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## Responses of *Anopheles minimus* to DDT residual spraying in a cleared forested foothill area in central Thailand

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### Summary

*Anopheles balabacensis balabacensis* and *Anopheles minimus* are the main malaria vectors in Thailand. In a cleared forested foothill area in the central part of the country *A. minimus* was the most prevalent anopheline species found, only 6 specimens of *A. b. balabacensis* being collected over a 3-year period. Cattle were scarce in the area, tractors being largely used for working in the fields. This situation contributed to high man-vector contact.

*A. minimus* occurred throughout the year, with a major peak of density in the dry cool season and a smaller peak in the wet season. The contact of *A. minimus* with man was much higher outdoors than indoors, and studies showed the species to be an early biter, especially in the dry season, thus increasing the chance of man-vector contact.

DDT spraying appeared to reduce considerably the estimated vectorial capacities, however, this effect was not maintained and malaria transmission was not interrupted. Trials with supplementary or alternative attack measures are therefore indicated in this particular ecological situation.

**Key words:** *Anopheles minimus*; malaria in Thailand; DDT house spraying; vector control; deforested foothills.

### Introduction

*Anopheles balabacensis balabacensis* and *Anopheles minimus* are the main malaria vectors in the forested hilly areas in most parts of Thailand. Under forest clearance the ecological conditions become unfavourable for the breeding of *A. b. balabacensis* and more favourable for *A. minimus*. As a result, the first vector disappears and the second becomes more prevalent.

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The malaria eradication project has been facing difficulties in interrupting transmission in forested hilly and cleared forested foothill areas. The responses of *A. b. balabacensis* and *A. minimus* to attack measures with DDT residual house spraying were studied in a typical forested hilly area in northern Thailand [5, 6]. On the other hand the responses of *A. minimus* to the same attack measures in its most favourable breeding sites, cleared forested foothill areas with slow streams, the subject of this paper, have been studied in a selected site in central Thailand.

Village 5 in Salangphan Canton, Muaklek District of Saraburi Province has been a persistent focus of transmission for a number of years. The focus was selected for these studies in December 1971, based on the results of a preliminary survey. Starting in January 1972, the village was sprayed thoroughly with DDT and from February 1972 until December 1974, monthly entomological observations were conducted regularly.

### **The study area, background information on malaria**

The study area is located in a narrow valley bounded by several mountains and hills. It is about 60 km<sup>2</sup> in area, with a total population of 587 living in 124 houses or huts distributed in 7 hamlets. One main water stream and several tributaries cross the different hamlets. Previously the whole area was covered by forest. Forest clearance has been taking place gradually in the valley and the surrounding mountains and hills. At the beginning of the studies the area was deforested.

Farming is the main occupation of the villagers. Corn, cotton, castor oil plants, fruits and vegetables are the most common crops. Farmers in this village are relatively rich. Some are in possession of tractors which replaced cattle in field work. As a result cattle became very scarce in the village.

The study area has a long history of malaria. It has been under the attack phase of the malaria eradication programme for a number of years. Spraying with DDT residual insecticide started with the control programme in 1952, covering only some of the existing villages at that time. Since 1965 the malaria programme was converted to eradication and spraying continued, with 2–3 cycles per year, except in 1971 when the village was missed. For some years the study area has been under active case detection, one visit per month. The slide positivity rate reached 13.4% (65/484) in 1970 and 19.2% (68/355) in 1971 with *P. falciparum* being the predominant malaria parasite.

### **Methods**

#### *Entomological*

Entomological techniques consisted of mosquito collections on human bait and dissections for parous rate [1]. Collections of mosquitos were carried out indoors and outdoors, on four consecutive nights, in the early part of each month. Four sites were selected for this purpose and observations were carried out for one night in each site every month. Collections of mosquitos were carried

Table 1. Climatological data as recorded from the meteorological station in Pakchong. Average of 5 years, 1970–1974

