Susceptibility of Anopheles aquasalis and An. darlingi to Plasmodium vivax VK210 and VK247

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The susceptibility of Anopheles aquasalis (F4 generation) and An. darlingi (F1 generation) to Plasmodium vivax circumsporozoite protein phenotypes from a limited number of blood samples of malaria patients in Belém, state of Pará, Brazil, was examined. A polymerase chain reaction was used to determine the P. vivax phenotypes in blood samples and the blood-fed infected mosquitoes were dissected and tested by ELISA. In all patient infections, more infected An. aquasalis and An. darlingi were positive for VK210 compared with VK247.

Key words: malaria - Plasmodium vivax - VK210 - VK247 - Anopheles aquasalis - Anopheles darlingi - Brazilian Amazon

In Belém (the capital of the state of Pará) two species have been incriminated as important malaria vectors (Davis 1931, Galvão et al. 1942, Póvoa et al. 2003): Anopheles (Nyssorhynchus) aquasalis Curry 1932, a coastal Neotropical species (Zimmerman 1992), considered to be the primary coastal malaria vector of Plasmodium vivax in Venezuela (Berti et al. 1993) and as far as Southeastern Brazil (Forattini 1962); and An. darlingi Root 1926, a Neotropical species that is highly susceptible to Plasmodium and the primary vector in the Amazon (Deane et al. 1948, Klein et al. 1991).

In Brazil overall, most malaria cases are caused by P. vivax (Akhavan et al. 1999), but the proportion of P. vivax to P. falciparum varies regionally (Brazilian Ministry of Health 2000). In Pará, from 1985 to 1999 the number of malaria infections by P. vivax and P. falciparum differed significantly ($p = 0.003$) with the incidence of P. vivax being considerably higher (Póvoa et al. 2003). From 1993-1999, in Belém, the proportion of malaria cases identified by Giemsa-stained blood smears as P. vivax fluctuated from a low of 85% in 1994 to a high of 97% in 1997 and 1998 (Póvoa et al. 2003). In all likelihood, in Belém, these parasites were transmitted by An. aquasalis and An. darlingi (Póvoa et al. 2003).

Based on the repeat units of the circumsporozoite (CS) proteins, three P. vivax variants have been identified: VK210 (amino acid sequence is GDRA(D/A)GQPA) (Arnot et al. 1985), VK247(amino acid sequence is ANGA(G/D)(N/D)QPG) (Rosenberg et al. 1989), and P. vivax-like (amino acid sequence is APGANQ(E/G)GGAA) (Qari et al. 1993). All three variants are present in the Brazilian Amazonian region, including Belém (Arruda et al. 1996, 1998, Machado & Póvoa 2000). However, it is rare to detect VK247 as a single infection; this variant is generally found in mixed infections with either VK210 or with both VK210 and P. vivax-like (Machado & Póvoa 2000, Machado et al. 2003).

Recent studies of Neotropical anophelines have described differential susceptibilities of An. albimanus and An. pseudopunctipennis to P. vivax variants VK210 and VK247 (González-Ceron et al. 1999, Rodriguez et al. 2000), but there have been no previous studies of either An. aquasalis or An. darlingi susceptibilities to P. vivax variants. Klein et al. (1991) found that An. darlingi from Rondônia in western Amazonian Brazil (among several other anopheine species) was very susceptible to P. vivax infection. More recently, F. An. konderi, An. oswaldoi, and An. darlingi collected in Acre and the state of Rondônia, Brazil, were all shown to be susceptible to infection by P. vivax (Marrelli et al. 1999). However, in both studies the authors did not know which P. vivax circumsporozoite protein phenotypes the mosquitoes species were dealing with. Our objective was to examine the susceptibility of An. aquasalis and An. darlingi from Belém to P. vivax VK210 and VK247.

MATERIALS AND METHODS

Patients - Seven subjects diagnosed with P. vivax malaria by Giemsa-stained blood smears at Instituto Evandro Chagas (IEC) or at Pará health clinics, were the source for mosquito infection. All individuals were older than ≥ 21 years old, infected with circulating asexual stages of P. vivax, parasitemia higher than 3000 parasites/mm³ and with history of previous malaria or not. The exclusion criteria were: individuals with debilitating symptoms such as vomit, diarrhea, dehydration caused by the infection, pregnant women, children, and Indigenous people.

The history of previous malaria episodes, approximate date of initial symptoms, and probable site where malaria transmission occurred were obtained from each patient. Treatment with chloroquine and primaquine, alone or combined was provided to each patient as soon as the mosquito feeding was completed.
This study was approved by the Ethical Committee on Human Subjects at the IEC, protocol number CEP/IEC 0699.

Infected mosquitoes - A maximum of 70 females, 3-6 days old laboratory reared An. aquasalis -F1 (Silva et al. 2006) and An. darlingi (F1), both obtained from females collected in Belém, Pará, were fed simultaneously for up to 30 min on the arms and legs of a same patient between 2-11 days after the first report of symptoms. Mosquitoes were not fed during 12 h prior to infected blood feeding. After complete engorgement, the mosquitoes were maintained at 23-28°C and 70-80% RH, and provided feeding. After complete engorgement, the mosquitoes were never found alone, only in mixed infections (patients 4-6; Table). All were susceptible to infection with *P. vivax* VK210 except *An. aquasalis* on patient 5, who had a parasitemia of 10,000 parasites/mm³. There was a higher proportion of *An. aquasalis* and *An. darlingi* infected with VK210 (2.89) versus VK247 (0.96) in mixed infections (patients 4-6; Table). However, in patient 4, who had a parasitemia of 7500 parasites/mm³ and 50 gametocyte forms, both *An. aquasalis* and *An. darlingi* became infected with VK210 and VK247 (Table).

