

PREVALENCE AND LEVELS OF IgG AND IgM ANTIBODIES AGAINST  
*PLASMODIUM FALCIPARUM* AND *P. VIVAX* IN BLOOD DONORS FROM  
RONDÔNIA, BRAZILIAN AMAZON

MARCELO URBANO FERREIRA<sup>†</sup>; LUÍS MARCELO ARANHA CAMARGO; MARIA ESTHER  
DE CARVALHO\*; REGINA TOMOKO NINOMIA\*; LUÍS ANTÔNIO VIEIRA GARCIA\*\* &  
FRANCISCO ROBERTO DOS SANTOS\*\*

Departamento de Parasitologia, Instituto de Ciências Biomédicas, Universidade de São Paulo,  
Av. Prof. Lineu Prestes, 1374 Cidade Universitária, 05508-900 São Paulo, SP, Brasil \*Laboratório de Sorologia,  
Superintendência de Controle de Endemias (SUCEN), São Paulo, SP \*\* Centro de Hematologia e Hemoterapia  
de Rondônia (HEMERON), Porto Velho, RO, Brasil

*Antibodies of IgG and IgM isotypes reacting with Plasmodium falciparum and P. vivax thick-smear antigens were searched for by the indirect fluorescent antibody test (IFAT) in a random sample of 230 blood donors at the transfusion centre of Porto Velho (HEMERON), Rondônia State, western Brazilian Amazon. A high prevalence of IgG seropositivity (32% against P. falciparum, 24% against P. vivax and 37% against either P. falciparum or P. vivax antigens) was detected among them, despite the fact that candidates reporting recent (< 12 months) malaria attacks were not eligible. Only a small proportion of them had also detectable IgM antibodies to these antigens. These data suggest an intense, relatively recent exposure to malaria in such an urban population sample. However, parasitaemia (as detected by microscopical examination of Giemsa-stained thick smears) was patent in only one prospective donor. The antibody profile of blood donors was compared with that of healthy subjects of all age groups, living in a close endemic area (Candeias village, 21 km east of Porto Velho). The villagers were classified into two groups according to their history of a recent (< 12 months) or a remote (> 12 months) past malaria attack due to either P. falciparum or P. vivax. Extensive overlapping was observed when the distribution of antibody titres of healthy subjects from Candeias village with a recent and remote malaria history was compared. In conclusion, subjects with a recent or a remote malaria history could not be distinguished by serological criteria alone.*

Key words: malaria – blood donors – indirect fluorescent antibody test – Brazilian Amazon –  
*Plasmodium falciparum* – *Plasmodium vivax*

Prevention of transfusional malaria depends on epidemiological and laboratory criteria for screening potentially infected blood donors. In most nonendemic regions, volunteers who had left a malarious area three to five years before blood donation are accepted, despite the possibility of persistent infection in asymptomatic semi-immune carriers (Ambroise-Thomas et al., 1971; Miller, 1976; Bruce-Chwatt, 1982). As detection of *Plasmodium* in blood smears may be difficult in asymptomatic carriers with low parasitaemias (Carvalho et al., 1985; OMS, 1989), serological tests for antimalarial IgG antibodies have been proposed as a suitable

diagnostic alternative for blood donors in either nonendemic countries (Faugère & Ranque, 1976; Wells & Ala, 1985; International Forum, 1987; Chataing, 1988), or malaria-free areas within malarious countries (Espinal & Olaya de Morales, 1984; Olaya de Morales et al., 1986). However, in endemic regions a large proportion of healthy prospective donors may have significant titres of anti-malarial IgG antibodies due to past *Plasmodium* infections (OMS, 1989). In certain situations, neither the presence of seropositivity nor the high antibody titres might suggest acute or recent malaria attacks.

During the last two decades, the incidence of transfusional malaria has been growing in several countries, due to the large-scale migration of subjects originating from malarious rural

<sup>†</sup>Corresponding author.

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areas to malaria-free cities and towns. The official reports from the World Health Organization for the years 1973-80 quoted 1,069 malaria cases induced by transfusion of blood components (whole blood, erythrocytes, plasma, leukocytes or platelets), and the number of such cases was particularly high in four countries: India, Bangladesh, Brazil and Mexico (Bruce-Chwatt, 1982). A few cases have been reported in Brazil since 1980 (Barata et al., 1989), but only a small proportion of actual cases are usually recorded by the health authorities in most countries (Saleun et al., 1981; Bruce-Chwatt, 1982) and therefore the true incidence of transfusional malaria in Brazil is unknown.

