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STUDIES ON THE BIOLOGY OF ANOPHELES CAMPESTRIS REID (DIPTERA, CULICIDAE) AND ITS RESPONSE TO RESIDUAL SPRAYING WITH DDT, CARRIED OUT IN EXPERIMENTAL HUTS IN PENANG, MALAYSIA

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INTRODUCTION

ANOPHELES campestris Reid is an important vector of malaria along the coastal plains of Peninsular Malaysia, and of filariasis caused by the periodic form of *Brugia malayi*. It is a member of the *A. barbirostris* group and was formerly known as the 'dark winged form' of *A. barbirostris*, later raised to specific rank by Reid (1962).

In earlier studies in experimental huts, Reid and Wharton (1956) reported that residual spraying with DDT at 2 gm/m^2 did not have the required effect on this species after the first two months. Later however, Moorhouse and Chooi (1964) reported that *A. campestris* disappeared after two cycles of DDT spraying at 2 gm/m^2 in a Malaria Eradiction Pilot Project (MEPP) carried out in a 500 sq. mile area in Selangor state of Peninsular Malaysia from 1960 to 1964.

A Malaria Eradication Programme (MEP) was inaugurated in Peninsular Malaysia in 1967 starting from the north western part of the country and proceeding south and east in the following years. The island of Penang received its first cycle of spraying with DDT water dispersible powder (wdp) at 2 gm/m^2 in the second semester of 1968, followed thereafter by two cycles a year. After two years of spraying, *A. campestris*, the main vector of malaria in the south western part of the Island, not only did not disappear as was expected after the MEPP experience, but continued to transmit malaria at a low level in the area.

From the very inception of MEP spraying on the island, there were objections from the house-holders to the whitish deposit of the DDT wdp, resulting in high refusal rates, as high as 10-15%. Even in houses that accepted spraying, many areas like the roof and parts of the house were not allowed to be sprayed. Spraying, therefore, was mostly partial and added to this, many house-holders systematically washed or wiped off the DDT soon after spraying which was evident in as many as 80% of the houses in one village. Therefore, neither the coverage nor the DDT residue left on the walls was in any way near the very good coverage achieved by the MEPP with the emulsion formulation. The failure to interrupt transmission appeared to be mainly due to the inadequacy or the absence of DDT residue in the houses. But there were suggestions that continued transmission was due either to the development or resistance to DDT in *A. campestris* and/or to some behaviour changes in the vector by which it avoided coming into sprayed houses but maintained outdoor transmission. The reasons for these suggestions were (a) the area had been sprayed with DDT as an anti-malaria measure sporadically for about ten years before the beginning of the programme. DDT had also been used in agriculture against rice pests, and rice fields are common breeding places for this species. Both uses might have contributed to the development of resistance, if any, even before the advent of MEP. (b) most of the *A. campestris* collected in the area after spraying were from man-biting collections outdoors and very few from indoor collections, which some thought was due to the avoidance of the DDT by the vector or to a

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change in behaviour from indoor to outdoor biting, thereby maintaining an outdoor transmission.

The present study was therefore undertaken to see if there was a change of behaviour in *A campestris* and if it avoided coming into sprayed houses and if transmission could be interrupted in the area by DDT residual spraying. At the same time, susceptibility of the vector to DDT and other aspects of its biology which would add to the knowledge of the species, were also studied.

MATERIALS AND METHODS

Study area

The study was undertaken in the South West District of the Island of Penang which lies off the west coast of the mainland on the northern extremity of the Straits of Melaka. The Island has a total area of 108 sq. miles (300 sq. km) with a central range of hills. The study kampong (village), Jalan Baru is situated in the south west part of the island in the district of Balek Pulau, a narrow strip of flat land cultivated with rice and coconut, ideal for *A. campestris* breeding. The inhabitants were mostly malay living in wooden houses usually arranged on either side of the main road. Most of the houses were of a better quality with wooden walls and galvanised iron or attap (woven nipah palm leaves) roof with a wooden floor raised about O.6m (2 ft) to 1.2m (4 ft) above ground level. The houses usually had many windows and other openings for ventilation and would be bright inside during daytime. The walls were often painted with wood preservative and sometimes even with paint and generally the houses were well kept.

Jalan Baru is a typical kampong in the area with about 315 houses and 1,450 population. It was selected for the study as malaria cases were being regularly reported from the kampong and it was therefore considered a problem. Entomological investigations had also shown a reasonable density of *A. campestris* in the kampong.

Experimental huts

To study the entry and exit behaviour of vectors, window traps fitted to kampong houses would be ideal. But the types of houses in this country with such a large number of openings, do not lend themselves to window trap study, as most of the mosquitoes would escape by other routes. The next best would be to make the observations in experimental huts, built to simumate local houses as far as possible, where entry and exit of mosquitoes could be controlled. In spite of the report by Moorhouse and Wharton (1965) that most species in this country were reluctant to enter trap huts, it was decided to give it a try at Jalan Baru with *A. campestris.*

Two huts of the type shown in Figure 1 were built in 1971 among kampong houses in Jalan Baru. Each hut was 3.5m (11.5 ft) long, 2.6m (8.5 ft) wide, had 2.4m (8 ft) high walls with a roof 3m (10 ft) high at the highest point. The wooden floor was raised O.76m (2.5 ft) above the ground. The walls were of wooden plank and roof of attap. Each hut had a door 2m (6.5 ft) x 1m (3.3 ft) and five entry louvres each 1.8m (6 ft) x O.6m (2 ft) with louvres at an angle of 30^{o} to the vertical and 3.8cm (1.5 in) apart. Four exit traps of the cone type each 38cm (15 in) cube were fitted one to each side of the hut.

