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On the Use of Classic Epidemiological Formulae for Estimating the Intensity of Endemic Malaria Transmission by Vectors in the Amazon

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Abstract
Although various reports have described entomological inoculation rates of malaria vector species, most were limited to providing descriptive field data. Here, we report biting rates and survival data for two important malaria vectors in the Amazon, *Anopheles darlingi* (Root) and *Anopheles albitarsis* E (Lynch-Arribalzaga) (Diptera: Culicidae), in the state of Roraima, Brazil. We calculated theoretical sporozoite infection rates and critical vector biting rates for these species during 1 year, comprising six bimestrial collections. *Anopheles darlingi* had higher sporozoite rates and lower critical biting rates, indicating that it would be the more efficient vector at the beginning of epidemic malaria transmission. Our data, together with compiled information from the literature in the Amazon, suggest that epidemic malaria transmission may be initiated by the primary vector, such as *A. darlingi*, while secondary vectors, such as *A. albitarsis* E, may only become epidemiologically important when there is an increase in the prevalence of human malaria. We propose that mathematical modeling may be able to quantify the relative importance of secondary vector species in malaria epidemiology.

Introduction

Malaria is the most important vector-borne disease in the Amazon Basin. In the Brazilian Amazon, *Anopheles darlingi* (Root) has been for long implicated as the most important vector (Póvoa *et al* 2000a, Tadei & Thatcher 2000, Galardo *et al* 2007), while other species have been pointed as important local vectors (Arruda *et al* 1986). Several studies have determined sporozoite infection rates, i.e. the proportion of infected mosquitoes (Beier *et al* 1990) in this region (Vasconcelos *et al* 2002, Flores-Mendoza *et al* 2004, Póvoa *et al* 2006, Magris *et al* 2007, Gil *et al* 2007, Girod *et al* 2008, Moreno *et al* 2009). These studies have also identified other species as potential local vectors, such as *Anopheles albitarsis* E (Lynch-Arribálzaga) (Póvoa *et al* 2006), *Anopheles marajoara* (Galvão & Damasceno) and *Anopheles neomaculipalpus* (Curry) (Moreno *et al* 2009). These same studies have also estimated entomological inoculation rates (EIR) of malaria-infected species. The number of infectious bites received per day by a human EIR is the product of the sporozoite rate and the man-biting rate (WHO 1975) and describes the intensity of transmission (Burkot & Graves 1995). The sporozoite rates are usually directly obtained by examining the human landing catches with immunological assays, such as enzyme-linked immunosorbent assay (ELISA), Vec-Test™ antigen panel assay (Medical Analysis Systems, Inc., Fremont, CA, USA) or other techniques. The man-biting rate can be measured by direct captures on human bait. Reports have traditionally been limited to summarizing data on the malaria vector

species. These studies usually have described higher EIR in *A. darlingi*. The efficiency of other species as malaria vectors in the Amazon has been questioned. In the state of Amazonas, for example, human transmission was only found in areas infested with *A. darlingi* (Tadei & Thatcher 2000).

In Boa Vista, Roraima, in the northern Brazilian Amazon, both *A. darlingi* and *A. albitarsis* E are considered important vectors of malaria (Vasconcelos *et al* 2002, Póvoa *et al* 2006). In here, classic epidemiological models using biting rates and survival data of anopheline species obtained from field data enabled the determination of theoretical sporozoite infection rates that would be expected in low transmission zones. Critical vector biting rates represent the man biting rates above which a species will increase the number of human malaria cases, considering a low prevalence of malaria in the area (Macdonald 1957). In this paper, an attempt is made to use these theoretical infection rates to compare the role that each vector species would have in malaria dissemination within low transmission zones.

Material and Methods

Study area

The study site has been previously characterized (Barros & Honório 2007). Briefly, the study site is located in Roraima, northernmost state of Brazil (Fig 1). Ecoclimatic and geomorphological maps have been elaborated for this state and the influence of such variables on the distribution of anophelines has been studied (Barros *et al* 2007a, Rosa-Freitas *et al* 2007). The study area, named Jardim das Copaíbas, is located 5 km south of the center of Boa Vista (02°45′28″N, 60°42′18″W), the capital of Roraima. The entire municipality of Boa Vista, the most populated of Roraima, had an annual parasitological index (API, malaria cases per 1,000 persons per year) of 23.4 in 2010 and 3.9 in 2004, at the time of study. The API in 2004 was approximately 230 in the study area, where malaria is hypoendemic. Spleen rates were <5%, in children aged 2–9 years (unpublished information). House spraying was not routinely performed and the last dose had been applied more than 6 months before the collections for the present study started.