In Porto Velho, the capital town of the State of Rondônia (180,000 inhabitants), in northwestern Brazil, 9,524 autochthonous malaria cases due to either *P. falciparum* or *P. vivax* were recorded in 1990 (National Health Foundation, unpublished data). However, serological tests for anti-malarial antibodies are not made as a routine for blood donor screening in the Porto Velho transfusion centre (HEMERON). As a consequence, selection of blood donors is based on parasitological (one negative standard thick-smear) and epidemiological (no malaria history in the last 12 months, as reported by the candidate) criteria. Nevertheless, epidemiological data on recent transfusion malaria cases are not available in Rondônia.

In this study, prevalence and levels of IgG and IgM antibodies to *P. falciparum* and *P. vivax* antigens, as determined by the indirect fluorescent antibody test (IFAT), were investigated in a random sample of prospective blood donors from Porto Velho. These data were compared with the antibody profile of subjects living in a neighbouring endemic area (a peri-urban village) classified according to their history of a recent (< 12 months) or a remote (> 12 months) *P. falciparum* or *P. vivax* infection.

#### MATERIALS AND METHODS

*Blood donors from the Rondonian Transfusion Centre (HEMERON)* – Between April and May 1991, blood samples were collected from 230 randomly chosen prospective donors (90% of all candidates presenting during this period), for serum separation and microscopical examination of Giemsa-stained blood smears. A short

epidemiological questionnaire was applied to all candidates. Their age, sex and number of past malaria attacks (as reported by the candidates) were recorded. Blood samples were collected only from eligible candidates, i.e. those reporting neither malaria attacks in the last 12 months nor fever in the last 30 days. They were also clinically assessed by a physician of our team (M.U.F. or L.M.A.C.) and none of them had axillary temperature higher than 37 °C or any other sign or symptom suggesting malaria. Giemsa-stained thick smears were examined for malaria parasites in a minimum of 100 immersion (1000 X) microscopical fields in HEMERON; for seropositive subjects, further 600 microscopical fields (on three different slides) were examined later by a second microscopist (M.U.F. or L.A.V.G.).

*Control sera* – All control sera used throughout this study were collected after informed consent from subjects of all age-groups during a clinical and sero-epidemiological malaria survey conducted in the village of Candeias do Jamari, about 21 km east of Porto Velho, between August and November 1990. Basic epidemiological information about this malaria-endemic peri-urban village has been presented elsewhere (Cardoso et al., 1992). Serum samples were divided into four groups: (a) negative controls (n = 108) from healthy subjects without a history of past malaria infections and negative by standard Giemsa-stained thick smears (100 microscopical fields); (b) positive controls for *P. falciparum* malaria (n = 45) or *P. vivax* malaria (n = 48), collected from patients with clinical symptoms and positive thick smears at the time of bleeding; (c) healthy subjects with a history of a recent (< 12 months) *P. falciparum* malaria (n = 103) or *P. vivax* malaria (n = 150), but asymptomatic and negative by thick smears at the time of bleeding; (d) healthy subjects whose last malaria attack, due to *P. falciparum* (n = 97) or *P. vivax* (n = 103), occurred more than 12 months previously ("remote" malaria history) and who were asymptomatic and negative by thick smears at the time of bleeding. Data about the last malaria attack and the *Plasmodium* species implicated were reported by the patients, and therefore some cases of misinformation could not be ruled out.

*Indirect fluorescent antibody test (IFAT)* – IFAT was performed essentially as previously described (Carvalho et al., 1992). *P. falciparum* and *P. vivax* thick-smear antigens (Sulzer et



al., 1969) were prepared with blood from patients with high asexual parasitaemia from Rondônia and stored at -20 °C for up to 90 days until used. Serum samples were at first assayed at 1:2 dilution and the positive ones were further serially diluted until 1:16,384. Commercial fluorescein-conjugated rabbit anti-human IgG and IgM were supplied by Behring (Germany) and individually titred for optimal working dilution. All reactions were read under an Olympus FLM fluorescence microscope by the same microscopist (M.E.C.) at 400 X magnification (Ferreira & Carvalho, 1973). Quantitative results were expressed as titres (reciprocals of the last sample dilution having fluorescence).

