

## A Seroepidemiological Survey of *Plasmodium falciparum* Infection in Malaria Endemic and Sub-endemic Areas in Colombia

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### Abstract

Sera were collected in malaria endemic areas in Colombia. An enzyme-linked immunosorbent assay revealed that a considerable proportion of the sera was seropositive for *Plasmodium falciparum*. Although a wide variety was shown in the titers, the positive rate for IgG antibody was higher in older people. The IgG titer of the positive population tended to be higher in the age range between 20 and 40 than at other ages. While the IgM antibody was detected more often in the younger population.

**Key words:** *Plasmodium falciparum*, ELISA, IgM, IgG, epidemiology

### Introduction

An increasing number of malaria cases is an important public health problem in tropical areas of developing countries (WHO, 1989). In Colombia, *P. vivax* is the dominant parasite species, but drug resistant *P. falciparum* appears to be becoming more widespread (Espinal et al., 1985, Instituto Nacional de Salud, Colombia, 1988, and WHO, 1987). To design anti-malarial measures effectively, it will be important to reveal incidence rate in the past at endemic areas. However, in Colombia, more data of serological examination for anti-malarial antibody have been needed to reveal past prevalence. In this study, we surveyed anti-*P. falciparum* IgM and IgG antibodies by an enzyme-linked immunosorbent assay (ELISA) in malaria endemic areas in Colombia.

### Materials and Methods

**Sera:** A total of 745 samples was collected in Tolu, September, 1988, and Villavicencio and its vicinity, February, 1989. Tolu locates at the Caribbean coast, while Villavicencio at the

Oriental Plains of Colombia. The sera were obtained from people who visited temporary or permanent health centers after the rainy season. Table 1 shows number and discrimination of the sera tested in this survey.

***P. falciparum* and *P. vivax* parasites:** The FCB-1 strain of *P. falciparum* was cultured by the candle jar method (Trager and Jensen, 1976). A mature trophozoite enriched preparation (>70%) was obtained by centrifugation of the culture on a 55% Percoll gradient. Blood from *P. vivax* patient was cultured by the candle jar method for 24 hr, and a fraction of mature trophozoites (>40%) was obtained by Percoll centrifugation.

**ELISA:** This was performed according to Voller and Bidwell (1986). A preparation of  $10^4$  parasitized erythrocytes/ml of carbonate-bicarbonate buffer was used to sensitize ELISA plates (Titer Tech, CA, U.S.A.). Biotinated anti-human  $\mu$  and anti-human  $\gamma$  rabbit antibodies, and avidine-alkaline phosphatase conjugate were purchased from Sigma (St. Louis, Mo, U.S.A.). Sera were obtained from patients with primary and repeated infections of *P. falciparum* and used as positive references for IgM and IgG antibodies, respectively. These patients had no recorded history of *P. vivax* infection. Normal Colombian donors were screened and negative controls were obtained.

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Table 1 Number and discrimination of sera tested

Age	Number of sera		
	Male	Female	Total
<b>Tolu</b>			
10-20	119	91	210
21-30	55	66	121
31-40	27	44	71
41-50	13	31	44
51-60	28	17	45
61-86	28	16	44
<b>Total</b>	<b>270</b>	<b>265</b>	<b>535</b>
<b>Villavicencio and vicinity</b>			
10-20	16	15	31
21-30	20	59	79
31-40	14	38	52
41-50	16	14	30
51-74	12	6	18
<b>Total</b>	<b>78</b>	<b>132</b>	<b>210</b>

## Results

Because *P. vivax* antigen obtained was insufficient in amount, sera collected in the endemic areas were tested only by *P. falciparum* ELISA. In a preliminary test, specificity of the ELISA was confirmed with reference sera. The positive reference sera for IgM and IgG antibodies to *P. falciparum*, when diluted to 1:100, gave optical densities (OD) of about 0.25-0.35 at 405 nm against *P. falciparum* antigen plate. A high IgM antibody titer was seen in patients with primary infections, whereas those with repeated infections tended to give a high IgG antibody titer. The same references gave 0.1 or lower OD against *P. vivax* antigen plate. *P. falciparum* antigen plate gave a low level of cross-reaction with sera from *P. vivax* patients (OD 0.15-0.25 when diluted to 1:50) who had no recorded history of *P. falciparum* infection. The negative controls showed OD 0.05-0.15 after dilution to 1:5. From these it was considered that this assay can be put into practical use for specific detection of anti-*P. falciparum* antibody.