Methods of collection

For indoor and outdoor biting collections, two collectors sitting down with their trousers rolled up to the knee, collected mosquitoes biting them, working 40 mins each hour and resting 20 minutes. To study the numbers entering the huts, local boys were hired to sleep in the hut for a small fee, the doors closed at 7 pm and hourly collections were made from window traps as well as the dead mosquitoes on the floor in the sprayed hut. For the study of the resting position and resting duration, the walls and roof were marked out into 1.2m x 1.2m (2 ft x 2 ft) numbered squares. Each hut was examined for resting mosquitoes by two collectors at 30 minute intervals and each mosquito seen was noted by the number of the square in which it rested and followed half-hourly till it left that position. This was done throughout the night and separate records were kept for fed and unfed mosquitoes.

All *A. campestris* collected were dissected for parous rates and all parous mosquitoes were dissected for gut and gland infections. Susceptibility tests on adults were carried out by the standard WHO test kit.

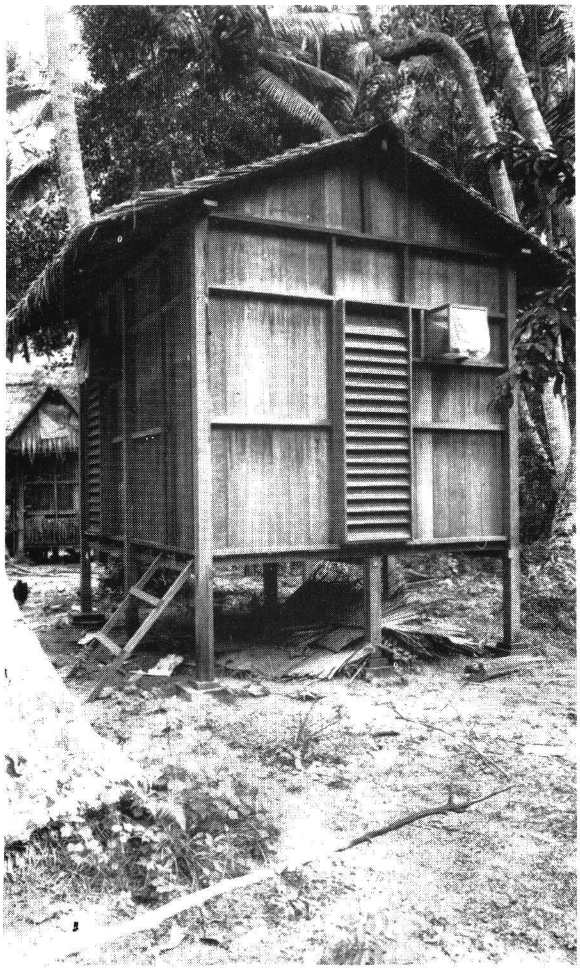

Fig. 1. Experimental hut in Jalan Baru, Penang.

BIOLOGY OF *A. CAMPESTRIS*

Most of the observations on the biology of *A. campestris* reported here were made in experimental huts which present an artificial situation to the vector and would not entirely represent conditions obtaining in nature. As this was the best possible that could be done the results are of value in understanding the behaviour of the vector. Unlike the observations made by Moorhouse and Wharton (1965), *A. campestris* readily entered the huts and during the study period of nearly two years, over two thousand mosquitoes were captured inside the experimental huts, this species forming nearly 95% of all the anophelines captured in the area.

Seasonal prevalence

Two years' results available from Jalan Baru on seasonal fluctuations of *A. campestris* are presented in Figure 2. In spite of the fact that the kampong was routinely sprayed at six monthly intervals as shown in the figure, fluctuation in density seen was not associated with the timing of spraying but rather to the rainfall. Rainfall figures from the Bayan Lepas airport which is a few kilometers away from Jalan Baru are also presented in Figure 2. May to August were the high density months for *A. campestris*, with peaks in June and July when 29.5 and 28.5 mosquitoes were collected per man-night, respectively, in 1972 and 10.5 and 14.5 for the same months in 1973. Another smaller peak was seen during December/ January. It could be observed that the peak density of *A. campestris* follows one to two months after the heavy rainfall months of April/May and September to December. The heavier rainfall of September to December produces a smaller peak in mosquito density and the shorter rainfall during April/May produces higher densities of vector.

The main breeding place in the area is a swamp skirting the kampong and a sluggish waterlogged drain running through it. Density fluctuations could be correlated to the state of water in the swamp and drain. During the very dry months there is little or no water at all for breeding and during the very wet months, with flooding and continuous flow of water, breeding is again considerably reduced. When there is just enough water with little movement as happens immediately after the rainy months, breeding increased as was confirmed by larval surveys, resulting in high densities of vector.

Malaria figures from the South West District (C2) of Penang in which Jalan Baru is situated and the figures for the kampong itself for the years 1971 to 1973 are given in Table I. The table shows an increase in cases from May up to about September which corresponds to the increase in vector density during the same period.