Malaria cases increase during the middle of the wet season (July) and can remain high until November (Vasconcelos *et al* 2002, Póvoa *et al* 2006). Although savanna is the predominant vegetation in northeastern Roraima, dense riparian gallery forests occur. Riversides become partly flooded during the rainy season. Temperatures are permanently high throughout Roraima, with little variation during the year, i.e., around 27.8°C (60-year average: 1939–1999) (Barbosa *et al* 1997). Relative humidity is also steady year-round. Relative humidity is around 73.8% (±3.6) in the savanna.

Meteorological variables

Data on rainfall was obtained from a station located 9.6 km away from the study site. Rainfall was 1,181 mm from August 2003 to July 2004, but markedly concentrated from May to July, with heavier rains occurring. Temperature and relative humidity were also monitored in the field on every night of collection. Mean daily temperature varied from 26°C in July to 29°C in January (Table 1). Mean relative humidity was 54.8% (±5.4).

Fig 1 The study site, near Boa Vista, state of Roraima, in the northern Brazilian Amazon. Jardim das Copaíbas is located at the margins of the Branco River.

Table 1 Total monthly rainfall (mm/m^2), mean daily temperature and mean night humidity from August 2003-July 2004.

Month	Mean daily temperature (°C)	Mean night humidity (%)	Monthly rainfall (mm/m^2)
Aug	27.0	57.6	55.1
Nov	26.1	61.1	33.3
Jan	26.6	55.3	4.8
Mar	27.1	53.6	13.3
May	25.9	58.4	280.5
Jul	25.7	61.6	300.1

Entomological survey

Adult collections were conducted during six bimestrial field collections from August 2003 to July 2004, including November 2003, January 2004, March 2004 and May 2004. Mosquitoes were captured on the act of landing in 10 mosquito collection stations distributed within the village. Mosquitoes from all collection stations and from all sampling times were dissected. Collectors were placed inside houses, and at 30 and >50 m away from the nearest house. Collectors exposed their arms and legs and mouth-aspirated landing mosquitoes, under a protocol approved by the Ethics Committee of the "Fundação Oswaldo Cruz". Twelve-hour collections were performed in the peridomicile for two nights. For the 12 h collections, a team of three collectors captured mosquitoes for six continuous hours. These collectors were substituted by another team of three collectors that carried out sampling for the remaining 6 h. On another two nights, mosquitoes were collected indoors and in the peridomicile from 18:00 to 22:00 h. The number of collectors per night varied from six to 10. A minimum of 120 man-hour of collections were performed on each collection period. Adult mosquitoes were identified using taxonomic keys (Consoli & Lourenço-de-Oliveira 1994). Further methodological details regarding mosquito collections have been described elsewhere (Barros & Honório 2007).

Epidemiological models: sporozoite rates and entomological inoculation rates

The mathematical models were based on Macdonald (1957) to facilitate their use by non-statisticians. Although more complex equations have been proposed (Molineaux *et al* 1988), the results provided are basically the same. The likelihood of a mosquito being infectious (S=sporozoite rate) can be derived by the equation: $S = \frac{P^n \mathrm{ax}}{\mathrm{ax} - \log_e P}$, where P=the probability of survival during 1 day; n=the duration in days of the extrinsic cycle of the parasite in the mosquito (e.g. usually nine or 10 for *Plasmodium vivax*); a is the average number of bites per day of a single vector (e.g. 0.5, if it feeds every 2 days); x=the proportion of bites on infective persons which result in infection of the vector. The expected EIR was calculated by multiplying the human biting rate by the sporozoite rate.

