SEROEPIDEMIOLOGY OF MALARIA IN NORTHERN THAILAND

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INTRODUCTION

This study was designed to evaluate the usefulness of the indirect immunofluorescent antibody test (IFAT) as an aid in monitoring malaria incidence in a National Malaria Programme. The feasibility of employing this test depends on several criteria: a) its ability to warn against the reappearance of malaria in areas from which it had been eradicated, (b) its ability to evaluate control measures in areas which had a high incidence of malaria in the past but in which it is now greatly reduced, c) the cost-effectiveness of the test in relationship to the available budget, and d) the ability to evaluate and interpret the data quickly enough to allow the information to be applied advantageously for planning control policy in the area surveyed.

The IFAT has been used successfully in a number of surveys and the advantages and disadvantages compared to other serological tests have been reviewed by Lobel and Kagen (1978). It is not meant to replace thick blood film slides for malaria detection, but rather to be used with parasitological and entomological findings to plan and evaluate control measures. An objective of this continuing study is to define the specific age groups which best reflect recent malaria experience in Thai communities of different epidemiological nature so that surveys can be done most efficiently on these groups.

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STUDY AREAS

Sarapee District, located on a wide valley floor near the capital of Chiang Mai Province, was chosen as a study area best representing one from which malaria has been eradicated. In 1949 it was selected by the World Health Organization as a demonstration area for the control of malaria by residual DDT spraying. In a 1949 survey prior to the initiation of DDT spraying, the spleen rate was 63.8 and the slide positivity rate (SPR) was 47.3 (Table 1). Within a few years after DDT application, *An. minimus*, the main vector, disappeared and malaria transmission ceased.

When Thailand launched its Eradication Programme in 1965, Sarapee was put into the Consolidation Phase and later into the Partial Integration Phase which relied only upon passive case detection for monitoring malaria. During this period of Partial Integration, only few cases have been reported and all of these were imported from other areas.

Li District, located in Lamphun Province about 100 km from Sarapee, is situated in the foothills of a narrow valley surrounded by forested mountains. The main occupations are agriculture and wood cutting outside of the villages. *An. dirus (balabacensis)* and *An. minimus* are the main vectors in this area. When DDT spraying was started in 1953, malaria was soon reduced, but has persisted at a low level due both to the occupational pursuits of the residents and to the habit of outdoor biting by the main vectors. The area was selected to represent one with a

Table 1

Background information of Sarapee and Li Districts.

	Sarapee	Li
Population (1981)	67,577	61,939
Malaria data before Control Programme (1949):		
Number examined	569	415
Slide positivity rate	47.3	24.8
Spleen rate	63.8	71.1
Infant parasite rate	29.5	13.7
Years under protection by DDT:	1949-52 1963	1953-57 1960-82
Vectors:		
Past	*An. minimus*	*An. minimus*, *An. dirus*
Present	not found	*An. minimus* *An. dirus*

Table 2

Parasitological data of Sarapee and Li Districts.

Parasite		Sarapee		Li	
		No. cases	%	No. cases	%
1949:	*P. falciparum*	190	92.4	73	92.4
	P. vivax	16	7.6	4	5.0
	P. malariae	0	0	1	1.3
	mixed	0	0	1	1.3
1981:	*P. falciparum*	3	60	345	59.4
	P. vivax	1	20	232	40.0
	P. malariae	0	0	4	0.7
	mixed	1	20	0	0

persistently low rate of infection. Additional epidemiological and parasitological information about these two areas is summarized in Table 1, 2 and 3.

MATERIALS AND METHODS

A random sample (Henderson and Sundaresan, 1982) of 30 villages in Li and Sarapee

Table 3

Slide positivity rates (SPR) for Sarapee and Li Districts.

	Sarapee					Li				
Year	Blood Exam.	Positive	SPR	Case Classification		Blood Exam.	Positive	SPR	Case Classification	
				Indigenous*	Imported**				Indigenous*	Imported**
1977	3,081	11	0.35	0	11	14,392	1,115	7.71	353	762
1978	3,097	5	0.46	0	5	17,335	1,163	6.70	359	804
1979	3,878	2	0.05	0	2	16,686	432	2.58	171	261
1980	3,042	2	0.06	0	2	15,412	245	1.58	46	199
1981	4,915	5	0.10	0	5	19,602	581	2.96	101	480

* Indigenous cases are those who acquired malaria transmission within their village.
** Imported cases are those who acquired malaria transmission outside of their village.

was surveyed. Sufficient households also were randomly selected to yield approximately 40 subjects aged 1-70 in each village with the aim of having about 1200 samples from each District. Effort was made to obtain samples from each eligible person in the household and in this we had over 95% success. Eligibility was based on age and whether this was the main residence of the subject.

