice, and virus isolates were identified by the immunofluorescent antibody assay. Experimental hamster and rabbit sera were tested for POW antibodies using the hemagglutination inhibition test. Our results indicated that all stages of *I. scapularis* became infected with POW virus after feeding on viremic hosts; all tick stages transmitted POW virus to clean (uninfected) hosts orally; and transstadial and transovarial transmission occurred.

Until now, POW encephalitis has shown a low incidence; however, if *I. scapularis* is an efficient vector in nature, the epidemiology of this disease could change. Field studies should be conducted in areas of North America where this tick species and POW virus distributions overlap. Furthermore, the possibility of POW virus being present in northern Mexico should be investigated.

Remote education: an alternative for personnel training in vector control

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Vertical programs to control malaria and dengue (Servicio Nacional de Erradicación de la Malaria, SNEM) have been decentralized and are now managed by municipalities and state health authorities in most Latin American countries. This new concept implies a reformulation of personnel policies, as seen in Colombia. In that country, for instance, more than 50% of the SNEM personnel were dismissed or given early retirement, whereas the remaining employees were hired by the municipalities and states along with new, untrained personnel. In this decentralized structure, human resources urgently require refresher and training courses in vector control. However, the high endemicity of the vector-borne diseases combined with the small budgets to control them do not allow control personnel to be withdrawn from their jobs and sent to training programs. For this reason, the Universidad del Valle in Cali developed a modular training course in vector control using remote education strategies. The modules include the following areas: management and strategic planning applied to vector control programs; leadership, communication strategies, and role of the entomologist and epidemiologist in primary health care in endemic areas; epidemiologic surveillance and entomologic parameters in vector-borne diseases; and ecology and biology of the principal disease vectors and control methods. Each student prepares a strategic plan for vector control to be developed in his/her working area. Every 8 wk the student meets with tutors to discuss and resolve questions. The cost for each student is U.S. $600 for the 10-month program; this amount includes all handouts and some visual material. Course expenses are covered either by local or state health agencies, alternatively the student pays directly to the university. Currently, there are 22 participants coming from most malaria and dengue endemic areas in Colombia.

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