MALARIA TRANSMISSION POTENTIAL BY *ANOPHELES* MOSQUITOES OF DAJABON, DOMINICAN REPUBLIC[1]

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ABSTRACT. A field and laboratory study was conducted to determine some of the parameters relevant to malaria transmission by *Anopheles* mosquitoes in Dajabon Province, Dominican Republic. Although all 4 species occurring in the area, i.e., *An. albimanus*, *An. crucians*, *An. grabhamii* and *An. vestitipennis*, were included in the investigations, most of the work focused on the first and last named species because of their abundance. Gonotrophic cycles were determined to be 2.6 and 3.2 days for *An. albimanus* and *An. vestitipennis*, respectively. Mean parity rates for the 2 species were 37.3 and 20.7%, respectively, in outdoor samples. The human blood index, as determined by ELISA, was 0.08 for *An. albimanus* and 0.12 for *An. vestitipennis*. Only *An. albimanus* was confirmed positive for *Plasmodium falciparum* circumsporozoite protein, using ELISA. The vectorial capacity of *An. albimanus* was determined to be 0.019 and that of *An. vestitipennis* 0.005.

INTRODUCTION

The most pertinent factors that determine the competence of an *Anopheles* species as a vector of malaria include its genetic susceptibility to infection by the parasite, feeding contact with man, longevity and biotic potential (Russell et al. 1963). However, within the same species and even within the same population, there can be individual and strain differences in susceptibility to infection. Pampana (1969) warned against assuming an *Anopheles* species to be a vector of malaria in an area just because it had been found to transmit the disease in another area. He advocated securing local evidence of vectorial role.

Anopheles albimanus Wied. is recognized as a major vector of malaria wherever it occurs (Horsfall 1972). Although the anopheline fauna of the Dominican Republic consists of *An. albimanus*, *An. crucians* Wied., *An. grabhamii* Theobald and *An. vestitipennis* Dyar and Knab, *An. albimanus* is regarded as the only vector of malaria (Martin et al.).[6] However, there are no records of any *Anopheles* species having been found naturally infected with malaria parasites in the country.

Although 3 species of malaria parasites existed in the Dominican Republic, *Plasmodium falciparum* Welch is virtually the only one encountered since 1968 (Sulsona et al. 1985). The number of malaria cases declined from 1964 to 1968 due to the efforts of the National Malaria Eradication Service (SNEM) (Sulsona et al. 1985). Since then, the number has gone up. In 1986 and 1987, 1,360 and 1,206 cases, respectively, were reported from the country (Rosa Cespedas, SNEM Director, unpublished data). During the same 2 years, 138 and 114 malaria cases were diagnosed from Dajabon Sector of SNEM (Santiago Carrasco, Dajabon Sector Chief, unpublished data). The sector is known to be one of the most malarious sectors in the country (Martin et al. 1982,[6] Sulsona et al. 1985).

The purpose of this study, conducted during July 1987 to October 1988, was to gain an understanding of entomological parameters relevant to malaria transmission by local *Anopheles* mosquitoes. Such knowledge is essential for the design and execution of a cost-effective control program. This report includes observations on the gonotrophic cycle, parity rate, human blood index, malaria infection rate and vectorial capacity (VC) of *An. albimanus* and *An. vestitipen-*

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[6] Martin, G. G., J. Ayalde, F. J. L. Antuñano, R. M. Rodríguez, D. Gañan, D. R. S. Cury, E. J. Medina and P. Mencia. 1982. Informe (general) de la Evaluación del Programa de Malaria en La República Dominicana. 66 pp. + annexes (Trip report).

nis, the 2 most abundant anophelines in the study area.

MATERIALS AND METHODS

Study site: The study area in Dajabon Province, on the northwest border of the Dominican Republic, is described by Mekuria et al. (1990). Mosquitoes were collected from 3 locations (Calle Duarte, Colonia Japonesa and La Bomba), in the town of Dajabon or within 4 km of it.

Determination of gonotrophic cycle (GC): The time from bloodmeal to egg maturation and oviposition was observed using field-collected *An. albimanus* and *An. vestitipennis*. Mosquitoes were captured as they attempted to feed on humans, making sure they did not feed at the time of capture. At the end of the collection period the mosquitoes were allowed to blood-feed for 30 minutes.

