EPIDEMIOLOGICAL AND ECOLOGICAL ASPECTS RELATED TO MALARIA IN THE AREA OF INFLUENCE OF THE LAKE AT PORTO PRIMAVERA DAM, IN WESTERN SÃO PAULO STATE, BRAZIL

Almério de C. GOMES(1), Marcia B. de PAULA(1), Ana Maria R. de C. DUARTE(2), Maura A. LIMA(1), Rosely dos S. MALAFRONTE(3), Luis F. MUCCI(4), Sabina Lea D. GOTLIEB(1) & Delsio NATAL(1)

SUMMARY

A study was carried out in the area of influence of the Porto Primavera Hydroelectric Power Station, in western São Paulo State, to investigate ecological and epidemiological aspects of malaria in the area and monitor the profile of the anopheline populations following the environmental changes brought about by the construction of the lake. Mosquitoes captured were analyzed by standardized indicator species analysis (ISA) before and during different flooding phases (253 m and 257 m elevations). The local human population was studied by means of parasitological (thin/thick blood smears), molecular (PCR) and serological tests. Serological tests consisted of Enzyme Linked Immunosorbent Assay (ELISA) with synthetic peptides of the circumsporozoite protein (CSP) from classic *Plasmodium vivax*, *P. vivax* variants (VK247 and "vivax-like"), *P. malariae* and *P. falciparum* and Indirect Immunofluorescence Assay (IFA) with asexual forms of *P. vivax*, *P. malariae* and *P. falciparum*. The results of the entomological survey indicated that, although the *Anopheles darlingi* population increased after the flooding, the population density remained very low. No malaria, parasite infection or DNA was detected in the inhabitants of the study area. However, there was a low frequency of antibodies against asexual forms and a significant prevalence of antibodies against *P. vivax*, *P. vivax* variants, *P. falciparum* and *P. malariae*; the presence of these antibodies may result from recent or less recent contact with human or simian *Plasmodium* (a parallel study in the same area revealed the existence of a sylvatic cycle). Nevertheless, these results suggest that, as in other places where malaria is present and potential vectors circulate, the local epidemiological conditions observed could potentially support the transmission of malaria in Porto Primavera Lake if infected individuals are introduced in sufficient numbers. Further studies are required to elucidate the phenomena described in this paper.

KEYWORDS: Malaria; Porto Primavera Hydroelectric Power Station; Entomology; Antibodies to Circumsporozoite Protein; Antibodies to *Plasmodium* blood-stage antigens.

INTRODUCTION

It is now well documented that the destruction of forests affects arthropod-vector populations[43]. In the case of malaria in Brazil, the presence of the disease is closely related to the occupation of new areas, although old transmission areas continue to be a factor in the increasing incidence of the disease. In the past, the disease was widespread outside the Legal Amazon; however, it is now under control in this region where less than 0.5% of cases are now observed outside this region. An example of this is the São Paulo State, where the number of autochthonous cases recorded since 1940[4,9] has declined sharply with the use of DDT and the growth in agriculture during this period, as a result of which the disease has tended to become limited to isolated foci and sporadic cases[11].

The declining trend seen in Brazil in the 50s[8] continued in the São Paulo State in the 70s. In the west of the state, transmission of the disease was nearly always associated with the Paraná River's high-water season,

and the disease, for which the main contributory factor was the appearance of conditions favorable to *An. darlingi*, was known as "riverbank malaria". Outbreaks continued to occur in the region for a number of years, and from a total of 20,000 cases notified from 1983 to 1992, only 2% were autochthonous[44], with the last recorded case being in 1993[38,39].

FORATTINI[20] reported that members of the *An. darlingi* population managed to survive in minimum ecological conditions even after deforestation.

With the increasing number of hydroelectric power stations and environmental imbalance, there is the possibility that *An. darlingi* may reappear. The Itaipu dam in the Paraná State is a good example of an area where malaria returned[8,21,41].

In this context, the lake at Porto Primavera, a project developed in an area with a history of malaria, puts the region at risk of increased

density of anophelines, in particular *An. darlingi,* a common species on the banks of dams with arboreal vegetation[12]. This phenomenon, together with migratory movements, could increase the risk of *Plasmodium* transmission, leading to the need for surveillance and measures to prevent malaria foci becoming established in the São Paulo State.

It was with this in mind that the present authors had as their goal to investigate the potential for malaria transmission associated with *An. darlingi* and the ecological and epidemiological conditions found in the area of influence of the Porto Primavera dam in Presidente Epitácio municipality. The approach adopted in the study involved entomological research, with an emphasis on anophelines, and investigation of the parasitological and serological prevalence of malaria.

MATERIALS AND METHODS

Study area: The study area is located on the left of the Paraná River in Presidente Epitácio municipality in the São Paulo State[30]. This area was originally the ecological reserve known as "Reserva Ecológica Lagoa São Paulo" or "Complexo Lagoa São Paulo" formed by the São Paulo, Comprida, Tremendal and Jota lakes[2].

The original vegetation in the area, which consisted of "cerrado", dense woodlands and semi-deciduous seasonal forest interspersed with riparian forest and freshwater swamp vegetation, was replaced with a landscape of plantations and pastures with fragments of native vegetation irregularly distributed as a result of human and bovine activity. With the formation of the lake, in December 1999, the vegetation was further reduced.

According to the Köppen classification, the weather is classified as Aw tropical with a dry winter and warmest and coldest month average temperatures of around 26 °C and 19 °C, respectively. The annual rainfall index varies between 1,000 and 1,400 mm[2]

Human population: The human population studied consisted of individuals who lived in two separate areas: Campinal (a subdistrict in the Presidente Epitácio municipality) and Reassentamento Lagoa São Paulo (RLSP), an area neighboring Campinal, which was built in 1980 by the "Companhia Energética do Estado de São Paulo" (CESP).

Part of the population in the area lived in the Campinal, and the population in the year 2000 was of 2,476 inhabitants[22]. Another part lived in farms in the swampy areas in the old "Reserva Biológica da Lagoa São Paulo" and islands on the Paraná River. This population was transferred to Reassentamento Lagoa São Paulo from 1980 onwards, at various times which we were unable to establish.

Because of the delay in building the hydroelectric power station, large numbers of squatters and "landless" people gradually invaded the unoccupied areas of RLSP. As a result, the population in the area became quite heterogeneous, both in terms of where they originated from and the time for which they had lived in the area. No specific population data for the area was found.

When the dam was built, a tourist center based on leisure and fishing was set up, with visitors coming from other areas in the São Paulo State and the West Central region of Brazil.

Entomological study: The entomological study was carried out during two periods - the pre-filling period, when the elevation of the Paraná River was 247m above sea level (before December 1998), and the post-filling period, when the elevation was increased first to 253m (in January 1999) and then to 257m (in March 2001) (Fig. 1).

Two Shannon traps were installed in the following two rural properties: JB Farm (52º00'25"W/21º38'45"S), which represents the two changes the environment underwent (flooding to 253m and 257m), and SA Farm (51º55'38"W/21º34'07"S), which was included as a supplement to this study, where captures were only made at the 257m elevation.