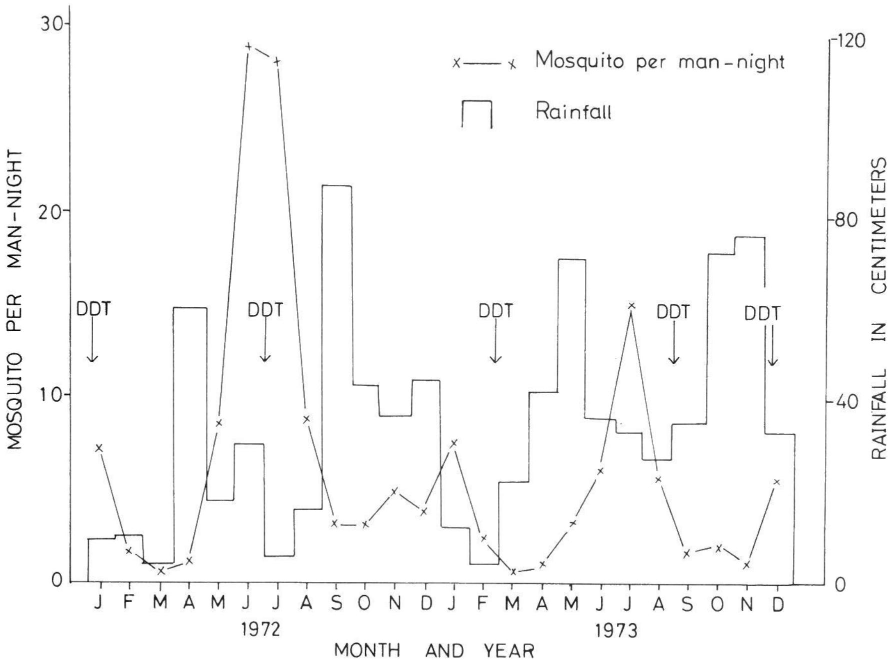

Fig. 2. Rainfall figures from Bayan Lepas and seasonal density of *A. campestris* obtained from Jalan Baru, Penang during 1972 and 1973.

Table I. Showing the number of malaria cases from Southwest District of Penang and Kpg. Jalan Baru for the years 1971, 1972 and 1973.

Year	Month / Area	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
1971	South West Dt. Penang	17	7	11	11	42	51	56	118	54	17	16	20	420
	Jalan Baru	0	0	0	1	11	10	15	16	5	2	0	0	60
1972	South West Dt. Penang	13	18	11	8	22	36	21	16	11	11	7	3	177
	Jalan Baru	2	0	0	0	5	3	3	0	0	1	0	0	14
1973	South West Dt. Penang	2	12	8	9	18	16	38	40	35	8	21	4	211
	Jalan Baru	1	0	0	0	3	2	2	1	3	0	0	1	13

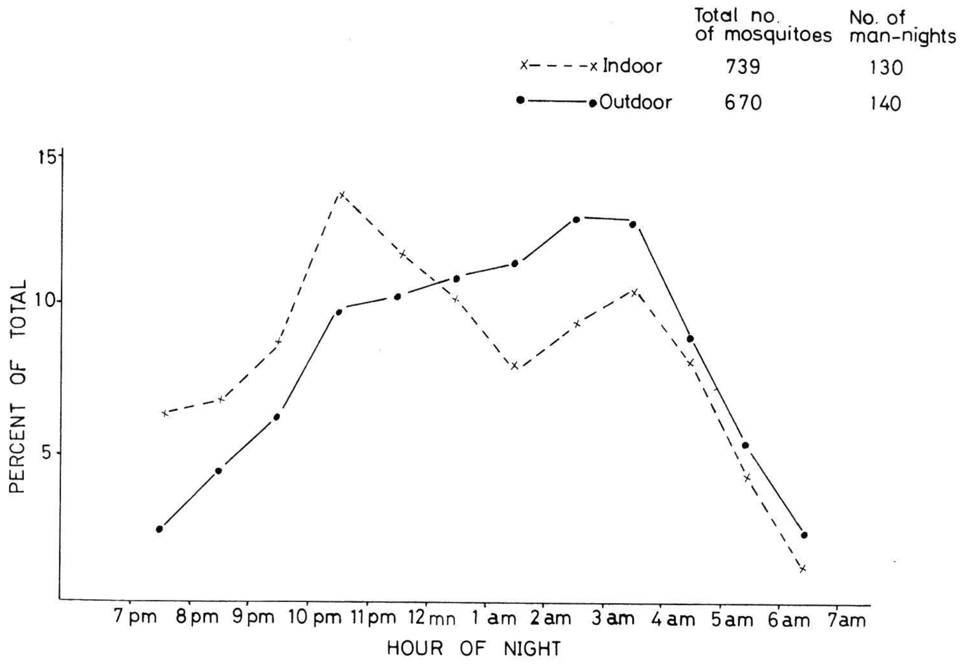

Fig. 3. Indoor [in experimental hut] and outdoor man-biting rhythm of *A. campestris* obtained by hourly catches at Kpg. Jalan Baru, Penang during 1972 and 1973.

Indoor and outdoor biting

Studies were carried out during 1972 and 1973 in about 140 man-nights in experimental huts and the results are presented in Figure 3. During this period, indoor biting collection of *A. campestris* was 739 (5.7 per man-night) and outdoor collection was 640 (4.8 per man-night). The indoor: outdoor biting ratio was 1.2 : 1 with a total of nearly 1,400 mosquitoes, showing a slight preference to indoor biting. Moorhouse and Chooi (1964) with about 100 mosquitoes collected in three nights had a ratio of 4.3 : 1 which shows a high degree of preference to indoor biting, where indoor biting was carried out in kampong houses. At the beginning of the present study, there was usually more outdoor biting than indoor biting in experimental hut, after which indoor biting increased considerably. This was probably due to the huts being new at the beginning gradually becoming more attractive after being slept in for several nights. It is probable that indoor biting : outdoor biting would be higher in kampong houses, than the present results indicate.

Biting rhythm

All night man-biting studies were carried out to see the biting rhythm of *A. campestris.* The results also seen in Figure 3 indicates that it bites right through the night, indoor biting gradually increasing to reach a peak between 2 am and 4 am. Outdoor biting starts off at a higher level from 7 pm and shows a fairly sharp peak between 10 pm and midnight and then again a smaller peak between 2 am and 4 am. These results are the average of about 140 man-nights' collections and a total of about 1,400 *A. campestris.* Moorhouse and Wharton (1964) also reported a peak biting between 8 pm and 2 am.

