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Malaria and dengue vector biology and control in Latin America

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Abstract

Malaria and dengue are major public-health problems in Latin America. Malaria is transmitted in 21 countries in the region with over 885,000 cases in 2002. Ninety-five percent of the cases occur in the Amazonian countries, mainly in Brazil. The main malaria vectors in Mexico, Central America and Northern South America are *Anopheles albimanus* and *An. pseudopunctipennis*, and *An. darlingi* and *An. nuñeztovari* in the Amazon and Venezuela. With the exception of *An. darlingi*, these mosquitoes are zoophagic, present low sporozoite indices and have low survival rates. These characteristics make them poor malaria vectors, supporting only seasonal transmission when mosquito abundance peaks. Over 500 million people live at risk of infection with dengue in the region, and all four dengue virus serotypes are in circulation. Over 482,000 clinical cases and 9,893 dengue hemorrhagic fever (DHF) cases were reported in the region in 2003. *Aedes aegypti* was introduced in colonial times and extended to most parts of the continent. After eradication from most part of the continent in the late 1950s and 1960s, re-infestation soon occurred. *Ae. albopictus* was introduced in the region in 1985 and dispersed from the Southern United States into Mexico, Central America and Brazil. The participation of this mosquito in dengue transmission in the area awaits assessment. Several populations with variable vectorial capacities have been identified in Mexico.
Keywords: malaria; dengue; *Anopheles*; *Aedes*; *Plasmodium*; transmission; control; gene flow

Malaria situation in the Americas

The use of DDT spraying for malaria control in the region began in 1941, and by 1948 its efficacy in eliminating transmission in some areas and reducing malaria cases in others, prompted the initiative to eradicate the disease, a strategy adopted until 1955. With DDT indoor residual spraying (IRS) as the spearhead, anti-malarial activities were mainly directed to attack mosquito vectors, but the progressive limitation in success and difficulties to maintain intensive operations were determinant for abandoning eradication for epidemiology-based specific control goals in 1992. In this new strategy, vector control is part of sustained preventive methods selectively applied. This strategy has been maintained to the present, and new elements related to improving health-services decentralization, drug-treatment

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surveillance and evaluation, and the integration of resources networks were added in 1998, as part of the worldwide Rollback Malaria initiative advocated by the World Health Organization.

Nowadays, malaria transmission has been interrupted in Canada, the United States and all Caribbean countries except Haiti and the Dominican Republic, but transmission still remains in 21 of the 37 countries of the region, while 915 imported cases were reported in the 18 countries without local transmission. It is estimated that out of 849 million inhabitants of the region, 175 million live in areas with some risk of malaria infection (PAHO 2003).

In 2002, a total of 885,000 malaria cases were reported in a population of 175 million people living in risk areas in the Americas, of which 28.3% were infections with *Plasmodium falciparum* and 71.2% with *P. vivax*. Cases produced by *P. malariae* occurred mainly in Brazil and Surinam. Brazil leads with more than 349,000 of the cases (40%) in the region, followed by Colombia (22%), Ecuador and Peru with 10% each. Along with Guatemala, Guyana, Honduras, Surinam and Venezuela, these countries reported 95% of the regional cases (PAHO 2003).

Malaria control activities

Apart from deploying strategies for better malaria detection and diagnosis, the main vector control activities include environmental sanitation, application of larvicides, IRS (with pyrethroids) and outdoor malathion application. To a lesser extent, biological control using *Bacillus thuringiensis israelensis* and *B. sphaericus* as well as larvivorus fish are also applied in a few countries. Also, a network incorporating the eight Amazonian endemic countries was established in 2001 for drug resistance surveillance.

Particular success was achieved in the control of malaria in Mexico (Chanon et al. 2003). Malaria in this country is unstable with areas of persistent transmission resilient to control interventions. Since the implementation of the global eradication campaign in the fifties, control activities were carried out using mostly IRS (with DDT), and relaxation of these activities was frequently followed by outbreaks that originated from persistent foci. New control strategies with the participation of the endemic communities, based on elimination of filamentous algae from *An. pseudopunctipennis* larval breeding sites, the elimination of the parasite reservoir (*P. vivax*) by repeated treatment of patients in order to eliminate relapses, and the application of low-volume pyrethroid insecticides, have controlled malaria to historically low levels without the use of DDT during the last four years. New insecticide-spraying techniques use less insecticide, act faster and are cheaper. This strategy is now in the process of being tested in other parts of Central America.

Malaria vectors

The main malaria vectors in Mexico and Central America are *Anopheles pseudopunctipennis* and *An. albimanus*, while *An. darlingi*, *An. aquasalis and An. nuñeztovari* are the principal vectors in the Amazonian countries (Zimmerman 1992). Malaria transmission by *An. albimanus* and *An. pseudopunctipennis* occurs in South America with other anophelines like *An. vestitipennis* and *An. punctimacula* as secondary vectors in Mesoamerica, while *An. aquasalis* plays a secondary role in transmission in South America. Only the main vectors are reviewed here.

Anopheles albimanus is widely distributed at low altitudes in the tropics and subtropical regions of the Americas, from the South of the Southern United States to Northern Colombia, Ecuador, Peru and Venezuela, including the Caribbean Islands

(Faran 1980). Their larvae generally prefer exposed areas in the sun, such as shores of lakes, lagoons and small streams, and rain pools, but also swamps or brackish waters (Breeland 1972; Savage et al. 1990).