Month	Temperature (° C)			Relative humidity			Rainfall (mm)
	mean max.	mean min.	mean	mean max.	mean min.	mean	
January .....	29.6	12.9	21.3	98.2	37.6	68	2.5
February .....	31.6	16.4	24.1	97.2	36.2	66.8	12.2
March .....	33.0	19.5	26.3	97.2	40.8	69.8	106
April .....	32.6	21.1	27.3	99.3	45.8	72.0	127.2
May .....	33.3	22.1	27.7	99.5	53.0	75.0	150.6
June .....	32.1	22.5	27.2	98.2	53.2	75.4	51.4
July .....	31.6	22.6	27.1	96.8	54.2	75.4	66
August .....	30.8	22.3	26.6	99.2	56.6	77.8	114.2
September .....	30.9	21.8	26.4	99.2	58	78.6	253.6
October .....	30.0	20.9	25.1	99.2	58.2	78.8	158.6
November .....	28.3	20.6	23.5	98.8	53.6	76.6	35.4
December .....	27.7	15.9	22.1	97.6	48.2	73.0	10.9
Mean or total . . .	31.0	19.9	25.4	98.4	50.1	73.9	1076.4

out from 18.00 h until 6.00 h by insect collectors who worked in pairs, one indoors and one outdoors and on two shifts for 6 h each. They collected mosquitos alighting on their bare legs for 50 min and took 10 min rest each hour. Due to the day-time exophilic habit of the vector no effort was made to collect mosquitos resting indoors, during day-time.

The susceptibility level of *A. minimus* to DDT was checked once a year by using the WHO standard adult susceptibility test kit.

#### Parasitological

Active case detection was carried out regularly once a month by a house visitor. In addition a total of eight mass blood surveys were carried out during the period of study. All malaria cases were investigated and classified accordingly. Presumptive treatment was given to suspected cases and radical treatment to confirmed ones. The treatment followed the procedure of the malaria project. All confirmed *falciparum* cases were treated with 1000 mg sulfadoxine, 50 mg pyrimethamine, as a single dose, and 75 mg primaquine, since October 1971, while *vivax* cases were treated with 1500 mg chloroquine and 75 mg primaquine, as a 5-day regimen. All aforementioned doses are adult doses. Due adjustments were made for the younger age-groups.

#### DDT spraying

The study area received 3 cycles of DDT spraying in 1972 and 2 each in 1973 and 1974. Following each cycle, a mop-up team visited the area for spraying missed and new houses or structures. DDT residual spraying was carried out as usual with a dosage of 2 g/m<sup>2</sup>. Subsequent to these studies this area has continued to be sprayed twice per year to date.

Records on rainfall, atmospheric temperature and relative humidity were taken from the nearest meteorological station which is located in the municipal area of Pakchong, about 30 km from the study site.

Climatological conditions in the study area differed from those in the forested hilly area in northern Thailand where Ismail et al. [5, 6] conducted their observations on *A. b. balabacensis* and *A. minimus*. Average of 5-year records, from 1970 to 1974 (Table 1) showed that temperatures were usually higher, relative humidity lower and rainfall less in this study area than in the north.

## Results

### *Anopheline species*

During the period of study a total of nine anopheline species were encountered. *A. minimus* was the most prevalent species representing 91.1% of all mosquitos collected as follows: 5772 *A. minimus*, 6 *A. b. balabacensis*, 190 *A. maculatus*, 7 *A. aconitus*, 95 *A. philippinensis*, 118 *A. hyrcanus* group, 81 *A. barbirostris* group, 61 *A. vagus*, 7 *A. tessellatus*.

Beside *A. b. balabacensis* and *A. minimus* only *A. aconitus* has been incriminated as a malaria vector in Thailand [4]. *A. maculatus*, *A. philippinensis* and *A. campestris* (a member of *A. barbirostris* group) have been considered as suspected vectors in some parts of the country. However, their prevalence in low numbers did not favour an active role in the transmission of malaria during the period of study.

### *Man-vector contact with A. minimus*

Results of man biting collections showed that *A. minimus* was contacting man much more outdoors than indoors (Fig. 1). Its outdoor contact amounted to 5.8 (1010/173), 5.0 (2134/425) and 4.4 (1657/373) times as much as indoors in 1972, 1973 and 1974 respectively. *A. minimus* was encountered all the year round but appeared in higher densities during two periods: early part of the dry season and the wet season. The first peak was always the highest and more regular while the second peak was smaller, irregular and varied considerably in time and duration. In spite of DDT spraying *A. minimus* continued to be prevalent at high densities as shown from the results of its contact with man. Nevertheless there was an apparent decrease in the vector population in the wet season of 1974 which, however, was probably due to climatological factors, namely shortage of rain.

### *Biting cycle of A. minimus*

Results of the biting cycle of *A. minimus* in the dry and wet season differed considerably. In general biting was earlier indoors than outdoors (Fig. 2).

