

# Studies on the Relationship between Fluorescent Antibody Response and Ecology of Malaria in Malaysia \*

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*The fluorescent antibody (FA) technique was used to detect the presence of malarial antibody in populations living in 3 different ecological areas of Malaysia. Serum samples were tested using Plasmodium falciparum, P. vivax, P. malariae and P. fieldi antigens. An area of hyperendemic malaria had a good correlation between the antibody responses and active parasitaemias. The percentage and intensity of responses increased with the age of the individuals. In an area of hypoendemic malaria, each of 17 sites had ecological conditions which would favour or discourage the transmission of malaria. The reasons for high FA responses in some villages and low responses in others were readily apparent. The effect of even limited control programmes on the malarial ecology could be measured by an examination of the antibody responses. An aboriginal population receiving suppressive drugs had FA responses indicating both past experience and the effect of the drug programme.*

In recent investigations on various aspects of serological response in malaria, a series of methods, in addition to the classic examinations of blood-films, have been introduced to evaluate the status of malaria in given populations. The fluorescent antibody (FA) technique has been used extensively in the laboratory for various studies where the detection of the presence of malaria antibody is needed. In addition, several investigators have carried out studies on sera from individuals living in malarious areas of Liberia (Voller & Bray, 1962), Gambia (McGregor et al., 1965) and Nigeria (Collins et al., 1967).

Early in 1966, studies on the ecology of primate malaria were carried out in 3 different environmental areas in Malaysia. Sera for FA studies were collected from approximately 2000 people as part of these studies. The results of these serological investigations are presented in this report and are also

compared with the known parasite rates and with previous efforts at antimalarial treatment in the same populations.

## MATERIALS AND METHODS

Blood samples were collected by venepuncture and an aliquot was placed on a clean glass slide. From this pool of blood, both thick and thin blood film preparations were made. These smears were stained with Giemsa stain and examined microscopically to determine the species of *Plasmodium* present. From the same pool, approximately 0.1 ml of blood was drawn into a small pipette and transferred to a piece of Whatman No. 3 filter-paper. After they had been dried, the filter-papers, interleaved with wax paper, were sent by air-mail to our laboratory in Chamblee, Ga., USA, where they were stored at  $-20^{\circ}\text{C}$  until examined.

The procedures and equipment used in these tests are essentially the same as those used in our previous studies (Collins et al., 1967). Antigen was obtained from patent infections of *P. falciparum*, *P. malariae* and *P. vivax* induced in human volunteers at the Laboratory of Parasite Chemotherapy, Unit on Human Malaria, Atlanta, Ga., USA, and from infections of *P. fieldi* induced in *Macaca mulatta* monkeys

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in our own laboratory. Antigen slides were prepared when the majority of the parasites were mature trophozoites. The stages of *P. falciparum* were mature enough to exhibit pigment under dark-field microscopy. The number of parasites ranged from 1 to 3 in the field of the microscope under a magnification of  $\times 800$ . Antigen slides were stored without fixation at  $-70^{\circ}\text{C}$  until they were used.

The blood spots were cut from the filter-paper with scissors, rolled and put into a 2-inch (5-cm) section of plastic tubing, the bottom of which was closed with a clamp. To this was added 1.0 ml of phosphate buffered saline of pH 7.1 and the mixture was allowed to stand for 30 minutes. The contents of the tube were squeezed out by means of a hand vice into a clean vial. This sample was designed the 1 : 10 dilution and serial 2-fold dilutions were then made from it.

Fluorescence was arbitrarily graded from 1+ to 4+. A reading of 2+ was considered positive

and reproducible. The highest serum dilution giving a 2+ reading was taken as the FA serum dilution end-point. Each of the samples examined was tested simultaneously against all 4 antigens.

## RESULTS

### Area I

All samples from this area were collected from 9 villages (*kampongs*) located in the State of Perlis in north-western Malaysia. In this area, as well as in areas II and III, a minimum of 60% of the individuals in a village were sampled. The exact location of the study area is shown in the accompanying map and the sites studied are identified by number. Three villages, Padang Mawar, Kok Mak and Sena (site 4) are adjacent and are considered together so that there are only 6 sites located on the map. This area is characterized by monsoon forests which are replaced by very thick secondary

