DIFFERENCES IN ANOPHELES COMPOSITION AND MALARIA TRANSMISSION IN THE VILLAGE SETTLEMENTS AND CULTIVATED FARMING ZONE IN SARAWAK, MALAYSIA

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Abstract. *Anopheles* mosquitos were surveyed using three trapping technics in four longhouse settlements and their respectively farming zone in western Sarawak, Malaysia. The study area was mountainous with tropical rain forest. *An. leucosphyrus* and *An. donaldi* were predominant in the farm huts. *An. tessellatus* and *An. subpictus* were more abundant in the village settlements. In both ecotypes, human baited traps yielded a significantly greater proportion of *Anopheles* mosquito than CDC light traps and landing biting catches. Circumsporozoite antigen positively rate, mosquito survival rate and parasite rate showed that malaria transmission is more intense in farm huts than in longhouse settlements. The entomological inoculation rate of *An. donaldi* and *An. leucosphyrus* in farm huts was 0.035 and 0.023, respectively. No sporozoite infections were observed in the main settlements.

INTRODUCTION

Levels of malaria endemicity, transmission rate and survival rate of anopheline mosquitos may differ between villages situated within a few kilometers of each other (Burkot *et al*, 1984; Charlwood, 1986). These differences may affect heterogeneity in the microepidemiology of malaria (Greenwood, 1989). Indeed, this phenomenon has been shown in a study of *Anopheles leucosphyrus* in Sarawak (Chang *et al*, 1995). This species exhibits differences in biting rate, parity rate and malaria infections between villages and farm huts located 0.5 km from the villages. In many Southeast Asian countries, forest and forest fringe areas are recognized to be the focal points for malaria transmission (Meek, 1995; Pradya *et al*, 1998). In Sarawak, malaria foci are often associated with farm huts in agricultural zones (Ooi, 1994) while there is relatively low risk of malaria infection in village settlements. Despite the significance of farm huts in malaria epidemiology, no entomological study has been done to investigate transmission dynamics in these areas. In conjunction with the environmental impact assessment of the Bakun Hydro-electric project in upper Belaga District, a study was undertaken to determine the species composition, age structure, malaria infections and biting patterns of *Anopheles* mosquitos in farm huts and village settlements in the region during the farming season in July, 1994.

MATERIALS AND MATHODS

Study area

Belaga district is located in the western part of Sarawak State, East Malaysia. The district is hilly, covered with tropical rain forest and sparsely populated. Communication is mainly by river since all the longhouse settlements are situated along the Balui River and its tributaries. Ethnically, the people are mainly Kayan, a native community of Sarawak. Other minor groups include Penan and Okit. Subsistence is shifting cultivation, hunting and employment by logging companies. Four longhouse settlements, Lg Belangan, Lg Liko, Lg Pangai and Lg Murum, and their respectively farm huts were selected as study sites. The longhouses are located in similar ecological setttings along the Balui River, approximately 20 km from each other. The farm huts are isolated and located at the forest fringe, some 3 to 5 km from the main villages. One farm hut from each village was selected for study. The farm huts are semipermanent and occupied by farmers and their dependants during the padi weeding and harvesting seasons in July and March, respectively. Cash crops such as cocoa and tapioca are also cultivated in close proximately to the farm huts. DDT residual spraying has been used in the villages and farm huts as an antimalarial control measure biannually since 1972.

Field collections and dissections

Two-night mosquito collections were made in each of the village settlements and farm huts. Human-baited net traps (WHO, 1992; Services, 1993) were used to capture *Anopheles* mosquitos from 18.00 to 06.00 hours. Simultaneous collections were made with four CDC light traps placed outside selected houses in the village and farm huts. Outdoor landing collections were made by two collectors situ-

ated approximately 500 m from the human bait traps.

Mosquitos collected from the study sites were identified using the keys of Reid (1968) and dissected to determine parity rates (Detinova, 1962). The head and thorax (abdomen was removed) of the identified specimens were placed in individual polypropylene micro centrifuge tubes (1.5 ml) and dried over silica gel.

Identification of infected mosquitos

Dried specimens were tested for the presence of malaria parasites by enzyme-link immunosorbent assay (Burkot *et al*, 1984; Wirtz *et al*, 1985, 1987). Monoclonal antibodies, 2A10 and NSV3, directed against circumsporozoites (CS) protein of *Plasmodium falciparum* and *P. vivax*, respectively, were run on every ELISA test plate. Positive controls consisted of 100 pg and 10pg of a recombinant *P. falciparum* CS protein and 500 ng and 50 ng of *P. vivax* CS protein. A laboratory colony of *An. donaldi* was used for negative controls in all assays. In confirmatory tests, samples were considered positive for sporozoite antigens only if the 30 minutes absorbance values from the plate reader exceeded the mean plus 4SD of the eight negative control mosquitos.