*Dry season.* The peak of biting of *A. minimus* in the dry season was recorded between 18.00 h and 19.00 h, with a rapid fall which continued until 23.00–24.00 h, by which time 82.2% indoors and 82.4% outdoors of the *A. minimus* caught had attempted to feed. Biting continued throughout the rest of the night but in comparatively small numbers.

*Wet season.* During the wet season biting commenced one hour later with an initial peak between 20.00 h and 21.00 h. Biting continued at approximately the same rate until 02.00–03.00 h when it fell sharply and by which time 91.2% indoors and 88.7% outdoors of *A. minimus* caught had attempted to feed. Biting continued for the rest of the night but again at a very low level.

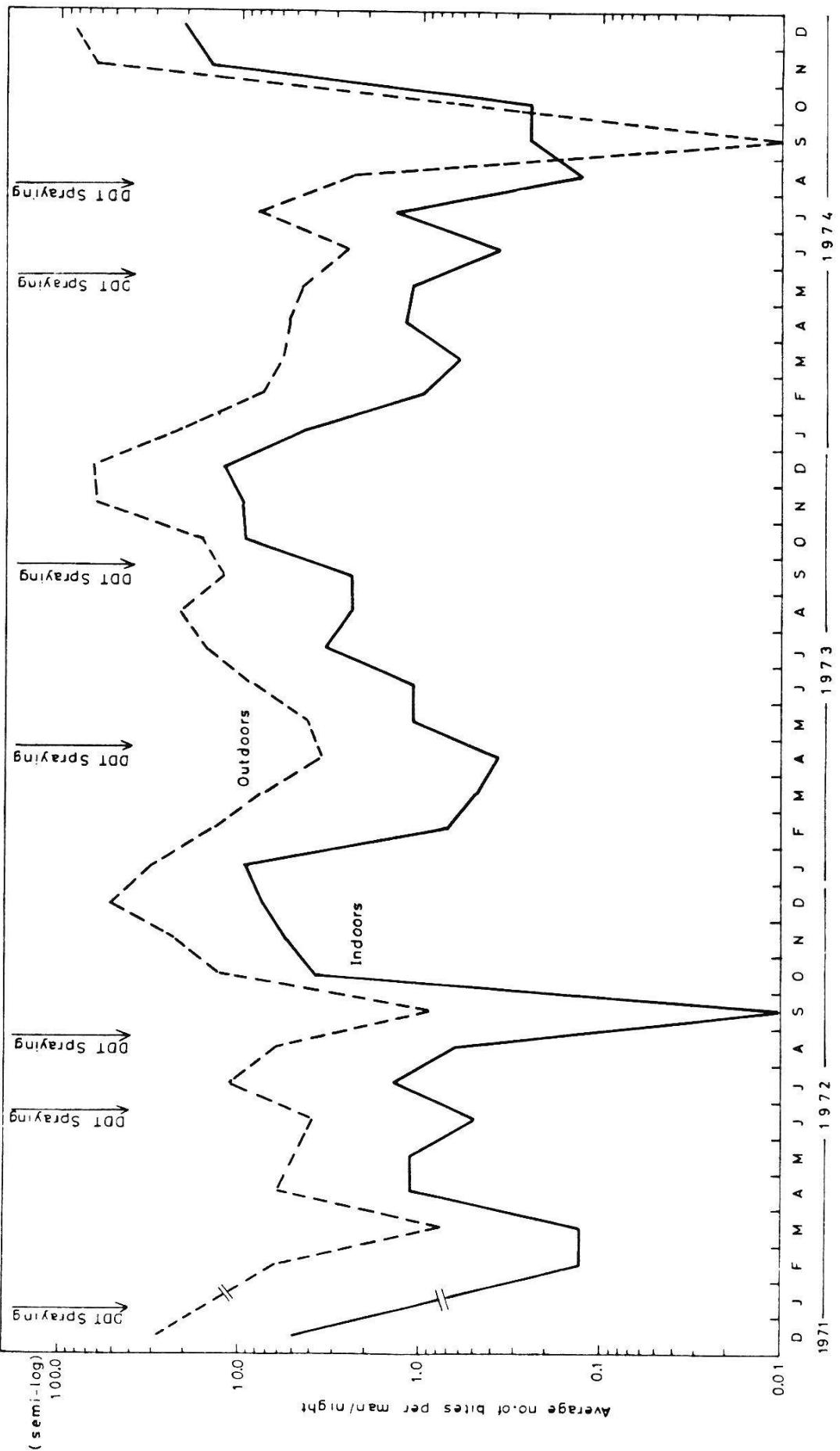


Fig. 1. Man-vector contact with *A. minimus*, indoors and outdoors.