## STUDY AREAS IN MALAYSIA

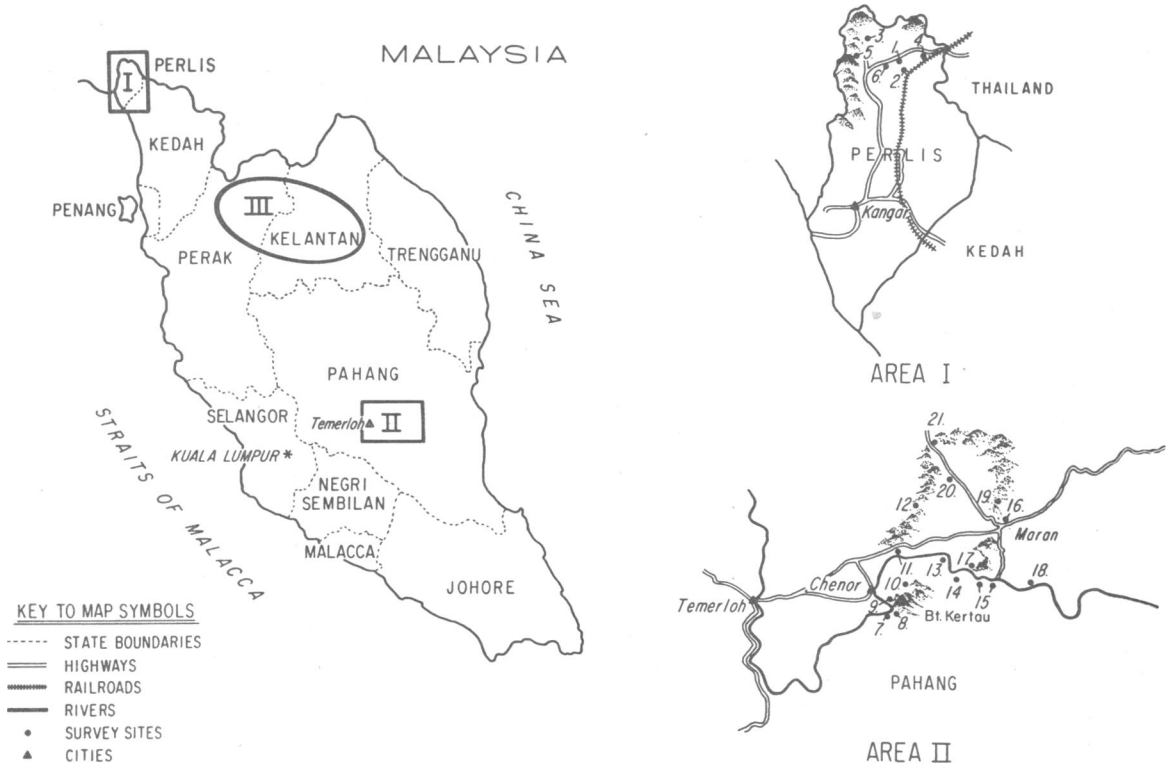


TABLE 1  
DISTRIBUTION OF FA TITRES FROM PERLIS, MALAYSIA (AREA I),  
USING 4 *PLASMODIUM* ANTIGENS

Antigen	Age-group (years)	FA serum dilution end-point								No. of sera	Mean titre
		<1:10	1:10	1:20	1:40	1:80	1:160	1:320	1:640		
<i>P. falciparum</i>	≤5	5	9	6	7	1				28	1:15
	6-10	9	11	14	25	15	5	1		80	1:30
	>10	23	25	31	76	66	46	18	4	289	1:48
	Total	37	45	51	108	82	51	19	4	397	1:40
<i>P. malariae</i>	≤5	3	14	8	3					28	1:13
	6-10	14	32	16	13	4		1		80	1:15
	>10	18	66	64	51	59	25	6		289	1:30
	Total	35	112	88	67	63	25	7		397	1:25
<i>P. vivax</i>	≤5	11	12	4	1					28	1:9
	6-10	10	32	25	11	2				80	1:14
	>10	29	72	81	68	29	9	1		289	1:21
	Total	50	116	110	80	31	9	1		397	1:19
<i>P. fieldi</i>	≤5	1	7	9	7	4				28	1:23
	6-10		10	17	27	18	7	1		80	1:39
	>10		34	38	66	82	50	19		289	1:55
	Total	1	51	64	100	104	57	20		397	1:48

growth near the villages. The flat, heavily cultivated land is frequently broken by precipitous limestone outcroppings and a ring of rugged limestone hills dominates the northern part of our study area. The rainy season usually begins late in October and extends through February with a secondary wet period in April or May. The primary vector of human malaria is apparently *Anopheles balabacensis balabacensis* with *A. maculatus* and *A. campestris* contributing to the problem as secondary vectors. Since *A. b. balabacensis* breeds in transient pools on the jungle floor, mosquito populations are markedly dependent upon the extent and duration of the rains. Malaria transmission can be expected to begin 3-4 weeks after the onset of the rainy season and remains intense until about 1 month after the rains have ceased. The dry season is free of malaria transmission and this period may be lengthened or

shortened according to the monsoons. The studies here were carried out in February 1966, when anopheline populations were high. There was no routine administration of prophylactic therapy in this area although chloroquine was available by purchase or was administered on a treatment basis at the local health clinic. The distribution of the FA responses obtained from the sera from this area is presented in Table 1. Here the results have been grouped according to 3 different age-groups, i.e., 5 years of age or less, 6-10 years, and older than 10 years.