The method of obtaining eggs was similar to that of Rabbani et al. (1976). Single blood-fed mosquitoes were held in plastic tubes, the bottoms of which were lined with filter paper wetted with pond water and sprinkled with a pinch of larval food (liver powder: brewer's yeast, 3:1). The tubes were then placed in a bucket-like container and kept in the shade outside the laboratory. Observations for deposited eggs and dissections of ovaries to determine Christophers' stages of egg development were made at intervals of ca. 6 hours.

Determination of parity: Ovarian tracheoles of wild-caught anophelines were examined to determine parity rates (PR) (Detinova 1962). The PR was used in computing the probability of survival through one day (p), which is a factor in the computation of vectorial capacity (Garrett-Jones and Grab 1964).

Man-mosquito contact (bloodmeal ELISA): Engorged *Anopheles* specimens were obtained from inside and outside dwellings including corrals, using light traps, animal-baited net traps and aspirators. Specimens were dried under a 60-W lamp for ca. 30 min, placed in capped vials and kept in ziplock plastic bags containing anhydrous calcium sulfate (Drierite™). In the laboratory at Santiago, specimens were stored at −20°C until tested. Bloodmeal source was determined by an enzyme-linked immunosorbent assay (ELISA) (Beier et al. 1988). In each 96-well microtiter plate, 8 wells were used as controls; 2 each with homogenates of mosquitoes fed on human and non-human hosts, respectively, and 4 with only the reagents used in the ELISA.

Human blood index (HBI) was computed by taking the simple means of the proportions positive for human blood from each type of sampling site (Garrett-Jones 1964). Most of the tested mosquitoes were collected from dwellings because more blood-fed females were trapped indoors than outdoors.

Detection of sporozoites (sporozoite ELISA): *Anopheles* specimens obtained by light trap collection, man-biting capture, animal-baited net trapping and by aspiration from resting sites at corrals were tested for *P. falciparum* circumsporozoite (CS) protein by ELISA (Wirtz et al. 1987). Specimens were identified and their nutritional state determined. They were then handled in the same manner as specimens for bloodmeal ELISA, until tested. While, as explained above, blood-fed specimens were triturated singly and were tested individually for bloodmeal source and for CS antigen (Beier et al. 1988), non-blood-fed specimens were triturated in batches of ca. 5 and tested for CS protein (Wirtz et al. 1987). Two wells of each microtitration plate were used for positive controls (100 and 10 pg of recombinant *P. falciparum* CS protein). Four wells with uninfected mosquito homogenates and 2 in which only blocking buffer was placed were used as negative controls.

Positivity was determined visually 30 min after the addition of substrate. Positive homogenates were transported on wet ice by air to the Walter Reed Army Institute of Research where they were retested and the results read using an ELISA plate reader. Samples that gave a minimum optical density of the mean plus 5 SD of 7 negative samples (controls) were considered positive. A specimen that was positive in the Santiago laboratory but negative upon retesting was taken as an unconfirmed positive.

RESULTS

Gonotrophic cycle: The time required by *An. albimanus* and *An. vestitipennis* to develop eggs following a bloodmeal and their oviposition rate at various post-prandial intervals are presented in Tables 1 and 2. Oogenesis generally took longer in *An. vestitipennis* than in *An. albimanus* (Table 1). The proportion of *An. albimanus* that had mature eggs at 41.8 h post-feeding in August, for example, was significantly higher than that of *An. vestitipennis* ($\chi^2_1 = 5.52$, $P = 0.02$). The cumulative oviposition rates (Table 2) by the end of 72.8 h, however, were not significantly different for *An. vestitipennis* and *An. albimanus* ($\chi^2_1 = 0.85$, $P = 0.36$). To determine a mean oviposition time, females which laid eggs at any one observation event were considered to have oviposited midway between the preceding and the current observation (Table 2). Thus, 29% of the *An. albimanus* females were found to have

Table 1. Observations on egg development following a blood meal by *Anopheles albimanus* and *An. vestitipennis* in Dajabon Town, Dominican Republic.

