**NOTES**

**Differential Susceptibilities of *Anopheles albimanus* and *Anopheles pseudopunctipennis* to Infections with Coindigenous *Plasmodium vivax* Variants VK210 and VK247 in Southern Mexico**

LILIA GONZALEZ-CERON, MARIO H. RODRIGUEZ, CUAUITEMOC VILLARREAL, JOSE C. NETTEL, KEVIN C. KAIN, AND JUAN E. HERNANDEZ

Centro de Investigación de Paludismo, Centro de Investigaciones Sobre Enfermedades Infecciosas, and Department of Informatics, Instituto Nacional de Salud Publica, Cuernavaca, Morelos, Mexico, and Tropical Diseases Unit, The Toronto Hospital and The University of Toronto, Toronto, Canada

Received 27 May 1998/Returned for modification 24 July 1998/Accepted 5 October 1998

*Plasmodium vivax* is the primary agent of malaria in Mexico, producing 98% of all cases (22), and the main vectors are *Anopheles albimanus* in the coastal areas and *Anopheles pseudopunctipennis* in the foothills (10). Two *P. vivax* variants have been identified based on the repeat units of their circumsporozoite (CS) proteins: variant VK210 [GDRA(A/D)GQPA] (1) and variant VK247 (ANGAGNQPG) (23). Both variants are detectable infections in at least one of the mosquito species. Of these, four feedings corresponded to variant VK210 (infections 2 to 5 listed in Table 1), four corresponded to VK247 (infections 6 to 9), and one was a mix of both variants (infection 1), which was excluded from all calculations (Table 1).

The number of surviving mosquitoes at day 7 after the infected blood meal varied among mosquito lots, but no differences in survival in relation to the parasite polymorph were detected (α = 0.05 and P = 0.7; likelihood ratio test) (15). Because fewer *A. pseudopunctipennis* mosquitoes fed in the artificial membranes, more *A. albimanus* mosquitoes (n = 160) than *A. pseudopunctipennis* mosquitoes (n = 85) were examined. Mosquito infections were associated with CS protein

---

* Corresponding author. Mailing address: Centro de Investigaciones Sobre Enfermedades Infecciosas, Instituto Nacional de Salud Publica, Av. Universidad 655, Col. Sta. Maria Ahuacatitlan, 62508 Cuernavaca, Morelos, Mexico. Phone: 52 (73) 138969. Fax: 52 (73) 175485. E-mail: mhenry@insp3.insp.mx.
variants, and VK210 infected predominately *A. albimanus* (MIR was 0.62 and MOD ± standard deviation was 29.74 ± 17.03) but infected few *A. pseudopunctipennis* (MIR was 0.13 and MOD was 6.8 ± 12.96) mosquitoes. In contrast, VK247 infected all *A. pseudopunctipennis* (MIR was 1.0 and MOD was 27.85 ± 20.23) but few *A. albimanus* (MIR was 0.05 and MOD was 2.75 ± 1.89) mosquitoes.

Because all *A. pseudopunctipennis* mosquitoes were infected with VK247 and therefore there was no variability within this group, it was not included in the logistic model. The fitted proportions of infections with VK210 in *A. albimanus* and *A. pseudopunctipennis* (0.62 and 0.13, respectively) and the proportion of infection with VK247 in *A. albimanus* (0.05) obtained in the model were very close to the observed ones. The proportions infected with VK210 were different between species (*P* < 0.001 and *α* = 0.05). Also, the proportion of *A. albimanus* mosquitoes infected with VK247 was significantly lower than that of those infected with VK210 (*P* < 0.0001 and *α* = 0.05). The expected mean oocyst counts per mosquito in the Poisson model were also very similar to the observed ones, and there were significant differences by species and CS protein polymorphs: the counts were 29.74 and 6.79 oocysts per mosquito for *A. albimanus* and *A. pseudopunctipennis*, respectively, for VK210 infections (*P* < 0.0001 and *α* = 0.05) and 22.46 and 8.85 oocysts per mosquito, respectively, for VK247 infections (*P* < 0.0001 and *α* = 0.05).

This is the first time that the CS protein of any *P. vivax* strain has been associated with its infectivity to any of its documented vectors. These observations have implications for two aspects of malaria research. First, they open up new opportunities to study vector-parasite interactions in order to identify new targets for interrupting transmission by using transgenic technology (6). Second, the preferential transmission of variants provides new insights into our understanding of malaria epidemiology in the region, where malaria control has been more difficult in areas where *A. pseudopunctipennis* is the main vector (22).