Parasites rates

Malariological blood smear surveys of the villagers and farms huts populations were conducted by local malaria teams. Positive cases were treated using the standard regime of chloroquine and primaque.

Data analysis

Analysis of variance (Anova) was used to compare the relative abundance of mosquitos between village settlements and farm huts. The probability of daily survival was computed using Davison's (1954) formula based on a three – day gonotrophic cycle. An estimation was made of the probability of females surviving for nine days at the duration of the sporogonic cycle at 25°C (Macdonald, 1973). Southwood's (1978) index of association between species was calculated I = 2 (J/(A+B) – 0.5), where J is the number of individuals of both species in samples where they occur together, and A and B are the total numbers of individuals of the two species in all samples (Services, 1993). The entomological inoculation rate (EIR) was calculated as the number of anophelines /man-night x sporozoites rate.

RESULTS

Species composition and abundance

There were differences in the *Anopheles* species captured in the two different ecological zones for all collection methods. *Anopheles donaldi, An.*

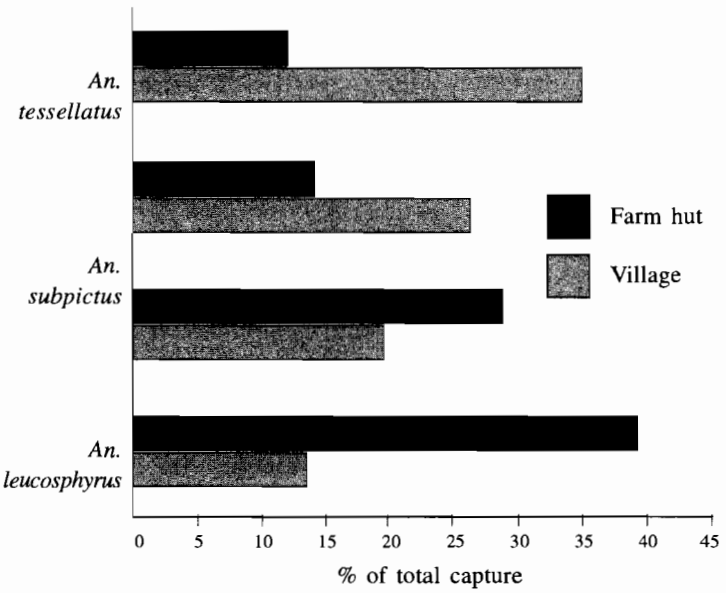

Fig 1–Prevalence of anopheline mosquitos in villages and farm huts, Belaga district, Sarawak, Malaysia.

Table 1
Measure of association between *Anopheles* in village settlements and farm huts in Belaga district, Sarawak (all collections combined).

Anopheles	*An. donaldi*	*An. subpictus*	*An. tessellatus*
An. leucosphyrus	0.94 (0.89)	0.64 (0.42)	- 0.32 (-0.26)
An. donaldi		0.80 (0.50)	0.48 (0.33)
An. subpictus			0.66 (0.18)

leucosphyrus, An. subpictus and *An. tessellatus* comprised 41.5%, 30.4%, 15% and 12.9% respectively, of 2,385 *Anopheles* captured in farm hut collections. Of 1,557 *Anopheles* collected in the four village settlements, *An. tessellatus, An. subpictus, An. leucosphyrus* and *An. donaldi* represented 37%, 27.8%, 20.8% and 14.3% of the total, respectively (Fig 1). The differences in distribution and species composition were consistently noted between the villages and farm huts. *An. donaldi* was the most abundant species in farm hut and the least abundant in village settlements. In the villages *An. tessellatus* was the most abundant species. In both ecotypes, the strongest association was between *An. donaldi* and *An. leucosphyrus*, with an index of 0.94 and 0.89 in the villages and farm huts respectively. This was followed by *An. donaldi* and *An. subpictus* at 0.8 and 0.5. There was a negative association (-0.32) between *An. leucosphyrus* and *An. tessellatus* (Table 1).