Critical mosquito biting rates and vectorial capacities

To better understand the relation of the sporozoite infection rates and malaria transmission, we studied the critical vector biting rates, obtained from the malaria reproduction indices (z; Macdonald 1957). The malaria reproduction index reflects the number of secondary cases of malaria that result from a primary case, i.e. the number of humans that will be infected from a single case. In low-transmission areas (hypoendemic malaria), the index can be calculated as follows: $z = \frac{\mathrm{ma}^2 b P^n}{-r \log_e P}$, where **ma** is the vector density relative to humans (i.e. the man biting rate), estimated through the human biting rate, b=proportion of bites that are infectious to humans, r=the human recovery rate (the reciprocal of the number of days for recovery of an infected individual). Malaria reproduction rates >1.0 would enable the expansion of infections in a population, while those <1.0 indicate a decline in infections. Critical biting rates would be those which would result in a malaria reproduction rate of 1.0. Critical biting rates ($\mathrm{ma}^{\mathrm{crit}}$) can be determined by a rearrangement of the malaria reproduction rate equation: $\mathrm{ma}^{\mathrm{crit}} = \frac{-r \log_e P}{\mathrm{abx} P^n}$.

The vectorial capacities (VC), which describes the potential for transmitting malaria, were determined for each vector on every occasion by: $\mathrm{VC} = \frac{\mathrm{ma}^2 P^n}{-\log_e P}$ (WHO 2002). The VC is the same as the malaria reproduction rate considered on a daily basis. When two or more vectors are transmitting the same disease, the daily reproduction rate is equal to the sum of their individual VCs (WHO Expert Committee on Malaria 1966).

Parameter estimates: mosquito dissections for survival rates and gonotrophic cycle duration

Dissections were performed by the same trained entomologist during the collection period (FSM Barros). Parity was determined by verifying the state of the terminal tracheoles of the ovaries of adult mosquitoes and by determining the presence of follicular stalk dilatations through the Polovodova's technique (Detinova 1962). During the first oogenesis cycle of a mosquito, the terminal tracheole branches move further apart, as the ovary grows. This stretching is irreversible and allows for the identification of parous females by microscopic examination. Daily survival rates (P) were calculated by Davidson's method as follows: $P - \sqrt[g]{\mathrm{parity}}$,

where parity represents the ratio between the number of parous mosquitoes and the total number of females collected, and g=the duration of the gonotrophic cycle in days (Davidson & Draper 1953). Daily survival rates correspond to the proportion of mosquitoes surviving 1 day. The duration of the gonotrophic cycle (g), i.e. the time elapsed from one blood feeding to the next, can be calculated by adding the time elapsed from oviposition to blood feeding (estimated by examining the state of contraction of the terminal sections of the ovarioles after passage of mature eggs) and the time for egg development as indicated in Charlwood and Alecrim (1989) & Charlwood *et al* (1997). Mosquitoes sacrificed more than 6 h after their capture were not considered because this could invalidate accurate estimates of the gonotrophic cycle duration (Barros *et al* 2007b). The time for egg development was taken from the literature (Charlwood 1980). Further methods for age-grading mosquitoes and more methodological details have been previously described (Barros *et al* 2007b).

Other parameter estimates

The average daily number of bites on men of a single vector (a) was determined by the percentage of mosquitoes biting men, instead of other animals, divided by the duration of the gonotrophic cycle (Molineaux *et al* 1988). The percentage of mosquitoes biting men instead of other animals (a) was assumed to be 35% for both species, derived from the literature (Rachou 1958).

The susceptibility of mosquitoes to gametocyte infection and development of sporozoites in their salivary glands (x) was calculated from laboratory dissection studies in the published literature (Klein *et al* 1991). For determining the proportion of bites that are infectious to humans (b), all infected mosquitoes were assumed to transmit malaria effectively, i.e. only the value of x was used in the formula. The duration of the sporogonic cycle (n) was estimated by the Moshkovsky method (WHO 1975). First, the mean daily temperatures during the period of study are determined. The duration of the sporogonic cycle for *P. vivax* corresponds to the amount of days required accumulate 105° per day. The value of the human recovery rate (z) was taken from the literature and assumed to be the same for both species. Since it takes an average of 3–7 days to recover from malaria, the duration of sickness/illness can be calculated as r=1/7=0.143 (Kamugisha 1992).

Results and Discussion

A total of 180 *A. darlingi* and 726 *A. albitarsis* E were captured and dissected during six collecting periods and their biting rates and remaining parameters proposed for this work were calculated (Table 2).