The geometrical mean of the reciprocal titre was determined using the formula

$$GMRT = \text{antilog} [\sum_f (\log X) / N],$$

where *X* is the reciprocal titre, *N* is the number of sera tested and *f* is the number of sera with a given titre. The reciprocal of this number is then presented

TABLE 2  
ANTIGENS GIVING MAXIMUM RESPONSES TO SERA WITH A TITRE OF 1 : 20 OR MORE TO SERA FROM PERLIS,  
MALAYSIA (AREA I)

Site No. <sup>a</sup>	Village	No. of sera	Responses $\geq$ 1 : 20		Antigen					
					<i>P. falciparum</i>		<i>P. vivax</i>		<i>P. malariae</i>	
			No.	%	No.	%	No.	%	No.	%
1	Titi Tinggi Ulu	82	76	93	57	75	11	14	21	28
2	Titi Tinggi Hilir	38	38	100	31	82	0	0	14	37
3	Wang Kelian	50	47	94	47	100	3	6	9	19
4	Padang Mawar, Kok Mak and Sena	106	95	90	72	76	9	9	53	56
5	Kaki Bukit Rubber Estate	34	26	76	18	69	6	23	9	35
6	Chantek	87	65	75	49	75	12	18	20	31
	Total	397	347	87	274	79	41	12	126	36

<sup>a</sup> See map.

as the mean FA response. All those samples with a response of less than 1 : 10 were arbitrarily designated as having a level of response of 1 : 5 in the calculation of the mean FA titres for a group. The mean FA response of the youngest age-group to the *P. falciparum* antigen was 1 : 15. The intermediate age-group had a mean response of 1 : 30 and the oldest age-group had a mean response of 1 : 48. It is apparent that the mean FA response increased with age not only to this antigen but to the other antigens as well. The mean responses for the *P. falciparum*, *P. malariae* and *P. vivax* antigens regardless of the age were 1 : 40, 1 : 25 and 1 : 19, respectively. The *P. fieldi* antigen gave mean responses higher than any one of the human species (1 : 23, 1 : 39 and 1 : 55) for each of the 3 age-groups.

Since, in our experience, a level of response of 1 : 10 or less is quite questionable with regard to an indication of past or present malaria, a comparison was made of the responses of 1 : 20 or greater for the 9 villages examined. The results of this examination of the data are presented in Table 2. In addition, the number and percentage of the maximum responses<sup>1</sup> for the different human infecting antigens are listed. A total of 347 of the 397 serum samples (87.4%) gave responses to at least one of these antigens at a level of 1 : 20 or greater. Maximum responses to antigens were 79%

to the *P. falciparum* antigen, 12% were to the *P. vivax* antigen and 36% were to the *P. malariae* antigen. The presence of equal maximum responses accounted for the total in excess of 100% for the different antigens.

The prevalence of malarial infection, based on single examinations when the serum samples were collected, is shown in Table 3. It can be seen that the *P. falciparum* parasite was present most often; 148 of the 176 positive individuals were infected with this parasite. The second most common parasite was *P. vivax* and only 2 infections with *P. malariae* were observed. A total of 12 mixed infections with *P. falciparum* and *P. vivax* were found. A total of 57% of the individuals 5 years of age or less were infected; 65% of those 6-10 years of age were positive (which is the basis for terming this area as hyperendemic for malaria) and 42% of those over 10 years of age were infected. The overall infection rate was 45%. The percentage infection for the different villages ranged from 45% to 50% in all except the Kaki Bukit Rubber Estate (site 5), where there was a 27% infection rate. Both infections with *P. malariae* were in females from the village areas of Padang Mawar, Kok Mak and Sena (site 4). Although not presented in the tables, 62% of the number of infections found were in males. There were also slightly higher FA responses to all the antigens in the samples from males than in those from females.

<sup>1</sup> The test antigen giving the highest FA serum dilution end-point.

TABLE 3  
MALARIAL INFECTIONS IN INDIVIDUALS SAMPLED FROM 9 DIFFERENT VILLAGES IN THE STATE OF PERLIS,  
MALAYSIA (AREA I) AS DETERMINED BY BLOOD FILM EXAMINATIONS

Site No. <sup>a</sup>	Village	Infection				Total	
		<i>P. falciparum</i>	<i>P. vivax</i>	<i>P. malariae</i>	<i>P. falciparum</i> + <i>P. vivax</i>	Infected	Sampled
1	Titi Tinggi Ulu	30	5	0	3	38	82
2	Titi Tinggi Hilir	15	1	0	1	17	38
3	Wang Kellian	21	3	0	1	25	50
4	Padang Mawar, Kok Mak and Sena	31	10	2	5	48	106
5	Kaki Bukit Rubber Estate	9	0	0	0	9	34
6	Chantek	30	7	0	2	39	87
	Total	136	26	2	12	176	397

<sup>a</sup> See map.

The mean FA response with the *P. falciparum* antigen for those individuals in which no detectable parasitaemia was present was 1 : 37, indicating a considerable amount of past malarial experience in this group. The mean response of those presently infected with *P. falciparum* was 1 : 53 and of those having any species of malarial infection, the mean response was 1 : 44.