JB Farm is 20 km from the nearest urban center and has forest fragments, pastures and domiciles located in swampy areas by the water's edge. *Panicum maximum* (Guinea grass) and *Eichhornia crassipes* (common water hyacinth) are the predominant vegetation. SA Farm is 34 km from the nearest urban center and is located along the edge of the Peixe River, one of the most important tributaries of the Paraná River. It contains a narrow strip of secondary riparian forest and a long, swampy area that was covered by the dam water. There was a strip of bushes between the lake and the dwellings in this area.

Monthly mosquito captures were made before the flooding (at the 247m elevation) and after the flooding (at the 253m and 257m elevations).

The adult *Culicidae* were caught in Shannon traps (SHANNON, 1939) on the edge of the forest during the evening twilight, with the time of capture classified as follows: 1st pre-twilight, 2nd pre-twilight, twilight and 1st post-twilight. Captures on JB Farm took place between July 1997 and December 1998 (247m elevation); July 1999 and December 2000 (253m elevation); and July 2001 and December 2002 (257m elevation). Captures on SA Farm took place between April 2002 and February 2003, after the dam had been filled to 257m. Each of the four periods lasted the same time as the twilight (approximately 20 minutes). This time was estimated for the local coordinates, according to REBELO *et al.*[33]. All the captured mosquitoes were killed and taken to the laboratory for identification.

To calculate the adult mosquito abundance corresponding to each specified capture period, the standardized Index of Species Abundance (ISA) proposed by ROBERTS & HSI[34] was used.

Parasitological and serological studies: The first step in the study of the human population involved interviewing 861 residents of Campinal (496) and RLSP (365) who agreed to take part in the research as volunteers. A questionnaire was applied to gather information about personal data, length of residence in the study area, malaria episodes, travel to endemic areas, episodes of other diseases, etc. No clinical tests were carried out.

The size of the sample for blood collection was calculated based on the figure of 861 referred to above (non-probabilistic sample). The sample size obtained was 196. Using a figure of 5% for the 1st species error (α) and 20% for the 2nd species error (β), together with a possible refusal proportion of 10%, a sample size of 216 was obtained. These individuals were chosen using systematic random sampling with an interval size of 4 using the SPSS 10.0 program for Windows.
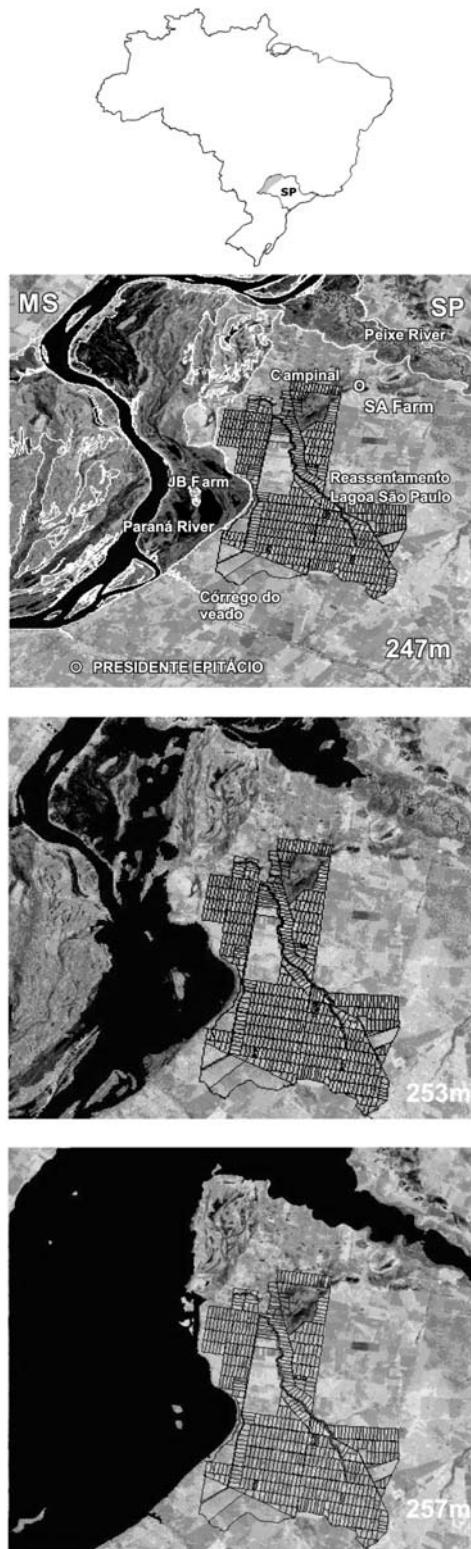
Fig. 1 - Flooding sequence on the Paraná River and the areas of interest in the study. Projections based on 1:10,000 scale maps (247m, 253m and 257m elevations) and boundary maps for the Reassentamento Lagoa São Paulo (RLSP) on a LANDSAT- TM 3/4/5 (RGB) composite image.

From the 216 members of the sample, 130 were residents of Campinal, and 86 residents of Reassentamento Lagoa São Paulo (RLSP).

In January 2000, the blood samples of the 216 individuals were collected in vacutainer tubes with and without anti-coagulant (EDTA). At the same time, thick-smear slides were prepared from each sample. All those who took part in the study signed the voluntary informed-consent form approved by the Ethics Committee of the School of Public Health.

The thin and thick-smear slide techniques were used, as advocated by the World Health Organization[6,45]. The slides were examined under an optical immersion microscope (100x).

Genomic DNA was extracted using the "Wizard Genomic DNA Purification Kit" (Promega-A-1125) according to the manufacturer's instructions.

Polymerase chain reaction (PCR) was performed to amplify the species-specific fragment corresponding to the plasmodium 18S ssrRNA gene according to the "Rubio" protocol[36]. Amplicons were analyzed under UV light following electrophoresis on a standard 2% agarose gel and staining with ethidium bromide.

Enzyme Linked Immunosorbent Assay (ELISA) was performed as previously standardized for detection of IgG antibodies against circumsporozoite protein (CSP) of *Plasmodium*[46] with some modifications[10,11]. We used synthetic peptides corresponding to CSP repeats: *P. vivax Type I (classic)* (GDRADGQPA)$_2$ (GDRAAGQPA)$_2$ (GDRADGQPA)[3,28] (Pvc); *P. vivax* Type II (VK247) (ANGAQNQPG)$_4$ [35] (Pvk); human *P. vivax*-like (APGANQEGGAA) [32] (Pvl); *P. malariae/P. brasilianum* [(NAAG)$_4$]$_4$ [24,25](Pm/Pb); and *P. falciparum* (NANP)$_8$ [46](Pf). These peptides were synthesized by Invitrogen.

Positive and negative controls for each peptide were included in each assay. The cut-off values were determined for each peptide by adding three standard deviations to the mean absorbance of 50 negative sera (from blood donor sera from the Fundação Hemocentro Blood Bank, Hospital das Clínicas, Faculty of Medicine, University of São Paulo-HC-FMUSP). The cut-off values were 0.127 for Pm/Pb, 0.135 for Pvc, 0.151 for Pvk, 0.179 for Pvl and 0.122 for Pf.