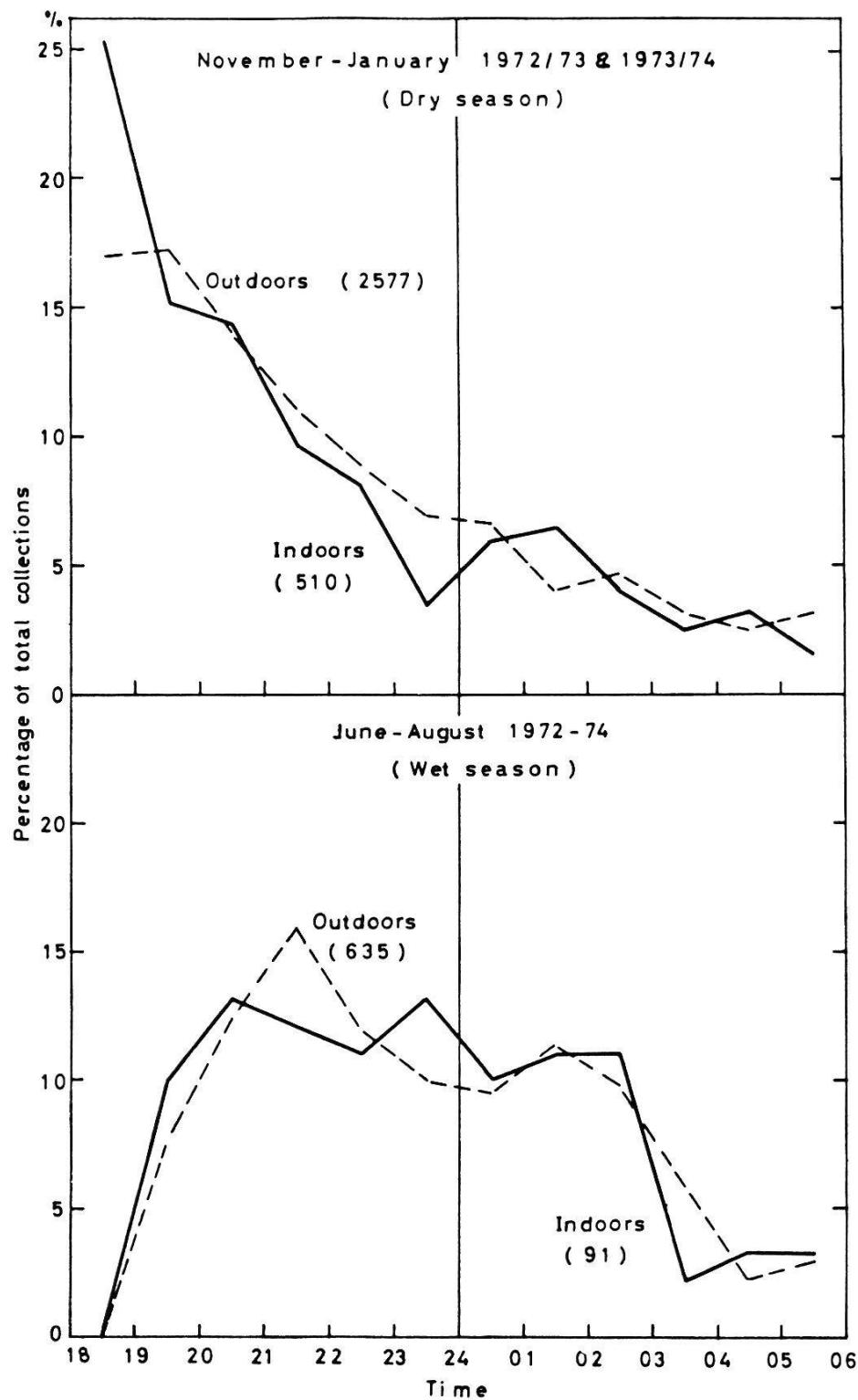


Fig. 2. Indoor and outdoor biting time of *A. minimus*. Figures in parenthesis represent number of mosquitos collected.