Anopheles albimanus is highly zoophilic (Arredondo-Jiménez et al. 1992) with human blood indices (HBI) ranging from 0.4 to 0.21 (Garrett-Jones 1964; Garrett-Jones, Boreham and Pant 1980; Loyola et al. 1993). However, host availability and ecological conditions could explain differences in the HBI observed among mosquito populations (Loyola et al. 1993) that readily feed on humans when big mammals are scarce. This anopheline is mainly exophagic, but indoor resting after outdoor feeding has been documented (Loyola et al. 1990; 1993).

Differences in ribosomal DNA structure (Beach, Mills and Collins 1989), egg morphology (Rodriguez et al. 1992b) and allozyme pattern (Narang, Seawright and Suarez 1991) have been documented among *An. albimanus* populations, but polytene chromosomes (Keppler Jr., Kitzmiller and Rabbani 1973; Narang, Seawright and Suarez 1991) and cross-hybridization experiments (Narang, Seawright and Suarez 1991) have failed to demonstrate subgroups within the species.

Estimation of gene flow among *An. albimanus* populations in Mexico using RAPD-PCR analysis indicated low variability (F_{st}= 0.169), distributed in two groups, one in the North and the other in the Southeast, including Guatemala. Nationwide, a migration rate of *Nm* = 1.2 was calculated, but in Southern Mexico, populations collected from five neighbouring different agro-ecological areas had *Nm* values of 1.9 to 2.4, with a mean F_{st} of 0.117 (Villareal-Treviño 2001).

An. albimanus is the main vector of *P. vivax* on the coastal plains of Mexico, Central America and Colombia; it has also been found infected with *P. falciparum* (Rodriguez and Loyola 1990; Herrera et al. 1987). Parasite indices are usually low (0.01-0.1%, Ramsey et al. 1994), and it is estimated that only 2% of mosquitoes live long enough to transmit malaria (Rodriguez et al. 1992a). These characteristics make this species a poor malaria vector. Yet, it supports seasonal transmission during the rainy season, when the abundance of breeding sites produces high mosquito densities.

Anopheles pseudopunctipennis is the most widely distributed anopheline in the Neotropical region (Bruce-Chwatt 1985), covering from Southern United States to Northern Argentina, including the Andean countries and extending to the lesser Antilles (Aitken 1945; Manguin et al. 1995). Throughout its geographical range, *An. pseudopunctipennis* habitats occur at altitudes over 200 m above sea level, where the rugged terrain determines the formation of larval breeding sites in pools, ponds and lagoons developing in the margins of rivers and streams when the rainy season ends (Aitken 1945; Fernandez-Salas et al. 1994).

An. pseudopunctipennis is traditionally considered an anthropophilic mosquito (Vargas 1938; Davis and Shannon 1928). However, although high HBI (between 29.5 and 54.7%) were documented in Southern Mexico, host preference estimates through forage-ratio analysis indicate that the HBI reflects host availability, and that this mosquito has a bias towards horses and dogs (Fernandez-Salas et al. 1993), as documented by higher mosquito collections in horses than in humans (Fernandez-Salas et al. 1993).

At least five subspecies and one variant of *An. pseudopunctipennis* were morphologically described in South America (Knight and Stone 1977). Later, using rDNA analysis and cross-mating experiments, a complex of two allopatric species was identified in Central Mexico and Peru and Bolivia, respectively (Estrada-Franco et al. 1993a; 1993b). Finally, Manguin et al. (1995), by using isozyme analysis, classified the entire population in three clusters, one located in the Antilles, one

extending from the Southern United States though Mexico and Guatemala and the third from South America through Central America to Belize.

An. pseudopunctipennis is frequently the only malaria vector found at altitudes higher than 600 m above sea level (Aitken 1945; Vargas, Casis and Earle 1941). Malaria sporozoite rates range from 2.6% in Peru (Hayes et al. 1987) to 3.16% in Mexico (Loyola et al. 1991). Mark-recapture studies indicate a daily survival rate of 0.875, and that about 60% of nulliparous females require a second blood meal to complete their gonotrophic cycle (Fernandez-Salas, Rodriguez and Roberts 1994). These data may explain why *An. pseudopunctipennis* supports malaria transmission with low population densities.

Anopheles darlingi is distributed in the South of Mexico, Guatemala, Honduras, Belize, Colombia, Venezuela, Bolivia, Peru, Ecuador, Paraguay, Brazil and Argentina (Forattini 1962). This mosquito breeds in shaded clean rainwater pools that are often man-made after forest clearing (Klein, Lima and Toda Tang 1992; Charlwood 1996). Furthermore, the species is frequently clustered in relatively small areas at the end of the rainy season (Charlwood 1980), while there is low mosquito abundance during most parts of the year. Although *An. darlingi* has a longer survival rate than other local anophelines in Rondônia, Brazil (Charlwood and Alecrim 1989), low mosquito densities have hindered extensive studies on survival rates.

Several studies indicate the preference of this mosquito for human blood: offered a choice of hosts *An. darlingi* preferred humans (Oliveira-Ferreira et al. 1992), and 59% of specimens collected in the Brazilian Amazon region were trying to feed on humans or in nearby human dwellings (Tadei et al. 1998). Although this mosquito readily enters houses to feed, it remains inside only for short times.