#### Area II

All samples from this area were collected from 17 sites located along the Pahang River in the Temerloh District of Pahang State in central Malaysia during March and early April 1966. The location of these sites is also shown in the accompanying map. The banks of the river are heavily populated and the flat land is used for wet cultivation of rice. However, this activity usually extends no more than 1 mile (1.6 km) from the river; swamp forests, broken by an occasional hill (*bukit*), are found quite close to the villages and farm areas. The first 4 villages, Tebing Tembah, Telok, Kandis and Paya Ladang (sites 7-10) are located in the vicinity of Bukit Kertau, a hill of 873 feet (266 m) elevation located on the east bank of "the great bend" of the Pahang River. Kuala Jenka (site 11) is located north of the point where the river again curves to the east and approaches the Temerloh to Kuantan highway. Baru Sungei Leng (site 12) is located north of the highway in hill country approximately 3 miles (about 5 km) from the river. Here the crop activities are primarily devoted to rubber

and fruit planting. Antimalarial activities have been greater here than in the villages near the river since this was a government rural development scheme and malaria control is part of the programme in these recently settled areas. Pijeng, Sekereta and Kuala Wah, Gunting, Papai (sites 13-15), Berkat and Batu Dinding (sites 17-18) are riverine villages which receive medical help from a health department boat. The secondary school and the primary school, Maran (site 16) are boarding-schools and drugs are readily available. The samples from the Health Centre, Maran (site 16) were taken from sick patients coming from neighbouring villages and Maran. Serengkam, Kuala Sentul and Batu 55 Jerantut Road (sites 19-21) are villages north of Maran and the Pahang River. This area is quite hilly and *A. maculatus* were present in much larger numbers than along the river. The Kuala Sentul Malay School (site 20) also had drugs readily available. Batu 55 Jerantut Road (site 21) was a school and a small village in which the children lived, located not far from Kuala Sentul. The village consisted of a group of aborigines who had been encouraged to come out of the jungle and live in a Malay-like village.

The distribution of the FA titres of sera from this area is given in Table 4. Three of the samples from individuals in the youngest age-group gave responses of greater than 1 : 10; 1 of 1 : 20 to *P. malariae* and 2 of 1 : 20 to *P. feldi*. In all, 8 of the 316 individuals in the intermediate age-group gave a response of 1 : 20 or higher with the

TABLE 4  
DISTRIBUTION OF FA TITRES OF SERA FROM THE TEMERLOH DISTRICT OF PAHANG STATE,  
MALAYSIA (AREA II)

Antigen	Age-group (years)	FA serum dilution end-point						No. of sera	Mean titre
		<1:10	1:10	1:20	1:40	1:80	1:160		
<i>P. falciparum</i>	≤5	11	5					16	1:6
	6-10	258	50	6	2			316	1:6
	>10	510	169	61	33	8	4	785	1:7
	Total	779	224	67	35	8	4	1117	1:7
<i>P. malariae</i>	≤5	10	5	1				16	1:7
	6-10	264	49	3				316	1:6
	>10	511	190	40	33	10	1	785	1:7
	Total	785	244	44	33	10	1	1117	1:7
<i>P. vivax</i>	≤5	12	4					16	1:6
	6-10	218	96	2				316	1:6
	>10	523	208	45	9			785	1:7
	Total	753	308	47	9			1117	1:7
<i>P. fieldi</i>	≤5	8	6	2				16	1:8
	6-10	127	154	35				316	1:8
	>10	362	312	90	18	3		785	1:8
	Total	497	472	127	18	3		1117	1:8

*P. falciparum* antigen; also at this level, 2 responded to *P. vivax*, 3 responded to *P. malariae* and 35 responded to the *P. fieldi* antigens.

A total of 106 of the samples from 785 individuals in the older age-group responded at a level of 1:20 or higher to the *P. falciparum* antigen; 84 responded to the *P. malariae* antigen; 54 responded to the *P. vivax* antigen; and 111 responded to the *P. fieldi* antigen at a level of 1:20 or higher. The mean FA responses regardless of age to the 4 test antigens were 1:7, 1:7, 1:7, and 1:8, respectively.

A comparison of the responses of 1:20 or greater with sera from the 17 sites examined is presented in Table 5. A total of 158 of the 1117 samples (14%) gave responses to at least 1 of these antigens at a 1:20 level or higher. Maximum responses to antigens were 64% to the *P. falciparum* antigen, 21% to the *P. vivax* antigen and 40% to the *P. malariae* antigen.

The prevalence of malarial infection when the samples were collected is given in Table 6. Only 28 individuals were shown to have malarial infections on the day the serum samples were collected. In contrast to the results from area I, there were more infections with *P. vivax* (13) than with *P. falciparum* (11). Four individuals had infections in which the species of parasite was not determined. There was no difference between the mean antibody titres of the infected individuals and those of the rest of the population.

