ENTOMOLOGICAL AND EPIDEMIOLOGICAL INVESTIGATIONS OF MALARIA TRANSMISSION IN RELATION TO POPULATION MOVEMENTS IN FOREST AREAS OF NORTH-WEST THAILAND

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Abstract. Transmission of forest-related malaria was observed entomologically and epidemiologically for 2 transmission seasons in 1990 and 1991 in 5 villages of Mae Sariang district, Mae Hong Son Province, north-west Thailand. The entomological study included collections of mosquitos and determination of infection rate by using enzyme-linked immunosorbent assay in the residential villages and the farm huts. The epidemiological study included fortnightly visits to 30% of the households to interview and record movement activities and illness of villagers. Circumsporozoite proteins, in most cases of *Plasmodium falciparum*, were detected in *Anopheles minimus* species A, *An. dirus s.l., An. maculatus s.s.* and *An. sawadwongporni* in residential villages and/or farm huts, suggesting transmission could occur there. Movement of people away from their residences occurred throughout the year for several reasons with a sharp peak in July for agricultural activity, mainly ploughing and planting for rice cultivation. The relative risk of infection for people engaged in agricultural activity was 3 times that of people living in the residential villages. Although a higher biting density of vectors was generally evident at the farm huts, the estimated inoculation rates in the 2 settings were similar. Movement for forest activity increased after harvesting rice in the cool dry season and carried the highest malaria risk, suggesting different epidemiological and probably entomological conditions which need further investigation. The significance is discussed of discrepancies between the case classification system used by this study and that used by malaria sector staff.

INTRODUCTION

Current information on malaria epidemiology in north-west (NW) or west Thailand adjacent to the Myanmar border has suggested that malaria transmission is closely associated with the forest, and with movement of the population leading to contact with intense foci of transmission (Singhanetra-Renard, 1986; Fungladda *et al*, 1987; Ketrangsee *et al*, 1991). In Mae Hong Son Province, for instance, during October 1988 to March 1990, less than 1% of about 6,000 cases were classified as indigenous, *ie* having acquired infection in the village of residence (Malaria Division, 1990, quoted by Kondrashin *et al*, 1991). This kind of information has suggested that most malaria transmission takes place in forests on both sides of the Thai-Myanmar border, and in farm huts where people move for rice cultivation. If this is the case, control of malaria transmission focusing only on the villages is likely to be of limited effectiveness. However, exactly how and where people become infected has yet to be clearly understood. This is because information concerning the site of transmission has been obtained mainly by interviewing malaria cases, which is a routine part of malaria control operations in Thailand and is normally carried out by malaria officers when a positive case is presented at a malaria clinic.

Molineaux (1991) pointed out that as cases usually occur among people who are both living in or close to forests and working in them, the attribution of a case to transmission in either village or forest is not always easy and may grossly overestimate the share of transmission in the forest, for the following reasons:

(i) A patient who has spent a short time (1 night) outside the village before the onset of symptoms (usually 1-3 weeks) is classified as an imported case, when in fact the infection may have been contracted in the village. Conversely, some patients who contracted malaria somewhere outside their villages may state that they have visited another place or have not been out of the village. They may say this because they have forgotten, or because they do not wish to report their activities (which may be illegal, such as wood cutting).

(ii) There are no interviews of non-cases, so comparisons of the relative risks are impossible.

This paper reports an attempt to obtain more reliable information, by a combination of direct epidemiological and entomological observations. The locations where and when people are exposed to transmission were studied by means of mosquito sampling in residential villages and farm huts and by observing human behavior associated with a high risk of transmission.