Under the above conditions, positive reference sera diluted to 1:100 were considered as one unit. Serum samples were regarded as positive when two or more units were detected after dilution to 1:5.

Fig. 1 indicates incidences of IgM and IgG antibodies in both areas. Because there seemed to be no significant sex difference in antibody titer and positive rate, data obtained from both sexes were combined in the figure. Although incidence of positive reaction for IgM and IgG antibodies varied widely depending on the area and age, several specific findings were noted in the profile of the positive rate.

First, an apparently higher proportion of inhabitants were considered to be seropositive in Villavicencio and its vicinity (Fig. 1C and D) than in Tolu (Fig. 1A and B). This tendency was remarkable for IgG positive cases.

Second, the positive rate for IgM was higher in the younger than the older population in both areas (Fig. 1A and C). Inhabitants of Villavicencio at age of <30 showed high positive rates for IgM. When IgM titers were analyzed among seropositive individuals, a wide variety was observed depending on the age ranges and no specific tendency was observed (data not shown).

Third, in contrast to IgM, proportion of the positive rate for IgG antibody was apparently higher in the older than in the younger population (Fig. 1B and D). In Villavicencio about 10% of the inhabitants showed positive ELISA by 20 years old. The seropositive rate for IgG raised further in proportion to age.

Fourth, when seropositive individuals were compared, the IgG titer tended to be higher in the age range between 20 and 40 than at other ages. In Table 2 mean IgG antibody units of seropositive individuals are shown. Although the number of positive sera was limited, the antibody level at age range between 21 and 30 was significantly higher than those at ages 41 or older.

## Discussion

The reported annual cases of malaria in Colombia varied between about 50,000 and 100,000 in the past decade. Although incidence