### Area III

Samples from this area were collected in late April 1966 in 9 deep-jungle sites from aborigines who ranged over much of Upper Perak and Ulu Kelatan. This area is mountainous and covered by primary rain forest. The forests are broken only in the immediate vicinity of aborigine villages

TABLE 5  
ANTIGENS GIVING MAXIMUM FA RESPONSES TO SERA FROM THE TEMERLOH DISTRICT,  
PAHANG STATE, MALAYSIA (AREA II)

Site No. <sup>a</sup>	Village	No. of sera	Responses $\geq 1:20$		Antigen					
			No.	%	<i>P. falciparum</i>		<i>P. vivax</i>		<i>P. malariae</i>	
					No.	%	No.	%	No.	%
7	Tebing Tembach	53	14	26	3	21	4	29	7	50
8	Telok	52	12	23	6	50	1	8	8	67
9	Kandis	48	10	21	7	70	0	—	4	40
10	Paya Ladang	104	31	30	20	65	6	19	11	35
11	Kuala Jenka	74	18	24	12	67	5	28	11	61
12	Baru Sungai Leng	88	4	5	3	75	0	—	2	50
13	Pijeng	112	12	11	6	50	0	—	9	75
14	Sekereta and Kuala Wah	131	4	3	4	100	0	—	0	—
15	Gunting and Papai	35	2	6	1	50	0	—	1	50
16	Secondary School, Maran	105	3	3	2	67	1	33	0	—
16	Primary School, Maran	91	5	5	3	60	2	40	1	20
16	Health Centre, Maran	12	5	42	4	80	1	20	1	20
17	Berkat	32	9	28	8	89	3	33	2	22
18	Batu Dinding	34	7	21	7	100	4	57	0	—
19	Serengkam	59	11	19	7	64	4	36	3	27
20	Malay School, Kuala Sentul	58	2	3	0	—	0	—	2	100
21	Batu 55 Jerantut Road	29	9	31	8	89	2	22	1	11
Total		1 117	158	14	101	64	33	21	63	40

<sup>a</sup> See map.

where the jungle has been cut down for shifting dry cultivation of rice and of tapioca. The people are all migrants and can be expected to remain at one village site for no more than 1 or 2 years. Some of the more primitive tribes have no permanent village sites. Malarial infections are characteristically high among these people. Seepages created by jungle clearings near the villages provide excellent breeding sites for *A. maculatus*, while in the north and western extremes of this area the jungle-breeding *A. b. balabacensis* is undoubtedly involved. Malaria transmission is less seasonal here than in area I; however, the monsoons coincide in the 2 areas and periods of intense transmission are similar in both. Studies reported here were carried out in late April 1966.

With respect to the prevalence of malaria, areas I and III were very similar until June 1964 when,

under the auspices of the Aborigine Medical Service, a programme of suppressive drug administration was begun in area III. Daraclor (a combination of pyrimethamine and chloroquine) was administered monthly to all individuals who visited a jungle station. It is estimated that 60%–70% of the aboriginal population of 40 000 received this monthly dose. The effectiveness of this programme is demonstrated by the fact that blood surveys conducted in the same way in the same population prior to the drug suppression programme in 1963 showed a crude parasite rate of 29% on a single examination; this proportion was down to less than 10% in the 1966 survey.

The distribution of the FA titres of sera from this area is presented in Table 7. Of the 34 samples from individuals in the youngest age-group, 66 samples from the intermediate age-group and 301

TABLE 6  
MALARIAL INFECTIONS IN INDIVIDUALS SAMPLED FROM 17 DIFFERENT VILLAGES IN THE TEMERLOH DISTRICT OF PAHANG STATE, MALAYSIA (AREA II) AS DETERMINED BY BLOOD-FILM EXAMINATIONS

Site No. <sup>a</sup>	Village	<i>P. falciparum</i>	<i>P. vivax</i>	Undetermined species	Total	
					Infected	Sampled
7	Tebing Tembah	1	0	0	1	53
8	Telok	0	3	1	4	52
9	Kandis	0	0	0	0	48
10	Paya Ladang	0	0	1	1	104
11	Kuala Jenka	0	0	0	0	74
12	Baru Sungei Leng	0	0	0	0	88
13	Pijeng	0	3	1	4	112
14	Sekereta and Kuala Wah	2	1	0	3	131
15	Gunting and Papai	1	0	0	1	35
16	Secondary School, Maran	1	1	0	2	105
16	Primary School, Maran	2	0	1	3	91
16	Health Centre, Maran	0	0	0	0	12
17	Berkat	0	0	0	0	32
18	Batu Dinding	3	0	0	3	34
19	Serengkam	0	5	0	5	59
20	Malay School, Kuala Sentul	0	0	0	0	58
21	Batu 55 Jerantut Road	1	0	0	1	29
Total		11	13	4	28	1 117

<sup>a</sup> See map.

samples from the older age-group, 11, 23 and 190, respectively, gave responses of 1:20 or higher to the *P. falciparum* antigen. The mean FA titre of the samples against the *P. falciparum* antigen was 1:16. The mean response to the *P. malariae* antigen was 1:15 and to the *P. vivax* antigen, it was 1:8. The *P. fieldi* antigen had a mean response of 1:19. The highest response obtained was at the 1:320 level with 1 individual sample against the *P. falciparum* antigen.