A questionnaire carrying the code number, name, age, sex, residence, occupation, and history of malaria and of travel outside the area was completed for each eligible household member.

A thick blood film on a coded slide was prepared, prestained and returned to Region II Malaria Centre at the end of each week to be read by Region microscopists. Two samples of approximately 70 microliters of blood were obtained in heparinized microhematocrit tubes from the same finger prick used to obtain the blood film and the blood was transferred to Whatman No. 2 filter paper bearing the code number. The papers were air dried and stored in plastic bags at room temperature until the end of each week and then frozen at −40°C. Duplicate samples were stored in separate packets.

The data for slide parasite positive cases for 1981 do not represent the total for Li, but only the ones for which we had ages available.

Filter papers were removed from the freezer and immediately placed in a desiccator for at least 30 min. The blood-containing area of paper was cut out and placed in a coded test tube, 0.7 ml. phosphate buffered saline (PBS, pH 7.2) was added to each tube and the tubes then refrigerated overnight. The paper was then removed and the tubes centrifuged for 10 min at 2000 rpm. The upper half of sample was removed and stored at −70°C. until tested. The plasma dilution was appropriately 1 : 20 after this procedure.

Antigen slides were prepared using a thin blood film of *in vitro* cultured *P. falciparum* with approximately 8% parasitemia, mostly in the schizont stage (kindly supplied by Dr. Savanat Tharavanij, Faculty of Tropical Medicine, Mahidol Univ., Bangkok). Fluorescin-labelled rabbit anti-human IgG (Behringwerke, Marburg, Germany) was used at 1: 25

dilution. Antigen slides and reconstituted fluorescin conjugate were stored at −70°C. The positive control serum had a titer of 1 : 5120. The negative control serum was negative at 1:40 and 1:80 dilution.

The IFAT procedure : Antigen slides were removed from the freezer, fixed for 10 min. in cold acetone and then air dried. The blood film was divided into twenty 4-5 mm squares with a waterproof pen. Each slide had a positive and negative control at 1:40 dilution and each survey sample was tested at 1:40 and 1:80 dilution. The slides were prepared by a method similar to that described by Collins *et al.*, (1964), mounted in glycerine and read under a fluorescent microscope (Olympus Model FIM 200674) by two individuals. If the reading did not agree at both dilutions, the test was repeated and read again. The duplicate samples were used for repeat tests or saved for future studies.

The data were analyzed by testing the difference between groups by the Chi Square method (Sigel, 1956). Probabilities of less than 0.01 were accepted as statistically significant.

RESULTS

Although 19.4% of the males and 13.7% of the females from both Districts had positive IFAT, this difference was not statistically significant and the data for both sexes were pooled for subsequent analyses.

Although the percent of positive IFAT for the total populations of Sarapee and Li were essentially the same, 16.8 and 16.3 respectively, the similarity breaks down when individuals from 1 to 40 years old were compared (Table 4). In both Sarapee and Li the difference between the percent of positive IFAT in those under and over 40 yrs. is statistically

Table 4

Positive IFAT in serum of individuals from Sarapee and Li in 1981.

Age group	Sarapee			Li		
	Total	Pos.	% Pos.	Total	Pos.	% Pos.
1–5	53	1*	1.89	128	1	0.78
6–10	87	0	0	155	6	3.87
11–15	114	0	0	164	6	3.66
16–20	114	2***	1.75	142	6	4.23
21–30	246	6*	2.44	204	20	9.80
31–40	88	1**	1.14	130	33	25.38
41–50	175	52	29.71	125	44	35.20
51–60	196	86	43.88	93	52	55.91
61–70	81	47	58.02	48	26	54.17
Total	1154	194	16.81	1189	194	16.32

* Negative 1982
** Confirmed 1982
*** Confirmed on 1981 duplicate.

significant ($p = < 0.001$). When the 1-40 yr. groups from Sarapee and Li are compared to each other, the percent of positive IFAT was significantly higher in Li ($p = <0.001$) and this difference was even greater when only the 21–40 yr. groups were compared because there was a sharp increase in positive tests in Li but not in Sarapee (Table 4 and Fig. 1). The greatest difference in percent positive IFAT in Sarapee compared to Li was in the 31–40 yr. group ($p = < 0.001$). Those in this age group were below 10 yrs old when DDT spraying brought about the eradication of malaria in Sarapee and they had only short exposure to malaria and reduced chance for multiple infection; so generally no anamnestic antibody responses would have been elicited. This difference was accentuated by the increase in positive tests in Li residents at this same age when many have been working outside the village for ten or more years. Likewise, the significant difference ($p=<0.001$) between the 21–30 and 31–40 yr. olds in Li probably also represents the increased anamnestic antibody response over a longer period of exposure in the older group. These trends are illustrated in Fig. 1.

