

## Correlation between positive serology for *Plasmodium vivax*-like/*Plasmodium simiovale* malaria parasites in the human and anopheline populations in the State of Acre, Brazil

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### Abstract

Antibodies against the *Plasmodium vivax*-like/*P. simiovale* malaria parasite circumsporozoite repeat peptide (APGANQEGGAA)<sub>3</sub> were determined by enzyme-linked immunosorbent assay (ELISA) in 120 sera randomly collected in 1994 from adults in 3 localities of the malaria endemic area in the State of Acre, Brazil; antibody was detected in 18 (15%). A 'sandwich' ELISA using monoclonal antibody (mab) Pam 172, directed against the same peptide, was carried out on 1207 *Anopheles oswaldoi*, 12 of which (1.0%) were positive, and 168 *A. deaneorum*, 2 of which (1.2%) were positive. This is the first report of serological detection of the *P. vivax*-like parasite in anophelines and the first report linking anopheline to human serology for this parasite in the same geographical area. It is an additional indication that *A. oswaldoi* is a malaria vector in Acre.

**Keywords:** malaria, *Plasmodium simiovale*, *Plasmodium vivax*-like species, seroprevalence, *Anopheles oswaldoi*, *Anopheles deaneorum*, Brazil

### Introduction

The circumsporozoite proteins (CSPs) of human malaria parasites have been used to identify *Plasmodium* species in anophelines (COLLINS *et al.*, 1984; ARRUDA *et al.*, 1986; BRANQUINHO *et al.*, 1993) and in seroepidemiological studies (NARDIN *et al.*, 1979; ARRUDA *et al.*, 1989; CURADO *et al.*, 1997), since they have a central region of tandemly repeated amino acid sequences, characteristic of the species (ZAVALA *et al.*, 1983).

Polymorphism of the CSP of the human malaria parasite *P. vivax* was reported initially by ROSENBERG *et al.* (1989) and QARI *et al.* (1991). In addition to parasites with the original amino acid sequence, designated *P. vivax* VK210 (ARNOT *et al.*, 1985), a variant, VK247, was also described.

QARI *et al.* (1993a) characterized the CSP genes of *P. vivax* samples obtained from patients in Papua New Guinea. The sequence of the repetitive region of some of these isolates differed from those of the 2 known variants of *P. vivax*, being identical to that of *P. simiovale*, a parasite of 'New World' monkeys. This variant was named 'human *P. vivax*-like', since its morphology was that of *P. vivax* (see QARI *et al.*, 1993a). The monoclonal antibody (mab) Pam 172 (UDHAYAKUMAR *et al.*, 1994), raised against the repeat sequence, recognized sporozoites of *P. simiovale* but not those of other malaria species, including the 2 known CSP variants of *P. vivax*.

The occurrence of the *P. vivax*-like malaria parasite has been detected, both by serology and the polymerase chain reaction, in samples of human blood from Papua New Guinea, Brazil, Indonesia and Madagascar (QARI *et al.*, 1993a, 1993b; CURADO *et al.*, 1997).

In a previous study by our group in the State of Acre, Brazil, 3056 anophelines were captured in 1991–1992 and they were tested only by 'sandwich' enzyme-linked immunosorbent assay (ELISA) with monoclonal antibodies specific for *P. vivax* VK210 and VK247, besides *P. malariae* and *P. falciparum* (see BRANQUINHO *et al.*, 1993). Sporozoites were detected in the salivary glands of one of 34 *Anopheles oswaldoi* in the same region in 1994 (BRANQUINHO *et al.*, 1996).

The aim of the present study was to evaluate the extent of naturally occurring anti-*P. vivax*-like antibodies in human sera from the same region of the State of Acre,

Brazil, and its correlation with the *P. vivax*-like infection rates in anophelines.

### Materials and Methods

#### Study area

The study was conducted with human sera and anophelines collected in 3 districts in the State of Acre, Brazil, where malaria is hypoendemic. Two of the districts, Senador Guiomard Santos and Plácido de Castro, have been settled rather recently and the population consists mainly of migrants with no previous experience of malaria. Rio Branco was the third district, most of the inhabitants of which had lived there for several generations.

#### Serum samples

Venous blood samples were obtained with informed consent from all individuals. A questionnaire concerning personal data (age, sex, occupation), epidemiological information on places of residence, malaria episodes, and clinical symptoms, was completed for each participant. Blood samples were collected in 1994 from 120 adults of both sexes, 80 from patients attending the local outpatient clinics and 40 from random visits to homes in the area. Most of these people had a history of past or present malaria episodes. Sera were stored at –20°C until used.

#### Enzyme-linked immunosorbent assay of sera

The ELISA was performed according to ZAVALA *et al.* (1986) and CURADO *et al.* (1997) with some modifications, using a synthetic peptide corresponding to repeats of the CSP of the human *P. vivax*-like (PvI) malaria parasite, (APGANQEGGAA)<sub>3</sub> (QARI *et al.*, 1993a).

ELISA plates (Maxisorp™, Nunc) were coated with 100 µL/well of a solution containing 10 µg/mL of the peptide, diluted in phosphate-buffered saline at pH 7.2 (PBS), and incubated overnight at 4°C in a humid chamber. They were then washed 6 times with PBS containing 0.05% Tween 20™ (PBS-T), and blocked for 2 h at 37°C with PBS containing 5% fat-free lyophilized milk. Following this, 100 µL of serum, diluted 1:50 in PBS containing 2.5% of milk and 0.05% Tween 20™, were added to each well and the plates were incubated for 1 h at 37°C. After additional washings with PBS-T, and incubation for 1 h with peroxidase-conjugated anti-human immunoglobulin G (Sigma) at 37°C, followed by 6 washings with PBS-T and one with PBS, the reactions were developed with 100 µL per well of

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