The results of the examination of the data with regard to responses of 1:20 or higher are given in Table 8. A total of 246 of the 401 samples (61%) gave responses to at least 1 of the antigens at the 1:20 level or higher. Maximum responses of this group to antigens were 70% to the *P. falciparum* antigen, 4% were to the *P. vivax* antigen, and 51% were to the *P. malariae* antigen.

In July 1963, the parasitological examinations of

the 346 specimens from this aboriginal area revealed that 29% of individuals were infected with malaria. Of 107 infections, 58 were with *P. falciparum*, 15 with *P. vivax* and 26 with *P. malariae*, and there were 8 mixed infections. The results of the present examinations are shown in Table 9. Out of the 401 individuals examined, 38 were infected (9%); 22 were infected with *P. vivax*, 12 with *P. falciparum* and 1 with *P. malariae*. Two individuals were infected with mixtures of *P. falciparum* and *P. vivax* and there was 1 infection in which the species of parasite was not determined. It can be seen that the drug therapy had greatly reduced the prevalence of malaria but had not eliminated the disease.

#### DISCUSSION

##### Area I

The correlation between the results of the microscopic examinations and the FA response appears,



TABLE 7  
DISTRIBUTION OF FA TITRES OF SERA FROM ABORIGINES MAINLY LIVING IN UPPER PERAK  
AND ULU KELANTAN, MALAYSIA (AREA III)

Antigen	Age-group (years)	FA serum dilution end-point						No. of sera	Mean titre	
		<1:10	1:10	1:20	1:40	1:80	1:160			1:320
<i>P. falciparum</i>	≤5	15	8	7	1	1	2		34	1:11
	6-10	22	21	12	9	2			66	1:12
	>10	53	82	67	66	28	4	1	301	1:18
	Total	90	111	86	76	31	6	1	401	1:16
<i>P. malariae</i>	≤5	11	15	3	3	1	1		34	1:11
	6-10	21	28	10	7				66	1:10
	>10	31	109	94	40	23	4		301	1:17
	Total	63	152	107	50	24	5		401	1:15
<i>P. vivax</i>	≤5	24	6	3	1				34	1:7
	6-10	43	20	2	1				66	1:7
	>10	143	113	32	12	1			301	1:8
	Total	210	139	37	14	1			401	1:8
<i>P. feldi</i>	≤5	3	15	10	4	1	1		34	1:16
	6-10	13	34	15	4				66	1:11
	>10	23	133	78	50	17			301	1:15
	Total	39	182	103	58	18	1		401	1:19

in general, to be close. The presence of the highest response with the *P. falciparum* antigen is apparently directly related to the high incidence of infection with this parasite. The response to the *P. malariae* antigen in contrast to its low incidence reflects the known tendency for this parasite to be present at levels too low to be detected by a single microscopic examination of a stained blood film, but none the less present to a measurable extent when examinations of the same individual are made over an extended period. The greater antigenic response of individuals in the older age-group is an indication of the cumulative malarial experience in people living in this hyperendemic area.

It was of interest that only rarely was the antibody response highest to the *P. vivax* antigen even in persons currently infected with this parasite; the exact reason for this is not at present apparent.

The fact that infections with *P. vivax* induce high antibody response has been amply demonstrated by a number of workers, e.g., Tobie et al. (1962) and Kuvin et al. (1962a, 1962b). However, in the present situation, infections with the other plasmodia, particularly *P. falciparum*, were present. In those individuals with mixed infections, the *P. falciparum* parasite may induce responses to a higher level than the *P. vivax* parasite does, so that maximum responses occur more often to the *P. falciparum* antigen. The results from the samples from the Kaki Bukit Rubber Estate (site 5) may provide some support for this possible explanation for the low responses to the *P. vivax* antigen. The manager of this estate had provided antimalarial drugs at irregular intervals to his plantation workers and this is reflected in the lower percentage of malaria prevalence here (27%) than in any other village.

TABLE 8  
ANTIGENS GIVING MAXIMUM RESPONSES TO SERA FROM ABORIGINES IN MALAYSIA (AREA III)

Village	No. of sera	Responses $\geq 1:20$		Antigen					
				<i>P. falciparum</i>		<i>P. vivax</i>		<i>P. malariae</i>	
		No.	%	No.	%	No.	%	No.	%
Fort Kemar	101	70	69	44	63	2	3	39	56
Banding	24	11	46	9	82	0	—	4	36
Kuah	23	18	78	11	61	0	—	11	61
Sira	17	11	65	4	36	0	—	9	82
Peltu	10	10	100	5	50	1	10	7	70
Fort Betau	96	62	65	53	85	3	5	21	34
Fort Chabai	48	28	58	21	75	1	4	11	39
Kuala Yai	48	26	54	21	81	2	8	15	58
Gamala	34	10	29	4	40	0	—	8	80
Total	401	246	61	172	70	9	4	125	51

This may indicate that a reduction in the total malarial experience in a population as a result of treatment may allow the antibody response to the *P. vivax* antigen, which would otherwise be masked by the predominant experience with *P. falciparum* infections, to be detected. The relapsing *P. vivax*

parasite would have a better chance of survival under these conditions of occasional drug treatment than would the non-relapsing *P. falciparum* parasite.

