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Artikel: Studies on malaria and responses of "Anopheles balabacensis balabacensis" and "Anopheles minimus" to DDT residual spraying in Thailand. Part II, Post-spraying observations

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Studies on Malaria and Responses of *Anopheles balabacensis balabacensis* and *Anopheles minimus* to DDT Residual Spraying in Thailand.[1]

Part II. Post-Spraying Observations.

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Abstract

Studies on malaria and on *A. b. balabacensis* and *A. minimus* responses to DDT spraying were conducted in a forested hilly area in northern Thailand. In a first phase, base-line data were collected from July 1970 to March 1972. In a second phase, the study area received five rounds of DDT spraying over a period of two years and at the same time all malaria infections received radical treatment. During this two-year period of field operations, entomological and epidemiological observations were continued.

The studies carried out in the second phase, showed that malaria transmission decreased under the applied optimum anti-malarial measures but was not interrupted. Human ecology and population movement inside the forest, especially during the dry season, contributed to a great extent to this result. The transmission occurring in the early part of the monsoon season clearly indicates the importance of the timing of DDT spraying. *A. b. balabacensis* appeared to be transmitting malaria all the year round in the deep forest but only in the monsoon season in the forest fringe. The vectorial capacity of both vectors was estimated separately for indoor and outdoor populations. The pre-spraying values obtained for *A. b. balabacensis* were much higher than for *A. minimus*. After DDT spraying *A. b. balabacensis* showed a decrease in vectorial capacity estimated at 31.5 times for the indoor population and 18 times for the outdoor population. *A. minimus*, on the other hand, showed a much smaller decrease, estimated at 6.8 and 1.9 times for the indoor and outdoor populations respectively.

1. Introduction

In the forested hilly areas of Thailand, where *A. b. balabacensis* and *A. minimus* are the main vectors, malaria transmission could not be interrupted despite several years of DDT spraying. In order to determine the factors responsible

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for this situation, a field research programme was launched in July 1970. A site in the northern part of the country had been selected as the study area, and from July 1970–March 1972 base-line data were obtained on malaria incidence and on the natural habits of the vectors in the absence of DDT spraying (ISMAIL et al., 1974). The study area was then sprayed with DDT and field observations continued for two years from April 1972 to March 1974. The present paper reports the findings made during this period of post-spraying observations.

2. Study area

2.1 Topography

The study area with its population and dwellings is divided into three zones: forest fringe, deep forest and cleared zone. The forest fringe includes the dwellings on both sides of the southern edge of the forest. This fringe zone has an approximate radius of 0.5 km extending outside the forest and 2 km penetrating inside the forest. COLLESS (1953) estimated the flight range of *A. b. balabacensis* from the margin of the jungle at 0.8 km. The deep forest zone extends beyond the forest fringe to the south for about 6 km and the cleared zone extends to the north of the forest fringe for about 3.5 km.

On the western and eastern sides of the study area forest clearance has reached the foothills, whereas on the southern side, where the field station is located, only limited clearance has been carried out.

2.2 Dwellings

The term dwelling is used here to refer to any construction where people reside, either permanently or merely temporarily during the monsoon season. The number of dwellings in the study area, like that of the inhabitants, changed somewhat during the period of post-spraying observations.

The dwellings in the area normally have one side completely or partially open and are made of wood and/or split bamboo for the flooring and walls and of thatch for the roof. During the dry season, seasonal forest settlers often live in very simple shelters which are made of various cheap materials such as thatch, split bamboo, tree leaves, plastic sheets, etc., and which consist of a roof, floor and walls covering one or more sides. The dwellings which are always raised about one metre above the ground were included in the spraying operations, but not the shelters which are constructed directly on the ground.

2.3 Movement of population

Population movement and new settlements inside the forest play an important role in the transmission of malaria in the area. Forest movement entailing overnight stays can be divided into two types:

(i) Movement during the monsoon season: this involved mostly local villagers and was for agricultural purposes.

(ii) Movement during the dry season: this involved both local and outside villagers and was for purposes which varied from one year to the next, i. e., woodcutting in 1971/1972, collection of tree bark in 1972/1973, and clearance for cultivation in 1973/1974. The activity of 1972/1973 was the most important in that it involved the greatest number of people and the longest stays in the forest.

Cattle were completely absent from the forest settlements, mostly due to the low financial means of the villagers. It is only when an area has been cleared of most of its wild vegetation and the villagers become more affluent that cattle are brought in.

2.4 DDT spraying

Spraying operations which had been interrupted in the study area in 1970 were resumed in 1972. The area received five rounds of DDT residual spraying with a dosage of 2 g/m^2; of these five rounds, two were carried out in 1972 and three in 1973. Spraying covered all dwellings in the cleared zone and in the forest fringe including the field station. No spraying was carried out in deep forest dwellings.

Timing of the spraying rounds in 1972 was based on the observed seasonal prevalence of the two vectors during the previous year. The first round was, therefore, carried out in the last three days of March to cover the full season of *A. b. balabacensis* and the wet season of *A. minimus,* while the second round was made in mid-October to cover exclusively the dry season of *A. minimus*. In 1972, however, the rainfall followed a different pattern from that of 1971 and this affected the seasonal prevalence of the vectors. In particular *A. b. balabacensis* reached its peak density in August which was a full four months after the spraying round of end March. In view of this situation, three spraying rounds were applied the following year, i.e., at the end of March, July and November. In addition, all newly constructed dwellings were sprayed regularly by a mop-up team which visited the study area once a month.

3. Methods

The procedures followed for the entomological and epidemiological surveys were similar to those applied in the surveys made during the pre-spraying period (ISMAIL et al., 1974). A change was made in the radical treatment of *vivax* malaria cases. These were treated with 1,500 mg chloroquine (base) and received a daily dose of 15 mg primaquine (base) for 14 days. All *falciparum* infections were treated with 1,000 mg sulfadoxine, 50 mg pyrimethamine and 75 mg primaquine (base). Drugs were administered daily under special supervision.

4. Results[5]

4.1 Entomological surveys

4.1.1 Seasonal prevalence

The variations in the rainfall pattern that took place in the study area from one year to the next, affected the seasonal prevalence of both vectors as recorded from the field station (Fig. 1). *A. b. balabacensis*

[5] In the presentation of the results, data from pre-spraying observations made during the year April 1971–March 1972 (ISMAIL et al., 1974) have also been included for easy reference.

