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Parasitological and serological surveys for malaria among the inhabitants of an aborigine village and an adjacent Malay village

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Summary

Malaria surveys in an Orang Asli (aborigine) and an adjacent Malay village showed significantly higher parasite rates in the age-group 0–9 years in the former. Parasite rates declined progressively from a maximum at 0–4 years in the Orang Asli to zero at 30–39 years while in the Malays it rose progressively with age. Indirect fluorescent antibody test (IFAT) titres against schizont antigens of *Plasmodium falciparum* and *P. cynomolgi* were higher in the Orang Asli in all age-groups with a statistically significant inverse relationship between IFAT titres and parasite rates. IFAT titres in the Malay population also increased with age but were very much lower. Antibody levels detected by the enzyme-linked immunosorbent assay (ELISA) using soluble schizont antigens were also much higher in the Orang Asli and values with *P. cynomolgi* were higher than those with *P. falciparum* antigens. These differences are attributed to the higher malaria transmission in the younger age-groups of the Orang Asli and presumably greater immunological experience to a wider diversity of antigens than the Malays, thus explaining the presence of "protective" antibodies in the former but not the latter group.

Key words: malaria serology; *Plasmodium falciparum; P. cynomolgi.*

Introduction

Malaria and filariasis are still common among Orang Asli (aborigine) and those living in some rural areas (Mak, 1978; Wharton et al., 1963). In 1983, the

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Sg. Lui Orang Asli village at Ulu Langat district, Selangor, was found to be endemic for malaria, with 28 cases, but none was found in the adjacent Malay village (Annual Report, Vector Borne Diseases Control Programme, Selangor). Remedial measures carried out were focal residual spraying with DDT at three-monthly cycles, active and passive case detection and radical and presumptive treatment of cases.

In June 1984, it was decided to carry out parasitological and serological surveys in the Orang Asli village at Sg. Lui and also the adjacent Malay village as it was suspected that the Orang Asli would be a source of infections for the Malays. It was also decided to compare the suitability and sensitivity of antigens prepared from in vitro cultured *P. falciparum* with those from *P. cynomolgi* maintained in experimentally infected monkeys in the immunodiagnostic tests.

Materials and Methods

The Orang Asli village at Sg. Lui (Kpg. Asli Sg. Lui) and the adjacent portion of the Malay village (Kpg. Sg. Lui), situated in Ulu Langat district, Selangor, were surveyed. Total coverage of all the 117 Orang Asli in 20 houses and about 200 Malays in the nearby 50 houses were targeted for.

A census was taken and thin and thick blood smears were prepared from finger-pricks. Blood was also collected on Nobutu filter paper strips according to the instructions of the manufacturer, for serological studies. Thick blood smears were dried overnight while thin smears were methanol fixed. Both smears were stained with diluted Giemsa and screened for malaria (200 fields under the 1000× magnification). For positive malaria smears, the number of parasites found in fields with a total of 500 leucocytes were determined and the parasite count per μl blood was estimated, assuming an average of 8000 leucocytes per μl blood. All children $\leq$10 years old were examined for spleen enlargement and splenomegaly classified according to Hackett's scale (WHO, 1963). Nobutu filter paper strips with blood smears were stored in sealed cellophane bags at –20° C until tested. Elutions from these were used in the indirect fluorescent antibody test (IFAT) and the enzyme-linked immunosorbent assay (ELISA).