So far, there is no clear evidence for the existence of subspecies or subpopulations in the *An. darlingi* taxon. Genetic variation is supported by isozyme analysis (Narang et al. 1979) and chromosomal polymorphism (Schreiber and Guedes 1961; Kreutzer, Kitzmiller and Ferreira 1972). However, although differences in biting behaviour were documented in three Brazilian populations, calculated genetic distances (the highest ≤ 0.049) using isozyme and hydrocarbon cuticular analysis only support intra specific variation (Rosa-Freitas, Deane and Momen 1990).

An. darlingi is the main *P. falciparum* and *P. vivax* malaria vector in the endemic areas of Amazonian countries (De Arruda et al. 1986; Klein et al. 1991; Zimmerman 1992; Tadei et al. 1998; Roper et al. 2000). This mosquito is present in the Lacandon forest in Southern Mexico and in restricted areas of Central America, but its participation in malaria transmission in these areas was only inferred in Guatemala because no other anopheline could be incriminated as a vector.

The geographic range of *Anopheles nuñeztovari* covers the regions from Panama to Northern South America, including Colombia, Venezuela, Guyana, French Guyana, Bolivia, Brazil, Ecuador and Peru (Forattini 1962). Larval habitats have been described in flooded areas left by subsided rivers or in flooded pastures. The nature of the breeding sites results in a peak of *An. nuñeztovari* densities during the dry season in Surinam (Zimmerman 1992) and in the rainy season in Venezuela (Rubio 1991).

Anopheles nuñeztovari readily feeds on humans, with HBI between 18.2 and 38.9 in outdoor collections from Venezuela (Rubio 1991). This mosquito is more exophagic than endophagic. Along with *An. aquasalis* it is the main vector of *P. vivax* in Western Venezuela (Rubio 1991) and Colombia (Herrera et al. 1987) and a secondary vector of *P. vivax* and *P. falciparum* in Brazil (De Arruda et al. 1986; Tadei et al. 1998). An isozyme analysis of four Brazilian and two Colombian *An. nuñeztovari* populations provided evidence for some degree of reproductive isolation

between the two groups (Scarpassa, Tadei and Suarez 1996), but no indication of subspeciation.

Anopheles vestitipennis is presented here as a special case, as this is the only malaria vector for which evidence for the existence of subpopulations has accumulated. *An. vestitipennis* ranges from central Mexico to Northern South America and the Great and Lesser Antilles (Wilkerson, Strickman and Litwak 1990). This mosquito has been incriminated as a vector of *P. vivax* in the Lacandon Forest (Loyola et al. 1991) and it probably also transmits malaria in Guatemala (Padilla et al. 1992).

Isozyme analysis of *An. vestitipennis* from Southern Mexico indicated differences between mosquito populations collected on humans and animal baits (D = 0.07) (Arredondo-Jiménez et al. 1996). These findings were later supported by differences in RAPD markers of specimens collected with animal and human baits (D = 0.25) (Murillo-Sánchez 2001). Further mark-recapture experiments confirmed the existence of subpopulations with different host preferences in the field (Ulloa et al. 2002). Interestingly these populations can be separated by differences in the ornamentation of their eggs (Rodriguez et al. 1999).

Special aspects of malaria transmission in Southern Mexico

The main malaria vectors on the Pacific Ocean coast of the State of Chiapas, Mexico are *An. albimanus* on the coastal plains and *An. pseudopunctipennis* in the foothills (Rodriguez and Loyola 1990). *Plasmodium vivax* is responsible for over 95% of malaria cases in the area. The two phenotypes of the circumsporozoite protein (CSP) identified in this parasite, VK210 and VK247 (Rosenberg et al. 1989; Arnot et al. 1985) occur in the area, but so far only VK210 has been identified in patients living on the plains, while both phenotypes, with a predominance of VK247, occur at higher altitudes. This parasite prevalence distribution follows the geographic distribution of the vector mosquitoes (Rodriguez et al. 2000).

Experiments using *P. vivax*-infected patient blood to infect *An. albimanus* and *An. pseudopunctipennis* demonstrated that the former was more susceptible to the phenotype VK210, while the latter was more susceptible to VK247 (Gonzalez-Ceron et al. 1999). Further studies indicated that *P. vivax* ookinetes VK247 either could not invade the midgut epithelium of *An. albimanus* or were killed during crossing it (Gonzalez-Ceron et al. 2001). On the other hand, VK210 parasites were unable to exit the blood-meal bolus and were destroyed when fed to *An. pseudopunctipennis* (Gonzalez-Ceron et al. in prep.).

This situation may not occur in other malarious areas of the Americas where *An. albimanus* transmits malaria. Thus, although a higher prevalence of antibodies to VK210 is observed in Colombia compared to those to VK247, more VK247 sporozoites were isolated later in experimental infections of *An. albimanus* (Gonzalez-Ceron et al. 2001). On the other hand, recent experiments indicate a shift in the infectivity of *An. pseudopunctipennis* by some VK 210 parasites from the foothills, but not by those from the coastal plains. (Gonzalez-Ceron et al. in prep.).