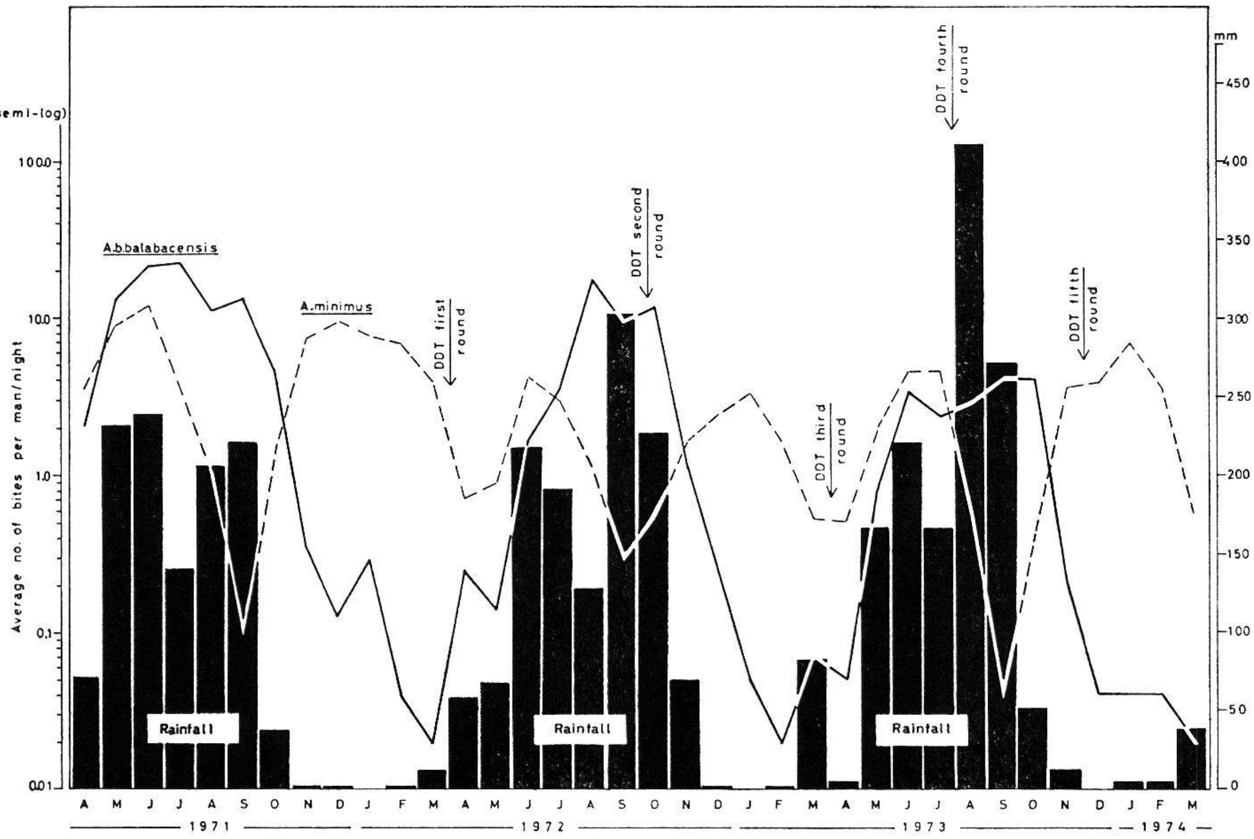

Fig. 1. Rainfall and seasonal prevalence of *A. b. balabacensis* and *A. minimus* in pre- and post-spraying observations.

was prevalent during the monsoon season and apparently disappeared or became difficult to find in the dry season. In other observations made in the deep forest zone during the same periods, *A. b. balabacensis* was encountered in considerable density. In February and March 1973 and 1974 this vector was collected in densities of three to nine per man/night. Specimens included young and old mosquitos. Moreover, two infected specimens with sporozoites were detected: one in December 1973, the other in early March 1974. These findings suggest that with the reduction of breeding places in the dry season *A. b. balabacensis* apparently disappears from the forest fringe and becomes restricted to areas deep inside the forest where ecological conditions seem to be more favourable, i.e., thicker vegetation, more shade, higher relative humidity and available (though limited) breeding places. However, the search for larvae in these breeding places proved negative for *A. b. balabacensis*. With the arrival of the rains, it is most likely that mosquitos from inside the forest invade the forest fringe where conditions have again become favourable and establish breeding in the newly formed breeding places. Results on the parous rate (section 4.1.8) support this suggestion, and it would therefore appear that *A. b. balabacensis* is transmitting malaria all the year round inside the forest and during only the monsoon season in the forest fringe zone.

Table 1. *A. b. balabacensis* and *A. minimus* man-biting densities per man-night, as recorded during post-spraying observations

Month	*A. b. balabacensis*						*A. minimus*					
	1972/1973			1973/1974			1972/1973			1973/1974		
	In-doors	Out-doors	Total	In-doors	Out-doors	Total	In-doors	Out-doors	Total	In-doors	Out-doors	Total
April	0.07	0.43	0.25	0.04	0.07	0.05	0.46	0.93	0.70	0.11	0.89	0.50
May	0.14	0.14	0.14	0.39	1.17	0.79	0.50	1.25	0.88	0.75	3.07	1.91
June	1.07	2.36	1.71	2.79	4.00	3.39	2.68	5.50	4.09	1.89	6.79	4.33
July	2.21	5.29	3.75	1.18	3.61	2.39	1.42	4.29	2.86	1.96	6.89	4.43
August	9.75	25.90	17.82	1.96	3.79	2.88	0.39	1.79	1.09	0.36	0.68	0.52
September	5.43	14.14	9.79	2.43	5.50	3.96	0.29	0.29	0.29	0	0.07	0.04
October	5.86	18.39	12.13	1.71	6.25	3.98	0.11	1.04	0.57	0.21	0.64	0.43
November	0.57	1.75	1.16	0.14	0.25	0.20	0.54	2.64	1.59	1.50	5.54	3.52
December	0	0.46	0.23	0	0.07	0.04	1.11	3.61	2.36	1.61	5.86	3.73
January	0.07	0.46	0.05	0.04	0.04	0.04	1.00	5.50	3.25	2.43	10.93	6.68
February	0.04	0	0.02	0	0.07	0.04	0.89	2.36	1.63	1.14	5.64	3.39
March	0.11	0.04	0.07	0	0.04	0.02	0.25	0.79	0.52	0.39	0.75	0.57
Total[a]	2.10 (709)	5.74 (1930)	3.93 (2639)	0.89 (299)	2.96 (696)	1.48 (995)	0.80 (270)	2.50 (839)	1.65 (1109)	1.03 (346)	3.98 (1337)	2.50 (1683)
Pre-spraying[b]	6.91 (2031)	6.71 (1974)	6.81 (4005)	–	–	–	4.42 (1299)	5.85 (1720)	5.13 (3019)	–	–	–

[a] Figures in parentheses represent total number of mosquitos collected.
[b] Pre-spraying data cover the period April 1971–March 1972.

4.1.2 Man-vector contact indoors and outdoors

After spraying a change occurred in the indoor and outdoor contact of *A. b. balabacensis* and *A. minimus* with man, as well as in the overall densities of both vector populations. This is shown in Table 1.

The excito-repellency of DDT deposits apparently led to a decrease in the indoor contact of both vectors with man (Figs. 2 and 3). As a result outdoor contact of *A. b. balabacensis* with man was 2.7 times higher than indoor contact in the first year of spraying and 2.3 times in the second (average 2.5). Somewhat similar results were found with *A. minimus* whose contact with man was 3.1 and 3.9 (average 3.5) times higher outdoors than indoors in the first and second years of spraying respectively.

In comparing man-vector contact before and after spraying (Table 2) it was noted that in the case of *A. b. balabacensis* this decreased in the

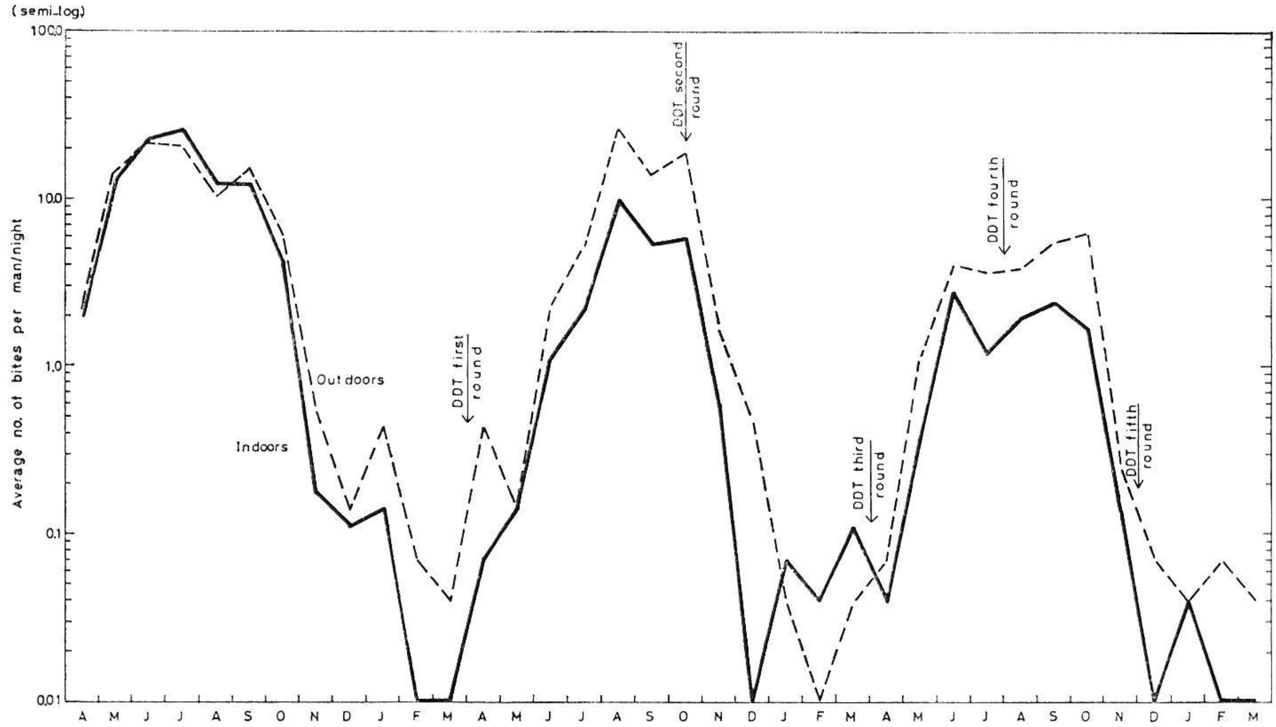

Fig. 2. Man-vector contact in *A. b. balabacensis*, indoors and outdoors in pre- and post-spraying observations.