The IFAT was carried out with *Plasmodium falciparum* (Gombak strain A) cultured by the in vitro method of Trager and Jensen (1976) and *Plasmodium cynomolgi* from infected *Macaca fascicularis*. Intact schizont antigens were used and the test carried out according to the method of Collins et al. (1964) using fluorescein isothiocyanate-conjugated antihuman immunoglobulin (IgG, A and M; Cappel Laboratories). For the ELISA tests, schizont stages from the above sources were separated essentially by the method of Saul et al. (1982); the specific gravity of Percoll being modified as determined by prior testing for the separation of *P. cynomolgi* schizonts. Soluble schizont antigens were prepared by the method of Spencer et al. (1979a). Peroxidase conjugated antihuman immunoglobulin (Ig A, M and G; Cappel Laboratories) was used with orthophenylenediamine as the substrate and the test was carried out according to the method of Voller et al. (1974). The microtitre plates (Immulon, Dynatech) were coated with 200 μl of soluble antigen (1:1000 dilution of *P. falciparum* antigen with 1.38 mg/ml of protein and 1:400 dilution of *P. cynomolgi* antigen with 0.92 mg/ml of protein). The serum dilution used for test samples, positive and negative controls for ELISA was 1:400. Initial serum dilution for IFAT was 1:25. For both tests, the positive control serum was from a patient with known *P. falciparum* infection while the negative control was a pool of 10 sera of uninfected subjects living in Kuala Lumpur (a non endemic area).

Table 1. Malaria parasitaemia* by age-groups in the Orang Asli and Malay villages, Sg. Lui, Selangor, 1984

Age-group (years)	Orang Asli village		Malay village	
	Examined	Positive (%)	Examined	Positive (%)
0–4	18	5 (27.78)	20	1 (5.00)
5–9	6	1 (16.67)	29	1 (3.45)
10–19	12	1 (8.33)	30	2 (6.67)
20–29	13	1 (7.69)	16	2 (12.50)
30–39	7	0	7	1 (14.29)
≥40	7	0	26	1 (3.85)
Total	63	8 (12.7)	128	9 (6.3)

* All due to *Plasmodium falciparum* infections except for two *P. vivax* infections in the age-group 0–4 years in the Orang Asli village.

Results

Parasitological findings

A total of 63 Orang Asli in Kpg. Asli Sg. Lui and 128 Malays in Kpg. Sg. Lui were examined. Of these 8 (12.7%) and 8 (6.3%) had malaria parasitaemia, respectively (Table 1). All had *P. falciparum* infection except for two Orang Asli who had *P. vivax* infection. In the Orang Asli, the parasite geometric mean count (GMC) was 259.9 (range 110–4160) per μl, while in the Malays, this was 150.6 (range 64–640) per μl.

In the Orang Asli, all malaria parasitaemic patients were ≤30 years old, with 44.5% ≤10 years old (Table 1). However, in the Malays, malaria positives were distributed among all age groups with 9.6% being in those ≥10 years old. The geometric mean ages for positives were 17.8 years in the Malays and 4.0 years in the Orang Asli. Spleen rates among those ≤10 years were 10.6% (5 out of 47) and 20.0% (3 out of 15) in the Malays and Orang Asli, respectively. Mean spleen sizes were 1.8 and 2.7, respectively.

Serological findings

With the IFAT using *P. falciparum* and *P. cynomolgi* schizont antigens all age groups had detectable antibody levels. However, antibody levels were higher with *P. falciparum* antigen. Pooled negative control serum had an IFAT titre less than 1:25 with both antigens.

In the Orang Asli, there was a progressive increase in the geometric mean titre (GMT) up to age 40 years which then declined slightly (Fig. 1). In the Malays, the GMT with both antigens were extremely low, especially in the younger age groups. There was, however, a progressive increase in the values at