Our results may have been influenced by the mosquitoes we used. Although the *A. pseudopunctipennis* strain is of recent colonization (26), the *A. albimanus* strain has been colonized for over 10 years and the white-striped strain has been selected for its high susceptibility to *P. vivax* (4). Nevertheless, their differences in susceptibility are consistent with our previous observations of higher prevalence of human infections with VK210 in the coastal areas and the presence of VK247 in the foothills (13). Also, in the present study, all patients with infections caused by VK210 were residents of coastal villages and those having infections caused by VK247 were from the foothills. This correlates with serological observations in military personnel (19) of higher prevalence of antibodies to VK 210 in areas where *A. albimanus* is common (Gulf of Mexico coast and Yucatan Peninsula) and higher prevalence of antibodies to VK247 in regions where *A. pseudopunctipennis* is common (Pacific coast north of Chiapas). However, our initial observations should be extended to infections with (F1) wild-caught mosquitoes.

How the CS protein variant may affect mosquito infection requires further investigation. CS proteins are first detected only after parasites reach the sporoblast stage (16). Parasites devoid of the CS gene are unable to develop beyond this stage (17). In our experiments, no degenerated or encapsulated oocysts were seen in mosquitoes that failed to become infected. However, if insect immune responses (18, 25) produced parasite lysis, or if this occurred early during development, their remnants would be difficult to detect with the technique we used.

Other, not yet identified proteins present at earlier parasite stages may also be different between *P. vivax* polymorphs. The

### Notes

**Table 1.** Infection rates and infection intensity in colonized *A. albimanus* and *A. pseudopunctipennis* infected with coinoculated *P. vivax* VK210 and VK247<sup>a</sup>

<table>
<thead>
<tr>
<th>Infection no.</th>
<th>CS protein variant</th>
<th>Mosquito species</th>
<th>n&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Proportion infected</th>
<th>Mean oocyst count ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>VK210 &amp; VK247</td>
<td><em>A. albimanus</em></td>
<td>25</td>
<td>1.00</td>
<td>82.40 ± 38.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>A. pseudopunctipennis</em></td>
<td>21</td>
<td>0.76</td>
<td>7.56 ± 8.37</td>
</tr>
<tr>
<td>2</td>
<td>VK210</td>
<td><em>A. albimanus</em></td>
<td>10</td>
<td>0.90</td>
<td>28.00 ± 8.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>A. pseudopunctipennis</em></td>
<td>12</td>
<td>0.08</td>
<td>1.00</td>
</tr>
<tr>
<td>3</td>
<td>VK210</td>
<td><em>A. albimanus</em></td>
<td>10</td>
<td>1.00</td>
<td>24.10 ± 18.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>A. pseudopunctipennis</em></td>
<td>11</td>
<td>0.36</td>
<td>8.25 ± 14.50</td>
</tr>
<tr>
<td>4</td>
<td>VK210</td>
<td><em>A. albimanus</em></td>
<td>20</td>
<td>0.35</td>
<td>34.85 ± 22.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>A. pseudopunctipennis</em></td>
<td>10</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>5</td>
<td>VK210</td>
<td><em>A. albimanus</em></td>
<td>10</td>
<td>0.50</td>
<td>18.50 ± 22.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>A. pseudopunctipennis</em></td>
<td>4</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>6</td>
<td>VK247</td>
<td><em>A. albimanus</em></td>
<td>25</td>
<td>0.08</td>
<td>3.00 ± 2.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>A. pseudopunctipennis</em></td>
<td>9</td>
<td>1.00</td>
<td>40.22 ± 19.63</td>
</tr>
<tr>
<td>7</td>
<td>VK247</td>
<td><em>A. albimanus</em></td>
<td>10</td>
<td>0.2</td>
<td>1.50 ± 0.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>A. pseudopunctipennis</em></td>
<td>3</td>
<td>1.00</td>
<td>32.70 ± 33.30</td>
</tr>
<tr>
<td>8</td>
<td>VK247</td>
<td><em>A. albimanus</em></td>
<td>25</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>A. pseudopunctipennis</em></td>
<td>2</td>
<td>1.00</td>
<td>8.00 ± 8.49</td>
</tr>
<tr>
<td>9</td>
<td>VK247</td>
<td><em>A. albimanus</em></td>
<td>25</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>A. pseudopunctipennis</em></td>
<td>13</td>
<td>1.00</td>
<td>21.23 ± 14.35</td>
</tr>
</tbody>
</table>

<sup>a</sup> Results of nine experiments in which the two mosquito colonies were fed with the same infected blood.

<sup>b</sup> Number of mosquitoes examined.
combination of these proteins with each mosquito species’ physiology (2, 3, 20) may determine parasite- and mosquito species-specific infectivity. These possibilities are currently under investigation.

REFERENCES


Editor: J. M. Mansfield