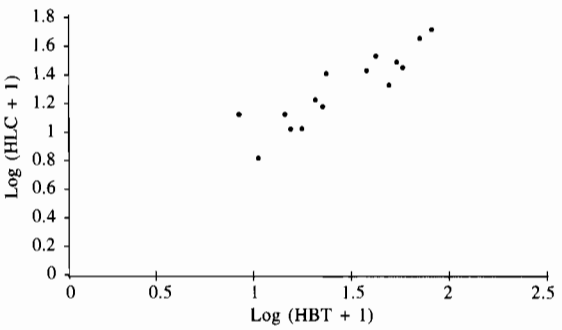

Fig 2–Number of *An. leucosphyrus* collected from human bait trap (HBT) and human landing catch (HLC) in villages and farm huts combined.

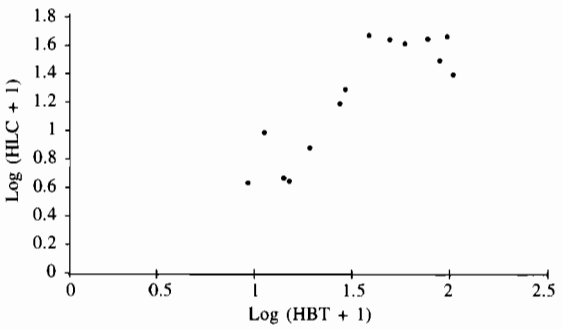

Fig 3–Combined numbers of *An. donaldi* collected from human-baited net trap (HBT) and landing catches (HLC) in villages and farm huts in Belaga district, Sarawak.

Methods of collection

A significantly higher number of *Anopheles* was collected by the three collection methods in the two collection zones ($F = 2.27$; $p < 0.05$). Human-baited traps were more effective than landing catches and CDC light traps. Comparison of human-baited traps and landing catches for *An. donaldi* and *An. leucosphyrus* were transfomed to [ln (x+1)] and plotted against each other (Fig 2, 3). There was no correlation between the two sampling techniques for either of these species ($r=0.85$, $p < 0.001$ and $r = 0.89$; $p < 0.001$, respectively).

Daily survival rate

Table 2 compares the daily survival rate of *Anopheles* females caught from the villages and farm huts. There were no significant differences in parity rate for all species collected in the farm huts ($\chi^2_{0.05} = 0.23$; G-test). Estimation of daily survivorship for each species was done separately based on the three-day gonotrophic cycle. The daily survivorship ranged from 0.901 for *An. subpictus* to 0.932 for *An. leucosphyrus* in the farm huts and from 0.879 for *An. subpictus* to 0.921 for *An. leucosphyrus* in the villages. The expected infective life in days or the mean number of days of life in the infective condition per mosquito was likewise estimated for both ecotypes. The calculated theoretical infective showed that *An. leuco-sphyrus* is more efficient in the transmission of malaria in both areas.

Malaria sporozoite rates and entomological inoculation rate in *Anopheles*

ELISA of 805 *An. leuco-sphyrus* and 1,040 *An. donaldi* from all collection methods combined

Table 2
Daily survival and expected infective life of *Anopheles* in village settlements and farm huts in Belaga district, Sarawak.

Species	Farm huts				Village settlements			
	1	2	3	4	1	2	3	4
An. donaldi	830	0.77	0.917	4.07	221	0.71	0.893	2.3
An. leucosphyrus	703	0.81	0.932	6.12	310	0.78	0.921	4.5
An. subpictus	360	0.73	0.901	2.70	433	0.68	0.879	1.65
An. tessellatus	308	0.75	0.909	3.30	1,541	0.73	0.90	2.68

1=Number dissected
2=Proportion parous
3=Daily survival rate (P)
4=Expected infective life ($1/-\log_e P$)

Table 3
Malaria sporozoite antigen positivity rate (number of sporozoite antigen positive mosquito tested) for *An. donaldi* and *An. leucosphyrus* in village settlements and farm huts in Belaga district, Sarawak (Figures indicate *An. leucosphyrus*).

Locality	No. assay	No. positive for sporozoite antigen	
		P. falciparum	*P. vivax*
Lg Belangan	30(60)	0(0)	0(0)
Farm huts	250(140)	0(0)	1(0)
Lg liko	30(30)	0(0)	0(0)
Farm huts	130(50)	1(0)	0(0)
Lg Pangai	40(45)	0(0)	0(0)
Farm huts	200(150)	0(0)	1(0)
Lg Marum	80(90)	0(0)	0(0)
Farm huts	280(240)	1(0)	0(1)

gave an average (*P. falciparum* plus *P. vivax*) circumsporozoite antigen po-sitivity rate (CSPR) of 0.27%. All positive samples were collected from the farm huts (Table 3). The specific circumsporozoites antigen positivity rate (CSPR) was detected from one *An. leucosphyrus* collected in landing catches from the farm hut of Lg Murum, and four *An. donaldi*, one each from the farm huts of Lg Liko, Lg Belangan, Lg Pangai and Lg Murum. All the samples were from human-baited net traps. Entomological inoculation rates in the farm huts were estimated to be 0.035 for *An. donaldi* and 0.023 for *An. leucosphyrus*.