Sera were tested in duplicate and considered positive if the average absorbance was equal to or greater than the cut-off value for the corresponding peptide.

Indirect Immunofluorescence Assay (IFA) was performed as previously standardized for detection of IgG antibodies against blood forms of *Plasmodium*[18]. We used erythrocytes from *P. vivax* primo-infected patients, *P. falciparum* from culture and *P. malariae* from an experimentally infected *Saimiri* monkey, washed in PBS pH 7.2, as antigens. Antigens were supplied by Dr. Salma G. Oliveira (Instituto Evandro Chagas, Brazil) and Dr. W.E. Collins (Centers for Disease Control, USA). Negative controls (sera from blood donors) and positive controls (sera from malaria patients) were included in all tests. Anti-human IgG fluorescent isothiocyanate (FITC) conjugate (Biolab) was tested at several dilutions with positive and negative sera. Each antigen was tested with 50 negative sera samples from blood donors. A 1:40 serum dilution was determined as the cut-off point for the three antigens. The

results were expressed as titers corresponding to the inverse of the last dilution of the serum with a positive reaction.

Statistical analysis: The prevalence of positive reactions obtained in ELISA and IFA tests were compared by chi-square test. Absorbance was compared by non-parametric Kruskall-Wallis test, and geometric mean titers by ANOVA.

RESULTS

Entomological analysis: A total of 3,519, 2,679 and 478 specimens of adult *Culicidae* were caught on JB Farm before, during and after the filling of the dam (247m, 253m and 257m respectively), and 12,478 on SA Farm at the 257m elevation.

The Culicidae specimens were from the genera *Aedeomyia, Aedes, Anopheles, Coquillettidia, Culex, Limatus, Mansonia, Psorophora, Sabethes, Uranotaenia, Wyeomyia*. Specimens from the genus *Anopheles* represented 28.36%, 15.42% and 6.07% of the totals caught on JB farm (247m, 253m and 257m respectively) and 37.15% of the total on SA Farm.

On JB farm, a total of 998 *Anopheles* specimens were collected at an elevation of 247m, no *An. darlingi* specimens were collected. At an elevation of 253m, the total number of *Anopheles* specimens was 413, of which 37 were *An. darlingi*. At 257m, the total number of *Anopheles* was 29, of which eight were *An. darlingi*. On SA farm, the total number of *Anopheles* specimens at an elevation of 257m was 4635, of which 983 were *An. darlingi*.

Figure 2 (a-d) shows the estimates for the standardized index of species abundance (ISA)[30] with the respective water elevations and areas where the species were collected.

When the ISA was applied to the twilight period on JB Farm before the dam was filled (247m), *Aedes scapularis* predominated, whereas *Anopheles albitarsis* was in 6th position. *Anopheles darlingi* was not present during this period of the study (Fig. 2a). When the elevation was 253m, *Mansonia humeralis* predominated, whereas *Anopheles albitarsis* was in 5th position and *Anopheles darlingi* in 8th (Fig. 2b). At an elevation of 257m, *Mansonia humeralis* still predominated, whereas *Anopheles arbitarsis* was in 8th position and *Anopheles darlingi* in 10th (Fig. 2c).

On the SA Farm, *Coquillettidia shannoni* predominated at an elevation of 257m, whereas *An. darlingi* was in 6th position and *An. albitaris* in 9th (Fig. 2d).

Parasitological and serological results: The parasitological tests were negative for malaria infection in both groups (thick and thin smears and PCR).

Thirty-eight sera were found to be reactive by ELISA, corresponding to 17.6% seropositivity (CI 95%). The prevalence of positive results for each peptide are shown in Table 1.

In the Campinal sample, the greatest prevalence of positive results was observed for the Vivax complex CSP (22.3%), with the greatest prevalence for Pvc (11.5%), followed by Pvl (7.7%) and Pvk (3.1%).

Table 1
Prevalence of IgG antibodies against circumsporozoite protein (CSP) repeat regions determined by ELISA with synthetic peptides. Sera from residents of Campinal and Reassentamento Lagoa São Paulo (RLSP), Presidente Epitácio, São Paulo, January 2000

CSP - ELISA	IgG antibodies - Positive (%)		
	Campinal 130 (100%)	RLSP 86 (100%)	TOTAL 216 (100%)
Pvc*	15 (11.5)	2 (2.3)	17 (7.9)
Pvk*	4 (3.1)	1 (1.2)	5 (2.3)
Pvl*	10 (7.7)	6 (7.0)	16 (7.4)
Vivax complex**	29 (22.3)	9 (10.5)	38 (17.6)
Pm/Pb*	5 (3.8)	2 (2.3)	7 (3.2)
Pf*	5 (3.8)	6 (7.0)	11 (5.1)

*Pvc - classic *P. vivax* (Type1); Pvk - *P. vivax* VK247 variant (Type 2); Pvl - human *P. vivax*-like/*P. simiovale*; Pm/Pb - *P. malariae/P. brasilianum*; Pf - *P. falciparum*.**vivax complex - total number of positive sera from *P. vivax* species (Pvc+Pvk+Pvl).

Positivity for Pm/Pb and Pf was also detected, although with a lower prevalence (3.8% for both).

In the RLSP sample, the greatest prevalence of positive results was observed for the Vivax complex CSP (10.5%), with the greatest prevalence for Pvl (7.0%), followed by Pvc (2.3%) and Pvk (1.2%); the prevalence for Pf was 7.0% and for Pm/Pb 2.3%.

Ten sera from Campinal (10/25 = 40.0%) and three from RLSP (3/13 = 23.1%) reacted with more than one peptide (data not shown).

Figure 3 shows the distribution of the absorbances of the sera for the residents of Campinal and RLSP. Comparative analysis of the average absorbances revealed a statistically significant difference for the Pvl peptide ($p < 0.0001$).

In IFA, 45 of the sera samples were reactive for anti-blood stage *P. vivax* IgG antibodies, corresponding to a 20.8% seropositivity (CI 95%) (Table 2). The titers of positive samples varied between 40 and 160.