Dengue situation in the Americas

The classic dengue fever (DF) together with their severe forms of dengue hemorrhagic fever (DHF) and shock syndrome (DSS) are serious health problems in many parts of the Americas. The fist dengue-like cases in the Americas were recorded in the Caribbean islands of Martinique and Guadeloupe in 1635. On the continent, this

occurred in 1780 in Philadelphia. The first reported epidemic with over 50,000 cases was recorded in Peru in 1818, and since then many outbreaks varying in intensity and distribution have occurred. Several pandemic outbreaks were recorded in many countries in the region: cases were reported from the United States, Cuba, Brazil and Peru during the pandemic of 1845-51; during the pandemic of 1870-73, cases were reported in Alabama and, notably, over 40,000 cases were reported in New Orleans; the 1901-07 pandemic extended to the Southern United States, the Caribbean, Panama and Colombia; the 1912-16 pandemic included cases in Central America, Northern South America and Puerto Rico. In the 1998 pandemic the more affected countries in the region were Venezuela, Peru, Colombia, Ecuador and Brazil with over 500,000 cases reported (Schneider and Droll 2003). Regional epidemics have affected the whole region, from the United States with over half a million cases in Texas in 1922.

Dengue serotype 1 was introduced into Jamaica in 1977 and the first important epidemic outbreaks produced by this serotype occurred in Bolivia, Brazil, Ecuador, Paraguay and Peru in 1982. Dengue serotype 3 was responsible for epidemics in the Caribbean countries in 1963 and, together with the serotype 2, it was presented again in epidemics occurring in these islands and Venezuela in 1968-1968.

In the late 1970s, serotypes 2 and 3 circulated in the Caribbean and in 1981 this serotype produced a major epidemic in Cuba with 344,203 cases, 10,312 hemorrhagic-fever cases and 158 deaths, (Guzmán et al. 1988). Serotype 4 arrived in the region in 1981 and since then it has been responsible for outbreaks in the Great and Lesser Antilles, Mexico, Central America and Northern South America, generating over 7,000 cases in Brazil in 1982. Serotype 4, in conjunction with serotypes 1 and 2, led to over 10,000 cases in Colombia between 1992 and 1995. Also, between 1980-1994 serotypes 1, 2 and 4 were responsible of all dengue activity in the region. Serotype 3 (Sri Lanka/India genotype) reappeared in Nicaragua and rapidly extended into Central America and Mexico.

Currently, over 500 million people live at risk of infection with dengue viruses and all four serotypes are in circulation in the Americas. Over 482,000 clinical cases (only 16,966 confirmed by laboratory diagnosis) and 9,893 DHF cases were reported in the region in 2003 (PAHO 2004).

The present increase in DF/DHF in the Americas follows the same pattern as seen in Southeast Asia and the occidental Pacific forty years ago, indicating the possibility of ever more important outbreaks with severe illness and higher mortality. The documentation of neutralizing antibodies to dengue virus serotypes 1 and 2 in 22% of a sample of fruit bats in Costa Rica, and to serotypes 2 and 3 in 30% in Ecuador (Platt et al. 2000) raises the possibility of the existence of feral reservoirs.

Dengue vectors

Both main worldwide recognized vectors of dengue, *Aedes aegypti* and *Ae. albopictus,* are present in the region. *Aedes aegypti* was introduced during colonial times (Christophides, Vlachou and Kafatos 2004; Tabachnick 1991) and invaded the whole continent from the United States, the Caribbean, Central and South America, down to Chile.

In 1947, a hemisphere-wide initiative was initiated to combat yellow fever through the eradication of *Ae. aegypti*. This succeeded in eliminating this mosquito in most territories from 1952 (Colombia) to 1963 (Mexico). However, re-infestation soon occurred after 1967, probably from areas where eradication had not been achieved. In spite of additional efforts to re-eliminate this mosquito in Panama, Brazil, Peru and Belize, re-infestation occurred again. Nowadays, *Ae. aegypti* is present in all

American countries except Bermuda, Canada, Chile and Uruguay (Gubler and Trent 1993). It is distributed in altitudes ranging from sea level up to 2,200 m above sea level in Colombia and up to 1,700 m in Mexico.

Aedes albopictus was introduced to the American continent, through the United States where it was fist detected in 1985, but it appeared almost simultaneously in Brazil in 1986. In the United States, it was initially reported in the State of Texas where it dispersed northwards, and by 1995 it was present in 23 states. In 1993, it was recognized in Santo Domingo. *Aedes albopictus* was recently identified in the Southern-Mexican state of Chiapas (Casas-Martinez and Torres-Estrada 2003) and in Guatemala, where, as in other areas (Hobbs, Hughes and Eichold II 1991), it is replacing *Ae. aegypti*. The importance of this vector in dengue transmission remains to be assessed.

Vector population studies

Population genetic analysis of ten *Ae. aegypti* collections from Northern Mexico, using mitochondrial DNA as markers, identified seven haplotypes in two clades. Polymorphic DNA (RAPD) markers indicated that populations are isolated by distance, but free gene flow occurs within a 90-250-km ratio (Gorrochotegui-Escalante et al. 2000). These studies were extended to 32 populations from the Gulf of Mexico to the Pacific Ocean. Twenty-five haplotypes were detected using single-strand conformation polymorphism analysis of the *Nicotinamide Adenine Dinucleotide Dehydrogenase subunit 4* mitochondrial gene. Three genetically different mosquito populations were identified, namely in Northeastern Mexico, the Yucatan Peninsula and the Pacific Ocean coast. These populations were isolated by distance but, again, free gene flow occurred within populations (Gorrochotegui-Escalante et al. 2002).