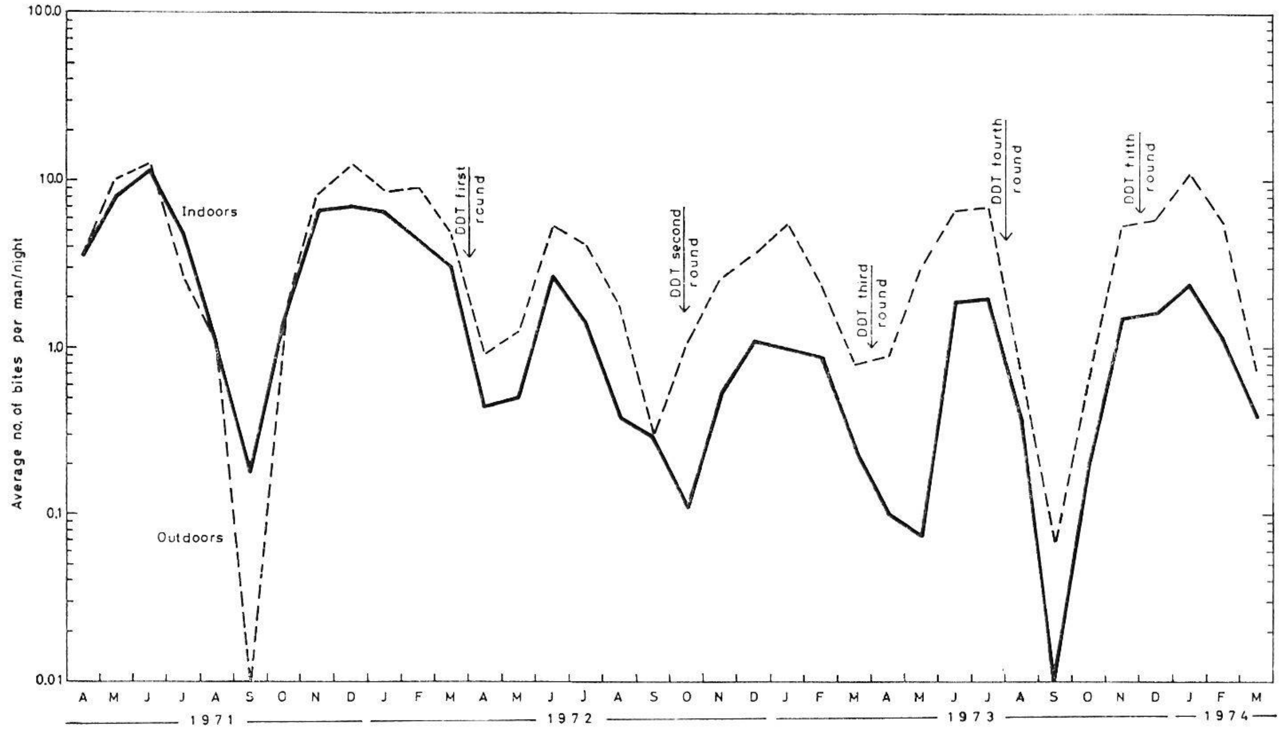

Fig. 3. Man-vector contact in *A. minimus*, indoors and outdoors in pre- and post-spraying observations.

Table 2. Annual changes in the contact[a] of *A. b. balabacensis* and *A. minimus* with man following DDT spraying

Period	*A. b. balabacensis*						*A. minimus*					
	Indoors		Outdoors		Total		Indoors		Outdoors		Total	
	Density	% increase (+) or decrease (–)	Density	% increase (+) or decrease (–)	Density	% increase (+) or decrease (–)	Density	% increase (+) or decrease (–)	Density	% increase (+) or decrease (–)	Density	% increase (+) or decrease (–)
Pre-spraying												
April 1971–March 1972	6.91		6.71		6.81		4.42		5.85		5.13	
Post-spraying												
April 1972–March 1973	2.11	–69	5.74	–14	3.93	–42	0.80	–82	2.50	–57	1.65	–68
April 1973–March 1974	0.89	–58	2.96	–48	1.48	–62	1.03	+29	3.98	+59	2.50	+52
Total change in density (%)		–87		–56		–78		–77		–32		–51

[a] Contact of vectors with man is presented by average numbers collected per man per night.

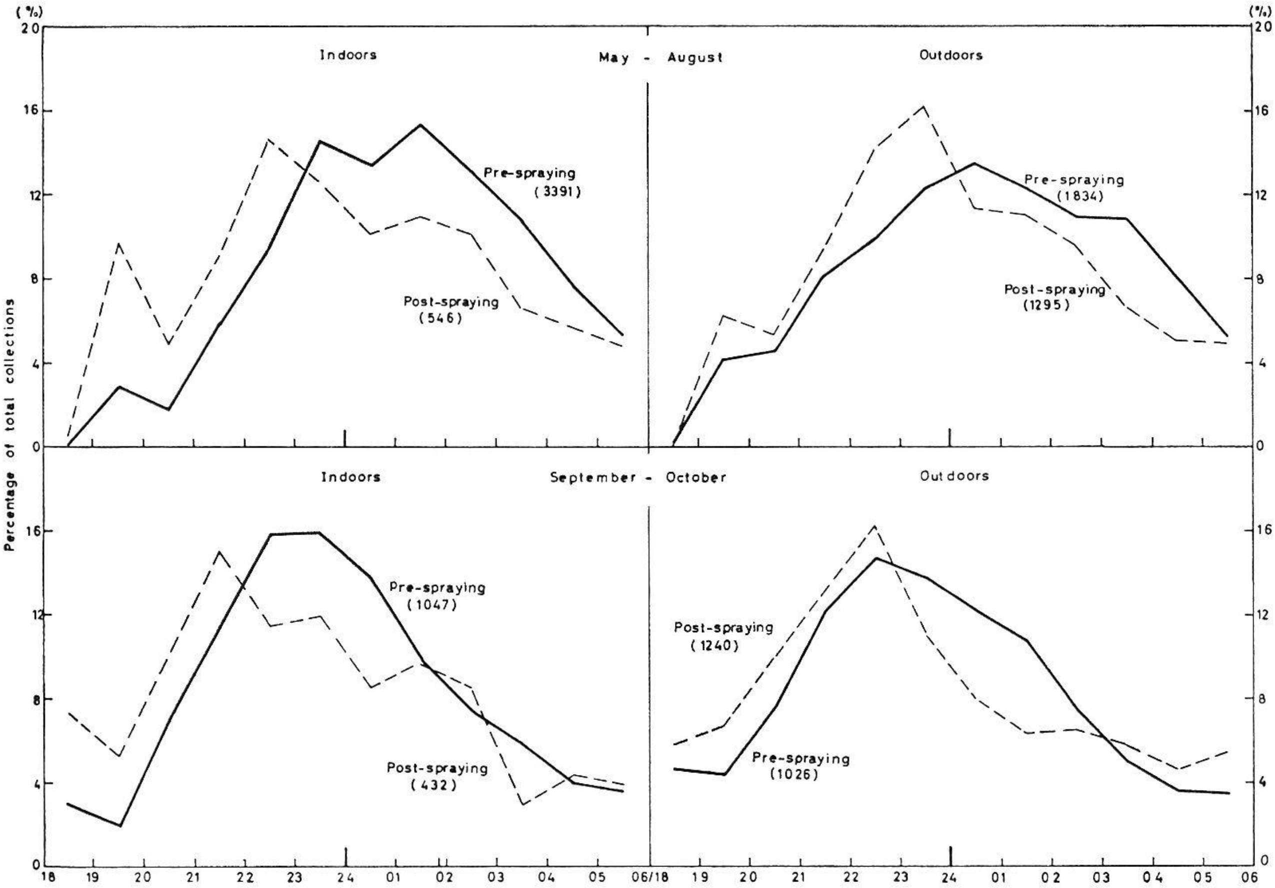

Fig. 4. Indoor and outdoor biting time of *A. b. balabacensis* in pre- and post-spraying observations.