Table 2
Prevalence of IgG antibodies against antigens of asexual blood forms of *P. vivax* by Indirect Immunofluorescence Assay (IFA)*. Sera from residents of Campinal and Reassentamento Lagoa São Paulo (RLSP), Presidente Epitácio, São Paulo, January 2000

Antigen - IFA	IgG antibodies - Positive (%)		
	Campinal 130 (100%)	RLSP 86 (100%)	TOTAL 216 (100%)
P. vivax	28 21.5)	17 (19.8)	45 (20.8)
P. falciparum	0 (0)	0 (0)	0 (0)
P. malariae	0 (0)	1 (1.2)	1 (0.5)

* *P. falciparum* antigen - sera from Campinal and RLSP were negative. *P. malariae* antigen - only one serum from RLSP was positive. Number of positive sera per titer: 38 sera for 1:40; 5 sera for 1:80; and 2 sera for 1:160.
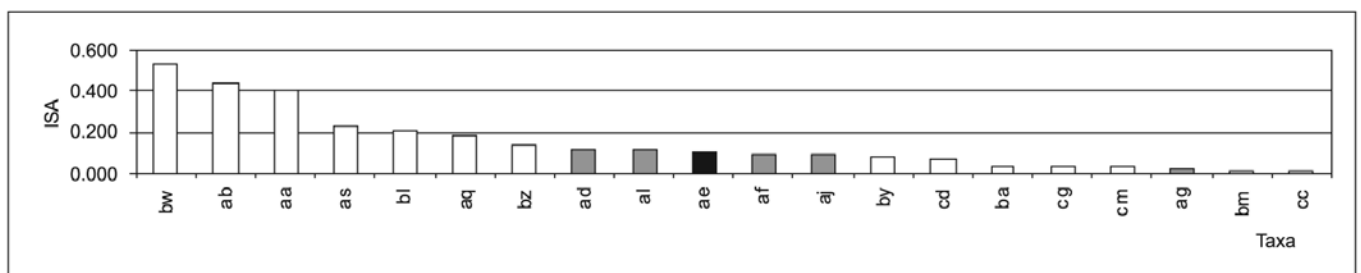
2a (247m elevation – JB farm).
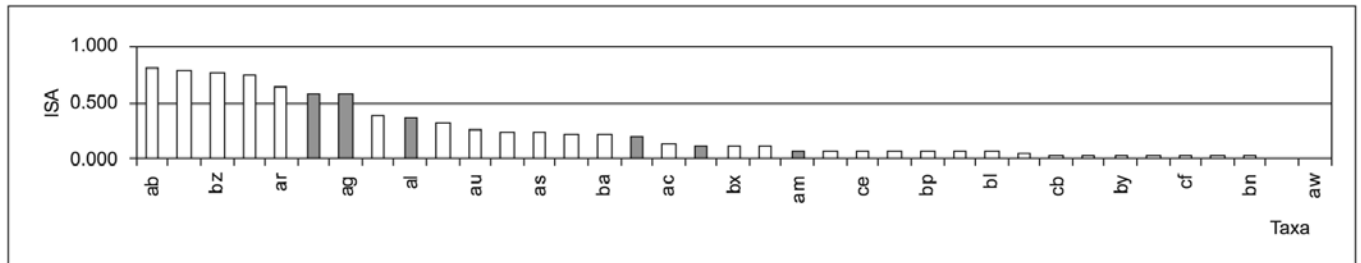
2b (253m elevation – JB farm).
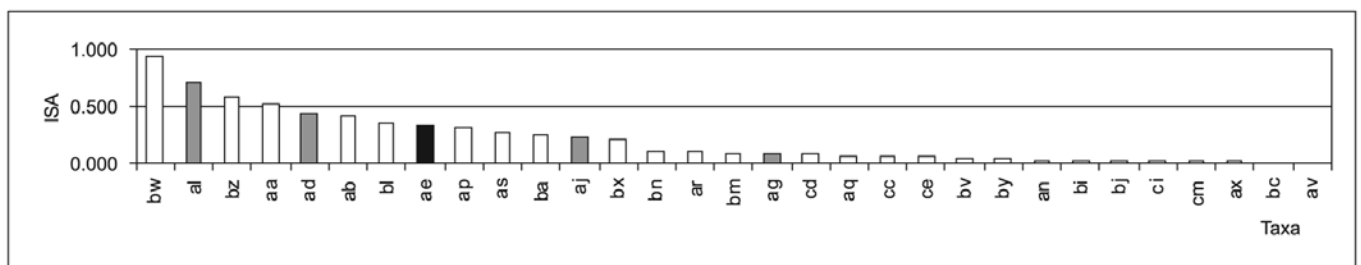
2c (257m elevation – JB farm).
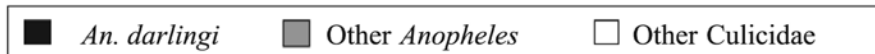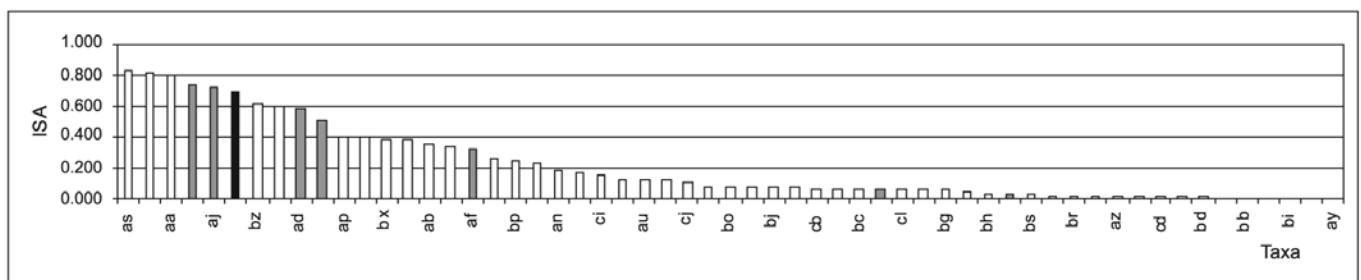
2d (257m elevation – SA farm).