An examination of Table 2 indicates that the Padang Mawar, Kok Mak and Sena area (site 4) had the highest level of response to the *P. malariae*

TABLE 9  
MALARIAL INFECTIONS IN ABORIGINES IN MALAYSIA (AREA III)  
AS DETERMINED BY BLOOD-FILM EXAMINATIONS

Village	<i>P. falciparum</i>	<i>P. vivax</i>	<i>P. falciparum</i> + <i>P. vivax</i>	<i>P. malariae</i>	Undetermined species	Total	
						Infected	Sampled
Fort Kemar	3	3	0	0	0	6	101
Banding	0	1	0	0	0	1	24
Kuah	1	0	0	0	0	1	23
Sira	0	1	0	0	0	1	17
Peltu	0	0	0	0	0	0	10
Fort Betau	3	9	2	0	0	14	96
Fort Chabai	3	2	0	0	1	6	48
Kuala Yai	0	5	0	1	0	6	48
Gamala	2	1	0	0	0	3	34
Total	12	22	2	1	1	38	401

antigen. This is coupled with the presence in this area of both of the detected infections with this parasite and may indicate that there is a higher rate of transmission of this parasite here.

The higher levels of FA response in those individuals with current detectable infections of malaria strongly suggest that these infections stimulate higher levels of response even in an area of hyperendemicity where, no doubt, the older individuals have experienced many episodes of malarial infection.

Although both the parasitological examinations and the FA responses indicated a higher level of infection with malaria in the males, the reasons for this are not completely clear. In an area of such high transmission, few would escape at least occasional infection over an extended period. In this area, there are 3 recognized vectors of malaria, i.e., *Anopheles balabacensis balabacensis*, *A. campestris* and *A. maculatus*. The first, probably the primary vector, tends to be exophagous and Malayan anophelines are all night-time feeders. Night-time activity by the villagers is much greater among the males than the females. Visiting from house to house and sitting around outside are typical masculine activities. Women move around much less at night and when they are outside they are covered literally from head to foot. Under these circumstances it would seem quite possible that a higher level of exposure might be found among the males than among the females. Since it has already been pointed out that the presence of a current infection results in a higher FA response, this seasonally higher incidence of infection in the males could be a direct factor in their higher parasitological incidence and the slightly higher levels of the FA response.

### Area II

Even though only 27 individuals (2.4%) had current parasitaemia, 158 (14%) had FA serum dilution end-points of 1 : 20 or higher against at least 1 of the human infecting antigens tested. This would appear to indicate that prior or undetected malaria was present in the population to a greater extent than can be ascertained by the examination of a single stained specimen. Quite possibly, the level of FA response is more indicative of the malarial activity in an area of hypoendemic malaria than is the parasitological examination.

As with the samples from the hyperendemic area, the highest percentage of maximum responses was

with the *P. falciparum* antigen followed by *P. malariae* and *P. vivax*. The continued absence of maximum responses with the *P. vivax* antigen remains unexplained. The possibility exists here, as with the other group, that the presence of infection with *P. falciparum* and with *P. malariae* (as shown by the high antibody responses to these antigens) tends to reduce the number of instances in which *P. vivax* gives the maximum response even when the current infection is with this parasite.

It was apparent that some groups had higher percentages of individuals with positive FA responses than did others. No malarial control measures were applied to the first 5 villages and the percentage of positive responses here ranged from 21 to 30. The highest percentage of positive response was obtained from the sera collected at the Health Centre, Maran (site 16) where many persons had recently experienced fevers, many of which were possibly due to malaria. In the last area with a relatively high percentage of FA response, Batu 55 Jerantut Road (site 21), were aborigines who had recently settled there from the jungle. Here, one would have expected a higher level of FA response. The other villages or schools had low positive responses ranging from 3% to 11%, which were probably a direct result of frequent antimalarial treatment. The reasons for high FA responses in some villages and low FA responses in others were readily apparent. Thus, the presence of even limited control programmes has an effect on the antibody levels of the population as a whole which can be easily differentiated by the examination of single serum specimens.

Although 14% of the people had levels of response of 1 : 20 or higher, the detection of this percentage may be the result of accumulated infections in individuals and not a reflection of single infections. Thus, one would expect those people who have had a number of episodes of malarial infection to have higher levels of FA response. This appears to be borne out by the findings in those areas where no malarial control efforts have been made.