Sporozoite rates and critical vector biting rates

Malaria control strategies require data to estimate relative reductions in mosquito mortality, emergence or human biting rates. Field data measuring EIR of Amazonian vector species have sometimes determined values that are so low that these parameters would not be practical indicators of the efficacy of control campaigns (Girod *et al* 2008, Moreno *et al* 2009). Therefore, it may be more advantageous to have consistent estimates of epidemiological parameters, such as survival rates, that permit estimating sporozoite rates, even if the "gold standard" of determining EIR from human landing catches provides a more accurate estimate (Smith & McKenzie 2004). We have previously demonstrated that parity rates can vary in different seasons (Barros *et al* 2007b). We also reported that malaria incidence may be better correlated with survival rates than adult mosquito density (Barros *et al* 2011c). It has been suggested that heavy rainfall has an effect on adult mortality (Bruyning 1952) and Barros *et al* (2011c) found a significant association between the number of wet days per month and mosquito parity.

In the present study, we determined that *A. darlingi* and *A. albitarsis* E had relatively similar survival rates (P) (Table 2). *Anopheles darlingi* had higher sporozoite rates and lower critical vector biting rates, indicating that lower numbers of mosquitoes of this species would be necessary for initiating epidemic malaria transmission. However, the VC of *A. albitarsis* E was higher than that of *A. darlingi* on every collection (Table 2). The higher estimated sporozoite rates for *A. darlingi* were primarily due to the differences in susceptibility to sporozoite infection (x). Because of its efficiency in transmitting both *P. vivax* and *P. falciparum*, very low biting rates of *A. darlingi* have been found to be enough for maintaining malaria transmission (Rubio-Palis & Zimmerman 1997, Lounibos & Conn 2000). In this study, *A. darlingi* also had a lower critical vector biting rate, indicating that lower numbers of this mosquito would be needed for initiating epidemic malaria transmission (Table 2). The results suggest that the initial autochthonous cases in our study area, at the time of sampling, would most likely be transmitted by *A. darlingi*. Similar results have been suggested by Tadei & Thatcher (2000). A systematically higher vectorial capacity at the study site means that *A. albitarsis* E contributes more to malaria reproduction rate than *A. darlingi*. Although *A. darlingi* has higher sporozoite rates, the higher densities of *A. albitarsis* E at the study area account for the higher EIR's of this species (Table 2).

Table 2 Parameter estimates and values of critical biting rates and sporozoite rates of *Anopheles darlingi* and *Anopheles albitarsis* E per collection period.

Month	Aug		Nov		Jan		Mar		May		Jul	
Anopheline species	*dar*	*alb*	*dar*	*alb*	*dar*	*alb*	*dar*	*alb*	*dar*	*alb*	*dar*	*alb*
Man biting rates (*ma*) in bites/man/hour	2.06	20.92	0.28	22.89	1.88	9.85	0.47	1.41	3.85	5.63	8.35	7.41
Probabilities of surviving one day (*P*)	0.81	0.80	0.83	0.80	0.81	0.78	0.91	0.86	0.88	0.87	0.80	0.83
Extrinsic cycle durations in days (*n*)	8.14	8.14	7.34	7.34	7.29	7.29	7.24	7.24	8.68	8.68	9.13	9.13
Gonotrophic cycle durations (*g*)	2.30	2.63	2.30	2.63	2.30	2.63	2.30	2.63	2.30	2.63	2.30	2.63
Proportion of bites on man infectious to mosquitoes (*x*)	0.82	0.58	0.82	0.58	0.82	0.58	0.82	0.58	0.82	0.58	0.82	0.58
Number of blood meals per vector per day (*a*)	0.15	0.13	0.15	0.13	0.15	0.13	0.15	0.13	0.15	0.13	0.15	0.13
Critical biting rates (mosquitoes/man/night) (ma^{crit})	1.36	4.52	0.90	3.40	1.57	5.94	0.27	1.51	0.62	1.41	1.98	3.44
Sporozoite rate *(S)*	0.08	0.04	0.11	0.05	0.07	0.03	0.28	0.11	0.15	0.11	0.05	0.05
Vectorial capacity (*VC*)	0.32	0.29	0.07	0.06	0.25	0.28	0.36	0.38	1.33	1.41	0.90	0.82
EIR	0.16	0.85	0.03	1.22	0.13	0.33	0.13	0.15	0.57	0.63	0.46	0.38

dar=*Anopheles darlingi*, *alb*=*Anopheles albitarsis* E.