Observation date	Temperature (°C)		Relative humidity (%)	
	Min	Max	Min	Max
July 1987	25.0	28.0	75	84
Aug. 1987	25.0	27.0	67	92
Dec. 1987	23.5	26.0	79	88

Observation date	Hours post-feeding	Number dissected	Number with Christophers' stage		% with stage V eggs
			IV	V	
		An. albimanus			
July 1987	58.1	13	0	13	100.0
Aug. 1987	34.8	5	5	0	0.0
	41.8	21	0	21	100.0
	48.0	20	0	20	100.0
Dec. 1987	42.0	27	16	11	40.7
	48.0	23	0	23	100.0
	78.8	7	0	7	100.0
		An. vestitipennis			
July 1987	58.1	9	0	9	100.0
Aug. 1987	34.8	5	5	0	0.0
	41.8	17	13	4	23.5
	48.0	28	4	24	85.7
Dec. 1987	42.0	26	21	5	19.2
	48.0	23	6	17	73.9
	54.0	13	0	13	100.0
	78.8	6	0	6	100.0

Table 2. Observations on time taken from bloodmeal to oviposition by *Anopheles albimanus* (AL) and *An. vestitipennis* (VE): Dajabon Town, Dominican Republic.

Date fed: Dec. 11, 1987 Time fed: 2315 h
Min. temp.: 23.5°C Max. temp.: 26.0°C
Min. RH: 79% Max. RH: 88%

Hours post-feeding	No. observed[1]		No. with eggs		Cumulative % with eggs	
	AL	VE	AL	VE	AL	VE
42.8	45	43	0	0	0.0	0.0
48.8	45	43	13	0	28.9	0.0
54.8	32	43	3	15	38.1	34.9
60.8	26	26	4	7	48.8	53.7
66.8	21	19	0	3	50.0	62.5
72.8	20	16	3	2	57.5	67.5
78.8	17	13	0	0	57.5	67.5

[1] One cohort of each species was followed to determine occurrence of oviposition. At every observation point, mosquitoes that had laid eggs or had died were excluded from further observation.

oviposited at a minimum duration of 1.9 days post-feeding. The mean ($\bar{x} = \Sigma f_i x_i / \Sigma f_i$) bloodmeal-to-oviposition time was 2.2 days for *An. albimanus* and 2.3 days for *An. vestitipennis*.

According to Detinova (1962), since most mosquitoes feed soon after oviposition, the gonotrophic cycle can be obtained by adding 24 h, which is the time required for location of an oviposition site, egg laying and obtaining a bloodmeal, to the time taken for egg maturation after a bloodmeal. Adding 24 h to the 1.6 days required by *An. albimanus* for egg maturation gives a gonotrophic cycle of 2.6 days. By a similar computation, the cycle of for *An. vestitipennis* is 3.2 days.

Age grading: Parity rates in outdoor man-biting and light trap samples of *An. albimanus* obtained concurrently from the same location

Table 3. Vectorial capacities (VC) of *Anopheles albimanus* and *An. vestitipennis* populations in Dajabon, Dominican Republic.

Expression[1]	Value for *albimanus*	Value for *vestitipennis*	Remarks (Sources)
ma	15.7	13.8	See text
GC	2.6	3.2	See text
HBI	0.08	0.12	See text
a	0.031	0.038	HBI/GC
PR	0.373	0.207	See text
p	0.684	0.611	$(PR)^{1/GC}$
n	11	11	Bruce-Chwatt (1985)
p^n	0.015	0.005	
$1/-\log_e p$	2.636	2.032	
C	0.019	0.005	$ma^2p^n/-\log_e p$

[1] p = The probability of a mosquito surviving through one day; n = number of days required for completion of sporogonic cycle; p^n = probability of a mosquito surviving through n days; $1/-\log_e p$ = Life expectancy of a mosquito. See text for additional explanation of terms.

were comparable. In Calle Duarte, for example, the rates for July 1987 (man-biting: $n = 205$, PR = 40.5%; trap: $n = 102$, PR = 50.0%) were not significantly different ($\chi^2_1 = 2.51$, $P = 0.11$). The overall PR for outdoor biting and trap samples of *An. albimanus* ($n = 566$) was 37.3% while that for *An. vestitipennis* ($n = 169$) was 20.7%. Parity determination was made on relatively few indoor samples; the PR for *An. albimanus* ($n = 21$) was 33.3% and for *An. vestitipennis* ($n = 56$) 35.7%.