Fig. 2 - **S**tandardized Index of Species Abundance (ISA) for Culicidae obtained by monthly Shannon-trap captures on the edge of the forest during the evening twilight on the JB and SA farms. Captures on JB Farm took place between July 1997 and December 1998 at the 247m elevation (a); between July 1999 and December 2000 at the 253m elevation (b); and between July 2001 and December 2002 at the 257m elevation (c). Captures on SA Farm took place between April 2002 and February 2003 after the dam had been filled to 257m (d). **aa** - *Aedeomyia squamipennis*, **ab** - *Aedes scapularis*, **ac** - *Ae. serratus*, **ad** - *Anopheles albitarsis s.l.*, **ae** - *An. darlingi*, **af** - *An. evansae*, **ag** - *An. galvao*i, **ah** - *An. oswaldoi*, **ai** - *An. parvus*, **aj** - *An. rondoni*, **ak** - *An. strodei*, **al** - *An. triannulatus*, **am** - *An. (Nyssorhynchus) sp.*, **an** - *Coquillettidia albicosta*, **ao** - *Cq. chrysonotum/albifera*, **ap** - *Cq. hermanoi*, **aq** - *Cq. juxtamansonia*, **ar** - *Cq. nigricans*, **as** - *Cq. shannoni*, **at** - *Cq. venezuelensis*, **au** - *Culex (Aedinus) amazonensis*, **av** - *Cx. (Culex) bidens*, **ax** - *Cx. (Cux.) chidesteri*, **aw** - *Cx. (Cux.) declarator*, **ay** - *Cx. (Cux.) maxi*, **az** - *Cx. (Cux.) mollis*, **ba** - *Cx. (Cux.) spp.*, **bb** - *Cx. (Melanoconion) adamesi*, **bc** - *Cx. (Mel.) bastagarius*, **bd** - *Cx. (Mel.) clarki*, **be** - *Cx. (Mel.) contei*, **bf -** *Cx. (Mel.) delpontei*, **bg** - *Cx. (Mel.) dunni*, **bh -** *Cx. (Mel.) eastor*, **bi** - *Cx. (Mel.) flabellifer*, **bj -** *Cx. (Mel.)* group Atratus, **bk** - *Cx. (Mel.) idottus*, **bl** - *Cx. (Mel.) ocossa*, **bm** - *Cx. (Mel.) oedipus*, **bn** - *Cx. (Mel.) pavlovsky*, **bo -** *Cx. (Mel.) rabelloi*, **bp** - *Cx. (Mel.) ribeirensis*, **bq -** *Cx. (Mel.) section Melanoconion*, **br** - *Cx. (Mel.) theobaldi*, **bs** - *Cx. (Mel.) vaxus*, **bt -** *Cx. (Mel.) zeteki*, **bu** - *Cx. (Phenacomyia) corniger*, **bv** - *Limatus flavisetosus*, **bx** - *Mansonia amazonensis*, **bw** - *Ma. humeralis*, **by** - *Ma. indubitans*, **bz** - *Ma. titillans*, **ca** - *Ma. wilsoni*, **cb** - *Psorophora (Grhabhmia) confinnis*, **cc** - *Ps. (Janthinosoma) albigenu*, **cd** - *Ps. discrucians*, **ce** - *Ps. ferox*, **cf** - *Ps. (Psorophora) ciliata*, **cg -** *Sabethes glaucodaemon*, **ch** - *Uranotaenia apicalis*, **ci** - *Ur. geometrica*, **cj** - *Ur. lowii*, **ck** - *Ur. nataliae*, **cl** - *Ur. pulcherrima*, **cm** - *Wyeomyia melanocephala*.
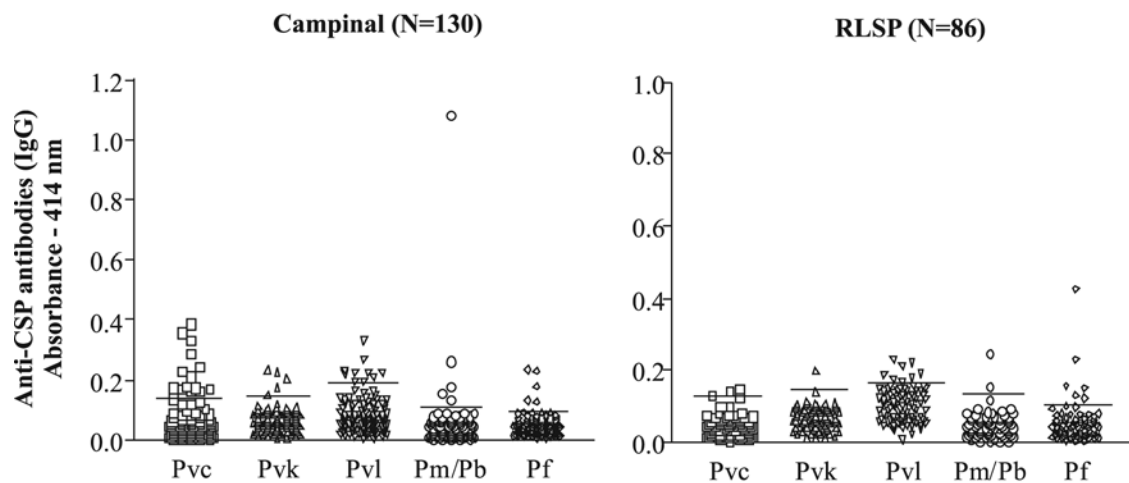
Fig. 3 - Results of ELISA (absorbance) with circumsporozoite protein (CSP) repeat peptides of "classic" *Plasmodium vivax* (Pvc), *P. vivax VK247* (Pvk), human *P. vivax*-like (Pvl), *P. malariae/P. brasilianum* (Pm/Pb) and *P. falciparum* (Pf) for sera samples from residents of Campinal and Reassentamento Lagoa São Paulo. Cut-off values: (Mean + Three Standard Deviations) indicated by horizontal lines (Pvc: 0.135; Pvk: 0.151; Pvl: 0.179; Pm/Pb: 0.127; Pf: 0.122).

The sera tested were not positive for the presence of IgG antibodies to *P. falciparum* antigen, and only one sample (RLSP) was positive for the presence of anti-*P. malariae* IgG antibodies up to a titer of 160.

Positivity rate for the *P. vivax* antigen was 21.5% (28/130) and 19.8% (17/86) among the residents of Campinal and RLSP, respectively (Table 2). Comparative analysis of the percentage of IFA-positive results for the Campinal and RLSP samples revealed a statistically significant difference ($p < 0.0001$).

Analysis of ELISA and IFA results and the epidemiologic information gathered did not reveal a statistically significant difference ($p > 0.05$) in the results obtained for the inhabitants of Campinal and RLSP in terms of sex and occupation (data not shown), but a statistically significant difference was observed in terms of length of stay for the inhabitants of Campinal and RLSP ($p < 0.0001$).

A low positivity rate was observed among residents of Campinal who had lived there for five years or less (classic *P. vivax* - Pvc; *P. vivax* VK247 - Pvk; human *P. vivax*-like/*P. simiovale* - Pvl; *P. malariae/P. brasilianum* - Pm/Pb and *P. falciparum* - Pf CSP peptides as well as the *P. vivax* antigen). Positivity increased among individuals who had lived there for longer (> 10 years), both for CSP and *P. vivax* blood forms (data not shown).

Of the RLSP residents who had lived in the area for up to five years, only one individual was positive for the Pm/Pb peptide and one for the *P. vivax* antigen. As in Campinal, positivity increased among individuals who had lived there for longer, both for CSP and blood forms. Among residents who had lived in the area for more than 21 years, most notable was the presence of anti-CSP antibodies for the Pvk peptide for both Campinal and RLSP; these antibodies were absent among residents who had lived in the two areas for less than 20 years (data not shown).

Positivity for the Pm/Pb peptide was observed in both Campinal and RLSP in residents who had lived there for one year (data not shown).

Some individuals, including inhabitants who had lived in the region for more than twenty years before the blood samples were collected, were found to be positive for the vivax variants *P. vivax* VK247 and P. vivax-like (data not shown).

No travel to areas with endemic malaria was reported by the individuals who were positive for ELISA and IFA in either location.

DISCUSSION

Following the formation of the lake at the Porto Primavera power station on the Paraná River in Presidente Epitácio municipality, the profile of mosquito species changed as a result of the reduction in the diversity and abundance of culicids at the two successive water elevations (253m and 257m)[30]. Within the Culicidae, it was observed that *Aedes scapularis* almost disappeared after the dam had been filled, *An. braziliensis* was no longer observed and there was a drop in the abundance of *An. triannulatus*, whereas the frequency of species such as *An. albitarsis* and *An. galvaoi* oscillated, indicating that they had become tolerant to the ecological conditions in the new habitats.