Method limitations: parameter estimates

Our study has a number of methodological limitations. Parameter estimates obtained in the study were designed only for comparative analysis between vector species and cannot be considered absolute values for comparison with other samples. The lack of experimental data available on some parameters meant that they had to be estimated from the literature.

One important parameter to be considered for critical density calculations is the proportion of mosquito bites that are infectious to humans (*b*). In the present study, it was assumed that all bites from sporozoite-positive mosquitoes were infectious to humans, effectively transmitting malaria. However, it has been suggested that transmission efficiency is much lower and that there is variation among *Plasmodium* species. In the Garki Project in West Africa, this parameter was estimated to be 0.097±0.017 (Molineaux & Gramiccia 1980). To our knowledge, the only available estimate of *b* with South American vectors was determined by Rubio-Palis *et al* (1992). Only 0.0032 of *P. vivax* CS protein-positive mosquitoes would result in clinically evident malaria, while 0.202 of bites from *Plasmodium falciparum* CS protein-positive mosquitoes would result in infection. No variation has yet been reported among mosquito species. If the critical vector densities are corrected with these values ($b=0.0032 \cdot x$), much higher critical biting rates would be obtained for both *A. darlingi* and *A. albitarsis* E. Another parameter, the human biting rate (*a*), was not controlled for and was assumed to be the same for both species. A percentage of only 35% of mosquitoes were assumed to be biting man instead of other animals. Since the area is rather densely inhabited and most of the forest has been destroyed, this represents a low value and possibly higher values would be more likely. Bustamante *et al* (1951) & Oliveira-Ferreira *et al* (1992) reported that around 65–70% of *A. darlingi* were found biting man. We are unaware of human biting rate determinations for *A. albitarsis* E. For that reason, we decided to use the same biting rate for both species. However, we have verified that, although both species are anthropophilic, *A. darlingi* is more anthropophilic than *A. albitarsis* E in this area (Barros *et al* 2010). If *A. darlingi* is indeed associated with higher values of *a*, as compared to *A. albitarsis* E, an even greater discrepancy in the sporozoite rates and critical biting rates of each species can be expected (Table 2).

The susceptibility of a mosquito species to sporozoite infections may be the most important factor determining its epidemiological importance. The susceptibility of mosquitoes to sporozoite infections (*x*) was obtained from the literature, where they were estimated under laboratory studies. To our knowledge, there are no studies that have corroborated these values in the field. Also, the study by Klein *et al* (1991) was performed on *A. albitarsis latu sensu* (l.s.) from Rondonia, Brazil, while we sampled *A. albitarsis* E. Another point to consider is the method for determining species-specific mosquito infection rates. We chose to use salivary gland dissection for determining sporozoite infection rates. It may be argued that ELISA or Vectest® are more sensitive than salivary gland dissections for the determination of sporozoite infection rates, but the latter may be more specific (Beier *et al* 1990). It is possible that some mosquitoes tested positive by ELISA may not be truly infectious, especially when processing both the head and thorax of the mosquito. This could be due to an insufficient number of infective sporozoite stages inside their salivary glands (Klein *et al* 1991, Vasconcelos *et al* 2002). Until a better understanding of the significance of the CS protein-positive mosquito pool is available, we believe the use of salivary gland

dissection could be favored in areas with high sporozoite infection rates while CS protein tests would still be more practical in areas with a low prevalence.

Method limitations: classic model simplification only for low transmission

The classic models provide a starting point for quantifying malaria transmission and for relating static and dynamic aspects of malaria infection in humans and mosquitoes, but the underlying epidemiology of malaria is more complicated. The Ross–Macdonald model was used in the present study due to its simplicity.

We believe that higher sporozoite rates or EIR found with a potential vector species during high transmission periods may not necessarily mean that the potential vector species is the more important vector in the studied region. The potential vector with higher EIR may be performing as a more efficient vector at that specific moment, but the same may not be true if there were changes in the biting rates or the prevalence of malaria in the human population. We caution against the use of the term "primary vectors" for these potential species, especially in areas with *A. darlingi*, until their role in malaria transmission dynamics is better characterized, as proposed with *A. marajoara* in Serra do Navio (Póvoa *et al* 2000b) and *A. albitarsis* E in Roraima (Póvoa *et al* 2006).