Further studies, including 22 collections from the same areas in Mexico and two collections from the Southern United States, were conducted to investigate their susceptibility to a dengue 2 virus strain. These studies evidenced differences in the vector competence among mosquito populations with a range between 24 and 83%, the Yucatecan mosquitoes being the most competent ones (Bennett et al. 2002).

Dengue control activities

Eradication efforts have been abandoned long ago in the region, and control is directed mainly to the abatement of mosquito populations. More and more control activities are directed towards the control of domestic breeding sites, and insecticides in the form of fogs are used outdoors during outbreaks. Although these activities are conducted in the face of epidemics, their effect on controlling transmission is uncertain. An excessive dependence on ultra-low-volume application instead of larval breeding control (Gubler and Clark 1995), and untimely and faulty application of insecticides (Gratz 1994) contribute to the inadequacy of the present interventions.

Most countries have limited success in deploying routine control interventions aimed at maintaining *Ae. aegypti* populations at low densities. The main efforts in these interventions seek to diminish larval breeding by treating domiciliary water containers. The application of temephos by public-health personnel often fails because of poor training of field technicians. Biological control using turtles (Borjas et al. 1993) and copepods (Marten et al. 1994), as well as larvicidal measures such as washing containers with chlorine bleach and detergent (Fernández et al. 1998), have the limitation of relying on community participation, even though the perception and attitudes of local people are not frequently taken into consideration.

References

Aitken, T.H.G., 1945. Studies on the anopheline complex of Western America. *University of California Publications in Entomology*, 7, 273-364.

Arnot, D.E., Barnwell, J.W., Tam, J.P., et al., 1985. Circumsporozoite protein of *Plasmodium vivax*: gene cloning and characterization of the immunodominant epitope. *Science*, 230 (4727), 815-818.

Arredondo-Jiménez, J.I., Bown, D.N., Rodriguez, M.H., et al., 1992. Tests for the existence of genetic determination or conditioning in host selection by *Anopheles albimanus* (Diptera: Culicidae). *Journal of Medical Entomology*, 29 (5), 894-897.

Arredondo-Jiménez, J.I., Gimnig, J., Rodriguez, M.H., et al., 1996. Genetic differences among *Anopheles vestitipennis* subpopulations collected using different methods in Chiapas state, southern Mexico. *Journal of the American Mosquito Control Association*, 12 (3 Pt. 1), 396-401.

Beach, R.F., Mills, D. and Collins, F.H., 1989. Structure of ribosomal DNA in *Anopheles albimanus* (Diptera: Culicidae). *Annals of the Entomological Society of America*, 82 (5), 641-648.

Bennett, K.E., Olson, K.E., Muñoz, M de L., et al., 2002. Variation in vector competence for dengue 2 virus among 24 collections of *Aedes aegypti* from Mexico and the United States. *American Journal of Tropical Medicine and Hygiene*, 67 (1), 85-92.

Borjas, G., Marten, G.G., Fernandez, E., et al., 1993. Juvenile turtles for mosquito control in water storage tanks. *Journal of Medical Entomology*, 30 (5), 943-946.

Breeland, S.G., 1972. Studies on the ecology of *Anopheles albimanus*. *American Journal of Tropical Medicine and Hygiene*, 21 (5), 751-754.

Bruce-Chwatt, L. J., 1985. *Essential malariology*. 2nd edn. Heinemann, London.

Casas-Martinez, M. and Torres-Estrada, J.L., 2003. First evidence of *Aedes albopictus* (Skuse) in southern Chiapas, Mexico. *Emerging Infectious Diseases*, 9 (5), 606-607.

Chanon, K.E., Méndez-Galván, J.F., Galindo-Jaramillo, J.M., et al., 2003. Cooperative actions to achieve malaria control without the use of DDT. *International Journal of Hygiene and Environmental Health*, 206 (4/5), 387-394.

Charlwood, J.D., 1980. Observations on the bionomics of *Anopheles darlingi* Root (Diptera: Culicidae) from Brazil. *Bulletin of Entomological Research*, 70 (4), 685-692.

Charlwood, J.D., 1996. Biological variation in *Anopheles darlingi* Root. *Memorias Do Instituto Oswaldo Cruz*, 91 (4), 391-398.

Charlwood, J.D. and Alecrim, W.A., 1989. Capture-recapture studies with the South American malaria vector *Anopheles darlingi* Root. *Annals of Tropical Medicine and Parasitology*, 83 (6), 569-576.

Christophides, G.K., Vlachou, D. and Kafatos, F.C., 2004. Comparative and functional genomics of the innate immune system in the malaria vector *Anopheles gambiae*. *Immunological Reviews*, 198, 127-148.

Davis, N.C. and Shannon, R.C., 1928. The blood feeding habits of *Anopheles pseudopunctipennis* in northern Argentina. *American Journal of Tropical Medicine and Hygiene*, 5, 443-447.