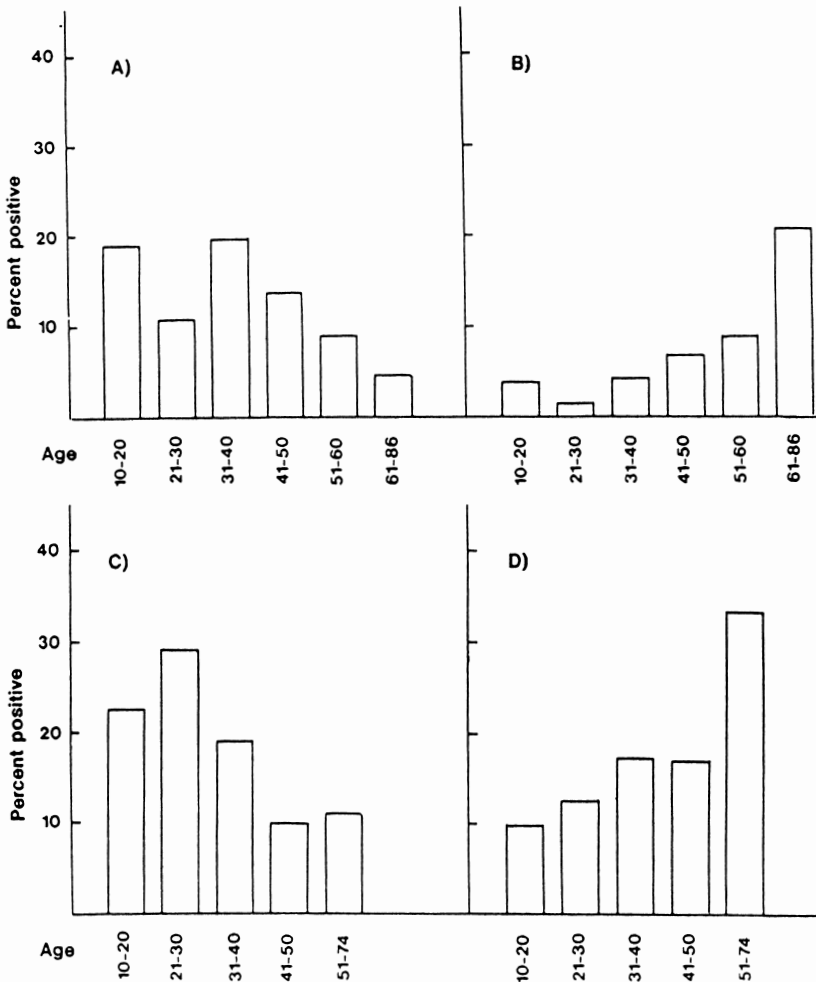


Fig. 1 Positivity rates for A) IgM in Tolu, B) IgG in Tolu, C) IgM in Villavicencio and its vicinity, and D) IgG in Villavicencio and its vicinity.

Table 2 Prevalence of IgG antibody in both areas and mean antibody titers among positive sera

Age	No.			Antibody unit	
	total	positive	(%)	mean	S.D.
10-20	241	11	( 4.6)	3.3	2.0
21-30	200	9	( 4.5)	16.3	16.9
31-40	123	11	( 8.9)	25.2	31.4
41-50	74	7	( 9.5)	9.2	8.2
51-83	107	19	(17.8)	3.7	1.7

Differences in mean antibody units are statistically significant between groups of (21-30) and (51-83), and (31-40) and (51-83) ( $p > 0.05$ ).

of the infection varied widely depending on area, year and season, the Oriental Plains which include Villavicencio has been considered to be meso- or hyper-endemic region (Instituto Nacional de Salud, Colombia, 1988, WHO, 1989). While degree of endemicity at the Caribbean coast including Tolu has been believed to be low in this country.

In this paper, serum samples were collected in Villavicencio and its vicinity and Tolu, and IgM and IgG antibodies against the schizont rich fraction of *P. falciparum* were detected by ELISA. There appeared only a low level of cross-

reaction between *P. falciparum* and *P. vivax* antigens, suggesting that data obtained in the present survey reflected the past prevalence of *P. falciparum* infection.

Results obtained in the present survey revealed a high prevalence rate of *P. falciparum* in Villavicencio and its vicinity. But noteworthy levels of antibody to *P. falciparum* were also detected in the sera from the Caribbean coast. These results suggested a wide distribution of falciparum parasite in these areas. Hematological examination indicated that *P. vivax* is the predominant parasite in this country (Nacional Instituto de Salud, 1988). However, drug resistant *P. falciparum* seems to be spreading widely in these areas (Espinal et al., 1983; Instituto Nacional de Salud, 1988). From these it was suggested that measures should be laid down in consideration of *P. falciparum* parasite in spite of that the present incidence by the parasite was estimated to be relatively low.

Interestingly, the positivity rate of IgG antibody was higher in the older than in the younger population in both areas. In contrast, the younger population showed a higher positivity rate of IgM antibody. This age dependent conversion of antibody class suggested that the inhabitants of these areas were under an increasing risk of bites by *P. falciparum* infected mosquitos in proportion to age. The most abundant malaria vectors at Tolu and Villavicencio were considered to be *Anopheles albimanus* and *A. darlingi*, respectively (Instituto Nacional de Salud, Colombia, 1988). Among the seropositive population, higher titers of IgG antibody were revealed at middle age than at younger ages. This finding may indicate a booster effect for the inhabitants at these areas by repeated bites by *P. falciparum*-infected mosquitos. The average titer of IgG

antibody reached plateau at ages between 21 and 40, and declined thereafter in proportion to age.

Because only serum samples were available in the present survey, test was carried out by ELISA to demonstrate the past incidence of the infection. Prevalence of *P. falciparum* parasite in the present time can be revealed by hematological examination. Both of these informations will help an effective and urgent anti-malarial measures be planned.

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