Longitudinal analysis of the study of Vasconcelos et al (2002)

During a malaria epidemic in the same area as the present study, Vasconcelos *et al* (2002) observed higher EIR for *A. albitarsis* E, with 6.9 infective bites/person/year as compared to 1.65 positive bites/person/year for *A. darlingi*. These rates were determined when malaria prevalence was high in the population. The higher sporozoite rates of *A. albitarsis* E may reflect the unusually high biting rates encountered at that time, representing approximately 67% of the total number of captured mosquitoes, while *A. darlingi* represented only 5.3%.

Analyzing the longitudinal determination of EIR reported by Vasconcelos *et al* (2002) in Boa Vista (Fig 2), it can be verified that, although *A. albitarsis* E reached higher infection rates after the epidemic peaks had started, *A. darlingi* infection preceded that of the former species on every occasion. Three malaria transmission peaks occurred during the 2-year observation period (unpublished data). If consideration is given solely to the EIR data, one could conclude that *A. albitarsis* E was the most important vector in the area. However, we suggest that *A. darlingi* may be important for initiating epidemic malaria transmission. When malaria becomes more prevalent in the human population, *A. albitarsis* E would assume a more important role, as a function of its higher biting rates.

Anopheline distribution in roraima and malaria prevalence, ELISA-positive species and A. darlingi

We have reported anopheline distributions in Roraima (Barros *et al* 2007b). This state differs from other regions in the Amazon because of dry-land colonization projects predominate instead of riverine settlements on the banks of large rivers. Frontier zone malaria appears to differ epidemiologically from alluvial or riverine malaria (Barros *et al* 2011a) and malaria transmission in the savanna may have its particularities. Despite the wide distribution of *A. albitarsis* l.s. over the entire savanna area, which covers most of the northeast corner of the state, the most densely populated area, malaria is almost completely absent in this ecosystem. Other species, such as *Anopheles triannulatus* l.s. (Neiva & Pinto) and *Anopheles nuneztovari* (Gabaldon) also breed abundantly in the savanna, while *A. darlingi* is completely absent in this ecosystem (Nagm *et al* 2007). *Anopheles darlingi* is restricted to areas with rivers and riparian vegetation (Rubio-Palis & Zimmerman 1997). All recorded important malaria foci in Roraima have occurred in these areas (Barros *et al* 2007b, Nagm *et al* 2007). Barros *et al* (2011b) have proposed that the species prefers shaded areas, proximity to human dwellings and microdams, and that the ideal conditions can be found at forested-deforested transitions. We believe that the same ideal breeding conditions may be present in savanna–forest interfaces. The preference for shade may be particularly important for explaining the absence of *A. darlingi* in the savanna.

The observed epidemiological picture of malaria in state of Roraima suggests that the density of *A. albitarsis* E necessary to initiate and maintain malaria transmission, independent of the presence of *A. darlingi*, may not occur in this region, although the latter is theoretically possible. Similarly, the potential vectors *A. nuneztovari* and *A. triannulatus* l.s., although giving positive results with the ELISA tests, may be incapable of causing outbreaks of malaria in the state of Amazonas in the absence of *A. darling* (Tadei & Thatcher 2000). Both *Anopheles oswaldoi* (Peryassu) and *Anopheles mediopunctatus* (Theobald) have also been reported to develop infective sporozoites in the laboratory (Marelli *et al* 1999, Klein *et al* 1991). However, *A. oswaldoi* develops infective sporozoites at very low rates (<7%). Although a high percentage of *A. oswaldoi* has been found naturally infected in the state of Acre in the Brazilian amazon basin, this was reported in the presence of very high biting rates, over 70 times that of *A. darlingi* in the same area (Branquinho *et al* 1996).