#### Gonotrophic cycle

Observations on *A. minimus* in northern Thailand [6] showed that it took 2 days for the completion of its gonotrophic cycle in the monsoon season when temperatures ranged between a monthly mean minimum of 21.8° C and maximum of 28.8° C. In the absence of observations in the dry, cool season and by

considering the temperature fluctuation compared with Thomson's results [10] from Assam, India, the authors estimated (as a rough approximation) gonotrophic cycle of 4 days average during that season. In our present observations efforts were made to study the gonotrophic cycle during the dry, cool season. In January 1975 a total of 398 specimens were observed for this purpose, out of which 304 or 76.4% completed the cycle in two days. Temperature records during that period showed that it was relatively higher than usual varying between a mean minimum of 19.2°C and maximum of 27.5°C (average of 23.4°C). Such temperatures were similar or closer to those normally recorded in November and February (Table 1). In December temperatures fell to 15.9°C minimum and 27.7°C maximum (average 22.1°C), and in January to 12.9°C minimum and 29.6°C maximum (average 21.3°C). Based on these data we estimated a gonotrophic cycle of 2-day average during the period from February to November, and 3-day average in December and January, with a feeding rhythm of 3 and 4 days respectively.

#### *Sporogonic cycle*

Considering monthly temperature records collected in this study area from 1970 to 1974, and applying this to the temperature/sporogonic cycle correlation for *P. falciparum* [8], the duration of the sporogonic cycle in this area for *P. falciparum*, the most prevalent species, may be considered as 20 days in January, 14 days in February, 12 days from March to September, 13 days in October, 15 days in November and 18 days in December.

#### *Host preference*

Due to the day-time exophilic tendency of *A. minimus*, efforts to collect blood-fed females for precipitin tests were directed only towards possible outdoor resting sites. Such efforts were carried out occasionally when mosquitos were prevalent in a high density. However, samples were always small and of no significant value for this purpose.

A survey of the domestic animals in the village revealed that cattle were very scarce. The significance of this in Thailand is that normally cattle are kept at night underneath the houses, thereby being in close proximity to man during the normal biting period of *A. minimus*. The marked scarcity of cattle, which have been replaced by tractors for agriculture work could have influenced the biting habits of the species more towards man.

In the absence of data on host preference and in view of the cattle situation in the study area we used a hypothetical value of 0.8 for the human blood index in our calculations on the vectorial capacity (section after next).

#### *Parous rate and life expectancy*

Values of the parous rate were utilized in deriving the probability of daily survival and life expectancy of *A. minimus* (Table 2). Only monthly data, with

Table 2. Parous rate\*, probability of daily survival\*\* and estimated life expectancy of *A. minimus*, 1972–1974

Month	1972			1973			1974		
	% parous	$p^{3&4}$	Life exp.	% parous	$p^{3&4}$	Life exp.	% parous	$p^{3&4}$	Life exp.
January . . . . .	—	—	—	60.5 (268)	0.885	8.19	65.8 (196)	0.90	9.52
February . . . . .	44.0 (50)	0.76	3.64	59.5 (74)	0.84	5.74	67.7 (68)	0.88	7.83
March . . . . .				64.9 (57)	0.865	6.90	72.3 (47)	0.895	9.03
April . . . . .	24.1 (54)	0.62	2.09				57.1 (49)	0.828	5.30
May . . . . .	73.9 (46)	0.905	10.0						
June . . . . .				70.4 (71)	0.89	8.59			
July . . . . .	52.0 (102)	0.805	4.61	54.3 (140)	0.815	4.89	68.6 (70)	0.882	7.97
August . . . . .	56.4 (55)	0.825	5.18	65.9 (173)	0.87	7.18			
September . . .				56.3 (112)	0.825	5.18			
October . . . . .	28.4 (127)	0.655	2.36	42.2 (180)	0.75	3.47			
November . . .	41.0 (183)	0.745	3.39	50.0 (466)	0.795	4.36	66.1 (560)	0.87	7.18
December . . . .	54.6 (242)	0.86	6.64	53.6 (530)	0.855	6.40	47.7 (625)	0.83	5.37
Mean	46.1 (859)	—	4.74	55.0 (2071)	—	6.09	59.0 (1615)	—	7.46

\* Monthly samples with less than 45 mosquitos are not included. No observations were conducted in January 1972. Figures between brackets represent total numbers dissected.

\*\* Probability of daily survival with the power of 3 during the months February–November and the power of 4 in December and January, Fig. 1, Garrett-Jones and Grab, 1964 [3].

more than 45 mosquitos dissected for parous rate, were used for this purpose. It should also be noted that no data are available for January 1972 when no observations were carried out. As a result values of the life expectancy were calculated for only some of the months in the 3-year period of study. With these reservations the range of fluctuation in the values of the life expectancy in the comparable months of February, July, November and December was:

- a) 3.39–6.64 days with a mean of 4.57 days in 1972,
- b) 4.36–6.40 days with a mean of 5.35 days in 1973,
- c) 5.37–7.97 days with a mean of 7.09 days in 1974.