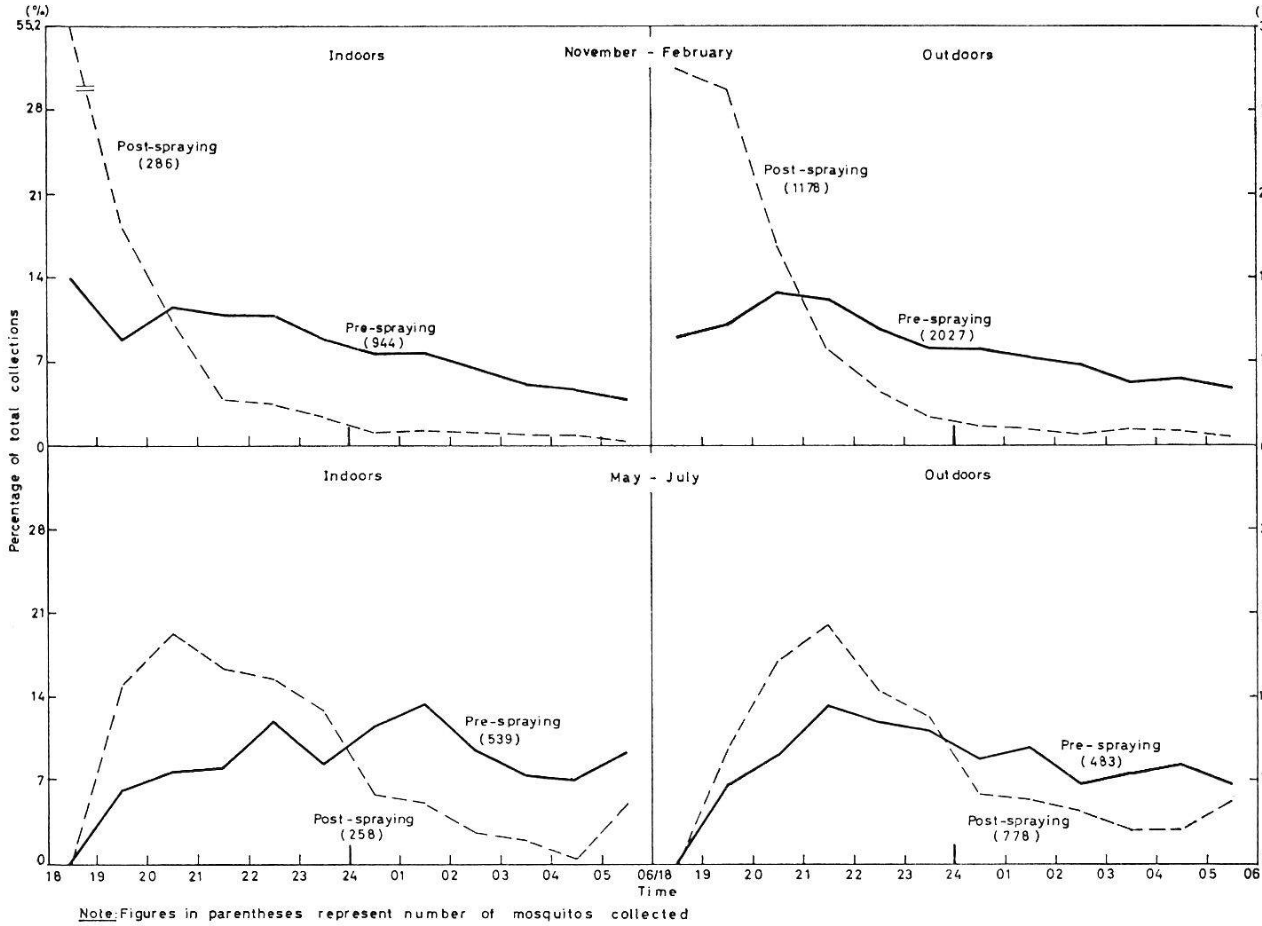

Fig. 5. Indoor and outdoor biting time of *A. minimus* in pre- and post-spraying observations.

first and again in the second year of spraying, giving an estimated net decrease of 87% indoors, and 56% outdoors, or a total of 78%. On the other hand *A. minimus* followed a different pattern as its contact with man decreased in the first year of spraying but increased in the second year and final results showed a net decrease of 77% indoors and 32% outdoors or a total of 51%. The decrease in contact of both vectors with man was more marked indoors than outdoors, especially with *A. minimus*. The overall decrease, however, was higher with *A. b. balabacensis* than with *A. minimus*.

4.1.3 Biting cycle

As in the first part of these studies, results of observations on the biting cycle are presented for two periods: May-August and September-October for *A. b. balabacensis,* and May-July (wet season) and November-February (dry season) for *A. minimus*.

Results with *A. b. balabacensis* during May-August and September-October, and comparison with pre-spraying results, are illustrated in Fig. 4. After spraying, the biting activity of this vector advanced towards earlier hours of the night. Indoors, the major biting peak occurred one hour earlier: 22.00–23.00 hours and 21.00–22.00 hours in the May-August and September-October periods respectively. A smaller, but marked, biting peak also occurred in the May-August period at 19.00–20.00 hours. A somewhat similar trend was noted outdoors, except that during the September-October period a greater percentage of the mosquitos was found to be biting earlier without change in the peak. Apart from these changes in both the indoor and outdoor populations, *A. b. balabacensis* continued to bite throughout the night, as was its habit prior to spraying.

Results with *A. minimus* during the dry and wet seasons, and comparison with pre-spraying results, are illustrated in Fig. 5. This vector showed a greater variation between its pre- and post-spraying biting activity than *A. b. balabacensis*. During the dry season 55.2% of its biting activity indoors occurred in the first hour (18.00–19.00). Biting decreased sharply in the second hour and continued to diminish until it reached a very low level in the second half of the night. Outdoor biting activity remained at a high level in the first two hours (18.00–20.00) and then during the rest of the night followed a similar trend to that of indoor biting. In the wet season biting started in the second hour (19.00–20.00) and most of it occurred in the first half of the night with a peak at 20.00–21.00 hours indoors and 21.00–22.00 hours outdoors. Perhaps these variations are due to the fact that *A. minimus* seems to aggregate near the host in the early hours of the night especially in the dry season.

The biting activity of both vectors after spraying suggests that the excito-repellency effect of DDT stimulated the mosquitos to bite earlier than usual, in particular *A. minimus*. Contact of *A. minimus* with sprayed surfaces before biting was therefore brief, and most probably shorter than in the case of *A. b. balabacensis*.

4.1.4 House frequenting behaviour as studied by the use of experimental huts

During the spraying rounds all the experimental huts, i. e. two verandah-trap huts and four portable-trap huts, were regularly sprayed with the same dosage of DDT. Procedures for operating the huts and recording the data were the same as those followed in the pre-spraying observations, with the exception that living mosquitos found indoors at 06.00 hours were collected instead of only counted as in the technique used by SMITH (1965 a). This modification was introduced because of the exophilic habit of the vectors. With rare exceptions only *A. b. balabacensis* were found in the huts. The overall decrease in the *A. b. balabacensis* population after spraying was also reflected in the results from the experimental huts. In the first year after spraying 1041 specimens were collected from the verandah-trap huts and 1011 from the portable-trap huts, while in the second year the yield dropped to 562 and 207 specimens respectively. The difference in the results between the two types of experimental huts was more marked in the second year, possibly because the more recent and frequent application of DDT had a greater deterrent effect (ZULUETA & CULLEN, 1963) in the more compact portable-trap huts.