**Statistical analysis** – Proportions of seropositive subjects were compared using Chi-squared statistic with Yates' correction for continuity. Antibody titres of different groups were compared by the nonparametric Mann-Whitney U-test (Mann & Whitney, 1947). Correlations between antibody titres and the number of past malaria infections, as well as the age of the blood donors, were calculated by the nonparametric Spearman rank test (Siegel, 1977). A commercial software (Systat version 5.0, Systat Inc., USA, 1990) was used in a PC-compatible microcomputer. P values < 0.05 were considered to be significant.

RESULTS

**Cut-off titres for IgG and IgM antibodies** – Cut-off titres were determined by studying the distribution of both IgG and IgM antibody titres in negative (n = 108) and positive controls (n = 45 for *P. falciparum* and n = 48 for *P. vivax*) (Fig. 1). Highest efficiency of the serological tests, as defined by Galen (1980), was obtained by using the following cut-off titres: eight for IgG antibodies to both antigens and two for IgM antibodies to both antigens. The sensitivity (Se%) and specificity (Sp%) estimates for our IFAT obtained by applying these cut-off titres are shown in Table I.

**Prevalence and levels of IgG and IgM antibodies in blood donors** – Seventy-three donors (32%) were IgG-positive by IFAT against *P. falciparum* and 55 (24%) against *P. vivax* antigen, while 86 donors (37%) were IgG-positive against either *P. falciparum* or *P. vivax* thick smear antigen. On the other hand, only nine donors (4%) were IgM-positive against *P. falciparum* antigen and three (1%) against *P.*

*vivax* antigen. Fig. 2 shows the distribution of IgG and IgM antibody titres to both antigens in this sample.