MATERIALS AND METHODS

Study areas

The study was conducted in Karen villages located at the forest-fringe, namely Mae Han, Mae Chon, Mae Top Nua and Mae Salap, and in 1 village located deeper in the forest, namely Huai Ngu, in Mae Sariang District, Mae Hong Son Province, NW Thailand. They were involved in the trial of pyrethroid-treated bed nets (Somboon *et al,* 1995). The populations in 1990 varied from 90 to 719 persons with annual parasite incidence (API) values in 1989 ranging from 80.7 to 279.7 per 1,000 persons. The forest fringe villages are situated in foothills surrounded by rice fields, scrub and secondary forest, with streams running through or alongside them. Farm huts, the temporary shelters to which villagers move during ploughing and harvesting periods, are scattered in rice fields in the valleys and on the mountains. The huts are built on posts 1-2 m above ground and are constructed of wood and/or split bamboo with the front side largely or completely open and a thatched roof made of teak leaves. The forest village is situated in a valley surrounded by relatively dense forest. According to Mae Sariang Malaria Sector, there have been no indigenous malaria cases reported for a number of years in any of the villages studied.

Besides rice cultivation, the main agricultural occupation, economic activities involve movement into the forest for wood cutting, hunting, collecting forest products, etc. In addition, movement across the border into Myanmar for cultural purposes and as wood cutters is also common.

Entomological data collection

Mosquitos were collected for 2 consecutive nights every month from May to December 1990 and in the same months in 1991. The collections were designed to cover the main transmission period in the area, according to previous records.

The methods of collection included human bait, animal bait and light trap catches. The human bait catches were performed by 2 collectors working indoors and 2 outdoors. The indoor collections were performed outside the sleeping room of villagers throughout the night but the outdoor catches (5-10 m away) were carried out only until midnight. Similar collections were carried out simultaneously at the farm hut areas of each forest fringe village (located 2-3 km away). In the deep forest village, collections were made only in the village, since the village was isolated with a small area of rice fields nearby.

Collections of mosquitos from animals were made in all the village areas from a large double-walled net (5 m^2 x 2 m high and made of mosquito netting) suspended on bamboo frames enclosing a water buffalo (or sometimes a cow). The edge of the inner wall was closed to the ground and that of the outer was about 25 cm above the ground. This allowed mosquitos to enter but not to take a blood meal. The distance between the inner and outer walls was about 60 cm. The netting was kept taut to prevent any wind from unduly ruffing it. A buffalo was normally used as bait and introduced into the net just before dusk and tethered there with enough fodder to keep it content until catching stopped at dawn. At the end of each hour, all *Anopheles* mosquitos resting between the inner and outer walls were caught by the aid of aspirators and torches.

In each village, a CDC miniature light trap was connected to a rechargeable battery from dusk until dawn. It was hung indoors beside a bed net occupied by a householder or a member of staff (Lines *et al,* 1991). The trap was set on the same nights as the human-bait collection but in another house. The mosquitos found in the bag of the light trap were transferred to plastic cups and brought to the field station in the morning.

After morphological identification, the known (*An. minimus s.l.* and *An. dirus s.l.*) and suspected malaria vector species (*An. maculatus* group) were dissected and the ovarian tracheoles were examined for parity (Detinova, 1962). The head/thorax portions of parous females were then separated and kept for testing for circumsporozoite (CS) antigens using an enzyme-linked immunosorbent assay (ELISA) (Burkot *et al,* 1984; Wirtz *et al,* 1987), with minor modifications. Since the sporozoites infection rate in mosquito vectors in Thailand is generally low, testing pooled specimens (up to 15) was preferred. For other anopheline species, the whole bodies (pooled up to 10) were tested. Samples for the ELISA were kept in labeled Eppendorf tubes with their lids open and dried in an incubator warmed up (42-45°C) by a 40 W light bulb for 18 hours. They were then sealed, packed and kept at room temperature for several days in the field and after that they were kept in a freezer at -40°C in the laboratory until being processed.

Epidemiological data collection

In the study area, the malaria transmission season usually occurs in the rainy season with peaks of cases in the early and late parts of the season. Attempts were made to observe the behavior of people during the transmission period, especially their movements.

Thirty percent of the households in the 5 villages were randomly selected. They were visited fortnightly and interviewed whether any household members had spent a night or more out of the village during the past 2 weeks. The malaria illness and the number of nights that each person had stayed away from the house during the previous fortnight were recorded.

Temperature, rainfall and relative humidity data were collected from the district weather station located in the town of Mae Sariang; the farthest forest fringe village studied was about 10 km from the station.