(a) Effect of DDT on feeding habits

Results from both types of experimental huts showed an increase in the proportion of unfed mosquitos after spraying. In the verandah-trap huts (Table 3) the average percentage of unfed mosquitos was:

Table 3. Number of fed and unfed *A. b. balabacensis* collected from DDT treated verandah- and portable-trap huts

DDT spaying round	Number of days after spraying	Verandah-trap huts				Portable-trap huts			
		Fed		Unfed		Fed		Unfed	
		No.	%	No.	%	No.	%	No.	%
First	99–198	719	74.7	243	25.3	699	73.7	250	26.3
Third	69–120	166	72.5	63	27.5	44	59.5	30	40.5
Fourth	8– 87	191	57.4	142	42.6	64	48.1	69	51.9

Table 4. Mortality rates in *A. b. balabacensis* collected from DDT treated verandah- and portable-trap huts

Number of days after spraying	Corresponding dates	Verandah-trap huts						Portable-trap huts					
		Fed		Unfed		Total		Fed		Unfed		Total	
		Number collected	% mortality	Number collected	% mortality	Number collected	% mortality	Number collected	% mortality	Number collected	% mortality	Number collected	% mortality
		First DDT spraying round											
99–120	8–29 July 1972	84	44.1	42	69.1	126	52.4	43	32.6	11	81.8	54	42.6
130–151	8–29 Aug. 1972	378	30.7	121	66.9	499	39.5	394	26.6	167	65.9	561	38.3
161–182	8–29 Sept. 1972	217	35.5	54	81.5	271	44.7	206	37.4	63	76.2	269	46.5
191–198	8–15 Oct. 1972	40	50.0	26	80.8	66	62.1	56	33.9	9	100	65	43.1
99–198	Total	719	34.8	243	72.0	962	44.2	699	30.8	250	70.8	949	41.3
		Second DDT spraying round											
7–38	22 Oct.–22 Nov. 1972	42	61.9	37	86.5	79	73.4	24	70.8	38	92.1	62	83.9
		Third DDT spraying round											
69–90	8–29 June 1973	101	72.3	36	91.7	137	77.4	28	78.7	13	100	41	85.4
99–120	8–29 July 1973	65	61.5	27	66.7	92	63.0	16	81.3	17	100	33	90.9
69–120	Total	166	68.1	63	81.0	229	71.6	44	79.6	30	100	74	87.8
		Fourth DDT spraying round											
8–29	8–29 Aug. 1973	75	77.3	60	85.0	135	80.7	16	87.5	13	100	29	93.1
39–60	8–29 Sept. 1973	65	72.3	38	100	103	82.5	19	89.5	17	94.1	36	91.7
66–87	5–26 Oct. 1973	51	86.3	44	90.9	95	88.4	29	79.3	39	100	68	91.2
8–87	Total	191	78.0	142	90.8	333	83.5	64	84.4	69	98.6	133	91.7

(i) 25.3% (range 19.9–39.4%) in the period between the 99th and 198th day after the first round of spraying.

(ii) 27.5% in the period between the third (69th day) and fourth (120th day) month after the third round of spraying, that is to say 26.4% in the third month and 29.4% in the fourth month.

(iii) 42.6% (range 36.9–46.3%) in the period between the 8th and 87th day after the fourth round of spraying.

Results from the portable-trap huts (Table 3) were somewhat higher.

In order to assess the effect of DDT on the feeding habits the figure of 4.3% representing the proportion of unfed mosquitos before treatment, was deducted from the values indicated in Table 3. Accordingly it appears that for the verandah-trap huts the excito-repellency effect of DDT had inhibited 21, 23.2 and 38.3% of the mosquitos collected from these huts from taking a blood meal when DDT deposits were respectively in their 99th to 198th day, 69th to 120th day and 8th to 87th day after spraying.

(b) Effect of DDT on mortality

The overall mortality rate, as shown in Table 4, was calculated from the number of dead mosquitos found on the floor and in the window trap of the portable- and verandah-trap huts and from the number found dead after a 24-hour holding period of all live mosquitos.

In blood-fed mosquitos the mortality rate reached an average of 34.8% (range 30.7–50%) from the verandah-trap huts and 30.8% (range 26.6–37.4%) from the portable-trap huts in the period of 99–198 days after the first DDT spraying round. With the shortening of intervals between spraying rounds and the accumulated residues of DDT deposits, the mortality rate from the verandah-trap huts increased considerably reaching an average of 68.1% (range 61.5–72.3%) in the period of 69–120 days after the third spraying round, and of 78.0% (range 72.3–86.3%) in the period of 8–87 days after the fourth round. Results from the portable-trap huts followed a similar trend.

In unfed mosquitos the mortality rate from both the verandah- and portable-trap huts was higher than in blood-fed ones. Even in the period of 99–198 days after the first spraying round the mortality rate reached an average of 72% (range 66.9–81.5%) and 70.8% (range 65.9–100%) from the verandah- and portable-trap huts respectively. Occasionally mortality rates of 100% were found.

(c) Effect of DDT on egress from verandah-trap huts

Mosquitos made more use of the window traps after spraying, when 24% (1450 specimens) of mosquitos collected left through the window traps and the rest through the eaves, whereas before spraying only 13.5% (1800 specimens) left through the window traps. Similar proportions were also obtained with blood-fed mosquitos alone. Apparently, mosquitos were irritated by DDT deposits and stimulated to make more use of the nearest outlet. This result differs from the findings of SMITH (1965 b) with *A. gambiae* and SMITH & WEBLEY (1969) with recently blood-fed *A. gambiae, A. funestus* and *Culex pipiens fatigans* who reported that mosquitos made more use of the eaves in their egress after treatment with insecticides.

4.1.5 Outdoor daytime resting places and the human blood index

Efforts to locate the outdoor resting places of the two exophilic vectors, *A. b. balabacensis* and *A. minimus,* were pursued during the monsoon season, but remained without success. Consequently, in the absence of data from wild-caught, blood-fed specimens, no conclusion could be reached on host preference and on whether or not DDT might exert a deviation to animal hosts. However, the observations of EYLES et al. (1964) from Cambodia and SCANLON & SANDHINAND (1965) from Thailand indicated that, besides man, *A. b. balabacensis* was highly attracted to monkeys but not to domestic animals. During the entire period of the present study no monkeys were seen in the vicinity of the field station or even deep inside the forest. Except for the two water buffalos in the field station, there were no other large domestic animals inside the forest. In mosquito collections made around the water buffalos before and after spraying, *A. b. balabacensis* was rarely found in spite of its being present in high densities in contact with man. On the other hand *A. minimus* was found in fair numbers around the animals.

4.1.6 Gonotrophic cycle

Field laboratory observations showed that *A. b. balabacensis* took an average of three days and *A. minimus* an average of two days for their ovarian development during the monsoon season, at mean monthly temperatures of 21.8 °C minimum and 28.8 °C maximum. One full blood meal was found to be sufficient for this purpose, and the absence of half gravid females from all the field collections supports this finding. In the dry and cool season from November to February temperatures varied greatly. Monthly records showed that the mean maximum and minimum temperatures ranged from 23.4–28.2 °C to 10.3–15.0 °C respectively. During this period no observations were made on the ovarian development of *A. minimus.* However, in the cooler climate of Assam, India, THOMSON (1941) recorded a gonotrophic cycle of four to six days in *A. minimus* when temperature ranged between 23.3 and 10.3 °C.

In the study area where such observations were lacking, the temperature fluctuation was considered in comparison with Thomson's results, and on this basis the gonotrophic cycle of *A. minimus* was estimated to last four days. Based on the above figures a feeding rhythm of four days was estimated for *A. b. balabacensis,* and of five and three days for *A. minimus* during the months of November to February and the remaining months respectively.

4.1.7 Sporogonic cycle

Records on temperature for nearly four years favoured a sporogonic cycle of 10 days for *P. vivax* and 13 days for *P. falciparum* from April to September. With the decrease in temperature during the rest of the year, particularly in December and January when temperature was at its lowest level, the cycles were presumably retarded.

4.1.8 Parous rate

The parous rates were utilized to calculate the probability of daily survival and life expectancy in both vectors. The *A. b. balabacensis* population appeared to follow a specific pattern throughout its season (Table 5), longevity being at its highest at the start of the season, then progressively declining before rising again late in the season. In the pre-spraying period the values of 15.63, 5.24 and 11.36 days were estimated for May, July and September/October respectively. However, variations in this trend were noted in September 1972, possibly caused by the unusual climatic changes which occurred during that period. Following DDT spraying there was a decline in the estimated life expectancy in the corresponding early, middle and late periods of the season. The mean monthly values were estimated at 6.18 and 5.13 days in the first and second years after spraying against 9.22 days in the pre-spraying observations.