De Arruda, M., Carvalho, M.B., Nussenzweig, R.S., et al., 1986. Potential vectors of malaria and their different susceptibility to *Plasmodium falciparum* and *Plasmodium vivax* in northern Brazil identified by immunoassay. *American Journal of Tropical Medicine and Hygiene,* 35 (5), 873-881.

Estrada-Franco, J.G., Lanzaro, G.C., Ma, M.C., et al., 1993a. Characterization of *Anopheles pseudopunctipennis* sensu lato from three countries of neotropical America from variation in allozymes and ribosomal DNA. *American Journal of Tropical Medicine and Hygiene,* 49 (6), 735-745.

Estrada-Franco, J.G., Ma, M.C., Gwadz, R.W., et al., 1993b. Evidence through crossmating experiments of a species complex in *Anopheles pseudopunctipennis* sensu lato: a primary malaria vector of the American continent. *American Journal of Tropical Medicine and Hygiene,* 49 (6), 746-755.

Faran, M.E., 1980. A revision of the albimanus section of the subgenus *Nyssorhynchus* of *Anopheles*. *Contributions of the American Entomological Institute,* 15 (7), 1-215.

Fernández, E.A., Leontsini, E., Sherman, C., et al., 1998. Trial of a community-based intervention to decrease infestation of *Aedes aegypti* mosquitoes in cement washbasins in El Progreso, Honduras. *Acta Tropica,* 70 (2), 171-183.

Fernandez-Salas, I., Roberts, D.R., Rodriguez, M.H., et al., 1993. Host selection patterns of *Anopheles pseudopunctipennis* under insecticide spraying situations in southern Mexico. *Journal of the American Mosquito Control Association,* 9 (4), 375-384.

Fernandez-Salas, I., Rodriguez, M.H. and Roberts, D.R., 1994. Gonotrophic cycle and survivorship of *Anopheles pseudopunctipennis* (Diptera: Culicidae) in the Tapachula foothills of southern Mexico. *Journal of Medical Entomology,* 31 (3), 340-347.

Fernandez-Salas, I., Rodriguez, M.H., Roberts, D.R., et al., 1994. Bionomics of adult *Anopheles pseudopunctipennis* (Diptera: Culicidae) in the Tapachula foothills area of southern Mexico. *Journal of Medical Entomology,* 31 (5), 663-670.

Forattini, O.P., 1962. *Entomologia medica. Vol. 1 . Parte geral, Diptera, Anophelini*. Faculdade de Higiene e Sáude Publica, Sao Paulo.

Garrett-Jones, C., 1964. The human blood index of malaria vectors in relation to epidemiological assessment. *Bulletin of the World Health Organization,* 30, 241-261.

Garrett-Jones, C., Boreham, P.F.L. and Pant, C.P., 1980. Feeding habits of anophelines (Diptera: Culicidae) in 1971-78, with reference to the human blood index: a review. *Bulletin of Entomological Research,* 70, 165-185.

Gonzalez-Ceron, L., Rodriguez, M.H., Nettel, J.C., et al., 1999. Differential susceptibilities of *Anopheles albimanus* and *Anopheles pseudopunctipennis* to infections with coindigenous *Plasmodium vivax* variants VK210 and VK247 in southern Mexico. *Infection and Immunity,* 67 (1), 410-412.

Gonzalez-Ceron, L., Rodriguez, M.H., Santillan, F., et al., 2001. *Plasmodium vivax*: ookinete destruction and oocyst development arrest are responsible for *Anopheles albimanus* resistance to circumsporozoite phenotype VK247 parasites. *Experimental Parasitology,* 98 (3), 152-161.

Gorrochotegui-Escalante, N., Gomez-Machorro, C., Lozano-Fuentes, S., et al., 2002. Breeding structure of *Aedes aegypti* populations in Mexico varies by region. *American Journal of Tropical and Medical Hygiene,* 66 (2), 213-222.

Gorrochotegui-Escalante, N., Muñoz, M. de L., Fernandez-Salas, I., et al., 2000. Genetic isolation by distance among *Aedes aegypti* populations along the northeastern coast of Mexico. *American Journal of Tropical Medicine and Hygiene,* 62 (2), 200-209.

Gratz, N.G., 1994. Education and employment of medical entomologists in *Aedes aegypti* control programmes. *Gaoxiong Yi Xue Ke Xue Za Zhi,* 10 (Suppl.), S19-S27.

Gubler, D.J. and Clark, G.G., 1995. Dengue/dengue hemorrhagic fever: the emergence of a global health problem. *Emerging Infectious Diseases,* 1 (2), 55-57.

Gubler, D.J. and Trent, D.W., 1993. Emergence of epidemic dengue/dengue hemorrhagic fever as a public health problem in the Americas. *Infective Agents of Diseases,* 2 (6), 383-393.

Guzmán, M.G., Kourí, G., Bravo, J., et al., 1988. Dengue in Cuba: history of an epidemic. *Revista Cubana de Medicina Tropical,* 40 (2), 29-49.

Hayes, J., Calderon, G., Falcon, R., et al., 1987. Newly incriminated anopheline vectors of human malaria parasites in Junin Department, Peru. *Journal of the American Mosquito Control Association,* 3 (3), 418-422.

Herrera, S., Suarez, M.F., Sanchez, G.I., et al., 1987. Uso de la tecnica inmuno-radiometrica (IRMA) en *Anopheles* de Colombia para la identificación de esporozoitos de *Plasmodium*. *Colombia Medica,* 18, 2-6.