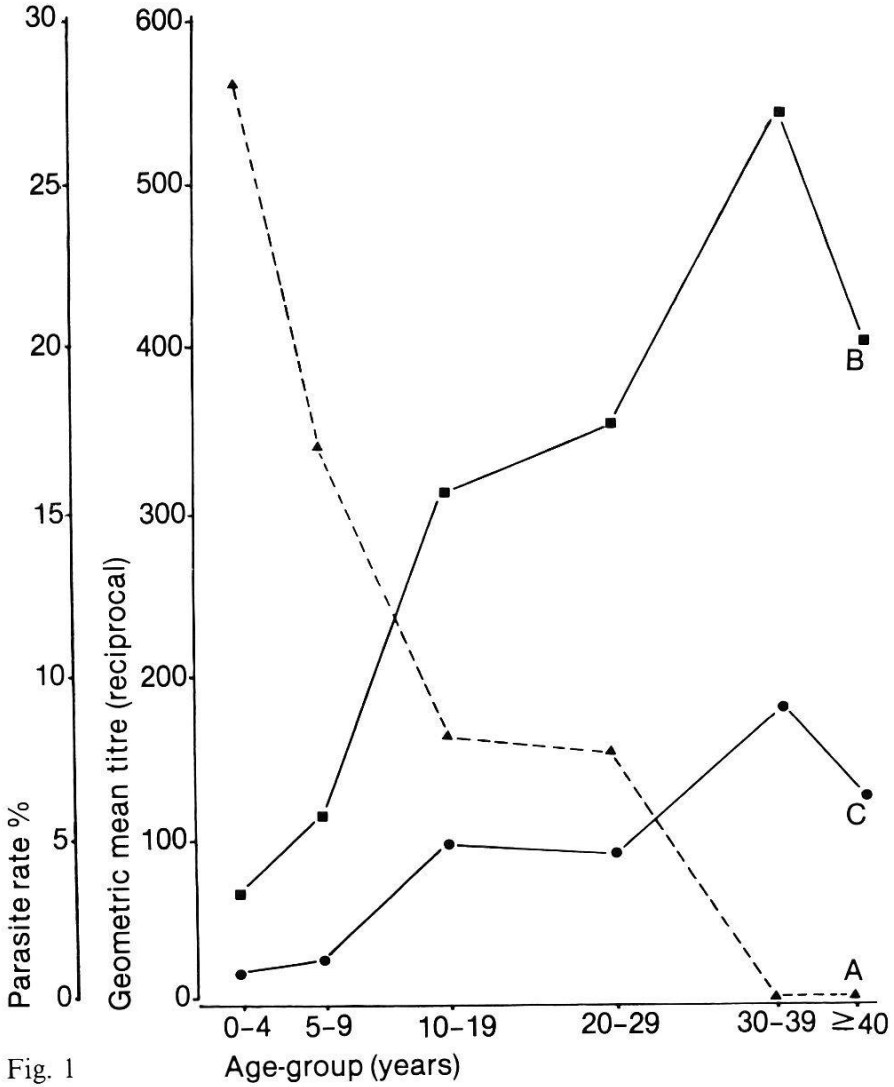

Fig. 1

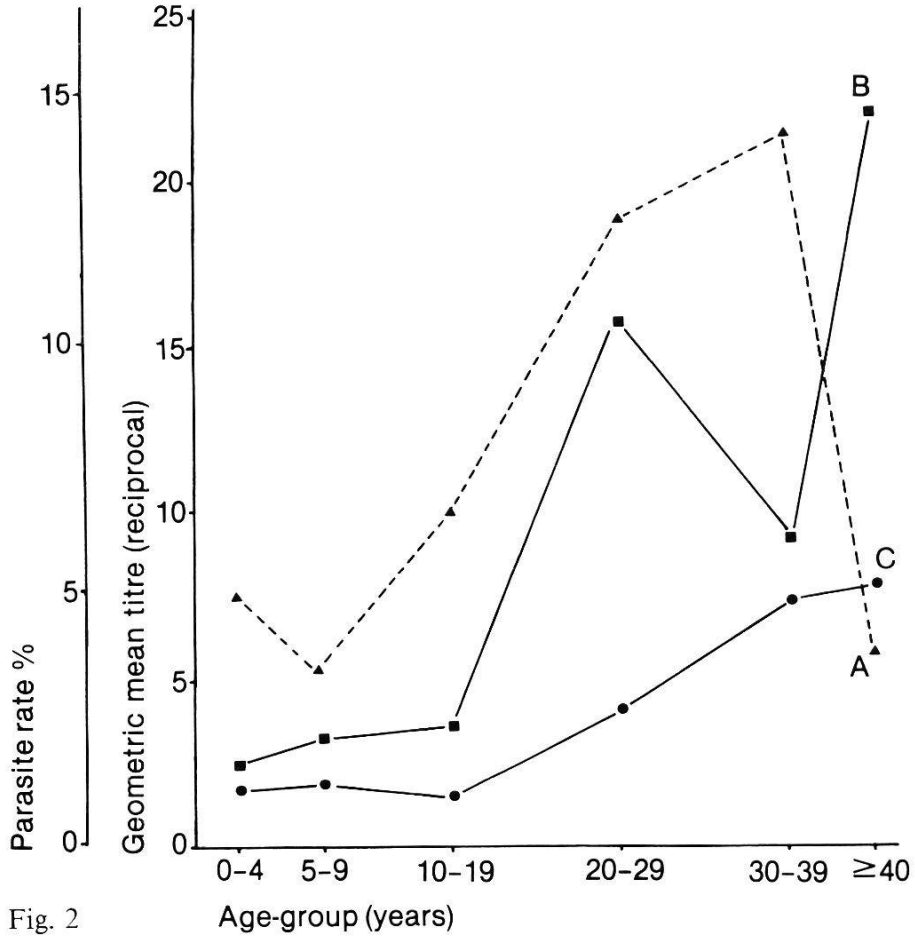

Fig. 2

Fig. 1. Malaria parasite rates (A) and indirect fluorescent antibody titres against *P. falciparum* (B) and *P. cynomolgi* (C) schizont antigens, in Orang Asli.

Fig. 2. Malaria parasite rates (A) and indirect fluorescent antibody titres against *P. falciparum* (B) and *P. cynomolgi* (C) schizont antigens, in Malays.

Table 2. Enzyme-linked immunosorbent assay mean ±SD optical density values at 492 nm using *Plasmodium falciparum* and *P. cynomolgi* soluble schizont antigens, by age-groups, in the Orang Asli and Malay villages, Sg. Lui, Selangor, 1984*

Age-group (years)	Orang Asli village			Malay village		
	Examined	*P. falciparum*	*P. cynomolgi*	Examined	*P. falciparum*	*P. cynomolgi*
0–4	14	0.84±0.35	1.10±0.24	19	0.21±0.18	0.59±0.32
5–9	5	0.56±0.17	1.18±0.11	24	0.34±0.28	0.78±0.38
10–19	11	0.88±0.21	1.30±0.17	29	0.27±0.15	0.75±0.33
20–29	11	1.36±0.06	1.31±0.25	15	0.42±0.30	0.97±0.36
30–39	7	1.04±0.33	1.26±0.26	6	0.34±0.22	0.63±0.30
≥40	6	1.02±0.34	1.35±0.29	25	0.31±0.22	0.69±0.36

* Readings with pooled negative control serum were <0.3 with *P. falciparum* antigens and <0.2 with *P. cynomolgi* antigens.

the older age groups (Fig. 2). Only five had titres ≥1:50 with *P. cynomolgi* and these were in the age groups 20 years and above. With the *P. falciparum* antigen, 13 out of 125 (10.4%) had titres ≥1:50 and, except for one in the age group 5–9 years, were seen mainly in those ≥20 years old. In the Orang Asli, 46 out of 55 (83.6%) had titres ≥1:50 and these were seen in all age groups. Similarly, with the *P. cynomolgi* antigen 35 out of 55 (63.6%) had this titre and this titre level was seen in all age groups.