Data on 539 *An. albimanus* were analyzed to determine if there was a significant difference in the distribution of parous females collected in the 4 quarters of the night. Parity rates (39, 45, 30 and 33%, respectively for quarters 1–4) were significantly different in samples taken during the 4 quarters of the night ($\chi^2_3 = 9.28$, $P = 0.03$). Parous females comprised a higher proportion of the samples captured in the first 2 quarters ($n_1 = 90$; $n_2 = 230$) of the night than in the last 2 ($n_3 = 135$; $n_4 = 84$).

Human blood index: The human blood positivity rates (HBPR) for indoor ($n = 1,232$), peridomestic ($n = 466$) and corral ($n = 136$) samples of *An. albimanus* were 13.3, 5.4 and 4.4%, respectively. The corresponding rates for indoor ($n = 143$), peridomestic ($n = 24$) and corral ($n = 4$) samples of *An. vestitipennis* were 9.8, 0 and 25%. In addition, 18, 11 and 9 specimens of *An. crucians* were tested from indoors, peridomestic sites and corrals, respectively, giving a combined HBPR of 2.6%. Three *An. grabhamii* tested were negative for human blood. The overall HBPR and HBI for *An. albimanus* were 10.6% and 0.08 and those for *An. vestitipennis* 8.8% and 0.12.

Infection with malaria parasites: A total of 16,703 *An. albimanus*, 4,391 *An. vestitipennis*, 831 *An. crucians* and 27 *An. grabhamii* were tested for *P. falciparum* CS protein. Two samples of pooled (ca. 5 specimens/pool) and 11 single *An. albimanus*; 1 single *An. vestitipennis*; 2 pooled *An. crucians* and 1 pooled *An. grabhamii* showed moderate to strong reaction in ELISAs conducted at Santiago and were sent to the Walter Reed Army Institute of Research for confirmation testing. Only 5 single *An. albimanus* were confirmed positive for *P. falciparum* CS protein (60-min absorbance range 0.11–1.43). All confirmed positives were collected by UV light traps.

Taking the confirmed, whole-mosquito ELISA positivity rate of 5 in 16,703 *An. albimanus*, and assuming as discussed below, that all positives are capable of transmission, 0.03% (p) of those tested would have been infective. It was shown by 15 man-nights of biting collection that a person spending the first 4 h of the night outdoors and the rest of the night indoors would receive 15.7 *An. albimanus* bites on an average night (Mekuria et al. 1990), equivalent to 5,731 bites per person per year (n). Since the expected distribution of human infection from mosquito bites is binomial (because every bite is infective or noninfective), the average number of infections per person per year would be given by np = 1.72 (i.e., 5,731 × 0.0003); a daily inoculation rate of 0.005. This is equivalent to a person being infected on the average once every 7 months. Accordingly, the probability that a person becomes infected at least once a year is $1-q^n = 1-(1-0.03\%)^{5,731} = 0.82$. This will hold true as long as the factors that control transmission dynamics do not vary widely through the year.

Vectorial capacity (VC): Garrett-Jones (1964) defined vectorial capacity as $ma^2p^n/-\log_e p$, composed of 3 parameters: man-biting rate (ma), man-biting habit (a) and expectation of infective life of the vector ($p^n/-\log_e p$). Data for computing VC are presented in Table 3. In Dajabon, where the annual mean temperature is 25.5°C (Garcia 1976), the sporogonic cycle for *P. falci-*

parum was estimated to be 11 days (Bruce-Chwatt 1985). Based on the VC of 0.019 and 0.005 for *An. albimanus* and *An. vestitipennis*, respectively, the daily inoculation rate from a single case of malaria would have been 0.024, assuming both species were capable of transmitting the parasite. Further, since a non-immune and untreated case of *P. falciparum* remains infective to mosquitoes for up to 80 days (Garrett-Jones and Shidrawi 1969), 1.9 inoculations could have been made from a case before the infection died out naturally.