All calculated monthly values were below that of December 1971 (19.16 days) which could be taken as a base-line.

#### *Vectorial capacity\**

Values of the vectorial capacity [2, 7] of *A. minimus* were calculated separately for its indoor and outdoor populations. In calculating the theoretical epidemiological indices the following values or hypothesis were utilized.

- a) Human blood index of 0.8,
- b) frequency of biting of 0.33 during February–November and 0.25 during December–January,
- c) "n" for *P. falciparum*, which was the most prevalent malaria parasite as mentioned in section "Sporogonic cycle".

Due to the lack of data on the longevity factor in some months, results of the vectorial capacity are presented in two different ways:

a) *Comparison of the corresponding monthly values for indoor and outdoor populations.* The results shown in Fig. 3 indicate that the vectorial capacity had its lowest monthly values in 1972 and increased gradually, in most cases, during the following two years. Nevertheless the highest values were those calculated for December 1971, from the result of the preliminary survey, with 7.78 and 43.9 for the indoor and outdoor populations respectively.

b) *Comparison of the sum of monthly values for corresponding periods.* Results of the sum of the monthly values of the vectorial capacity for the period October–March of 1972/73 and 1973/74, in which all monthly data are available, are presented in Table 3. Values for the indoor population were calculated as 2.493 in the first period and 3.42 in the second. The corresponding values for the outdoor population were calculated as 14.711 and 19.973 in the first and second periods respectively.

The results indicate that values of the vectorial capacity were nearly 6 times higher in the outdoor population than in the indoor population. Also there was an increasing trend in the vectorial capacity of both populations in the second period.

#### *Susceptibility to DDT*

During the course of these studies the susceptibility of *A. minimus* to DDT was checked periodically once a year during its dry season peak. Results of adult susceptibility tests in 1972/73, 1973/74 and 1974/75 showed that the vector continued to be susceptible to DDT. The LC<sub>50</sub> values were calculated as 0.33%, 0.40%, and 0.48% in the three annual tests respectively, with 100% mortality in the diagnostic tests for the standard 1-hour exposure period.