Mean ELISA optical density values at 492 nm (O. D.) using either *P. falciparum* or *P. cynomolgi* antigens generally increased with age, the highest values being seen in the age-group 20–29 years (Table 2). *P. cynomolgi* antigen appeared to give higher readings in the test compared to that with *P. falciparum.*

Discussion

The pattern of malaria infections in the various age groups in the two villages was markedly different, the highest infection rate in the Orang Asli being in the age group 0–4 years (27.8%), then declining progressively with age and no infection was seen after the age group 20–29 years. In contrast, parasite rates increased progressively with age in the Malays from 5.0% at the age-group 0–4 years to a maximum of 14.3% in the age-group 30–39 years and then declined to 3.9% in those ≥40 years. However, the parasite rates in the age-group 0–9 years were significantly different between the two villages, these being 25% and 4.1% in the Orang Asli and Malays, respectively (Chi square = 5.239; 0.05>P>0.02). The generally high parasite rates in all age-groups in the Malays as opposed to high rates only in the younger age-groups in the Orang Asli, may reflect differences in susceptibility to malaria.

In general, there was an inverse relationship between IFAT GMT and parasite rate in the Orang Asli, there being a progressive rise in the antibody levels from the age group 0–4 years to a maximum in the age-group 30–39 years (Fig. 1). This inverse relationship is statistically significant with the *P. falciparum* antigen ($r = 0.937$; $0.01>P>0.001$) and *P. cynomolgi* antigen ($r = 0.912$; $0.02>P>0.01$). In contrast, there was a progressive increase in the IFAT GMT levels as the parasite rate increased in the Malay population, but this positive correlation is not statistically significant ($r = 0.165$; $P>0.1$ for *P. falciparum* and $r = 0.375$; $P>0.1$ for *P. cynomolgi* antigens).

As in the Orang Asli, the highest GMT values in the Malays were in the older age-groups. We postulate, from the pattern of antibodies against *P. falciparum* antigen in the Orang Asli, that the IFAT which detects surface antibodies against schizont antigens, probably reflects population "immunity" towards the infection as evidenced by the inverse relationship between parasite rates and GMT levels. Although the rise in IFAT antibody levels as the parasite rates increased in the Malays does not apparently support this, it must be realized that these levels in the Orang Asli are of a very much higher magnitude than those seen in the Malays (between 10–70 fold for *P. cynomolgi* and 20–90 fold for *P. falciparum* antigens). Such antibodies which may include those with "protective functions", probably reflect the duration and intensity of exposure to a wide spectrum of antigenic epitopes.

Antibodies detected by the ELISA test were higher in population groups in malarious areas compared to those in areas where effective antimalarial measures were taken (Voller et al., 1974). These antibodies develop rapidly in semi-immunes in response to patent infections with *P. falciparum,* decline gradually with curative therapy and may persist for several years (Spencer et al., 1979a). In non-immunes, infection produces generally lower titres than in semi-immunes. In our study, the ELISA values were very much higher in the Orang Asli compared to the Malays and unlike the IFAT antibody levels, values with *P. cynomolgi* were higher than those with *P. falciparum* antigens. It appears therefore, that the *P. cynomolgi* soluble schizont antigens (mainly somatic), cross-react with antibodies against *P. falciparum* as did *P. knowlesi* antigens (Voller et al., 1975). The increase in ELISA antibodies with age probably reflects immunological experience with somatic antigens and not protective immunity.

In this study, there is no significant correlation between the IFAT and the ELISA results in the Malays ($r = -0.156$; $P>0.1$ for *P. cynomolgi,* and $r = 0.504$; $P>0.1$ for *P. falciparum* antigens) or in the Orang Asli ($r = 0.709$; $P>0.1$ for *P. cynomolgi* and $r = 0.626$; $P>0.1$ for *P. falciparum* antigens). A similar discordance between these two tests was also observed by Spencer et al. (1979b). There is also no significant correlation between the ELISA values using the two types of antigens ($r = 0.589$; $P>0.1$ in the Orang Asli and $r = 0.788$; $0.1>P>0.05$ in the Malays).

Acknowledgments

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