Results with *A. minimus* (Table 6) showed that in the wet season the highest longevity was noted early in the season, then progressively declined without a marked rise at the end of the season, as was the case with *A. b. balabacensis*. In the first year after DDT spraying there was no marked difference in the mean life expectancy while in the second year there was an increase from 4.81 days in 1972/1973 to 6.44 days. In the dry season higher values were noted than in the wet season. Estimated mean values on the life expectancy of *A. minimus* did not show a marked change in the first year after spraying while in the second year they fell from 13.3 days in 1972/1973 to 9.83 days.

That the life expectancy of *A. minimus* should have continued at the same level after spraying is most likely due to the highly exophilic

Table 5. Parous[a] and sporozoite rates[b], probability of daily survival[c] and estimated life expectancy of *A. b. balabacensis* in pre- and post-spraying observations

Month	Pre-spraying 1971 (A)			Post-spraying 1972 (B)			1973 (C)		
	% parous	p^4	% sporozoite	% parous	p^4	% sporozoite	% parous	p^4	% sporozoite
May	76.4 (335)	0.938	–	–	–	0 (8)			2.27 (44)
June	46.0 (322)	0.841	0.69 (725)	61.5 (96)	0.892	0 (93)	56.7 (187)	0.878	1.60 (188)
July	42.2 (1191)	0.826	0.54 (1295)	39.5 (205)	0.817	0 (207)	48.9 (131)	0.852	0.75 (134)
August	46.9 (606)	0.845	0.16 (612)	29.6 (989)	0.769	0 (983)	25.6 (161)	0.750	0 (161)
September	68.7 (728)	0.916	0.32 (626)	61.5 (540)	0.892	0.19 (538)	29.0 (217)	0.767	0 (220)
October	68.7 (252)	0.916	0.37 (270)	39.2 (669)	0.814	0.15 (656)	38.1 (223)	0.800	0 (222)
November	–	–	0 (19)	46.9 (64)	0.845	0 (64)	–	–	9.09 (11)
Mean	54.3 (3434)	–	0.45 (3547)	41.2 (2563)	–	0.08 (2549)	39.1 (919)	–	0.61 (980)

Life expectancy (in days)

	Range	Mean monthly values
(A) =	15.63–5.24	9.22
(B) =	8.77–3.80	6.18
(C) =	7.69–3.47	5.13

[a] Monthly samples with less than 50 mosquitos are not included.
[b] In the respective columns of the table, figures in parentheses represent the number of mosquitos examined.
[c] p^4 = probability of daily survival with the power of 4; probabilities were derived from GARRETT-JONES & GRAB (1964).

habit of this vector. However, the drop in the dry cool season from 13.3 days in 1972/1973 to 9.83 days in 1973/1974 could be attributed to climatological factors, as, in comparison with the previous seasons, temperature fell to its lowest level in 1973/1974. This may have incited a greater number of mosquitos to enter houses in search of warmth and consequently increased their contact with the insecticide.

4.1.9 Sporozoite rate

In the post-spraying period *A. b. balabacensis* infected with sporozoites continued to be detected (Table 5), though in the first year there was a drop to 0.08% from the pre-spraying rate of 0.45% and in the second year a rise to 0.61%. No infected *A. minimus* were detected as opposed to pre-spraying sporozoite rates of 0.16% and 0.12% in the wet and dry seasons respectively (Table 6).

4.1.10 Vectorial capacity

With the available data on biting and parous rates an attempt was made to calculate the theoretical epidemiological indices of the vectorial capacity of *A. b. balabacensis* and *A. minimus* using the following formula derived from MACDONALD (1957) and GARRETT-JONES (1964):

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

where m = mosquito density in relation to man
a = man-biting habit (frequency × human blood index)
(hence ma = man-biting rate)
p = probability of a mosquito surviving through one whole day
n = length (days) of extrinsic cycle of the parasite.

Under the conditions prevailing in the study area, data on the human blood index could not be obtained (see section 4.1.5), and therefore hypothetical values were used for this index: 0.9 for *A. b. balabacensis* (deviation to animal hosts after spraying considered to be nil) and 0.8 for *A. minimus* (allowing a deviation of 0.2 in favour of animal hosts after DDT spraying). The biting frequency was estimated at 0.25 for *A. b. balabacensis* and at 0.35 for *A. minimus* (in the wet season), while *n* for *Plasmodium falciparum,* which is the most prevalent parasite, was estimated at 13 days. For *m,* the mean values of the indoor and outdoor biting rates were used separately so that in turn the vectorial capacity of the indoor and outdoor populations could be estimated separately for each vector. Due to the wide variations in temperature during the dry, cool season, the vectorial capacity of *A. minimus* was estimated only for the wet season.

In order to cover an entire season, irrespective of its start or duration, the estimated vectorial capacity was calculated as being the sum of the monthly values, taking into account the results of all months in which samples of 50 mosquitos or more had been dissected for the parous rate (Table 7). The indoor population of *A. b. balabacensis* showed a decrease in its vectorial capacity of 10.6 times in the first year after spaying and a further three-fold decrease in the second year, the total

Table 6. Parous[a] and sporozoite rates[b], probability of daily survival[c] and estimated life expectancy of *A. minimus* in its wet and dry seasons in pre- and post-spraying observations

Month	Pre-spraying 1971/1972 (A)			Post-spraying 1972/1973 (B)			Post-spraying 1973/1974 (C)		
	% parous	$p^{3\ \&\ 5}$	% sporozoite	% parous	$p^{3\ \&\ 5}$	% sporozoite	% parous	$p^{3\ \&\ 5}$	% sporozoite
April	75.3 (85)	0.909	–	–	–	0 (10)	–	–	0 (28)
May	56.3 (199)	0.825	–	–	–	0 (43)	72.4 (105)	0.896	0 (105)
June	42.6 (136)	0.752	0.26 (384)	68.8 (215)	0.882	0 (212)	59.8 (239)	0.842	0 (238)
July	47.2 (199)	0.778	0 (200)	47.4 (156)	0.779	0 (160)	51.0 (245)	0.798	0 (245)
August	48.1 (52)	0.782	0 (60)	30.0 (60)	0.670	0 (60)	–	–	0 (29)
Mean	52.6 (671)	–	0.16 (644)	55.7 (431)	–	0 (485)	58.4 (589)	–	0 (645)
October	77.3 (75)	0.917	0 (58)	–	–	0 (32)	–	–	0 (24)
November	64.1 (454)	0.925	0.27 (377)	68.5 (89)	0.935	0 (89)	67.4 (196)	0.933	0 (195)
December	73.0 (534)	0.946	0 (534)	74.6 (126)	0.950	0 (126)	46.9 (207)	0.874	0 (209)
January	54.9 (233)	0.900	0 (218)	53.0 (181)	0.893	0 (181)	55.1 (374)	0.901	0 (372)
February	66.8 (358)	0.931	0.33 (301)	56.0 (91)	0.903	0 (91)	48.9 (188)	0.880	0 (188)
March	65.6 (215)	0.866	0 (215)	–	–	0 (29)	–	–	0 (32)
Mean	66.7 (1869)	–	0.12 (1703)	62.2 (487)	–	0 (548)	54.6 (965)	–	0 (1020)

Life expectancy (in days)

	Wet season Range	Mean monthly values	Dry season Range	Mean monthly values
(A) =	10.53–3.51	5.46	18.18–6.94	12.14
(B) =	7.94–2.49	4.81	19.61–8.85	13.30
(C) =	9.09–4.43	6.44	14.49–7.41	9.83

a Monthly samples with less than 50 mosquitos are not included.

b In the respective columns of the table, figures in parentheses represent the number of mosquitos examined.

c Probability of daily survival with the power of 5 during the cool months of November to February and the power of 3 for the other months; probabilities were derived from GARRETT-JONES & GRAB (1964).