Egg laying

Over 80 wild caught fed females were kept for egg laying, out of which 49 laid a total of 7,997 eggs for an average of 162 eggs per female (range - 15 to 302). Thirty out of the 49 (about 60%) laid between 150 and 250 eggs.

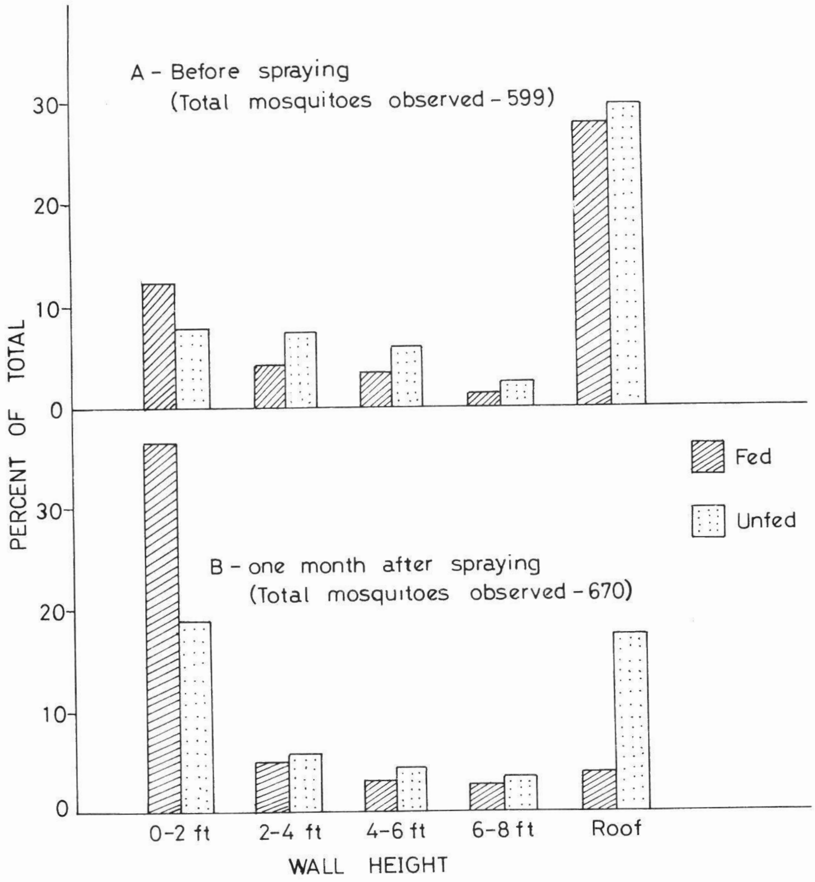

Fig. 4. Percentage of *A. campestris* resting at various heights of wall and roof of experimental huts at Kpg. Jalan Baru, Penang.

Gonotrophic cycle

The same 49 mosquitoes that laid eggs were also observed for the time taken from feeding to egg-laying, which is the gonotrophic cycle. The fed mosquitoes were collected from the experimental huts at three hourly intervals and egg-laying observed at 6 hourly intervals. It was therefore only possible to establish the time interval with a possible 9 hour error. Of the 49 mosquitoes, 37 (75%) laid eggs within 60 hours, 6 took 60 to 72 hours and the other 6 over 72 hours. As mosquitoes normally lay eggs in the early evening hours, it is reasonable to presume that the gonotrophic cycle for *A. campestris* is closer to two days than three.

Resting position

Experimental huts provide an artificial situation for this type of study where mosquitoes had to rest on walls or roof or sometimes on the floor, compared to the innumerable articles inside ordinary houses on which they could rest. The observations were made by noting the exact position of resting mosquitoes at half-hourly intervals. The results are presented in Figure 4 for observations made before and after spraying. Before spraying, 599 observations were made of which 56% were seen to rest on the roof, and 44% on the wall, the numbers on the wall decreasing progressively with height. This was repeated one month after spraying the hut, when

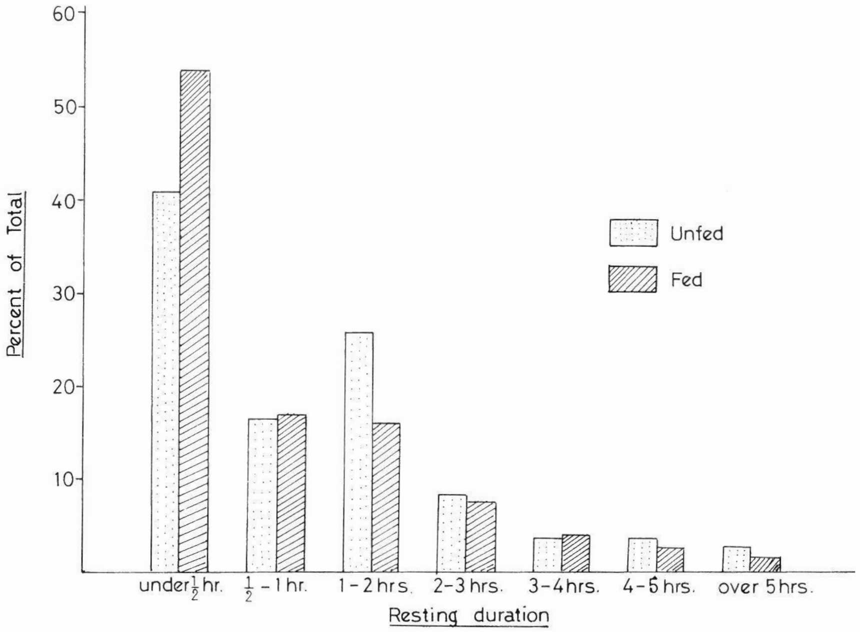

Fig. 5. Showing resting duration of *A. campestris* fed and unfed obtained during three nights in experimental huts in Jalan Baru, Penang during January 1972