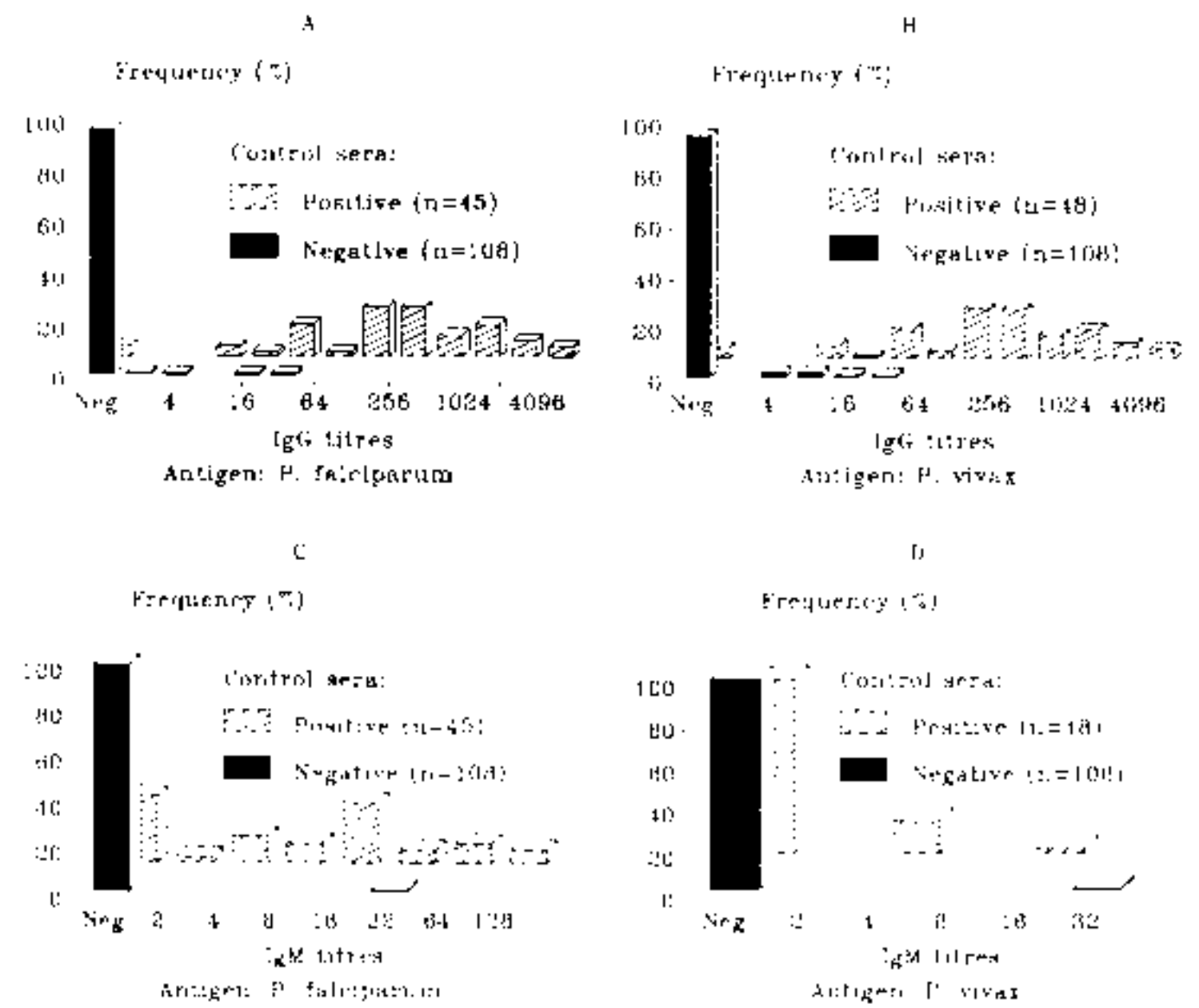


Fig. 1: frequency distribution of IgG (A and B) and IgM (C and D) antibody titres to *Plasmodium falciparum* (A and C) and *P. vivax* (B and D) thick-smear antigens in negative (n = 108) and positive (n = 45 for *P. falciparum* and n = 48 for *P. vivax*) control sera.

TABLE I

Sensitivity (Se%) and specificity (Sp%) for detection of IgG and IgM antibodies to *Plasmodium falciparum* and *P. vivax* thick-smear antigens by indirect fluorescent antibody test (IFAT), with 95% confidence intervals presented in brackets

Test	Antigen	Se%	Sp%
IFAT IgG	<i>P. falciparum</i>	96 (90-100)	98 (96-100)
IFAT IgM	<i>P. falciparum</i>	67 (53-80)	99 (97-100)
IFAT IgG	<i>P. vivax</i>	96 (90-100)	96 (93-100)
IFAT IgM	<i>P. vivax</i>	19 (7-30)	99 (97-100)

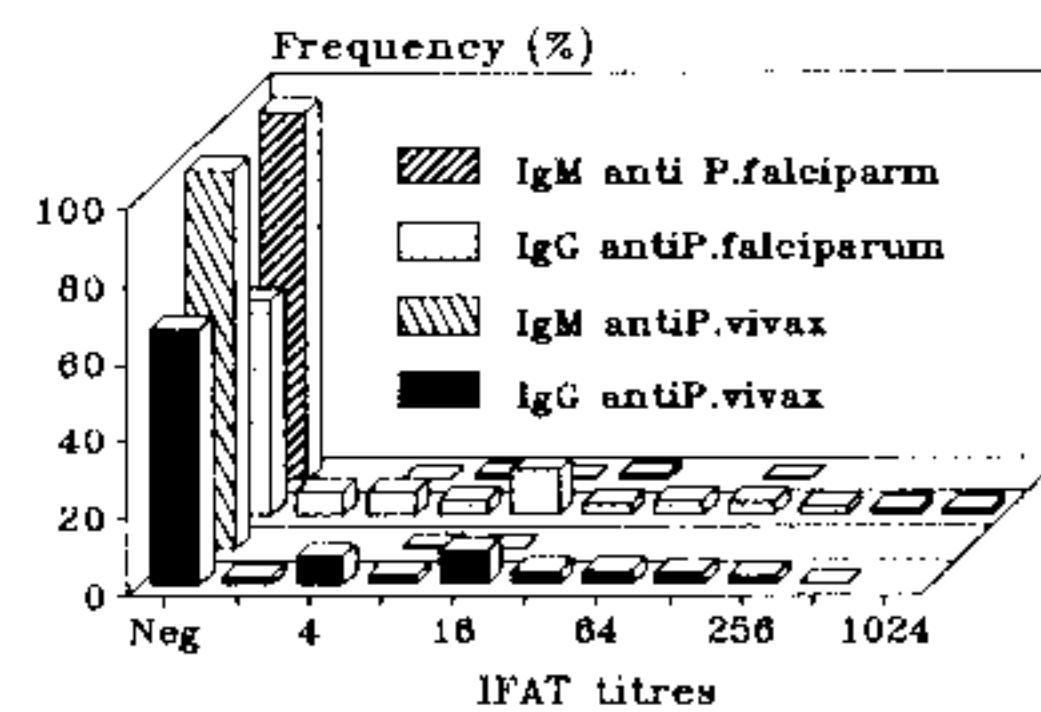


Fig. 2: frequency distribution of IgG and IgM titres to *Plasmodium falciparum* and *P. vivax* thick-smear antigens in 230 blood donors from Porto Velho, Brazilian Amazon.