Table 7. Estimated vectorial capacity of indoor and outdoor populations of *A. b. balabacensis* and *A. minimus* in pre- and post-spraying observations

	Pre-spraying	Post-spraying	
	1971 (A)	1972 (B)	1973 (C)
	A. b. balabacensis		
Corresponding period	May–Oct.	June–Nov.	June-Oct.
Vectorial capacity			
Indoor population	42.49	4.01	1.35
Outdoor population	46.08	10.51	2.56
Ratios	*(A) : (B)*	*(B) : (C)*	*(A) : (C)*
Indoor population	10.6 : 1	3.0 : 1	31.5 : 1
Outdoor population	4.4 : 1	4.1 : 1	18.0 : 1
	A. minimus		
Corresponding period	April–Aug.	June–Aug.	May–July
Vectorial capacity			
Indoor population	4.48	0.912	0.659
Outdoor population	4.66	1.92	2.52
Ratios	*(A) : (B)*	*(B) : (C)*	*(A) : (C)*
Indoor population	4.9 : 1	1.4 : 1	6.8 : 1
Outdoor population	2.4 : 1	0.76 : 1	1.9 : 1

decrease being 31.5 times. Corresponding values for the outdoor population were higher especially after spraying, with a total decrease estimated at only 18 times. On the other hand, the vectorial capacity of *A. minimus* was much lower than that of *A. b. balabacensis.* Before spraying similar values were obtained for the indoor and outdoor populations. After DDT spraying the indoor population showed a decrease estimated at 4.9 times in the first year and 1.4 times in the second year, the total decrease being 6.7 times. A lesser decrease was noted in the outdoor population where the final decrease was estimated to be only 1.9 times.

Calculation of the basic reproduction rate as proposed by MACDONALD (1957) showed that with the *A. b. balabacensis* indoor population a primary case will lead to 318, 32 and 9 new infections in the year before and the two years after spraying respectively. The corresponding values for the outdoor population were estimated to be 305, 89 and 20 new infections respectively.

4.2 Epidemiological surveys

4.2.1 Results of blood film examination

(a) Slide positivity rate

The number of persons examined increased from 392 in the pre-spraying observations to 592 and 595 in the two years after spraying, respectively. As shown in Table 8 the slide positivity rate decreased from 11.4% before spraying to 4.9% in the first year after spraying and in the second year remained at about the same level, i.e. 5.1%.

(b) Parasite formula

Results of blood film examination showed a parasite formula of 79.9% : 20.1% and 65.7% : 34.3% in the two years after spraying against 89.4% : 10.6% before spraying, all in favour of *P. falciparum* over *P. vivax,* but at the same time indicated a relative increase in the prevalence of vivax malaria. No significant change was noted in the gametocyte rate (Table 8).

(c) Parasite density index

The parasite density index (Table 8) did not appear to be affected by the spraying operations, remaining at the same class level of 5.1–5.4 throughout the pre- and post-spraying observations. The continued heavy infection is an indication of renewed transmission.

(d) Primary attacks

New cases continued to appear in the study area, with no marked change in the annual number detected. The primary attacks due to *P. falciparum* represented 80% in the first and 62% in the second year of post-spraying observations against 79% before spraying, while *P. vivax* represented the rest. This finding also indicates an increase in the relative prevalence of *P. vivax* as opposed to falciparum infections. This trend is probably due to the lower life expectancy of *A. b. balabacensis* following DDT spraying which consequently had a greater effect on the malaria parasite with the longer sporogonic cycle.

Because of the considerable amount of population movement, it was not possible to classify the malaria cases into six categories as in the pre-spraying observations (ISMAIL et al., 1974). Instead, cases were divided into three categories according to their place of residence, i. e. cleared zone, forest fringe and deep forest, as shown in Table 9. The populations of the cleared and fringe zones were completely covered by active case detection, while cases from the deep forest were mostly found when they came to the detection post. Except in the cleared zone, incidence of the disease did not show any marked change in the post-spraying observations.

Table 8. Blood-slide examination results, parasite density and number of primary attacks in the pre- and post-spraying observation periods

Period	Number of slides examined	Number of slides positive				Slide positivity rate	Number of slides with *falciparum* gametocytes	Parasite density index[a]	Number of primary attacks		
		P. falciparum	*P. vivax*	Mixed infection	Total				*P. falciparum*	*P. vivax*	Total
Pre-spraying											
4. 1971–3. 1972	1977	202	24	0	226	11.4	57	5.1	74	20	94
Post-spraying											
4. 1972–3. 1973	3116	123	31	0	154	4.9	20	5.3	86	21	107
4. 1973–3. 1974	3300	111	58	0	169	5.1	30	5.4	60	37	97

[a] As devised by BRUCE-CHWATT (1958).

Table 9. Incidence of malaria in the pre- and post-spraying observations

Period	Cleared zone			Forest fringe			Deep forest[a]			Total		
	Number of persons examined	Number positive	incidence %	Number of persons examined	Number positive	incidence %	Number of persons examined	Number positive	incidence %	Number of persons examined	Number positive	% incidence
Pre-spraying												
4. 1971–3. 1972	165	34	20.6	227	60	26.4	–	–	–	392	94	23.9
Post-spraying												
4. 1972–3. 1973	257	27	10.5	212	56	26.4	123	24	19.5	592	107	18.1
4. 1973–3. 1974	221	11	5.0	232	58	25.0	142	28	19.7	595	97	16.3

[a] In the pre-spraying observations no differentiation was made between people living in forest fringe and deep forest.

4.2.2 Role of the deep forest in malaria transmission during the dry season

During the post-spraying observations movement of population to the deep forest was highest in the dry season of 1972/1973. Out of the 107 malaria cases recorded from the study area in 1972/1973 as many as 42 were detected in the period November 1972–February 1973, and the majority came from the forest. This high number of cases indicates that active transmission took place inside the forest during the dry season. This finding was also supported by the results of the entomological investigations. Moreover it is worth noting that transmission occurred despite the low temperature prevailing at that time favouring the slow development of the gonotrophic and sporogonic cycles.

5. General Discussion and Conclusions

In the first part of this field research programme base-line data were collected on malaria and the habits of *A. b. balabacensis* and *A. minimus* in a forested hilly area in northern Thailand (Ismail et al., 1974). In this second part of the study 24 months of regular observations were made under adequate antimalarial measures, which included five rounds of DDT spraying with monthly mop-up and the detection, treatment and follow-up of malaria cases.

Entomological results showed a significant decrease in the population densities of both malaria vectors after spraying, especially *A. b. balabacensis* which decreased continuously throughout the two years after spraying, while *A. minimus* after a marked drop in the first year increased in the second. The contact of both vectors with man fell to a much lower level indoors than outdoors, so that outdoor contact exceeded indoor contact by about 2.5 times with *A. b. balabacensis* and 3.5 times with *A. minimus*. Before spraying, indoor and outdoor contact was equal with *A. b. balabacensis* and 1.6 times higher outdoors with *A. minimus*. Therefore spraying apparently gives some protection to people staying indoors.

Observations on the biting cycle showed that, with both vectors, biting occurred at an earlier hour after spraying. This change is apparently of no great epidemiological importance in the case of *A. b. balabacensis,* as earlier biting still occurred in the late hours of the night when most people are indoors. On the other hand, with *A. minimus* its earlier biting activity coincided, especially in the dry season, with the first hours of the night when many people are still outdoors.

The observations from the experimental huts showed that the excito-repellency effect of DDT appeared to stimulate a high number of *A. b. balabacensis* to leave without taking a blood meal. With the exception of the first spraying round, DDT deposits of up to four months from the date of spraying gave a mortality rate of 68–78% in blood-fed mosquitos and higher rates in unfed ones. The mortality rates following the initial round of spraying were lower. From the portable-trap huts particularly high mortality rates were found among unfed mosquitos, probably due to the compact size of these huts. These results alone cannot be applied to normal dwellings, as their structure obviously offers more and easier inlets and outlets and consequently fewer mosquitos are expected to pick up the lethal dose of insecticide. Moreover, houses and huts with fewer walls will attract more mosquitos as was observed by CHENG (1968) in Sabah, East Malaysia. Nevertheless, the results from the experimental huts do show that *A. b. balabacensis* is susceptible to DDT and indicate that the excito-repellency of DDT will stimulate a part of the mosquito population to leave dwellings without taking a blood meal, though most likely in a lower proportion than that obtained from the experimental huts.