670 observations were made and only 22% were found resting on the roof and 88% on the wall of which 54% were resting under 2 ft height. The results after spraying are normal as has been the experience in routine observations. The high percentage resting on the roof before spraying was probably due to the disturbance of the collectors and the flashing of the torch light when the mosquitoes might have been driven to the roof where there was less disturbance and they could go no further. It is relevant to mention here the number of observations do not represent the number of mosquitoes as the same mosquito would have been observed twice, once before feeding and once after feeding due to their moving to a different spot after feeding. If they had moved as a result of the disturbance, then the same mosquito could have been observed even more than twice. After spraying, when nearly 90% of the mosquitoes were killed in the hut and rested less than half an hour as presented later, the movement of individual mosquitoes would be minimal and the resting position observed gives a better picture of what happens in nature. The results for fed and unfed mosquitoes are presented separately and as was expected, 70% of the fed mosquitoes (35% of the total fed and unfed) were found resting below 2 ft after spraying.

Resting duration

To study the resting duration of fed and unfed mosquitoes, observations were made during three nights by noting them in their positions at half-hourly intervals. If it was seen at the same spot twice, then it would have rested at least for half hour and so on. Before spraying, a total of 494 observations were made. In the morning all mosquitoes in the huts and window traps were collected and the total collected during the same period was 230, which is a little less than half the total observations. If some mosquitoes had escaped from the hut, it is reasonable to presume that each mosquito was being observed at two different locations which is to be expected as each mosquito would change its first resting place after feeding.

The results presented in Figure 5 show that 41% of the fed and 54% of the unfed rested for less than half hour, 16% rested from half to one hour, 20% from one to two hours and becoming less and less for longer durations. Twelve unfed and four fed were observed to rest for over five hours. The average time computed for each mosquito was 54 minutes before feeding and 71 minutes after feeding, a total of 125 minutes (about 2 hrs) before and after feeding.

In about 670 observations made one month after spraying, one fed *A. campestris* was seen to rest between 1 to 1½ hours, 20 fed and four unfed between half to one hour and all the rest (over 96%) rested under half hour. During the six nights of observation, 437 *A. campestris* were collected from the sprayed hut of which 333 were found dead in the hut. This showed that a large number of *A. campestris* were being killed within half-hour of their coming to settle inside the hut.

Before spraying, the ratio of observations to the actual number of mosquitoes collected inside the hut was about 2:1 (490:230), whereas the same ratio was 1.5 : 1 (670:437) after spraying. This would mean that only half the mosquitoes were being observed twice after spraying unlike all the mosquitoes before spraying. This was confirmed by the fact that 57% of the *A. campestris* found dead in the hut were unfed showing that they were killed before they had a chance to feed, and were only observed only once.

The fact that no mosquito rested for over one and a half hours after spraying may indicate that they were being killed or left the hut within that time. Mortality figures in sprayed huts discussed later was a clear indication that a large percentage of vectors coming into the hut were picking up a lethal dose of DDT and being killed within the short period.

Daytime resting inside houses

A. campestris in this country has always been considered an endophilic (indoor resting) and endophagic (indoor feeding) vector. During six nights' observations in experimental huts it was found that only 32.6% of a total of 389 collected were found to rest inside the hut at 7 am, the majority leaving the hut through window traps during the night. Ordinary huts would probably be more attractive for the mosquitoes to stay indoors during the day, but Reid (1964) also states that although *A. campestris* has been known to be a highly endophilic species, probably the majority left houses to rest outside. The present findings support Reid's view.

Entry and exit

A. campestris was coming into experimental huts steadily right through the night as shown in Figure 3. In order to determine the time of exit, the window traps were emptied hourly, right through the night. Before spraying, 219 (about 80%) out of 263 collected in the window traps left the hut between 6 am and 7 am. During the other hours, a few were found to leave rather irregularly. After spraying, out of 381 collected from the window trap, only 45% left between 6 am and 7 am, the rest leaving at all hours of the night. It was also found that within two weeks of spraying, about 50% had left by 1 am and only 14% left after 6 am. Although most of those collected from the window traps died within 24 hours, the earlier time of exit may suggest some irritability to DDT which did not prevent them from picking up a lethal dose. There was no difference in the mortality rates of those leaving in the night compared to those leaving at the break of dawn.

Window traps had been set on each of the walls of the hut and the largest numbers left through the traps facing east.

Host preference

Reid and Weitz (1961) reported 63% of 27 *A. campestris* collected from day-time resting places had human blood. It has been almost impossible to collect *A. campestris* from day-time resting places to carry out precipitin tests. Therefore in 1973, a collection was made of fed mosquitoes from a net trap with two collectors and one cow acting as bait. The mosquitoes had an artificial but equal opportunity to feed on either man or cow. Of the 36 fed *A. campestris* collected from the net, only 8 (22%) had human blood and the rest bovid. Reid (1961) studying the attraction of mosquitoes to human and animal baits reported a man:calf ratio of 3.4:1 for *A. campestris.* Afifi (1965 unpublished) found that all 96 *A. campestris* collected during day-time from houses had human blood. In our experience too, in routine collections, most of the *A. campestris* are collected from man-biting collections, this species being very rare in animal bait collections where most of the other anophelines are abundant.