RESULTS

A total of 45,031 anophelines of 23 species were collected from all catching sites from 864 man-nights (an outdoor biting catch by 2 men until midnight is counted as 1 man-night), 156 animal trap-nights and 159 light trap-nights from May to December of the years 1990 and 1991. ELISA tests on 23,043 anophelines detected CS antigens in 5 of 672 pools (3,410 individuals) of *An. minimus* species A, 1 of 79 pools (114 individuals) of *An. dirus s.l.*, 1 of 177 pools (455 individuals) of *An. maculatus s.s.*, and 2 of 357 pools (1,506 individuals) of *An. sawadwongporni* (Table 1). Because the infection rate was low, it is reasonably assumed that there was only 1 positive mosquito in a positive pool. Therefore, the inoculation rate, calculated from human catches on this basis and assuming that the ELISA-positive mosquitos were infective, was estimeted as 0.005 infective bites/person/night in 1990 and 0.009 in 1991 (overall = 0.007). The overall rate (over 2 years) estimated from human catches in the villages was 0.008 and 0.005 at the farm huts.

Table 1

Malaria infection in mosquitos detected by ELISA: details of each positive pool.

Anopheles species	Malaria	No. of individual in positive pool	Village	Collecting site	Month/Year
minimus A	Pf	1	HN	human outdoors(V)	Jul/90
minimus A	Pf	5	MTN	light trap(V)	Oct/90
minimus A	Pf	8	MTN	light trap(V)	Jun/91
minimus A	Pf	12	MTN	human indoors(V)	Oct/91
minimus A	Pf	11	MTN	human outdoors(F)	Oct/91
dirus s.l.	Pf	1	MC	animal(V)	Jun/90
maculatus s.s.	Pf	3	MC	human outdoors(V)	Sep/91
sawadwongporni	Pv	1	MC	human indoors(V)	Jul/90
sawadwongporni	Pf	11	MSL	human indoors(F)	Jul/91

Pf = *P. falciparum*, Pv = *P. vivax*
HN = Huai Ngu, MTN = Mae Top Nua, MC = Mae Chon, MSL = Mae Salap
V = Village, F = Farm hut

Among 15,215 anophelines collected from human catches, *An. minimus* species A was the most prevalent species (35.8%) whereas *An. dirus s.l.* (2.1%), *An maculatus s.s* (4.6%) and *An. sawadwongporni* (14.5%) were collected in relatively smaller numbers; these species showed a higher biting density at farm hut settings than in the residential villages with the farm hut:village ratios of 2.3:1, 7.3:1, 3:1 and 2.6:1, repectively.

To test whether the parous rate varied with ecological settings (*ie* village and farm hut), the pairs of parous rates obtained by human baits in each setting were compared by Mantel-Haenszel χ^2 tests, stratifying by village. The parous rates of *An. minimus* species A consistently showed a significant tendency to be lower at the farm huts in both years (all $p < 0.001$); the overall rate (2 years) in the villages was 67.7% (n=1,566) and 48.3% (n=3,678) at the farm huts. For the other species, the parous rates were variable.

Fig la and 1b show the overall seasonal relationship between vector biting densities, malaria incidence, rainfall, and population movement. The monthly incidence of malaria cases among people in the 5 study villages collected by passive and active case detection systems of Mae Sariang Malaria Sector Office are shown with the monthly totals of mosquitos collected from human bait from all catching sites (Fig la). The peaks of malaria cases were similar to those observed in the whole district. Data of the second year are not shown as the number was low. Although there were no lag between appearance of mosquitos and of cases, as seen elsewhere, it is clearly seen that *An. minimus* species A was the most common species contacting man throughout the rainy season with peaks more closely associated with the human cases than the other species. *An. sawadwongporni* was the second most common species biting man and appeared in a single defined peak in the early rainy season. *An. maculatus s.s* and *An. dirus s.l.* were found in much lower densities. The patterns of their seasonal prevalence in the second year were similar (data not shown).