The index of stability (Bruce-Chwatt 1985) was estimated to be 0.08 in Dajabon, based on the data for *An. albimanus* (Table 3). The data on *An. vestitipennis* give a similar value. This indicates that malaria in Dajabon is highly unstable, with the potential for explosive outbreaks unless kept in check by preventive/control measures.

DISCUSSION

While *An. albimanus* is probably the primary vector of malaria in Dajabon, *An. vestitipennis* may play a secondary role in transmission. To establish the vectorial status of anophelines found positive by ELISA, however, it is necessary to dissect field-collected specimens and find sporozoites in the salivary glands. Antigenic deterioration due to thawing out of frozen samples in transit from the Dominican Republic or bacterial contamination at the time of initial testing may have accounted for the failure to confirm some of the positive specimens retested in the USA. The relatively high mean parity rate (37.3%) of *An. albimanus* compared with that of *An. vestitipennis* (20.7%) suggests that the former species may be more long-lived in Dajabon and hence epidemiologically more important.

The gonotrophic cycle of *An. albimanus* found in Dajabon is within the usual range of 2–3 days (Horsfall 1972). The unnatural ambient temperature in the oviposition tubes may have inhibited a high proportion of the females from ovipositing.

As determined by comparative analysis of quarter-nightly parity rates, older females of *An. albimanus* were captured in the first half of the night and not in the second half, as was suspected by Muirhead-Thomson and Mercier (1952).

Anopheles albimanus generally has a low HBPR (Garrett-Jones 1964). The disparity between indoor and outdoor HBPRs found in Dajabon is to be expected. The relatively high HBPR in corral samples is probably because corrals are usually within 50 m or less of human dwellings.

The finding of *P. falciparum* infection in *Anopheles* mosquitoes reported here is the first for the Dominican Republic. In neighboring Haiti, studies aimed at finding malaria parasites in anophelines had given negative results (Hobbs et al. 1986) until French workers encountered an unusually high sporozoite infection rate (13/642) in *An. albimanus* by dissecting salivary glands (Desenfant et al. 1988). Their claim, however, that this was the first time the species was shown to be a malaria vector "in the West Indies" is incorrect since Carley (1931) had found sporozoite infection in *An. albimanus* in Jamaica.

Although whole-mosquito ELISA overestimates the proportion of potentially infective mosquitoes (Wirtz et al. 1987), taking the 5 confirmed CS protein-positive *An. albimanus* as being capable of transmission is unlikely to inflate the inoculation rate determined for Dajabon because: a) the mean absorbance of negative controls + 5 SD was used as a cut off point for positivity instead of the more usual + 3 SD (Wirtz et al. 1987); and b) only confirmed positives were taken into account although, as explained earlier, some of the unconfirmed positives (of *An. albimanus* and other species) could also have had gland infection. However, knowledge of the proportion of *An. albimanus* which are gland-positive for sporozoites/CS protein as compared with those which are whole-mosquito ELISA-positive for CS protein will help better define the inoculation rate.

The determination of VC depends on a number of assumptions and on data obtained by field and lab methods that are not easy to standardize. However, one must agree with Dye (1986) that "the estimate C [VC] will be useful as a comparative index changing proportionally with the true Vectorial Capacity from site to site, from vector to vector, and within and between transmission seasons." Although VC is best estimated for a point in time and place, the estimate based on pooled, substantial data is probably more useful than that based on point, scanty data. Including a value for vector competence in the expression for VC would also improve its estimation (Meyer 1989). Experimental studies have shown *An. albimanus* to have a relatively low vector competence (Eyles and Young 1950).

In Dajabon, although the vectorial capacities of *An. albimanus* and *An. vestitipennis* were found to be low, the VC for *An. albimanus* was higher than that for some other *Anopheles* vectors (Shrestha et al. 1988). Considering the paucity of work done to determine the VC of *Anopheles* species in this part of the world, these preliminary findings may serve as a reference

point for further work. In the only other study that involved *An. albimanus*, VC ranged from 0.42 to 3.7 (Frederickson 1987).[7] High man-biting rate and a high probability of daily survival accounted for these elevated VC values.

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