Transmission of malaria and results of dissections

During the two year period of study, a total of 2,136 *A. campestris* were dissected without finding a single sporozoite or oocyst infection. This was not unexpected as Reid (1962) reported that up to that time in over 15,000 dissections of this species, the sporozoite rate was only 0.33% when the malaria rate in the country was probably 20-40%. It was Reid's conclusion that *A. campestris* can only maintain a low level of transmission. At the time of the study, when the malaria rate in the area was less than 1% it was not surprising that no infected mosquito was found.

RESPONSE OF *A. CAMPESTRIS* TO RESIDUAL SPRAYING WITH DDT

After some preliminary observations in the two experimental huts lasting about five months to study the attractiveness of the huts for *A. campestris* to enter, one of the huts, was sprayed with DDT wdp at 2 gm/m^2, during May 1972. The walls and roof were sprayed, care being taken not to spray the entry louvres. The other hut was maintained as untreated control. Subsequent sprayings were carried out during November 1972, July 1973 and January 1974 with DDT emulsion at the same dose, in keeping with the change of formulation by the MEP in 1972.

Man-biting collections were continued in the sprayed and unsprayed huts monthly for an average of two nights per month. In addition, baits were made to sleep in the huts during three other nights of the month and collections were made hourly of dead mosquitoes on the floor in the sprayed hut and from window traps of both huts. Mosquitoes collected from window traps as well as those found resting inside the hut at 7 am were kept for 24 hour survival test.

Entry of A. campestris into sprayed hut

From the results presented in Table III and Figure 6 for collections carried out simultaneously in the sprayed and unsprayed huts, there appeared to be no significant difference in the numbers entering the sprayed hut compared to the unsprayed hut. One month and six months after spraying, the numbers in the sprayed hut were slightly higher than the unsprayed and on the other occasions the numbers in the unsprayed hut were higher. Observations were carried out in the huts for two years during which time there were four applications of DDT. The number of *A. campestris* collected during this period from the sprayed hut was 1,881 compared to 2,347 in the unsprayed hut. With about 50% to 75% of the collections from the sprayed hut being found dead on the floor, there was a possibility that some of these could have been missed, and the difference between the huts could not be of significance. Added to the fact that such a large number was collected from the sprayed hut is proof enough that the *A. campestris* in the area had not changed its behaviour from an indoor biter to an outdoor biter and that it will readily enter DDT sprayed houses. The negligible number of vectors collected biting indoor during routine collections after spraying may be due to a combination of many factors like (a) the very low densities of this species generally after spraying (b) that the vectors coming in were being killed by the DDT before they have a chance to bite and (c) in a kampong house the mosquitoes have a choice between one or two collectors and five or more occupants inside, whereas outdoors the collectors sit alone.

Mortality of A. campestris in sprayed hut

It has been possible to observe mortality in the sprayed hut after two successive cycles of DDT spraying, and the results are presented in Table II and for the first cycle only in Figure 6. After the first spraying, over 75% mortality was obtained up to the 6th month after spraying. During four of the monthly observations, the mortality was over 85%. On the completion of six months, the mortality dropped to 51%, and the hut was re-sprayed, this time with DDT emulsion. After the second spraying, the mortality remained high up to the 7th month after which it dropped to 55.4% and after eight months mortality was only 9.4%.

Usually the mortality within the first two weeks after the spraying was not so high, being about 65 to 75%, but increased after this period. This was probably due to the irritant effect of the fresh DDT deposit which might have driven away the mosquitoes before they

Table II. Mortalities of *A. campestris* in experimental hut after spraying with DDT 2 gm/m^2, obtained at Jalan Baru, Penang during 1972 and 1973.

No. of months after spray	No. dead on floor			No. found alive in hut at 7 am			No. found in window traps			Total collection			No. Survived			Mortality %
	F	U	T	F	U	T	F	U	T	F	U	T	F	U	T	
0-½ *	25	6	31	4	4	8	10	10	20	39	20	59	10	4	14	76.3
1	125	224	349	9	3	12	48	50	98	182	277	459	45	11	56	87.8
2	24	100	124	4	2	6	27	24	51	55	126	181	23	5	28	93.0
3	61	13	74	4	1	5	18	8	26	83	22	105	9	3	12	84.8
4	30	6	36	0	0	0	4	3	7	34	9	43	4	2	6	86.4
5	11	8	19	0	0	0	8	1	9	19	9	28	7	0	7	75.0
6	39	12	51	1	2	3	29	17	46	69	31	100	30	19	49	61.0
0-½ a	8	1	9	0	0	0	3	7	10	11	8	19	11	8	19	68.4
1	44	3	47	1	0	1	10	4	14	55	7	62	8	1	9	85.5
2	15	2	17	0	1	1	3	2	5	18	5	23	2	1	3	86.9
3	8	0	8	0	0	0	3	0	3	11	0	11	3	0	3	72.3
4	1	0	1	0	0	0	1	0	1	2	0	2	0	0	0	100
5	4	0	4	0	0	0	2	2	4	6	2	8	0	0	0	100
6	0	0	0	0	0	0	11	0	11	11	0	11	0	0	0	100
7	9	2	11	0	3	3	30	12	42	39	17	56	20	5	25	55.4
8	0	0	0	0	0	0	31	1	32	31	1	32	29	0	29	9.4
Total	404	377	781	23	16	39	238	241	379	665	534	1199	201	59	260	

* Sprayed with wdp

a Sprayed with emulsion

F = Fed, U = Unfed, T = Total

could pick up a lethal dose, most of the survivors being from window trap collections. Reid and Wharton (1956) reported, from a similar experiment in Negri Sembilan, Malaysia, where DDT emulsion had been used, 72% mortality during the first month, 32% during the second month and that DDT failed to kill *A. campestris* in subsequent months. However, in the MEPP in Selangor, Moorhouse & Chooi (1964) reported that *A. campestris* totally disappeared from the area after only two cycles of spraying with DDT emulsion.