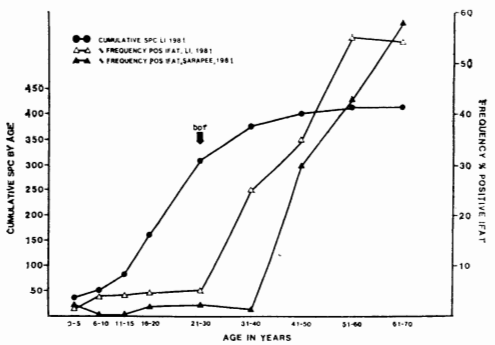

Fig. 1. Frequency per cent positive indirect immunofluorescent antibody tests (IFAT) in Sarapee and Li Districts by age and cumulative slide positive cases (SPC) by age in Li District. Arrow indicates approximate time of initiation of control measures in relation to age.

Age distribution of positive IFAT in relation to endemicity was analysed. Prior to the initiation of DDT spraying in 1949 in Sarapee and 1952 in Li, both Districts were hyperendemic with even more malaria in Sarapee than in Li (Table 1). The high number of positive IFAT in 41–70 yr–olds in both Districts reflects the high incidence of malaria in that population prior to spraying. Fig. 1 illustrates the precipitous drop in the number of positive tests in both districts following the introduction of control measures.

When the cumulative frequency of slide positive cases (SPC) by age for 1981 in Li District is plotted against the percent positive IFAT for the same year, the curves at age 20 through 40 are roughly parallel (Fig. 1). The SPC curve then levels off, indicating a possible protective effect of antibodies to malaria in the older age group, but decreased exposure of this age group might also contribute.

The thick blood films taken on all subjects in this study were parasite-negative, indicating that none of the subjects had malaria on the day the samples were taken.

DISCUSSION

An analysis of the age distribution of positive IFAT in Sarapee and Li shows that 40.9% of all tests in Sarapee and 45.9% of those in Li were positive in individuals over age 40. The high frequency of positive tests in this age range is a reflection of the hyperendemicity of malaria in both districts prior to initiation of control measures. Since Sarapee has been essentially free from malaria since 1950, it is obvious that these positive tests are due to persistence of antibodies for over 30 years in individuals having had prolonged and frequent exposure to malaria infection. Therefore, inclusion of this age group in surveys designed to give information on the current malaria situation would not be useful. Long persistence of antibodies to malaria

has been reported in other studies (Bruce-Chwatt *et al.*, 1972.) It is possible that cross-reactivity with antibodies to other organisms may also contribute to the high IFAT positivity in older individuals. In areas where effective radical treatment now is employed, antibody levels will probably be less persistent.

Both parasitological and epidemiological investigations show that no indigenous malaria was recorded in Sarapee since 1964 (Table 3) indicating that transmission stopped completely. In contrast, Li District still has quite a few indigenous cases but the slide positivity rate was only 1.5 to 2.9% during 1977-1981 compared to 24.8% in 1949. The interruption of transmission in Sarapee and the continued low transmission in Li was reflected in the serological findings, strengthening the validity of the three sets of observations. While only 1.4% of Sarapee residents under age 41 had positive IFAT indicating a few imported cases and/or false positive tests, 13.2% of Li residents of corresponding age had positive tests reflecting indigenous and imported cases and probably a few false positive as well. While the distribution of the few positive tests in the 1–40 yr. group is apparently random in Sarapee, in Li positive tests increase with age and most likely this represents the cumulative exposure to infection of this age group.

The high risk 21–40 age group in Li was the one in which we most often failed to get a sample, since these subjects often were staying outside the village in farm huts or in the forest. Thus, the frequency of positive tests at this age is probably underestimated. In a recent follow-up survey, return visits were made in an effort to decrease the number of non-available subjects and our results for 1982 should show if this assumption is correct.