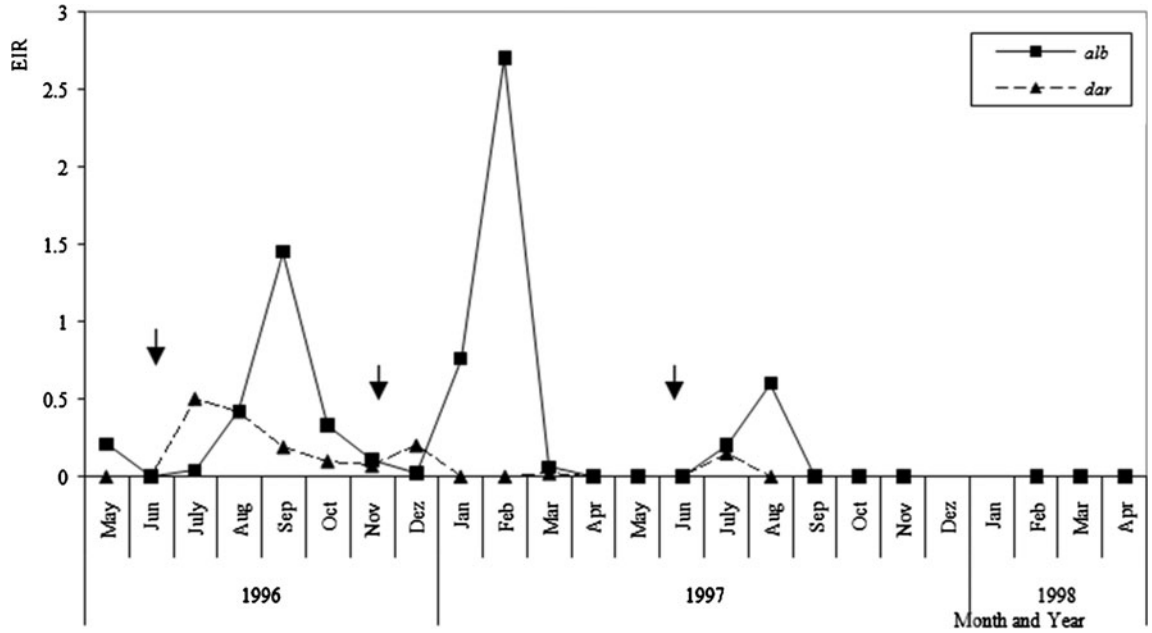

Fig 2 Entomological inoculation rates of *Anopheles darlingi* (*triangles*) and *Anopheles albitarsis* E (*squares*) in Boa Vista, Roraima, Brazil (modified from Vasconcelos *et al* 2002). *Anopheles darlingi* infection precedes *A. albitarsis* E infection at the three malaria transmission peaks (*arrows*).

Suggestions for future EIR studies

We suggest that researchers studying EIR's of potential vectors should correlate their results with ovarian dissection studies to determine the percentage of parous females and survival rates. This simple procedure would enable an estimation of critical man-biting rates of the vector species needed for initiating malaria transmission (Smith & McKenzie 2004). Studies that report EIR's should also describe the prevalence of human malaria at the time of sampling and vectors found positive in high malaria prevalence settings must be differentiated from the ones found in low prevalence situations.

Critical vector densities for control strategies

The knowledge of critical vector densities may have important implication for malaria control. We suggest that, through studies determining species-specific parameter estimates, the adult female biting rates at which each mosquito species becomes epidemiologically important can be determined and used to quantify the role of each species in a transmission area. These procedures could enable a better understanding of malaria transmission in endemic and epidemic situations.

Critical vector biting rates would not be static figures, but a species-specific function of mosquito survival, gonotrophic cycle duration and the presence of alternative sources of blood in the area. Control efforts could consist of increased surveillance of human malaria cases in areas where the biting rate of vector species are above their critical densities. Alternatively, the efficiency of control strategies, such as insecticide spraying or treating breeding sites of target species, can be monitored by using density and survival data to verify if mosquito biting rates have been decreased below their critical density levels. Even preventive measures become feasible.

The current data available suggests that the status of an anopheline species as a malaria vector in nature may be a function of the epidemiological setting. The terms "primary" and "secondary" or "potential" vectors in current use in the literature are relatively imprecise and have been used to indicate that some species have higher EIR than others. Our data, as well as that of other authors (Tadei & Thatcher 2000), suggest that *A. darlingi* may have an important role in "initiating" epidemic malaria transmission due to its ability in transmitting malaria at lower mosquito densities, when disease prevalence is low in the population. Other species, the "secondary" or "potential" vectors, could be able to maintain transmission levels when higher mosquito biting rates and/or disease prevalence become available.

The methods presented here may help to shed light in the importance of different anopheline species in malaria transmission in the Amazon. To our knowledge, this is the first proposition of the use of mathematical formulae to quantify the role of Neotropical secondary vectors in malaria transmission. We believe that more studies are necessary to verify these results.

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