\*  $\frac{ma^2 p^n}{-\log_e p}$

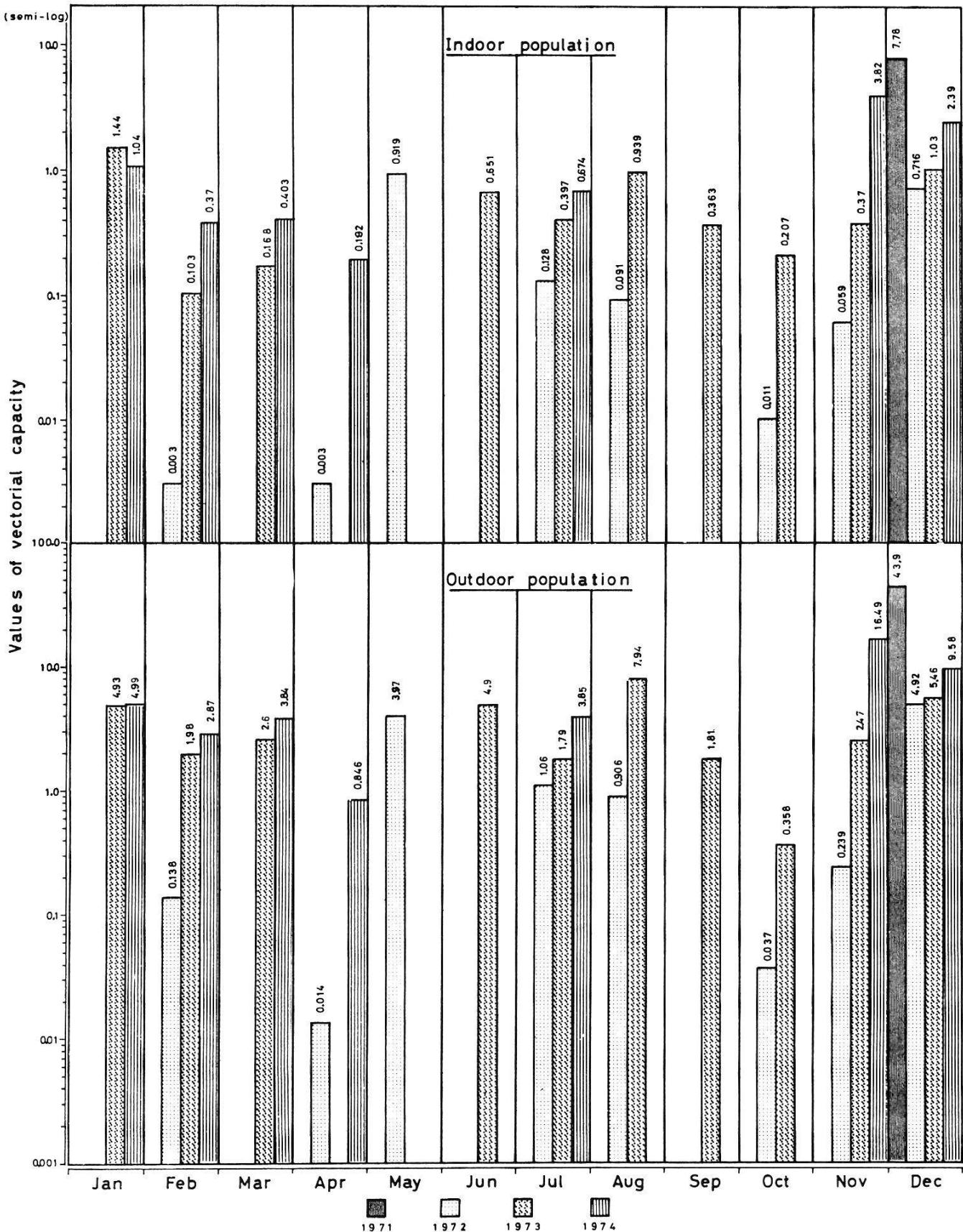


Fig. 3. Estimated monthly values of vectorial capacity in indoor and outdoor populations of *A. minimus*.

Table 3. Calculated vectorial capacity for indoor and outdoor populations of *A. minimus* in two corresponding periods

Month	Indoor population		Outdoor population	
	1972/73	1973/74	1972/73	1973/74
October .....	0.011	0.206	0.037	0.358
November .....	0.059	0.370	0.239	2.472
December .....	0.716	1.034	4.92	5.457
January .....	1.436	1.037	4.934	4.985
February .....	0.103	0.37	1.983	2.866
March .....	0.168	0.403	2.598	3.835
Total	2.493	3.42	14.711	19.973

Table 4. Result of blood slide examination in 1971–1974

Year	No. of slides	Positives and parasite species				Slide positivity rate	P.f.g. *
		P.f.	P.v.	Mx.	total		
1971 .....	355	57	11	0	68	19.2	16
1972 .....	1279	32	11	0	43	3.4	8
1973 .....	1623	34	7	0	41	2.5	11
1974 .....	509	16	7	0	23	4.5	4

\* P.f.g. = *P. falciparum* infections with gametocytes.

#### Parasitological data

The total number of blood slides collected per year and the result of their examination are shown in Table 4.

The slide positivity rate was 3.4% in 1972, 2.5% in 1973 and 4.5% in 1974. The number of cases decreased from 43 and 41 in 1972 and 1973 respectively to 23 in 1974, with an annual parasite incidence of 82.5 (43/521), 68.2 (41/601) and 39.2 (23/587) per thousand population for the three years respectively. If one considers the results of 1971, the year in which the area missed spraying, as a base-line it would appear that there was a marked decrease in the slide positivity rate, and the number of cases, during the year following DDT spraying. However, the decrease did not continue during the entire period of study (Table 4).

Infections with *P. falciparum* were more prevalent than those with *P. vivax*. They represented 83.8% of the cases in 1971, 74.5% in 1972, 82.9% in 1973 and 69.6% in 1974. Gametocytes appeared in 28.1%, 25%, 32.4% and 25% of the *P. falciparum* cases in 1971, 1972, 1973 and 1974 respectively.

Table 5. Malaria cases by sex and age 1972–1974

Sex	Age-group						Total	
	Infant	1–4	5–9	10–14	15–19	20+	No.	%
Male	0	9	13	9	8	16	55	51.4
Female	1	11	13	5	11	11	52	48.6
Total	1	20	26	14	19	27	107	100
	0.9%	18.7%	24.3%	13.1%	17.8%	25.2%	—	100

Results of the case investigations revealed that most of the cases, 40 in 1972, 37 in 1973 and 18 in 1974, were probably infected in the village.