Hobbs, J.H., Hughes, E.A. and Eichold II, B.H., 1991. Replacement of *Aedes aegypti* by *Aedes albopictus* in Mobile, Alabama. *Journal of the American Mosquito Control Association,* 7 (3), 488-489.

Keppler Jr., W.J., Kitzmiller, J.B. and Rabbani, M.G., 1973. The salivary gland chromosomes of *Anopheles albimanus*. *Mosquito News,* 33 (1), 42-49.

Klein, T.A., Lima, J.B., Tada, M.S., et al., 1991. Comparative susceptibility of anopheline mosquitoes in Rondonia, Brazil to infection by *Plasmodium vivax*. *American Journal of Tropical Medicine and Hygiene,* 45 (4), 463-470.

Klein, T.A., Lima, J.B. and Toda Tang, A., 1992. Vector incrimination and effects of antimalarial drugs on malaria transmission and control in the Amazon basin of Brazil. *Memorias Do Instituto Oswaldo Cruz,* 87 (Suppl. 3), 393-397.

Knight, K.L. and Stone, A., 1977. *A catalog of the mosquitoes of the world (Diptera: Culicidae). Volume 6*. 2nd edn. Entomological Society of America, The Thomas Say Foundation, Lanham.

Kreutzer, R.D., Kitzmiller, J.B. and Ferreira, E., 1972. Inversion polymorphism in the salivary gland chromosomes of *Anopheles darlingi* Root. *Mosquito News,* 32 (4), 555-565.

Loyola, E.G., Arredondo, J.I., Rodriguez, M.H., et al., 1991. *Anopheles vestitipennis*, the probable vector of *Plasmodium vivax* in the Lacandon forest of Chiapas, Mexico. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 85 (2), 171-174.

Loyola, E.G., Gonzalez-Ceron, L., Rodriguez, M.H., et al., 1993. *Anopheles albimanus* (Diptera: Culicidae) host selection patterns in three ecological areas of the coastal plains of Chiapas, southern Mexico. *Journal of Medical Entomology,* 30 (3), 518-523.

Loyola, E.G., Rodriguez, M.H., Gonzalez, L., et al., 1990. Effect of indoor residual spraying of DDT and bendiocarb on the feeding patterns of *Anopheles pseudopunctipennis* in Mexico. *Journal of the American Mosquito Control Association,* 6 (4), 635-640.

Manguin, S., Roberts, D.R., Peyton, E.L., et al., 1995. Biochemical systematics and population genetic structure of *Anopheles pseudopunctipennis*, vector of malaria in Central and South America. *American Journal of Tropical Medicine and Hygiene,* 53 (4), 362-377.

Marten, G.G., Borjas, G., Cush, M., et al., 1994. Control of larval *Aedes aegypti* (Diptera: Culicidae) by cyclopoid copepods in peridomestic breeding containers. *Journal of Medical Entomology,* 31 (1), 36-44.

Murillo-Sánchez, M.H., 2001. *Variabilidad genética de subpoblaciones de Anopheles vestitipennis (Diptera:Culicidae) mediante el uso de RAPD-PCR*. MSc Thesis Universidad Autónoma de Nuevo León, Monterrey, Mexico.

Narang, S., Santos, J.M.M., Garcia, J.C., et al., 1979. Genética de poulacoes de anofelinos. IV. Estudos electroforéticos das populacoes naturais de *Anopheles nuneztovari* e *Anopheles darlingi*: correlacao entre espécies. *Acta Amazonica,* 9, 529-542.

Narang, S.K., Seawright, J.A. and Suarez, M.F., 1991. Genetic structure of natural populations of *Anopheles albimanus* in Colombia. *Journal of the American Mosquito Control Association,* 7 (3), 437-445.

Oliveira-Ferreira, J., Lourenco-De Oliveira, R., Deane, L.M., et al., 1992. Feeding preference of *Anopheles darlingi* in malaria endemic areas of Rôndonia State, northwestern Brazil. *Memorias Do Instituto Oswaldo Cruz,* 87, 601-602.

Padilla, N.P., Molina, J., Juárez, J., et al., 1992. Potential malaria vectors in northern Guatemala. *Journal of the American Mosquito Control Association,* 8, 305-306.

PAHO, 2003. *Number of reported cases of dengue and dengue hemorrhagic fever DHF, region of the Americas (by country and subregion)*. Pan American Health Organization. [http://www.paho.org/English/AD/DPC/CD/dengue-cases-2003.htm]

PAHO, 2004. *Number of reported cases of dengue and dengue hemorrhagic fever DHF, region of the Americas (by country and subregion)*. Pan American Health Organization. [http://www.paho.org/English/AD/DPC/CD/dengue-cases-2004.htm]

Platt, K.B., Mangiafico, J.A., Rocha, O.J., et al., 2000. Detection of dengue virus neutralizing antibodies in bats from Costa Rica and Ecuador. *Journal of Medical Entomology,* 37 (6), 965-967.

Ramsey, J.M., Salinas, E., Bown, D.N., et al., 1994. *Plasmodium vivax* sporozoite rates from *Anopheles albimanus* in southern Chiapas, Mexico. *Journal of Parasitology,* 80 (3), 489-493.