Donors were on average 34 years old (range 18-59 years), most of them were males (87%) and lived in the urban area of Porto Velho (96%). Most (126 or 55%) reported no past malaria infections, while 85 (37%) reported one to five past malaria episodes and only 19 (8%) reported more than five past malaria infections.

Table II presents the geometric means of IgG and IgM antibody titres to both antigens according to the age distribution of the blood donors, revealing a minor trend for increasing levels of antibodies with age. A positive correlation between age and antibody titres was found only in relation to IgG against *P. falciparum* (Spearman's correlation coefficient  $r_s = 0.297$ ,  $P < 0.05$ ).

TABLE II

Age-dependent intensity (as measured by the geometric mean titres or GMT) of antibody reaction against *Plasmodium falciparum* and *P. vivax* thick-smear antigens in 230 blood donors from Rondônia

Test	Antigen	GMT by age group (years)		
		< 30 (n = 93)	30-50 (n = 116)	> 50 (n = 21)
IFAT IgG	<i>P. falciparum</i>	1.5	2.3	2.9
IFAT IgM	<i>P. falciparum</i>	1.0	1.1	1.3
IFAT IgG	<i>P. vivax</i>	1.4	1.9	2.0
IFAT IgM	<i>P. vivax</i>	0.0	1.1	0.0

However, strong positive correlations were observed between the titres of IgG, but not of IgM, antibodies to both *P. falciparum* and *P. vivax* thick-smear antigens and the number of past malaria infections reported by the prospective donors (Spearman's correlation coefficients respectively  $r_s = 0.438$  and  $r_s = 0.506$ ,  $P < 0.001$  for IgG and  $r_s = 0.155$  and  $r_s = 0.013$ ,  $P > 0.05$ , for IgM). Fig. 3 shows the differential profile of IgG reactivity of donors with (n = 104) and without (n = 126) a previous malaria history. Only one prospective donor was an asymptomatic carrier of malaria parasites (100 asexual *P. vivax* forms per microlitre of blood) at the time of bleeding, as detected by microscopical examination of thick smears. This subject presented the following antibody titres: against *P. falciparum* antigen, IgG = 64, IgM = 16; against *P. vivax* antigen, IgG = 256, IgM = 16. Interestingly, he had reported no past malaria infection.