We were unable to evaluate mosquito susceptibility to infection with *P. vivax* VK247 because these variants were never found alone, only in mixed infections in Belém (Machado & Póvoa 2000).

The dissection of the midgut of *An. darlingi* and *An. aquasalis*, demonstrated that both species developed infection by *P. vivax* but the proportion of infected *An. darlingi* (93.33%) and the mean number of oocysts (44,65 ± 42,52) was higher than *An. aquasalis* (52.94%) mean number (25.28 ± 23.94; Table). On some dissected mosquitoes of both species, infected by mixed infection carriers, we had observed degenerated oocysts (data not showed).

**Discussion**

Our results demonstrate for the first time, experimentally, that *An. aquasalis* from Belém are susceptible to *P. vivax* VK210 and VK247 and confirm the susceptibility of *An. darlingi* to *P. vivax* variants (Klein et al. 1991, Marrelli et al. 1999). We have previously demonstrated that the percentage of *An. aquasalis* naturally infected by *P. vivax* has increased in Belém from 0.26% (Galvão et al. 1942) to 1.18% (Póvoa et al. 2003). Our results suggest that additional regional comparative studies on *Anopheles* susceptibility to *Plasmodium* infections that include *An. darlingi* and the identification of *P. vivax* variants in both the patients and the infected mosquitoes would contribute significantly toward a better understanding of malaria epidemiology and parasite transmission rates in Brazil.

In mixed infections both *An. aquasalis* and *An. darlingi* appear to become more infected with VK210 rather than to VK247, but in one patient infected with VK210/VK247 (patient 4) we detected VK210 and VK247 in both mosquito species.

Our results may have been influenced by the small sample size. However, several studies have shown differences on the infectivity of anophelines to the variants. In Mexico, *An. albimanus* is more susceptible to infection with VK210, and *An. pseudopunctipennis* is more sus-
ceptible to VK247 (González-Ceron et al. 1999, 2001). Furthermore, in Southern Mexico, the prevalence of these two variants is closely associated with the distribution of these two mosquito species (Rodríguez et al. 2000). Our study is suggestive that a similar phenomenon, observed for An. albimanus (greater susceptibility to VK210) may occur in both An. aquasalis and An. darlingi, in Amazonian Brazil. We hypothesize that the greater probability of VK210 sporozoites development in An. aquasalis and An. darlingi compared with VK247 is either determined by the presence of a greater proportion of VK210 gametocytes circulating in patients or by other mechanisms (Vlachou & Kafatos 2005) such as ookinete destruction and/or oocyst development arrest as observed for An. albimanus that is resistant to infection with VK247 (González-Ceron et al. 2001). These mechanisms may explain in the preferential development of VK210 in An. aquasalis and An. darlingi.

Studies on mosquitoes experimentally infected in blood donors from regions of Colombia demonstrated that despite of the higher prevalence of anti-VK210 on these individuals, the mosquitoes produced more abundantly sporozoites VK247, suggesting that the anti-VK210 antibodies was blocking the development of sporozoites VK210 (González-Ceron et al. 2001). In contrast, our study do not allow us to conclude about the action of specific antibodies (anti- VK210 or VK 247) on the mosquitoes infection or the intensity of this infection, first because of our sample size and second, three (4-6) of our seven donors had no previous malaria. Further investigations are important to assess the variants immunogenicity as well as the infection in mosquitoes.

Susceptibility is a condition in which the body tissues of the insect can be successfully infected by the parasite (Sinden 2002), thus our findings indicate that An. darlingi and An. aquasalis were susceptible to variants P. vivax. However, we can not compare the intensity of the infection, since the number of mosquitoes analyzed was variable. In general, An. darlingi was proportionally more infected than An. aquasalis. Obviously, the mechanisms of interaction parasite/mosquito are different among the species (Sinden 2002), but we had observed difference in feeding time and blood engorgement (An. darlingi was more efficient than An. aquasalis) which may have further implications in their vector competence (Chadee & Beier 1995, Takken et al. 1998).

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REFERENCES

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### TABLE

**Plasmodium vivax** variants in patient blood samples detected by polymerase chain reaction (PCR) and in Anopheles aquasalis and An. darlingi detected by ELISA, and results of the mosquitoes dissection

<table>
<thead>
<tr>
<th>Patient Parasitemia</th>
<th>PCR variant identified</th>
<th>Species</th>
<th>Mosquito</th>
<th>Dissection</th>
<th>ELISA</th>
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<tbody>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>No. Oocysts</td>
<td>Mean no. (oocysts)</td>
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<td></td>
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<td>100</td>
</tr>
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<td></td>
<td>aqu</td>
<td>51</td>
<td>27</td>
<td>25,3</td>
</tr>
</tbody>
</table>

ND: test not done; dar: An. darlingi; aqu: An. aquasalis; a: VG P. vivax gametocyte; b: a monoclonal antibody for P. vivax-like was not available for the ELISA test; c: this number included one mosquito with a mixed infection of VK210 and VK247.


Brazilian Ministry of Health 2000. Epidemiological Survey of Malaria in Brazil, National Health Foundation, Brasília.