The data for the IFAT were related to the number of SPC reported in each age group in Li (Fig. 1). The number of SPC in individuals 1-20 yrs. old is relatively high in proportion to the number of positive IFAT, so that our first assumption that there is relatively little transmission in this age group may be wrong. Rather, it is likely that in young people. exposed to the parasites for the first time the antibody response is weaker and shorter-lasting than in older individuals in which two or more infections produce an anamnestic antibody response which is faster, stronger and more persistent. Because of this, the frequency of positive IFAT in individuals aged 1–20 in areas with a low infection rate represents only a very short period prevalence. Therefore, we feel that in areas of low endemicity such as Li, the total number of SPC plus the frequency of positive IFAT in the 20–40 yr. range would give the most useful information concerning period prevalence. Data for younger ages might indicate if there is a very recent change in malaria incidence and could be included for that reason. In monitoring an area such as Sarapee, the IFAT would be indicated only if there were an occurrence of SPC attributable to transmission within the area and in that case a large random sample of the 1–40 yr. population in the affected area would give the most useful information.

When comparing the SPC with the IFAT positive by age in Li (Fig. 1), the slope of the IFAT and that for the cumulative SPC are roughly parallel at age 20 to 40 when the SPC curve levels off quite abruptly. This plateau might indicate functional immunity in the older population, but in an area where the majority of cases are acquired outside the village might also indicate lower exposure of the older people. In areas such as Africa with high transmission, partial protective immunity might impede control measures since people with subclinical infection may harbor and transmit the malaria parasite (Miller, 1958; Jeffery, 1966; Carter *et al.*, 1979).

One disadvantage of the antigen used in this study is that it is only from *P. falciparum*. However, there is probably some cross-reaction with *P. vivax* and together with the blood film data it is sufficient to reflect trends in malaria epidemiology until a good source of *P. vivax* antigen becomes available.

We have found that titering serum at 1:40 and 1:80 dilutions with the IFAT gives an internal control and is sufficient to reflect the malaria situation without the additional time and cost required to titer each positive serum to its end point. This together with identifying the most sensitive age groups to sample in various populations makes such a survey cost–effective and realistic for countries with limited technical and financial resources.

These preliminary results together with the follow-up in Li District during 1982 and an additional survey in an area with a much higher endemicity should further establish the validity of our conclusions and the usefulness of this test in various types of populations within Thailand.

SUMMARY

The seroepidemiology of malaria in two areas of Northern Thailand was studied by means of the indirect immunofluorescent antibody tests (IFAT). In one area transmission has been interrupted completely for over 30 years, but in the other area transmission still occurs at a low level. Results of the survey for antibodies confirm existing parasitological findings and reflect both the recent and past history of malaria in both areas. The IFAT is less sensitive to recent malaria infection in very young people in an area of low endemicity than is the number of reported slide proven cases, but as age and cumulative exposure increase the IFAT reflects period prevalence in adults of ages 20–40. Thus, the serological data are complementary to other available information. This study also confirms the observation that malaria antibodies may last for over 30 years in people who formerly lived in hyperendemic areas and had multiple infections with the malaria parasite.

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REFERENCES

BRUCE-CHWATT, L.J., DODGE, J.S., DRAPER, C.C., TOPLEY. E. and VOLLER, A., (1972). Seroepidemiological studies on population groups previously exposed to malaria. *Lancet, 1* : 512.

CARTER, R., GWADZ, R.W. and GREEN, I., (1979). Naturally acquired immunity and antimalarial antibodies in relation to infectivity to mosquitoes in endemic *Plasmodium falciparum* malaria. In : *Immunodiagnostic Techniques in Malaria.* Transactions 3rd Meeting Scientific Working Group on the Immunology of Malaria, Panama, p 105.

COLLINS, W.E., JEFFERY, G.M. and SKINNER, J.C., (1964). Fluorescent antibody studies in human malaria. I. Development of antibodies to *Plasmodium malariae*. *Amer. J. Trop. Med. Hyg., 13* : 1.

HENDERSON, R.H. and SUNDARESAN, T., (1982). Cluster sampling to assess immunization coverage: a review of experience with a simplified sampling method. *Bull. WHO, 60* : 253.

JEFFERY, G.M., (1966). Epidemiological significance of repeated infections with homologous and heterologous strains of *Plasmodium. Bull. WHO, 35* : 873.

LOBEL, H.O. and KAGAN, I.G., (1978). Sero-epidemiology of parasite diseases. *Ann. Rev. Microbiol., 32* : 329.

MILLER, M.J., (1958). Observations on the natural history of malaria in the semi-resistant West African. *Trans. Roy. Soc. Trop. Med. Hyg., 52* : 152.

SIGAL, S., (1956). *Non-Parametric Statistics*, McGraw-Hill, New York.