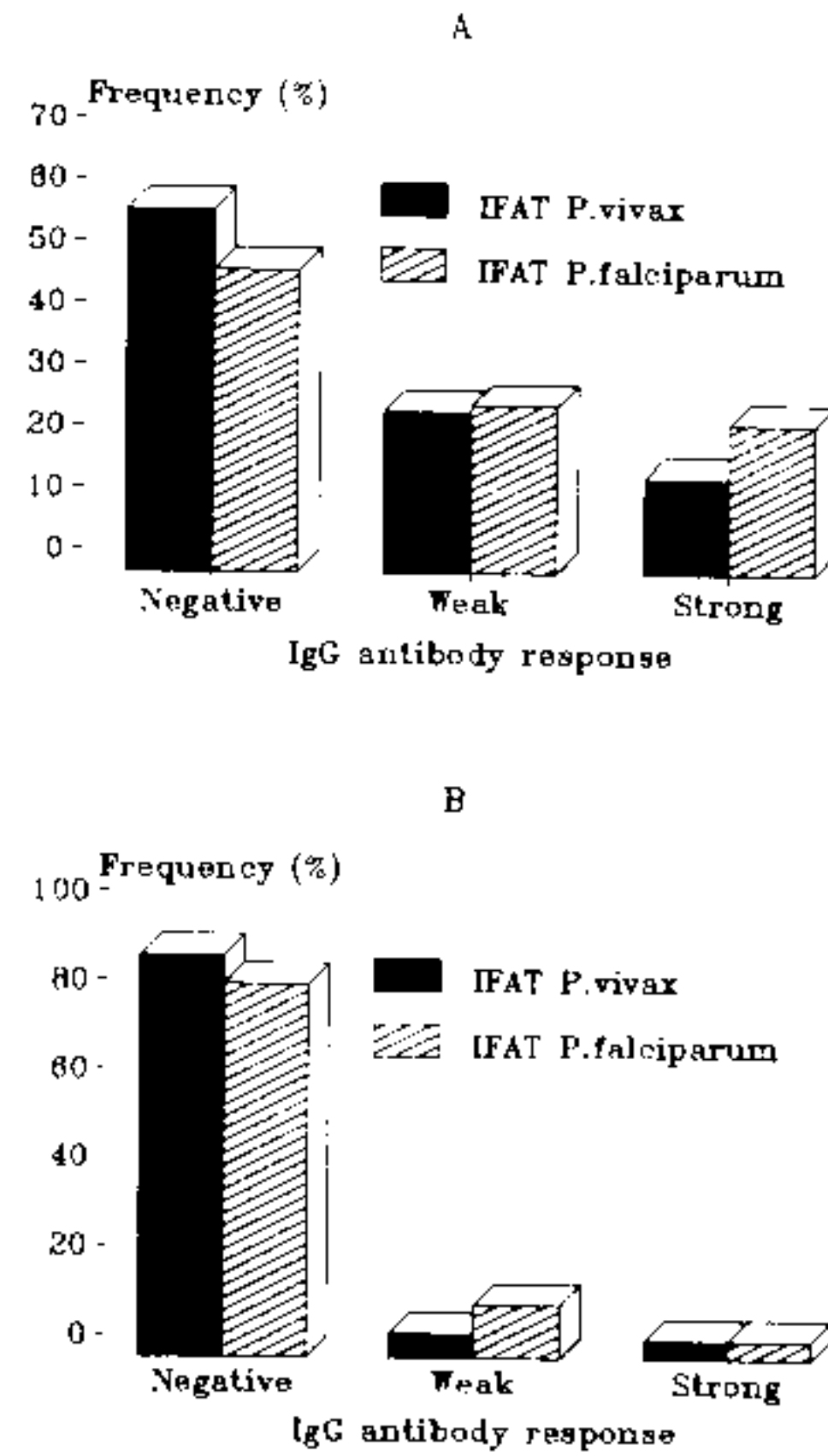


Fig. 3: frequency distribution of negative (titres < 8), weak (titres between 8 and 64) and strong (titres > 64) IgG antibody reactions to *Plasmodium falciparum* and *P. vivax* thick-smear antigens in blood donors from Porto Velho with (n = 104) (A) and without (n = 126) (B) a history of past malaria attacks.