Although temporary and permanent bodies of water were common before the lake was formed, *An. darlingi* was only present at low densities. During the same period, *An. albitarsis*, *An. galvaoi* and *An. triannulatus* were the most frequently captured anopheline species, with the first being the most abundant.

The results for the anopheline species on JB Farm showed that for the whole study period, which involved the three water elevations, *An. darlingi*, while not detected at 247m, became relatively abundant at the two post-filling levels. This finding suggests that flooding gave rise to factors that favored development of this species at that time. In contrast, the conditions before flooding favored the proliferation of *Ae. scapularis*, a finding that was expected in view of FORATTINI's[20] observation that this species is typical of swamp areas.

The presence of anophelines at different times when the lake was being filled, the finding that *An. darlingi* were present, the fact that the study area has a history of malaria, and the constant flux of humans through the study area (which acts as a link between Northern Brazil and the Southeastern and Southern states) lead us to believe that the area is potentially vulnerable to an outbreak of the disease.

These findings support a hypothesis that *plasmodia* may have been circulating in the area prior to the flooding and that this could be explained by the presence of secondary vectors, with *An. darlingi* playing a minor role as a rare species, as it was not collected during that phase.

In the 80's, approximately 70% of the cases of infection in the São Paulo State originated in the states of Mato Grosso and Rondônia, whereas the proportion of cases that originated outside the state in 1992 was 51.7%. These two states and the Pará State were the largest "exporters" of *plasmodia* to the São Paulo State[44]. These findings support the idea that *plasmodia* may have been introduced in the area.

Malaria transmission associated with the construction and formation of lakes at hydroelectric power stations has been reported in Tucuruí, Balbina and Itaipu, where the increase in the *plasmodia* vector populations culminated in a number of cases of the disease[7,21,40,41].

In spite of the negative results in the parasitological and molecular examinations of the sample population in the study area, a prevalence of antibodies against CSP protein in sera was observed, particularly for variants of *P. vivax*, suggesting that the inhabitants may have been exposed to *plasmodia* sporozoites. Of particular interest were the positive results for individuals who had not been living in the area for very long (up to five years before blood samples were collected) and did not report any trip to malarial areas, suggesting that, local contact with anophelines infected with *plasmodia* may have indeed taken place.

The older residents of Campinal and RLSP were found to have anti-CSP antibodies, a finding that was expected, as serological responses of this kind are known to persist for more than 10 years in some individuals[5,14,19]. This result was lent extra weight by reports from some residents of malaria episodes in the area in the 80s and 90s.

The serological investigation also showed that *P. malariae* and *P. falciparum* sporozoites were circulating. It can be inferred from the absence of clinical cases and the CSP positivity observed in the population that residents developed a serological response, possibly through exposure to mosquitoes with parasitic loads that were too low, or immature for the infection to evolve[29,31].

Some individuals, including inhabitants who had lived in the region for more than twenty years before the blood samples were collected, were found to be positive for the vivax variants *P. vivax* VK247 and *P. vivax*-like, suggesting that these strains may have circulated or may still be circulating locally; however, this remains to be confirmed. Serological responses against *P. vivax* variant CSP were found in populations in the states of Pará and Acre, and recent studies in areas with autochthonous malaria in the Atlantic Forest in the São Paulo State also reported the presence of anti-Pvk and Pvl antibodies[10,11].

Positivity for antibodies against blood forms of *P. vivax* in IFA in residents of Campinal and RLSP may indicate that these individuals developed malaria at some stage and that transmission occurred at some time in the past. Alternatively, these data may indicate that they have been exposed to partial parasitic development as a result of the parasite's inability to adapt to either vertebrate or invertebrate hosts for reasons other than immunological ones. However, the low titers observed cannot eliminate the possibility of cross reactions with other antigens[1] even though no parasitoses were reported by these individuals.

It should be stressed that similar epidemiological situations have been reported in parts of the Atlantic Forest[10,11] where it is known to have occurred asymptomatic and oligosymptomatic cases, raising issues about strategies for the surveillance and control of malaria not only in these regions but also in areas where the disease could potentially reemerge.

In this context, the potential circulation of *plasmodia* in the study area could involve more than one species of *Anopheles* and also possibly interact with the sylvatic transmission cycle observed in this area, as reported by the authors in another study[15].

The prevalence of antibodies against CSP in howler monkeys *(Alouatta caraya)* captured in Porto Primavera lake while it was being filled, was found to be 8.3% for Pf, 8.1% for Pvl, 8.0% for Pvk and 7.6% for Pvc and Pm/Pb. The prevalence of antibodies against asexual forms was 10.5% for *P. malariae*, 9.7% for *P. vivax* and 4.9% for *P. falciparum* antigen[15] in these animals. In the same study, similar serological results were found in simian populations in the "Cerrado" and Atlantic Forest, indicating that there is a sylvatic cycle in these ecosystems[15].

In the parasitological study of *Alouatta* at Porto Primavera, samples were found to be positive by multiplex PCR[26] with the following prevalences: 5.5% for *P. malariae/P. brasilianum*, 0.8% for *P. falciparum* and 0.4% for *P. vivax/P.simium*[16].

Other authors have reported the finding of antibodies against CSP and asexual forms of *P. vivax*, *P. malariae* and *P. falciparum* in monkeys in the Amazonian region of French Guiana, although only *P. malariae* were found in thick smears[17,42].

Little is known about the vectors of simian *plasmodia*, apart from a report incriminating *An. (Kerteszia) cruzii* as a vector for *P. simium* and P. *brasilianum* in some parts of the Atlantic Forest[13]. However, although anophelines, secondary vectors and *An. darlingi* were found in the study area, it was not possible to establish which of them were involved in the sylvatic cycle.

Our results for the monkey may suggest both that humans may be involved in the epidemiology of this zoonosis, and that the antibodies found in the human population might be related to human contact with simian parasites that are similar to human *plasmodia*.

In conclusion, this study shows two possible epidemiological situations linked to the presence of anopheline vectors in the area of influence of the flooding. One of them is the potential theoretical risk of human malaria returning to the region if intense migration from the Amazon to the Southeastern and Southern regions through this region continues. The other is the possibility that the human population may have contact with simian *plasmodia* and related vectors that have not yet

been identified, explaining the presence of anti-plasmidial antibodies recorded at low frequencies in the population. Further research is required to confirm the phenomena described in this article.