During the 14 months of observation, a total of 1,199 *A. campestris* were collected from the sprayed hut, of which 781 (65%) were found dead in the hut. Of these 404 (51.7%) were fed and 382 (48.3%) unfed. Of the ones caught in the window trap, about 80-85% died within the 24 hour holding period. The results therefore are a clear indication that *A. campestris* was being killed in sufficient numbers and for a sufficiently long period by the DDT spraying.

Tables III. Parous rates of *A. Campestris* obtained in Jalan Baru, Penang, during 1972 and 1973.

Month	1972		1973	
	No. diss.	% Parous	No. diss.	% Parous
Jan	126	37.3		-
Feb	90	62.2	53	30.2
Mar			8	37.5
Apr			12	33.3
May	78	39.7	40	60.0
Jun	442	26.2	57	54.4
Jul	439	40.1	79	41.8
Aug	127	44.1	48	35.4
Sep	137	37.2	13	23.1
Oct	106	38.7	18	33.3
Nov	89	58.4	9	66.7
Dec	89	31.5	76	26.3

Months of spraying of Jalan Baru -

Feb 71, Jul 71, Jan 72, Jul 72, Feb 73, Aug 73, Dec 73.

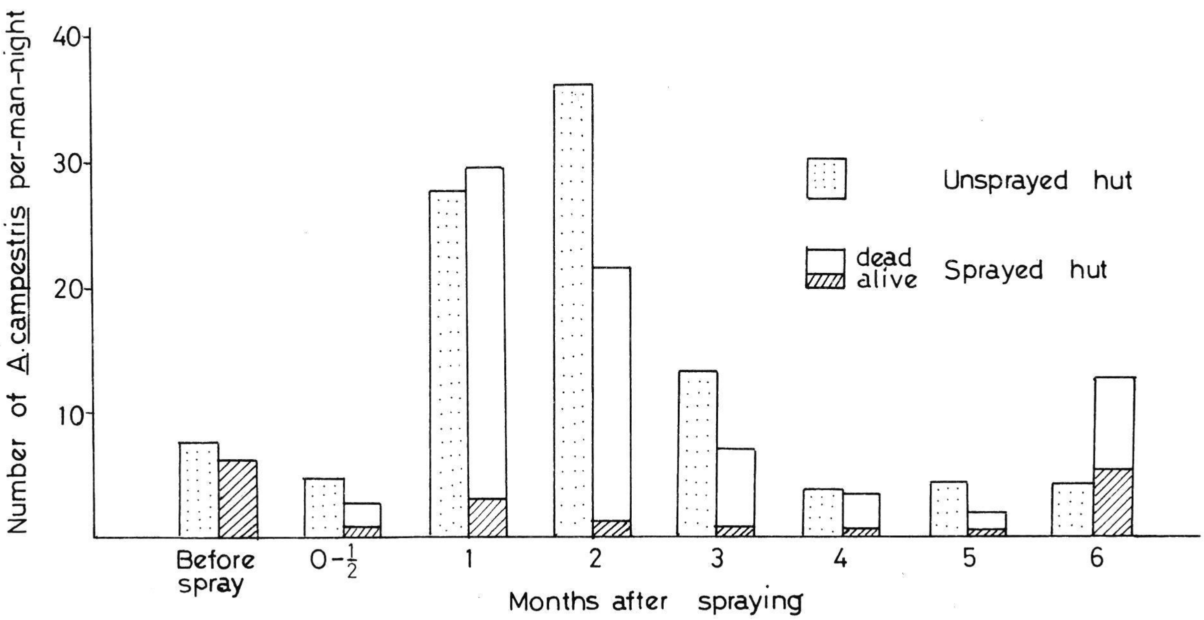

Fig. 6. Showing the number of *A. campestris* caught in sprayed and unsprayed huts at Jalan Baru, Penang.

SUSCEPTIBILITY OF *A. CAMPESTRIS* TO *DDT*

Several tests were carried out at Jalan Baru and in neighbouring kampongs from 1972 onwards to determine the susceptibility of *A. campestris* to DDT. When mosquitoes were available in sufficient numbers, a full series of tests were carried out exposing them to concentrations ranging from 0.25% to 4% DDT. When numbers were small, all were exposed to the discriminating dose of 4% DDT. When there were survivors to 4% DDT at one hour exposure, then 2 hour exposure with 4% DDT was tried. In mid 1972 several series of tests carried out at Jalan Baru with a total of 358 mosquitoes gave an LD_{50} of 1.4% DDT. No pre-spray figures are available from the area but Afifi (1966) reported an LD_{50} of 0.66% DDT for the species from Sabak Bernam, an unsprayed area in Selangor state. A test carried out during the present study, from the same area in 1972 (also before MEP spraying) although not with sufficient numbers, gave only 84.4% mortality with 4% DDT, similar to the figures from Jalan Baru. Being a rice growing area, there was probably extensive use of DDT between 1966 and 1972 in agriculture which may explain the later results. Wharton (1958) reported an LD_{50} of 1.3% DDT for laboratory bred *A campestris.*

From January 1972, in most of the tests carried out at Jalan Baru and neighbouring areas, there were a few survivors to one hour exposure to 4% DDT. Two hour exposure to 4% DDT did give 100% kill in all except in one instance in October 1972 when 3 out of 92 survived the 2 hour exposure.