Rodriguez, M.H., Bown, D.N., Arredondo-Jimenez, J.I., et al., 1992a. Gonotrophic cycle and survivorship of *Anopheles albimanus* (Diptera: Culicidae) in southern Mexico. *Journal of Medical Entomology,* 29 (3), 395-399.

Rodriguez, M.H., Chavez, B., Hernandez-Avila, J.E., et al., 1999. Description and morphometric analysis of the eggs of *Anopheles* (*Anopheles*) *vestitipennis* (Diptera: Culcidae) from southern Mexico. *Journal of Medical Entomology,* 36 (1), 78-87.

Rodriguez, M.H., Chavez, B., Orozco, A., et al., 1992b. Scanning electron microscopic observations of *Anopheles albimanus* (Diptera: Culicidae) eggs. *Journal of Medical Entomology,* 29 (3), 400-406.

Rodriguez, M.H., Gonzalez-Ceron, L., Hernandez, J.E., et al., 2000. Different prevalences of *Plasmodium vivax* phenotypes VK210 and VK247 associated with the distribution of *Anopheles albimanus* and *Anopheles pseudopunctipennis* in Mexico. *American Journal of Tropical Medicine and Hygiene,* 62 (1), 122-127.

Rodriguez, M.H. and Loyola, E.G., 1990. Malaria in Mexico. *In: Proceedings and papers of the 58th annual conference of the California Mosquito and Vector Control Association, Jan 28-31*. 49-52.

Roper, M.H., Torres, R.S., Goicochea, C.G., et al., 2000. The epidemiology of malaria in an epidemic area of the Peruvian Amazon. *American Journal of Tropical Medicine and Hygiene,* 62 (2), 247-256.

Rosa-Freitas, M.G., Deane, L.M. and Momen, H., 1990. A morphological, isoenzymatic and behavioural study of ten populations of *Anopheles* (*Nyssorhynchus*) *albitarsis* Lynch-Arribalzaga, 1878 (Diptera: Culicidae) including from the type-locality Baradero, Argentina. *Memorias do Instituto Oswaldo Cruz,* 85 (3), 275-289.

Rosenberg, R., Wirtz, R.A., Lanar, D.E., et al., 1989. Circumsporozoite protein heterogeneity in the human malaria parasite *Plasmodium vivax*. *Science,* 245 (4921), 973-976.

Rubio, Y., 1991. *Biology of Anopheles nuñeztovari and its role in malaria transmisión: final report*. National Academy of Sciences USA. Report no. MVR.VE1-97-81.

Savage, H.M., Rejmankova, E., Arredondo-Jiménez, J.I., et al., 1990. Limnological and botanical characterization of larval habitats for two primary malarial vectors, *Anopheles albimanus* and *Anopheles pseudopunctipennis*, in coastal areas of Chiapas State, Mexico. *Journal of the American Mosquito Control Association,* 6 (4), 612-620.

Scarpassa, V.M., Tadei, W.P. and Suarez, M.F., 1996. Allozyme differentiation among allopatric populations of *Anopheles nuñeztovari* (Diptera: Culicidae). *Brazilian Journal of Genetics,* 19 (2), 265-269.

Schneider, J. and Droll, D., 2003. *A timeline for dengue in the Americas to December 31, 2000 and notified first occurrences*. Pan American Health Organization, Washington.

Schreiber, G. and Guedes, A.S., 1961. Cytological aspects of the taxonomy of anophelines (subgenus Nyssorhynchus). *Bulletin of the World Health Organization,* 24, 657-658.

Tabachnick, W.J., 1991. Evolutionary genetics and arthropod-borne disease: the yellow fever mosquito. *American Entomologist,* 37 (1), 14-24.

Tadei, W.P., Thatcher, B.D., Santos, J.M., et al., 1998. Ecologic observations on anopheline vectors of malaria in the Brazilian Amazon. *American Journal of Tropical Medicine and Hygiene,* 59 (2), 325-335.

Ulloa, A., Arredondo-Jiménez, J.I., Rodriguez, M.H., et al., 2002. Mark-recapture studies of host selection by *Anopheles* (Anopheles) vestitipennis. *Journal of the American Mosquito Control Association,* 18 (1), 32-35.

Vargas, L., 1938. Observaciones sobre la preferencia alimenticia sanguinea del *A. pseudopunctipennis* en Temixco, Morelos. *Anales del Instituto de Biología México,* 9, 201-208.

Vargas, L., Casis, G. and Earle, W., 1941. *Anopheles psudopunctipennis* Theobald, a vector of malaria in Mexico. *American Journal of Tropical Medicine,* 21, 779-788.

Villareal-Treviño, C., 2001. *Flujo de genes de la metapoblación de Anopheles albimanus (Diptera: Culicidae) a pequeña escala en el ambito microgepgráfico en Chiapas y a gran escala en el ambito macrogeografico en Mexico*. PhD thesis Universidad Autonoma de Nuevo Leon.

Wilkerson, R.C., Strickman, D. and Litwak, T.R., 1990. Illustrated key to the female anopheline mosquitoes of Central America and Mexico. *Journal of the American Mosquito Control Association,* 6 (1), 7-34.

Zimmerman, R.H., 1992. Ecology of malaria vectors in the Americas and future direction. *Memorias Do Instituto Oswaldo Cruz*, 87 (Suppl. 3), 371-383.