In August 1994 blood smears were taken from 354 people in the four villages and 21 from the farm huts. All blood slides were examined for the presence of malaria parasites. The *P. vivax* rate was extremely low in the villages at 0.28% (1/354) and comparatively high in the farm huts at 9.5% (2/21).

DISCUSSION

For the measurement of biting rate in malaria entomological studies, two standard methods are widely used: landing catches on humans and human-baited net traps (WHO, 1975). Human-baited net trap collections are often used to compare the host preferences of a particular vector species. Quantitative comparisons between the two collection technics in this study demonstrated that human-baited net traps were superior to landing catches in all the collection sites. There was no correlation between the two sampling technics for any of the 16 collections made during the study.

Of the collection methods employed, light trapping without CO_2 is considered the least efficient technic in both farming areas and the village settlements. However Lines *et al* (1991) compared light traps and landing/biting catches and concluded that

a strong correlation exists between these two methods for the collection of *An. gambiae, An. funestus* and *Culex quinquefasciatus* in Africa. In their study light traps were hung close to bed nets which were occupied but untreated with insecticide. In our study, both traps, human-baited traps and landing catches were performed outdoors close to the farm huts or village dwellings.

The most significant observation involves difference in the *Anopheles* fauna found in the villages and the forested farming zone. *An. leucosphyrus* and *An. donaldi* were the dominant species in farm huts as opposed to *An. tessellates* and *An. subpictus* which were dominant in the main villages. This distribution is associated with ecological factors. *An. donaldi* and *An. leucosphyrus* are forested species (Reid, 1968). *An. tessellatus* and *An. subpictus* were, however, never incriminated as malaria vectors in Sarawak, despite their abundance in human dwellings (Reid, 1968).

Malaria transmission is comparatively more intense in the farm huts where it is maintained by *An. leucosphyrus*. ELISA analysis indicates that *An. donaldi* also plays a vectorial role in its maintenance in the forest areas of Belaga. Chang *et al* (1995) in an earlier study in areas along Baram River of Baram District showed that the sporozoite rate of *An. donaldi* varies from 0.14% in forested areas to 0.32% at the village perimeter. In terms of malaria endemicity in Sarawak, the Baram and Belaga Districts are categorized as malarious areas where the annual parasite incidence is greater than one per 1,000 population.

Although the occupation of farm huts by local people is transient, and coincides with the farming season, this result in an increase vector-man contact during the year. The temporary structure of the farm huts, their proximity to larval breeding sites and the abundance of adult resting sites of malaria vector species effectively increases the potential for malaria transmission. This is compounded by the general lack of personal protection measures, such as mosquito nets used by farmers and their families.

Existing information is insufficient to determine how malaria is initially introduced into the farm hut environment but it is probable that an infected person(s) who has contracted the disease elsewhere moves into the farm hut community where malaria is subsequently disseminated by the local vector species. The significantly high density of *An. donaldi* and *An. leucosphyrus*, coupled with heightened vector-man contact, would escalated transmission. It is assumed that the lack of domestic animals in the farm hut community as compared to the village settlements would contribute to an increase in the human-blood index of the vector species (*An. leucosphyrus* and *An. donaldi*). The study by Pradya *et al* (1998) in North-West Thailand of malaria transmission in relation to population movements in forest areas revealed both *An. minimus, An. dirus s.l., An. maculatus s.s.* and *An. sawadwongporni* in both villages and/or farm huts. Although a higher biting density of vectors was generally evident at the farm huts, the estimated inoculation rates in the 2 settings were similar.

Malaria incidence in Sarawak is relatively low with an overall parasite incidence of less than 1.5 per 1,000 during the past five years. However, the rate varies from district to district and sector to sector (Ooi, 1994). Periodical outbreaks associated with farming, logging and related forest activities have been recorded repeatedly in specific areas (Chang *et al*, 1995). This study illustrates the entomological factors that contributes to the periodic outbreaks of malaria in farming zones. The abundance of vectors and the circumsporozoite antigen positivity rate of their populations suggest that farm huts are a focal point of malaria transmission. A full epidemiological study on the transmission dynamic in the farm huts and forested areas need further investigation.

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