RESUMO

Aspectos epidemiológicos e ecológicos relacionados à malária na área de influência do lago da Represa de Porto Primavera, região oeste do Estado de São Paulo, Brasil

Foi realizada pesquisa na área de influência do lago da Usina Hidrelétrica de Porto Primavera, região oeste do Estado de São Paulo, para estudar aspectos ecológicos e epidemiológicos da malária na localidade e acompanhar o perfil das populações de anofelinos frente às mudanças decorrentes do impacto ambiental pela formação do lago. Mosquitos capturados foram analisados pelo Índice de Abundância de Espécies Padronizado (IAEP), antes e durante o enchimento do reservatório (cotas 253 e 257 m). A população humana local foi estudada por meio de teste parasitológico (gota espessa e esfregaço sangüíneo), testes moleculares (PCR) e testes sorológicos. A sorologia consistiu na reação de ELISA com peptídeos sintéticos correspondentes à porção repetitiva da proteína circumsporozoíta (CSP) de *Plasmodium vivax* clássico, e suas variantes VK247 e "vivax-like", *P. malariae* e *P. falciparum*; e reação de Imunofluorescência Indireta (RIFI) com formas assexuadas de *P. vivax*, *P. malariae* e *P. falciparum*. Os resultados do estudo entomológico indicaram que, embora a população de *Anopheles darlingi* tenha aumentando após o enchimento, permaneceu em baixa densidade. Não foi detectada malária nem a presença de parasitos ou de DNA parasitário nos habitantes estudados. No entanto, foi observada baixa freqüência de anticorpos contra formas assexuadas e significativa prevalência de anticorpos contra esporozoítos de *P. vivax* e suas variantes, *P. falciparum* e *P. malariae*, que poderiam decorrer de contatos prévios, recentes ou não, com plasmódios humanos ou símios (o ciclo silvestre foi evidenciado em estudo paralelo realizado na mesma área). Por outro lado, estes resultados sugerem que, como em outros lugares onde existem vetores potenciais da malária, as condições epidemiológicas poderiam potencialmente permitir a transmissão da malária na área de influência do lago de Porto Primavera, se indivíduos infectados fossem introduzidos em número suficiente. Estudos adicionais deverão ser realizados para elucidar os fenômenos relatados neste artigo.

ACKNOWLEDGMENTS

REFERENCES

1. ABRAMO, C.; FONTES, C.J.F. & KRETTLI, A.U. - Cross-reactivity between antibodies in the sera of individuals with leishmaniasis, toxoplasmosis and Chagas' disease and antigens of the blood-stage forms of *Plasmodium falciparum* determined by indirect immunofluorescence. **Amer. J. trop Med. Hyg., 53:** 202-205, 1995.

2. ALENCAR, F.M.A.; NOGUEIRA, J.C.B. & EMMERICH, W. - **A Lagoa São Paulo e alguns aspectos de sua fisiografia**. São Paulo, Instituto Florestal, 1976. (**Bol. Tec.,** (20), 1976).

3. ARNOT, D.E.; BARNWELL, J.W.; TAM, J.P. *et al*. - Circumsporozoite protein of *Plasmodium vivax* gene cloning and characterization of the immunodominant epitope. **Science, 230:** 815-818, 1985.

4. BARATA, R.C.B. - Malária no Brasil: panorama epidemiológico na última década. **Cadern. Saúde publ. (Rio de J.), 11:** 128-136, 1995.

5. BRAGA, E.M.; FONTES, C.J. & KRETTLI, A.U. - Persistence of humoral response against sporozoite and blood-stage malaria antigens 7 years after a brief exposure to *Plasmodium vivax*. **J. infect. Dis., 177**: 1132-1135, 1998.

6. BRUCE-CHWATT, L.J. - **Essential malariology**. 2. ed. London, William Heinemann Medical Books, 1985.

7. CONSOLIM, J. - O *Anopheles (Nyssorhynchus) darlingi* Root, 1926 e a malária no lago Itaipu, Estado do Paraná, Brasil. **Arq. Biol. Tecn., 1**(2): 263-286, 1991.

8. CORDEIRO, C.E.S.; FILOMENO, C.R.M.; COSTA, C.M.A. & COUTO A.A.R.A. - Perfil epidemiológico da malária no Estado do Pará em 1999 com base numa série histórica de dez anos (1989-1999). **Inf. epidem. SUS, 11:** 69-77, 2002.

9. CORRÊA, R. - Observações sobre *Anopheles (Nyssorhynchus) darlingi* Root 1926, no Estado de São Paulo. **Rev. Biol. Hig., 11:** 40-54, 1941.

10. CURADO, I.; DUARTE, A.M.R.C.; LAL, A.A.; OLIVEIRA, S.G. & KLOETZEL, J.K. - Antibodies anti-bloodstream and circumsporozoite antigens (*Plasmodium vivax* and *Plasmodium malariae/P.brasilianum*) in areas of very low malaria endemicity in Brazil. **Mem. Inst. Oswaldo Cruz, 92:** 235-243, 1997.

11. CURADO, I.; DOS SANTOS MALAFRONTE, R.; de CASTRO DUARTE, A.M.R. *et al*. - Malaria epidemiology in low-endemicity areas of the Atlantic Forest in the Vale do Ribeira, São Paulo, Brazil. **Acta trop., 100:** 54-62, 2006.

12. DEANE, L.M. - Malaria studies and control in Brazil. **Amer. J. trop. Med. Hyg., 38:** 223-230, 1988.

13. DEANE, L.M.; FERREIRA NETO, J.A.; DEANE, M.P. & SILVEIRA, I.P. - *Anopheles (Kerteszia) cruzi,* a natural vector of the monkey malaria parasites, *Plasmodium simium* and *Plasmodium brasilianum*. **Trans. roy. Soc. trop. Med. Hyg., 64:** 647, 1970.

14. DRUILHE, P.; PRADIER, O.; MARC, J.P. *et al*. - Levels of antibodies to *Plasmodium falciparum* sporozoite surface antigens reflect malaria transmission rates and are persistent in the absence of reinfection. **Infect. Immun., 53:** 393-397, 1986.

15. DUARTE, A.M.R.C.; PORTO, M.A.L.; CURADO, I. *et al*. - Widespread occurrence of antibodies against circumsporozoite protein and against blood forms of *Plasmodium vivax*, *P. falciparum* and *P. malariae* in Brazilian wild monkeys. **J. med. Primatol., 35:** 87-96, 2006.

16. DUARTE, A.M.R.C.; MALAFRONTE, R.S.; CERUTTI J.R. *et al* - Natural *Plasmodium* infections in Brazilian wild monkeys: reservoirs for human infections? **Acta trop., 107:** 179-185, 2008.

17. FANDEUR, T.; VOLNEY, B.; PENEAU, C. & DE THOISY, B. - Monkeys of the rainforest in French Guiana are natural reservoirs for *P. brasilianum/P. malariae* malaria. **Parasitology**, **120:** 11-21, 2000.

18. FERREIRA, A.W. & SANCHEZ, M.C. - Malária humana: padronização e optimização de testes sorológicos para diagnóstico individual e inquéritos soroepidemiológicos. **Rev. Inst. Med. trop. S. Paulo, 30:** 137-146, 1988.

19. FONTES, C.J.; BATHURST, I. & KRETTLI, A.U. - *Plasmodium vivax* sporozoite antibodies in individuals exposed during a single malaria outbreak in a non-endemic area. **Amer. J. trop. Med. Hyg., 44:** 28-33, 1991.

20. FORATTINI, O.P. - Comportamento exófilo de *Anopheles darlingi Root*, em região meridional do Brasil. **Rev. Saúde publ. (S. Paulo), 21:** 291-304, 1987.