The mortality regression lines for the Sabak Bernam tests in 1966 and the Jalan Baru tests in 1972 are given in Figure 7. The slope of the curve which is a rise per unit horizontal distance expressed as $2\ \frac{LC_{84}}{LC\ 50} + \frac{LC_{50}}{LC_{16}}$ is 0.51 for Sabak Bernam and 0.46 for Jalan Baru. The latter being less steep indicates a greater variability in the population and a shift to the right indicating an increase in tolerance. This in addition to the fact that 2 hour exposure to 4% DDT failed to give 100% mortality at least once, indicates a trend towards the species building up resistance to DDT in the area although still susceptible to DDT up to 1974.

DDT had been used as a malaria control measure in the South West District of Penang for over ten years before the MEP started, the spraying not being on a regular basis but carried out at times of malaria outbreaks. In addition, DDT would have been used extensively for agriculture on rice which is extensively grown here. Pre-spray figures for the area not being available, one cannot conclusively state that there is any change in the tolerance of *A. campestris* in the area to DDT although the results are suggestive of this fact, and the change if there was any was probably due to the earlier use of DDT for malaria control as well as in agriculture.

PAROUS RATES AND DAILY MORTALITY

Dissection for parous rates were carried out regularly during the two years and the results are presented in Table III. In over 2,000 dissections carried out during the period, the overall parous rate was 40.8 compared to 65% before spraying. It was difficult to correlate the monthly rates with the timing of spraying but generally the rates were higher 3 to 4 months after spraying. The daily mortality obtained by the formula $1-\sqrt[2]{\text{parous rate}}$ for a two day gonotrophic cycle was about 37% in 1972 compared to 24% in 1971.

There was an improvement in the spraying coverage in the area in mid-1972 which was mainly due to the change in formulation of DDT from wdp to emulsion which also resulted in far less wipe off of the DDT by the people. This was probably the main reason for the increased mortality in 1972 and 1973. The malaria situation had also shown a marked improvement in Jalan Baru in 1972 and 1973 as seen in Table I. In 1971 there were 63 cases which came down to 14 in 1972, 13 in 1973 and 2 in 1974. There was therefore reason to conclude that with a good spray coverage with DDT, transmission of malaria by *A. campestris* could still be interrupted in the area.

SUMMARY

Studies on the biology of *A. campestris* and its response to residual spraying with DDT in experimental huts at Jalan Baru, Penang, are presented. High density months were found to be May to August with peak in June/July and another smaller peak in December/January.

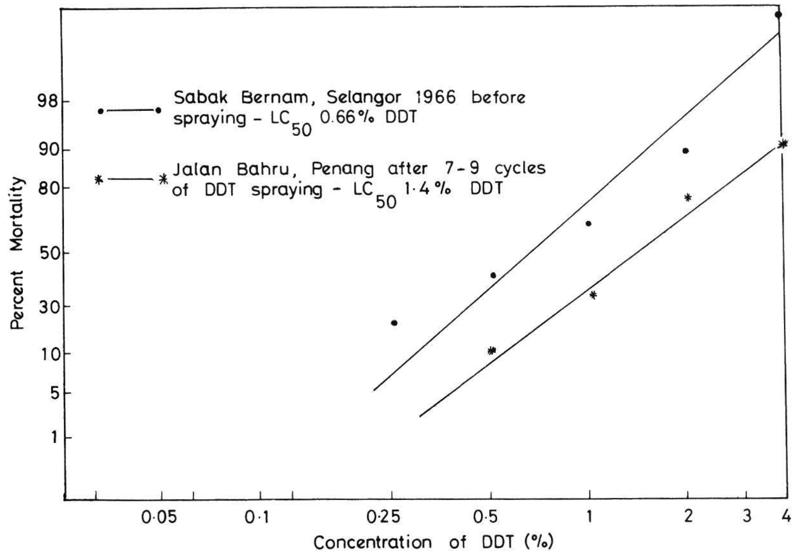

Fig. 7. Dosage mortality curve of *A. campestris* from Sabak Bernam, Selangor before spraying and from Jalan Baru, Penang after 4 years of spraying.

Indoor to outdoor biting ratio was 1.2 : 1 obtained with about 1,400 campestris during 140 man-nights. Indoor biting was fairly uniform right through the night with no marked peak, but increasing after midnight up to 4 am. Outdoor biting had a distinct peak from 9 pm to midnight and a smaller peak between 2 and 4 am. Average number of eggs laid by each female was 163 and the gonotrophic cycle was about two days.

Before spraying, it was seen that 56% rested on the roof and the rest on the wall, decreasing progressively with height. One month after spraying only 22% rested on the roof and 54% under 2 ft of the wall. Before spraying unfed mosquitoes rested for an average of 54 minutes and fed mosquitoes for 71 minutes. After spraying, only a few rested for more than half hour.

Of the *A. campestris* entering the hut, only 32.6% were found to remain inside the hut at 7 am the rest having left the hut by that time, the majority leaving between 6 am to 7 am before spraying. After spraying, about 45% left between 6 am and 7 am and 50% left before 1 am. Given the choice between cattle and human blood, 22% chose human and the rest cattle.

DDT at $2 gm/m^2$ gave a good kill of about 75% up to the end six months after which the mortality declined. There was no difference in the numbers entering the sprayed and unsprayed huts. The LC_{50} was about 1.4% DDT after 10 cycles of spraying with an odd mosquito surviving two hour exposure to 4% DDT. There was no evidence of any resistance in *A. campestris* at the time of reporting although there appears to be an increased tolerance. Dissections for parous rates are reported which could not be correlated with the DDT application in the area.

It is therefore concluded that *A. campestris* continues to enter sprayed houses to bite and would still be killed by residual spraying with DDT in the area up to the time of reporting.

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