In analysing the malaria cases according to sex and age (Table 5) it was noted that 51.4% were males and 48.6% were females. The highest positivity rate was in the age groups 5–9 years, and 20 years and over.

## Discussion and conclusions

In forested hilly areas of Thailand where both *A. minimus* and *A. b. balabacensis* are prevalent, the former plays a secondary role to that of the latter in the transmission of malaria. On the other hand *A. minimus* takes over the main role in the foothills, once the area has been cleared of the forest.

In the study site, which is a cleared forested foothill area, *A. minimus* was the most prevalent species. Other anophelines were found in very small numbers. The finding of only 6 specimens of *A. b. balabacensis* in a period of 3 years is an indication that this vector, most probably, did not play a role in transmission. The same also applies to the suspected vectors which may only be important when present in considerably high numbers.

Entomological findings revealed that *A. minimus* was prevalent most of the year with a major peak in the dry, cool season and a smaller, irregular peak in the wet season. The irregularity in the second peak is presumably due to seasonal variations in climatological conditions and possibly due to variations in the timing of DDT spraying rounds.

Records on the contact of *A. minimus* with man were considerably higher in relation to records obtained by the malaria project from other similar areas in the country. A survey on the number of domestic animals in the study area revealed a scarcity of cattle. In trials with CDC light-trap from the same area, it was noted that *A. minimus* was more or less equally attracted to man and cattle outdoors. In conclusion mosquitos apparently deviated more to man in the absence or scarcity of cattle.

The contact of *A. minimus* with man was much higher outdoors than indoors, where it amounted to 5.8, 5 and 4.4 times as much in 1972, 1973 and 1974

respectively. At the same time *A. minimus* continued to be highly susceptible to DDT but with a small increasing trend in the LC<sub>50</sub> from 0.33% to 0.48%.

Results of the biting cycle studies showed that *A. minimus* is an early biter, especially in the dry season. This coincides with the period when many people stay late outdoors, for various activities connected with their harvested crops. Naturally this situation increases the chances of vector-man contact. However, low temperatures which often occur during this period would slow down transmission due to its effect on the sporogonic and gonotrophic cycles.

A similar attempt to that of Ismail et al. [6] was also made by using the entomological indices in calculating the vectorial capacity in the indoor and outdoor populations of *A. minimus*. It is clear that the vectorial capacity, with its variable components, has its limitations in assessing the responses of the malaria vectors to the attack measures [9]. However, in the absence of other means its use may be considered for this purpose.

The vectorial capacity of the outdoor population exceeded by far, nearly 6 times as much, that of the indoor population. From the epidemiological point of view such difference might not be of great importance in the dry season when the peak of biting is in the early hours of the night while many people are still outdoors. Variations in values of the vectorial capacity were noted during the year. After the resumption of DDT spraying in January 1972, values of the vectorial capacity decreased considerably. Previously, in December 1971 it was calculated as 7.78 in the indoor population and 43.9 in the outdoor population, whereas one year later, in December 1972, it was calculated as only 0.72 and 4.92 in both populations respectively. These results represent a decrease of nearly 11-fold in the indoor population and 9-fold in the outdoor population. However, this decrease in the value of the vectorial capacity did not continue throughout the entire period of study. On the contrary there was a gradual increase in the values from 1973 onwards.

Parallel parasitological data showed that after the initial round of DDT spraying, transmission of malaria, represented by the number of cases and slide positivity rate, decreased considerably, possibly partly due to increased case detection and treatment. Nevertheless it was maintained at more or less the same level later on. The finding of equal numbers of cases from both sexes and the high infection rate in younger ages confirms that many cases were infected within the study area.

From these results the following main conclusions can be made in relation to the prevailing conditions in the study area:

1. In the absence or scarcity of cattle *A. minimus* deviated in high numbers to man.
2. The exophagic tendency of *A. minimus* combined with its early biting habit, especially in the dry, cool season, increases the chances of its contact with man while he is still outdoors.

3. DDT house spraying seems to have a considerable effect on the reduction of the vectorial capacity of both indoor and outdoor populations of *A. minimus*, but it would appear that this alone is not sufficient to lead to the interruption of malaria transmission by this vector. Moreover there are some signs that in the long run the effect of DDT declines gradually. With the present strategy of a long range control programme in those areas the above signs are not encouraging. A close check on the malaria situation and responses of *A. minimus* to DDT spraying should be made regularly.
4. Trials with supplementary or alternative attack measures should be encouraged.

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