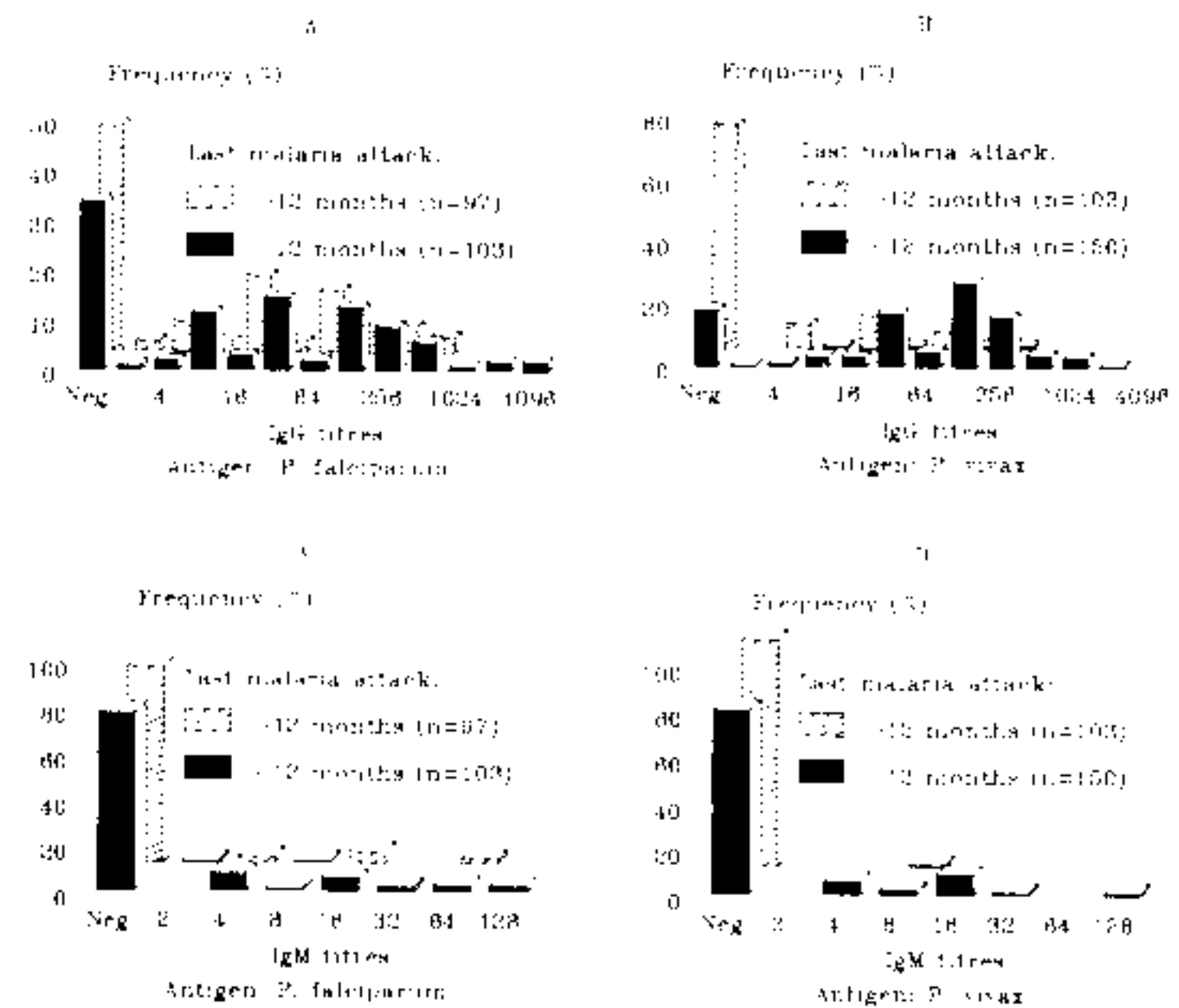


Fig. 4: frequency distribution of IgG (A and B) and IgM (C and D) antibody titres to *Plasmodium falciparum* (A and C) and *P. vivax* (B and D) thick-smear antigens in healthy subjects from Brazilian Amazon with a history of recent (< 12 months) malaria history (n = 103 for *P. falciparum* and n = 150 for *P. vivax*) or remote (> 12 months) malaria history (n = 97 for *P. falciparum* and n = 103 for *P. vivax*).

*Persistence of IgG and IgM seropositivity* -- Fig. 4 shows the differential profiles of IgG and IgM antibody titres to *P. falciparum* antigens (Fig. 4A, C) and to *P. vivax* antigens

TABLE III

Prevalence of IgG and IgM antibodies to homologous and heterologous antigen, as detected by indirect fluorescent antibody test (IFAT), in healthy subjects from Candeias village with a recent (< 12 months) or remote (> 12 months) history of malaria attack due to *Plasmodium falciparum* or *P. vivax*

Test	Antigen	Prevalence of antibodies (%)			
		<i>P. falciparum</i>		<i>P. vivax</i>	
		recent (n = 103)	remote (n = 97)	recent (n = 150)	remote (n = 103)
IFAT IgG	Homologous <sup>a</sup>	63	46	80	20
IFAT IgM	Homologous <sup>b</sup>	22	14	19	1
IFAT IgG	Heterologous <sup>c</sup>	49	31	30	34
IFAT IgM	Heterologous <sup>c</sup>	6	2	7	9

Comparisons between recent and remote malaria patients by Chi-squared statistic with Yates' correction: *a*  $P < 0.0001$  for *P. vivax* patients and  $P = 0.02$  for *P. falciparum* patients; *b*  $P < 0.0001$  for *P. vivax* patients and  $P > 0.05$  for *P. falciparum* patients; *c*  $P > 0.05$  for both *P. falciparum* and *P. vivax* patients.

(Fig. 4B, D) in subjects from Candeias village with a recent (< 12 months) or remote (> 12 months) history of malaria attack due to one of these parasites, whereas Table III presents the respective percentages of IgG and IgM seroprevalence in the same subjects against both homologous and heterologous antigens.

Significant differences in IgG antibody titres to homologous antigens were observed when subjects with a recent malaria history were compared with those with a remote history (respectively  $U = 5886.0$ ,  $P = 0.024$  for *P. falciparum* and  $U = 13084.5$ ,  $P < 0.0001$  for *P. vivax*, Mann-Whitney *U*-test). On the other hand, IgM titres to homologous antigen were significantly lower in subjects with a remote *P. vivax* malaria history ( $U = 9139.5$ ,  $P < 0.001$ ), but no statistically significant difference was observed between IgM titres to *P. falciparum* antigen in subjects with a recent or remote history of infection due to this species ( $U = 5392.5$ ,  $P = 0.15$ ).