Movement and malaria illness were observed among 494 persons (172,929 man-nights) from July 1990 to January 1991, and from June 1991 to January 1992. As the patterns of movements in the second year appeared to be similar to those of the first year, the results of only the first year are presented and the movement rate in June 1990 was estimated from that in June 1991 (Fig 1b). The observed proportion of people staying outside their villages was about 11% of the total population (measured in terms of person-nights). Analysis of movement duration, by age and sex, reveals that adult males of 15-59 years were predominant among those who were moving. A rapid increase of villager movements away from their residences for agricultural activity was evident in July. This activity was mainly ploughing and planting for rice cultivation. Harvesting of rice started in late October and continued until early December. There was no sharp peak of agricultural movements during harvesting period, although agriculture still ranked higher than other activities. Movements for forest activities were relatively low during the rainy season but started to increase in the dry season just

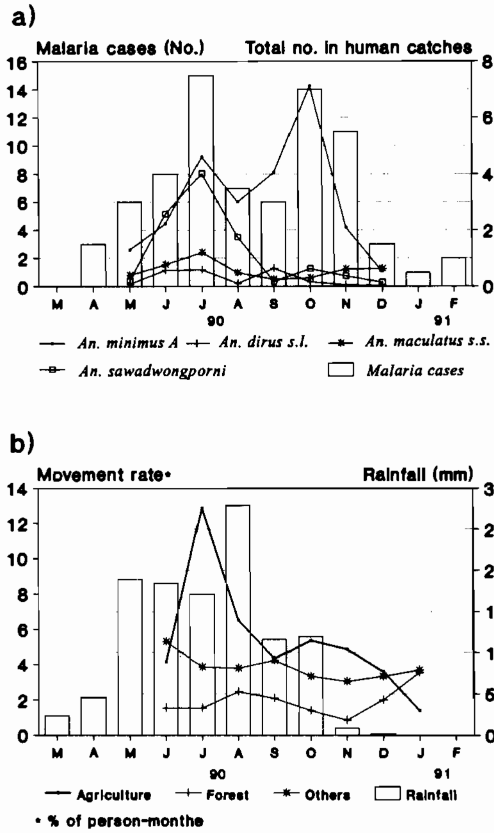

Fig 1– a) Monthly distribution of malaria cases in the 5 study villages (population = 1,304) and the total number of potential vectors collected from human catches at all sites. There were 54 man-nights/ month. b) Rainfalls and movement rate of villagers for agricultural, forest and other activities.

Table 2

Incidence rates of malaria episodes observed on 494 persons classified by activity, during July 1990 to January 1991 and June 1991 to January 1992.

Activity	Person-night	No. of episode	Incidence rate#	Relative risk (95% C.I.)
Outside village:				
Forest@	3,341	5	44.90	12.75 (4.74-34.33)
Agriculture	7,924	3	11.36	3.23 (0.95-10.96)
Other	8,049	2	7.45	2.12 (0.49-9.14)
Stay inside village:	153,615	18	3.52	1.00
Total	172,929	28	4.86	

Number of episodes/1,000 person-months.
@ Forest on both sides of the border.

after harvesting. The rising trend aparently continued into the dry season when the observation of movements ended in January. Other movements included activities such as visiting relatives, labouring in town, etc, and occurred throughout the observed periods.

With regard to the malaria illness during the 15 months over 2 transmission seasons (July 1990 to January 1991, and June 1991 to January 1992), Mae Sariang Malaria Sector reported 73 episodes in the 5 villages studied, all of which were classified as imported cases. Among them, 28 were also detected by the epidemiological study, but by our interviews, 18 (64%) of these had no history of any movement before the onset of symptoms and were therefore classified as indigenous cases. The relative risks of infection for people engaged in different activities are given in Table 2.

DISCUSSION

The report of Mae Sarang Malaria Sector that none of the 73 malaria episodes from the 5 study villages during the study period of our epidemiological study were classified by interview as indigenous cases contrasted with the epidemiological study results which suggested that 64% of the cases were indigenous and with the fact that in both 1990 and 1991 infected mosquitos were collected in the villages. After talking with malaria officers and observing their work, the discrepancy between the 2 interviews was apparently due to several factors:

(a) Some patients did not present themselves at the malaria clinic but went to hospitals. The records of these cases were collected by malaria officers on a weekly basis. However, follow-up interviews with the patients usually took place 1-2 months after they recovered. By that time, it was likely that the patients might have forgotten some details. In addition, some cases were not present at home when malaria workers visited, so the information about these patients was obtained from neighbours or the head of the village. This information was likely to be inaccurate. By contrast, the epidemiological team visited fortnightly, so it was easier for the patients to recall their activities.