*Plasmodium fieldi* was used as a test antigen since our previous studies (Collins et al., 1966, 1967) had indicated that this species cross-reacted at a high level with sera from individuals with a history of malarial infection with any species of *Plasmodium*. The reactions were, of course, not indicative of infection with this parasite. The continued presence of a high level of response to the *P. fieldi* antigen points out the usefulness of this antigen in the study

TABLE 10  
SUMMARY OF FLUORESCENT ANTIBODY RESPONSES FOR THE 3 AREAS OF MALAYSIA  
WITH DIFFERENT MALARIAL ECOLOGIES

Area	No. of samples	Positive for parasites	FA responses $\geq 1:20$		Mean FA responses			
			No.	%	<i>P. falciparum</i>	<i>P. vivax</i>	<i>P. malariae</i>	<i>P. feldi</i>
I. Hyperendemic area, Perlis State	397	176	347	87	1:40	1:19	1:25	1:48
II. Hypoendemic area, Pahang State	1 117	28	158	14	1:7	1:7	1:7	1:8
III. Jungle aborigine area	401	38	246	61	1:16	1:8	1:15	1:19
Total	1 915	242	751	39				

of antibody responses to *Plasmodium* sp., even in areas of hypoendemicity.

### Area III

One of the most obvious results of our study was the almost complete absence of infection with *P. malariae*. The incidence of this parasite is known to be quite high in the aboriginal groups in Malaysia (Sandosham, 1959). The high percentage of maximum FA responses (51%), however, would indicate either a high prevalence of current infections at a level too low to be detected in the examination of a single specimen or the persistence of antibody after the elimination of the infection by the drug.

As with the other 2 groups, the *P. vivax* antigen failed to give a high prevalence of maximum responses, even though it was the most common infection encountered. Again, the presence of infections with other plasmodia may have masked the presence of high responses to this antigen.

A summary of the results obtained by the examination of sera from these 3 different ecological areas is given in Table 10. Here it can be seen that each group presents patterns of FA response with all 4 antigens which are characteristic. The hyperen-

demic area had high rates of parasitaemia coupled with high mean FA responses. The hypoendemic area had low rates of parasitaemia coupled with very low mean responses. In area III, where control measures had been in effect, the rate of parasitaemia was low whereas the FA responses were high in regard to the percentage positive (1:20 or higher); the mean titres were lower, however, than those of the hyperendemic group.

The usefulness of the FA technique as a tool in studies on the epidemiology of malaria is well recognized. The presence of positive serological responses gives a clearer indication of the number of individuals in the population who have actually experienced malaria. The FA technique is at present the best means, other than continual blood-film examinations of the population at regular intervals, for determining this experience rate. The high rate of FA response in the jungle aborigine group indicated a level of experience approaching that of the group in the hyperendemic area of Perlis. However, the difference between the parasitological and serological results indicated the effectiveness of the antimalarial drug programme. Changes in mean FA titres of a population also appear to be a useful means of determining the effect of antimalarial activities.

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## RÉSUMÉ

Dans le cadre d'une étude écologique du paludisme des primates, on a procédé au début de 1966 à l'examen immunologique et parasitologique de 1915 échantillons de sang prélevés chez les habitants de trois régions distinctes de Malaisie. L'épreuve des anticorps fluorescents a été effectuée en utilisant des antigènes de *Plasmodium falciparum*, *P. malariae*, *P. vivax* et *P. feldi*. On a recherché la parasitémie sur étalements de sang et sur gouttes épaisses.

Dans l'Etat de Perlis, où le paludisme est hyperendémique, une corrélation satisfaisante est apparue entre les titres d'anticorps fluorescents et les données parasitologiques. La proportion et l'intensité des réponses positives en immunofluorescence augmentaient avec l'âge des sujets examinés; avec l'antigène *P. falciparum*, la moyenne des titres IF était de 1:15 dans le groupe d'âge inférieur à 5 ans, de 1:30 dans le groupe d'âge de 6 à 10 ans et de 1:48 dans le groupe d'âge supérieur à 10 ans.

Dans l'Etat de Pahang, où l'infection paludéenne est hypodémique, l'enquête hématologique a montré la présence de parasites chez 2,4% seulement des sujets examinés, alors que l'on décelait des titres IF de 1:20 ou plus chez 14% d'entre eux. Il semble que dans ce groupe l'atteinte paludéenne, antérieure ou non suspectée,

ait été plus marquée que ne l'aurait laissé supposer l'étude de la parasitémie. La réponse à l'épreuve d'immunofluorescence apparaît donc comme un témoin plus fidèle de l'activité du paludisme hypodémique. Par l'analyse des titres IF dans différents villages, on a pu percevoir aisément l'influence des traitements antipaludiques, même de peu d'envergure.

Enfin, dans la 3<sup>e</sup> région, l'examen en immunofluorescence de 401 sérums d'aborigènes a montré une réponse positive à des titres de 1:20 ou plus dans 246 cas (61%), alors que l'examen parasitologique ne révélait que 38 cas (9%) de paludisme. Cette divergence accentuée des données fournies par la recherche des parasites et des résultats de l'épreuve d'immunofluorescence s'explique par la mise en œuvre dans la région d'un programme de lutte antipaludique pendant 3 ans.

L'épreuve aux anticorps fluorescents est un procédé précieux d'étude épidémiologique du paludisme. L'analyse des taux de positivité permet d'établir aisément la proportion des individus qui, dans une population donnée, ont été en contact avec le parasite. Les fluctuations des titres IF peuvent servir à évaluer l'efficacité des mesures antipaludiques.

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