21. GUIMARÃES, A.E.; MELLO, R.P.; LOPES, C.M.; ALENCAR, J. & GENTILE, C. - Prevalência de anofelinos (Diptera: Culicidae) no crepúsculo vespertino em áreas da Usina Hidrelétrica de Itaipu, no município de Guaíra, Estado do Paraná, Brazil. **Mem. Inst. Oswaldo Cruz, 92:** 745-754, 1997.

22. IBGE. Fundação IBGE - **População residente, por situação do domicílio e sexo, segundo as Mesoregiões, as Microregiões, os Municípios, os Distritos e os Bairros, Estado de São Paulo, 2000.** [on line] URL:http://www.ibge.gov.br/home/ estatistica/ população/ censo2000/ tabelas grandes-regiões 211.shtm.

23. KREMSNER, P.G.; NEIFER, S.; ZOTTER, G.M. *et al*. - Prevalence and level of antibodies to the cirumsporozoite proteins of human malaria parasites, including a variant of *Plasmodium vivax,* in the population of two epidemiologically distinct areas in the state of Acre, Brazil. **Trans. roy. Soc. trop. Med. Hyg., 86:** 23-27, 1992.

24. LAL, A.A.; DE LA CRUZ, V.F.; CAMPBELL, G.H. *et al*. - Structure of the circumsporozoite gene of *Plasmodium malariae*. **Molec. Biochem. Parasit., 30:** 291-294, 1988.

25. LAL, A.A.; DE LA CRUZ, V.F.; COLLINS, W.E. *et al*. - Circumsporozoite protein gene from *Plasmodium brasilianum*. Animal reservoirs for human malaria parasites**? J. biol. Chem., 263:** 5495-5498, 1988.

26. MACHADO, R.L. & PÓVOA, M.M. - Distribution of *Plasmodium vivax* variants (VK210, VK247, *P. vivax*-like) in the three endemic areas of the Amazon region of Brazil and their correlation with chloroquine treatment. **Trans. roy. Soc. trop. Med. Hyg., 94:** 377-381, 2000.

27. MARRELLI, M.T.; BRANQUINHO, M.S.; HOFFMANN, E.H.E. *et al*. - ELISA with *Plasmodium vivax*-like/*P.simiovale* CS repeats in human sera and in anophelines from the State of Acre, Brazil. **Mem. Inst. Oswaldo Cruz, 92**(suppl. 1): 268, 1997.

28. McCUTCHAN, T.F.; LAL, A.A.; CRUZ, V.F. *et al*. - Sequence of the immunodominant epitope for the surface protein on sporozoites of *Plasmodium vivax*. **Science, 230:** 1381-1383, 1985.

29. NUSSENZWEIG, R.S. & NUSSENZWEIG, V. - Development of sporozoite vaccines. **Phil. Trans. B, 307:** 117-128, 1984.

30. PAULA, M.B. & GOMES, A.C. - Culicidae (Diptera) em área sob influência de construção de represa no Estado de São Paulo. **Rev. Saúde publ. (S. Paulo), 41:** 284-289, 2007.

31. PHILPOTT, J.; KEYSTONE, J.S.; REID, A. *et al*. - Effect of malaria chemoprophylaxis on the development of antibodies to *Plasmodium falciparum* in expatriates living in west Africa. **Amer. J. trop Med. Hyg., 42:** 28-45, 1990.

32. QARI, S.H.; SHI, Y.; GOLDMAN, I.F. *et al*. - Identification of *Plasmodium vivax*-like human malaria parasite. **Lancet, 341**(8848): 780-783, 1993.

33. REBELO, J.M.M.; SILVA, A.R.; FERREIRA, L.A. & VIEIRA, J.A. - *Anopheles* (Culicidae, Anophelinae) e a malária em Buriticupu-Santa Luzia, Pré-Amazônica maranhense. **Rev. Soc. bras. Med. trop., 30:** 107-111, 1997.

34. ROBERTS, D.R. & HSI, B.P. - An Index of species abundance for use with mosquito surveillance data. **Environ. Ent., 8:** 1007-1013, 1979.

35. ROSENBERG, R.; WIRTZ, R.A.; LANAR, D.E. *et al*. - Circumsporozoite protein heterogeneity in the human malaria parasite *Plasmodium vivax*. **Science, 245**(4921): 973-976, 1989.

36. RUBIO, J.M.; BENITO, A.; ROCHE, L. *et al*. - Semi-nested, multiplex polymerase chain reaction for detection of human malaria parasites and evidence of *Plasmodium vivax* infection in Equatorial Guinea. **Amer. J. trop. Med. Hyg., 60:** 183-187, 1999.

37. SHANNON, R. - Methods for collecting and feeding mosquitoes in jungle yellow fever studies. **Amer. J. trop Med. Hyg., 19:** 131-140, 1939.

38. SUCEN. Superintendência de Controle de Endemias - **Avaliação do Programa de Controle de Malária. Estado de São Paulo - período de 1992 a 1994.** São Paulo, SUCEN, 1995.

39. SUCEN. Superintendência de Controle de Endemias - **Casos autóctones de malária, por ano e local provável de infecção. Região do Planalto Ocidental, 1990 a 2003.** [on line] 2007 Jul 16.URL http://www.sucen.sp.gov.br/doencas/index.htm.

40. TADEI, W.P.; MASCARENHAS, B.M. & PODESTA, M.G. - Biologia de anofelinos amazônicos. VIII. Conhecimentos sobre a distribuição de espécies de *Anopheles* na região de Tucuruí-Marabá (Pará**). Acta amaz. (Manaus), 13:** 103-140, 1983.

41. TEODORO, U.; GUILHERME, A.L.F.; LOZOVEI, A.L. *et al*. - Culicídeos do Lago de Itaipu, no Rio Paraná, sul do Brasil. **Rev. Saúde públ. (S. Paulo), 29:** 6-14, 1995.

42. VOLNEY, B.; POULIQUEN, J.F.; DE THOISY, B. & FANDEUR, T. - A sero-epidemiological study of malaria in human and monkey population in French Guiana. **Acta trop., 82:** 11-23, 2002.

43. WALSH, J.F.; MOLYNEAUX, D.H. & BIRLEY, M.H. - Deforestation: effects on vector-borne disease. **Parasitology 106**(suppl.): S55-S75, 1993.

44. WANDERLEY, D.M.V.; SILVA, R.A. & ANDRADE, J.C.S.R. - Aspectos epidemiológicos da malária no Estado de São Paulo, Brasil, 1983 a 1992. **Rev. Saúde publ. (S. Paulo), 28:** 192-197, 1994.

45. WHO. World Health Organization. - **Procedimentos laboratoriais em parasitologia médica**. 2. ed. São Paulo, Livraria Santos, 1999.

46. ZAVALA, F.; TAM, J.P. & MASUDA, A. - Synthetic peptides as antigens for the detection of humoral immunity to *Plasmodium falciparum* sporozoites. **J. immunol. Meth., 93:** 55-61, 1986.