(b) Some malaria officers, but not the epidemiological team, tend to classify village houses located away from the central cluster of houses in the villages as farm huts, although the distance between them was not so far (*ie* 100-200 m).

(c) Some malaria officers, but not the epidemiological team, defined cases as imported if there was a history of "short-night" activities (*ie* returning home late after working in the field or after visiting relatives or friends at neighbouring villages, frog hunting, etc). These short-night activities are common for Karen people. However, there is little information to indicate that these activities are important for malaria transmission.

(d) Some officers working in Malaria Sectors may have been afraid that reporting an indigenous case could reflect badly on their activities and could lead to blame from higher authorities, *ie* Malaria Unit or Malaria Center (now Office of Vector-Borne Diseases Control).

These factors could lead to an overestimation of number of imported cases and underestimation of indigenous cases.

The finding of ELISA- positive mosquitos at the farm huts (in 1991) suggests that transmission at the farm huts is also possible. The inoculation rates over the 2 year study estimated from human catches between the villages and the farm huts were quite similar (*ie* 0.008 and 0.005 infective bites/man/night, respectively). However, the relative risk of contacting malaria following agricultural activities (Table 2) was 3 times higher, which with the increased activity in July would account for the peak of malaria in this month (Fig la and 1b). The parous rate of the vector populations, especially *An. minimus* species A which was probably the most important vector in the area, was significantly lower at the farm huts than in the villages (see above). Although the exact reason for the difference of parous rates of this mosquito species between the 2 settings is unknown, it may imply shorter longevity which can reduce the vectorial capacity of mosquito populations (Garrett-Jones and Grab, 1964), further accentuating the importance of moving reservoirs of infection (people) in transmission.

Another site of transmission based on the epidemiological data appeared to be forest foci. Movement for forest activities carried a malaria risk about 4-6 times higher than other activities and about 13 times higher than staying in the villages (Table 2). The higher risk of getting malaria from the forest has also been documented elsewhere in Thailand (*eg* Butraporn *et al,* 1986; Fungladda *et al,* 1987). Such a high risk of malaria in forest foci compared with farm huts suggests a difference in malaria epidemiology and probably entomology which needs further investigations. The deep forest village, Huai Ngu, may not give a representative picture of forest foci of transmission since the risk of getting malaria there was as low as in the other villages studied (data not shown).

However, the movement rate for forest activities was much lower than that for agricultural activities during the rainy season and did not relate to the peaks of malaria cases or the patterns of vector densities in the study area (Fig la, 1b). Of the 28 cases detected by our team, 5 were classified as forest cases and 3 as farm hut cases. 18 were classified as indigenous cases. Although the daily risk of getting malaria in the villages was low, about 89% of person-nights were spent staying in the villages. Therefore, overall the residential villages were relatively more important than other foci as sites of transmission.

In south-eastern Thailand, where *An. dirus* species A is the primary vector, Rosenberg *et al* (1990) showed that malaria transmission in their study area occurred within the village, which was located in groves of rubber and fruit trees. In another area of that region, Prasittisuk *et al* (1989) reported that transmission probably occurred in the forest and rubber plantations rather than rice field villages. In NW region, there have been 2 other studies carried out in Tak Province, about 200 km south of Mae Sariang, showing that active transmission occurred in the villages (Green *et al,* 1991; Harbach *et al,* 1987). In the present study area, entomological and epidemiological evidence suggest that residential villages, farm hut settings and forests are sites of transmission, but little is still known about the epidemiology and entomology of forest foci of transmission. Control of vector populations and/or use of improved methods of personal protection should be applied not only when people are moving to engage in their activities outside the villages but also when they are staying in their residential areas.

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