The entomological indices were used to estimate the vectorial capacity in the indoor and outdoor populations of both vectors. Values obtained for *A. b. balabacensis* were much higher than those for *A. minimus.* During the monsoon season, the vectorial capacity of *A. b. balabacensis* was at its highest in the early part of the season and at its lowest in the middle part. The highest vectorial capacity of *A. minimus* was also in the early part of the wet season. In the post-spraying observations, *A. b. balabacensis* showed a total decrease estimated at 31.5 times in its indoor population and 18 times in its outdoor population. *A. minimus* showed a much smaller decrease estimated at 6.8 times indoors and 1.9 times outdoors. This result is probably due to the higher exophilic habit of *A. minimus.* The decrease in the vectorial capacity of the vectors was not met by a parallel reduction in the incidence of the disease. However, while the vectorial capacity was estimated for the mosquito population within the limited area of the field station, the parasitological results covered a larger area with different ecological conditions.

In relating malaria cases to the population living in cleared, forest fringe and deep forest zones, a decrease in the incidence of malaria was found in the first zone but not in the others. Population movement inside the deep forest, especially in the dry season and pre-monsoon period, contributed largely to this result. During that period *A. b. balabacensis* was apparently rare and transmission low in the forest fringe, whereas in the deep forest the vector was prevalent and maintaining transmission. Though *A. minimus* was also prevalent in both zones at that time, its role in maintaining transmission was inferior to that of *A. b. balabacensis.* The finding of a higher sporozoite rate in *A. b. balabacensis,* even after spraying, and a higher vectorial capacity supports this conclusion. From the above results two main deductions can be made:

(1) It appears that *A. b. balabacensis* is maintaining transmission all the year round inside the deep forest and only in the monsoon season in the forest fringe and its surrounding area. Favourable environmental conditions (e.g. dense vegetation, which ensures a high relative humidity and shade, coupled with the presence of permanent breeding sites) appear to persist deep inside the forest during the dry season. MASHAAL (1973)[6] reported cerebral malaria cases from inside the forest, along the border with Burma, during the dry season. He suspected *A. b. balabacensis* as the responsible vector. However, the low temperature prevailing during this period, especially in December and January, should slow down transmission.

(2) The gametocyte reservoir in the deep forest is expected to infect the prevalent vectors. It is likely that these infected mosquitos are responsible for starting transmission in the forest fringe and its surroundings, once conditions there become favourable in the early part of the monsoon season. The high vectorial capacity in both vectors during that period is an important contributing factor in the intensity of transmission.

The values obtained for vectorial capacity demonstrate the impact of DDT spraying on the mosquito populations, especially *A. b. balabacensis* and, in particular, indoors. Ecological changes, mainly rainfall and forest clearance, cannot be dismissed as possible additional factors of lesser influence, if any, on the results obtained. Forest clearance though limited, may nevertheless have had some effect as it leads to the disappearance of *A. b. balabacensis*. However, it favours the prevalence of *A. minimus* and with the development of cleared areas in the foothills *A. minimus* alone is expected to maintain transmission, though at a much lower level.

In conclusion, the present studies demonstrate that malaria eradication in the forested hilly areas of Thailand is not feasible with the available anti-malarial measures. These studies support the decision taken by the malaria project to change the strategy in these areas to a long range programme aiming at the reduction of the malaria reservoir and the protection of gains already achieved in the plain areas. For the implementation of this strategy the following recommendations are proposed:

(1) DDT spraying should be carried out on a total coverage basis in the forest fringe and on a selective basis in the deep forest. The detection and spraying of the individual and widely scattered huts in

[6] Assignment report on the malaria eradication programme in Thailand. WHO project: Thailand 0065 (unpublished WHO document SEA/MAL/95).

the deep forest obviously do not produce the required impact on the large population of exophilic *A. b. balabacensis*. Under these conditions selective spraying should be directed towards groups of dwellings used by a large number of people, labour camps, habitations in mining areas and stop-over sites along the main forest routes especially in border areas.

(2) Two spraying cycles per year are recommended to cover the season of *A. b. balabacensis* and *A. minimus* during the monsoon period. The first cycle is of great importance and should be completed no later than April, just before the beginning of the rains. The second cycle should follow after an interval of no more than four months.

(3) Because of the importance of the excito-repellency effect of DDT in reducing man-vector contact, it is suggested that spraying be intensified for the protected portions of the outside surfaces of dwellings, such as the eaves.

(4) Following clearance of the forest in the foothills, a spraying barrier of no less than 2 km wide should be maintained from the foothills towards the plains in order to strengthen the control of *A. minimus*.

(5) In order to allow treatment of the sick, reduce mortality and lower the risk of imported malaria cases in the plains, active and passive case detection together with administration of presumptive treatment should be intensified in the forest fringe and cleared foothill areas. Radical treatment should be carried out without delay.

(6) A close watch should be kept on any development of resistance in malaria parasites to the drugs in use, and appropriate measures taken if and when indicated.

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Zusammenfassung

In einem Gebiet bewaldeten Hügellandes im nördlichen Thailand wurde die Wirkung der DDT-Anwendung auf die Malaria und auf die Überträger *Anopheles balabacensis balabacensis* und *A. minimus* untersucht. In einer ersten Phase, Juli 1970 – März 1972, wurden Bezugs-Daten gesammelt. In einer zweiten Phase, die sich über 2 Jahre erstreckte, wurde DDT fünfmal appliziert und wurden alle Malariainfektionen einer Radikalbehandlung unterzogen. Zugleich wurden die entomologischen und epidemiologischen Erhebungen weitergeführt.

Die Ergebnisse der zweiten Phase zeigen, dass die Malaria-Übertragung infolge der optimalen Anti-Malaria-Massnahmen zwar eingedämmt, jedoch nicht unterbrochen werden konnte. Die Gründe dafür sind vor allem in der Human-Ökologie und in Wanderungen im Waldgebiet, namentlich während der Trockenzeit, zu sehen. Die Beobachtungen zur Übertragung in der frühen Monsunzeit erweisen, wie wichtig der Zeitpunkt der DDT-Applikation ist. *A. b. balabacensis* scheint die Krankheit im Waldgebiet während des ganzen Jahres zu übertragen, in der Zone des Waldrandes hingegen nur zur Monsunzeit. Die Fähigkeit zur Übertragung wurde für beide Mückenarten in und ausserhalb der Häuser getrennt untersucht. Die erhaltenen Werte vor der Anwendung des Insektizids waren für *A. b. balabacensis* wesentlich höher als für *A. minimus*. Nach Einsatz von DDT sank die Vektor-Kapazität für *A. b. balabacensis* 31,5 × in den Häusern, und 18 × ausserhalb derselben. Dagegen sanken die entsprechenden Werte für *A. minimus* um einiges weniger ab, nämlich 6,8 × in bzw. 1,9 × ausserhalb der Häuser.

Résumé

Les auteurs ont étudié les effets des pulvérisations de DDT sur le paludisme et sur les populations d'*Anopheles balabacensis balabacensis* et d'*A. minimus* dans une région de collines boisées du nord de la Thaïlande. Dans une première phase, de juillet 1970 à mars 1972, des données de référence ont été rassemblées. Dans une deuxième phase, cinq cycles de pulvérisations de DDT ont été effectués en

deux ans tandis que tous les cas de paludisme étaient soumis à un traitement radical. Au cours de ces deux années, des enquêtes entomologiques et épidémiologiques suivies ont été faites.

Les études de la deuxième phase ont montré que la transmission du paludisme avait diminué sous l'effet des mesures antipaludiques jugées optimales, mais qu'elle n'avait pas été interrompue. L'écologie humaine et les mouvements de population dans la zone de forêt, notamment pendant la saison sèche, ont très largement contribué à ce résultat. La transmission intense observée au début de la mousson montre clairement combien il importe de bien choisir le moment des pulvérisations. Il semble que *A. b. balabacensis* transmette le paludisme toute l'année dans la forêt dense, mais seulement pendant la mousson à la périphérie de la forêt. La capacité vectorielle des deux espèces a été évaluée séparément pour les populations endophiles et exophiles. Les valeurs observées avant les pulvérisations étaient beaucoup plus élevées pour *A. b. balabacensis* que pour *A. minimus*. Pour *A. b. balabacensis,* il est apparu que, sous l'effet des pulvérisations de DDT, la capacité vectorielle était devenue 31,5 fois plus faible dans la population endophile et 18 fois plus faible dans la population exophile. La diminution était beaucoup moins marquée pour *A. minimus,* elle était 6,8 fois plus faible pour la population endophile et 1,9 fois plus faible pour la population exophile.