#### DISCUSSION

There is scant published information about the prevalence and levels of IgG and IgM antibodies to *Plasmodium* antigens in blood donors from towns within malarious regions in Brazil. Moreover, most sero-epidemiological studies have included only *in vitro* cultured *P. falciparum* antigens, despite the high prevalence of *P. vivax* infections in such malarious areas. As previously shown, heterologous antigens may be unreliable for screening *P. vivax* infections by IFAT (Carvalho et al., 1992). In this study, we at first analyzed titre distributions of positive and negative control sera

obtained by our IFAT using both IgG and IgM conjugates and *P. falciparum* and *P. vivax* antigens. Cut-off titres were defined from these preliminary data. This approach permitted us to estimate the sensitivity and specificity of the serological test, confirming our previous observations of high diagnostic sensitivity for IgG detection and low sensitivity for IgM antibodies (Carvalho, M. E., unpublished data). There is scarce comparative data on IgM response to homologous thick-smear antigens during acute malaria attacks due to either *P. falciparum* and *P. vivax*, and the reasons for the lower sensitivity of IgM detection in *P. vivax* infections, observed in our study, remain unknown. On the other hand, the competitive effect exerted by high concentrations of anti-*P. falciparum* IgG antibodies in sera from infected patients with intense past malaria exposure has been proposed as an explanation for the low sensitivity of IgM detection by standard immunoassays (Dziegiel et al., 1992).

High prevalences of IgG antibodies to both *P. falciparum* and *P. vivax* antigens were detected by IFAT in noninfected prospective blood donors from Porto Velho, but only a small proportion of them had detectable IgM antibodies to one or both antigens. Interestingly, a not negligible proportion of blood donors without a malaria history ( $n = 126$ ) were IgG positive to *P. falciparum* antigens (10%) or *P. vivax* antigens (16%). These findings are possibly due to imprecise malaria histories reported by prospective donors or to past malaria episodes either misdiagnosed or asymptomatic, suggesting that purely epidemiological criteria may not be adequate for screening blood donors in malarious areas. On



the other hand, a large proportion of prospective donors with one or more past malarial infections but negative by Giemsa-stained thick smears ( $n = 104$ ) remained seroreactive to *P. falciparum* (51%) or to *P. vivax* (41%), despite the minimum interval of 12 months after the last reported clinical malaria attack.

A large overlapping was observed when we compared the distribution of antibody titres to both antigens in healthy villagers with a recent and a remote malaria history. Significant differences were detected by comparing respective IgG titres, but on a serological basis alone it was not possible to allocate an individual subject from this population to the group with recent or remote malaria infection. Proportions of IgG-responders to *P. falciparum* thick-smear antigens were recently reported by Chougnet et al. (1991) to be similar among African subjects living in France for less than 12 months ( $n = 23$ ) and for more than 12 months (and without returning to malarious areas,  $n = 18$ ).

Strong positive correlations between antibody titres and cumulative exposure to malaria have been usually reported, and anti-malarial antibodies have been detected several years after the last malarial attack in subjects previously exposed to intense transmission (Bruce-Chwatt, 1982; OMS, 1989). Recent data from Madagascar suggest that some level of immune protection may persist for almost 30 years after the last malaria exposure in a previously hyperendemic area (Deleron & Chougnet, 1992). On the other hand, our longitudinal studies in Amazonian patients have shown that short-lived anti-malarial antibodies (specially of IgM isotype and IgG3 subclass) may be undetectable only a few months after a clinical malaria attack (Ferreira, M. U., Master's dissertation in preparation).

Serological data from routine blood donor screening have been used as an estimate of the overall prevalence of several infectious diseases, specially Chagas' disease and hepatitis B, in Brazilian urban communities. However, prospective blood donors constitute a biased sample of a given human population, as only apparently healthy subjects are included (Martelli et al., 1991). Therefore, the true prevalence of anti-malarial seropositivity in the adult population of Porto Velho is not possible to infer from our data, that are predictably underestimated. Nevertheless, such a high level

of seropositivity among blood donors living in Porto Velho, despite this population bias, discloses a previously unknown aspect of the malaria epidemiology in Brazilian Amazon, namely a considerable level of relatively recent exposure to malaria transmission of adult subjects from an Amazonian urban centre.

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