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NOTES

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

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The susceptibilities to coindigenous *Plasmodium vivax* of colonized *Anopheles albimanus* and *Anopheles pseudopunctipennis* from southern Mexico were investigated by simultaneous feeding with infected blood obtained from patients. The genes encoding circumsporozoite protein variant types (VK210 and VK247) in blood samples were determined by PCR and oligonucleotide probe hybridization. *A. albimanus* was more susceptible to VK210, and *A. pseudopunctipennis* was more susceptible to VK247.

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 distributed worldwide (5, 12), including in Mexico (11).

In a previous study, we found that all infections in patients living in the coastal plain of the state of Chiapas, Mexico, were caused by VK210, whereas 50% of the cases occurring in the foothills were caused by VK247 (13). We hypothesized that the VK247 variant was preferentially transmitted by *A. pseudopunctipennis*. We present herein a comparison of the susceptibilities of colonized *A. albimanus* and *A. pseudopunctipennis* to infection with both variants of coindigenous *P. vivax*.

Two to 6 days postemergence *A. albimanus* (white-striped strain) mosquitoes (4) and mosquitoes of a strain of *A. pseudopunctipennis* (26) were fed simultaneously, through parafilm membranes, heparinized blood from infected patients. To avoid possible effects from immune factors present in patient sera (14), the original serum was replaced with an equivalent pool of nonimmune sera from subjects with no history of malaria and without anti-*Plasmodium* antibodies (8). Engorged mosquitoes were maintained at 23 to 28°C and 70 to 80% relative humidity (in an air-conditioned insectary) and were provided a 10% sucrose-soaked cotton pad. Surviving mosquitoes were dissected 7 days after receiving the infective blood meal, and oocyst numbers were recorded by using a light microscope (7). All mosquitoes fed with one patient's blood were considered as one lot.

Blood samples blotted onto Whatman no. 2 filter paper were air dried and stored at −20°C until they were shipped to The Toronto Hospital. To determine the CS protein type, a portion of the *P. vivax* CS gene from each sample was amplified by PCR as described previously (9, 13). Positive and negative blood and DNA samples were included in each amplification run. Cross-contamination was prevented as previously described (14). Samples of each amplification reaction mixture were slot blotted and hybridized to ^{32}P-labeled oligonucleotide probes for VK210 and VK247 as described previously (9, 13). Genotyping analysis was performed by individuals who were blinded to the results of entomological and demographic investigations.

A logistic regression analysis was carried out to model the proportion of infected mosquitoes (mean infection rate [MIR]) by species and by CS protein variant, and a Poisson model was used to analyze the oocyst counts per mosquito (mean oocyst density [MOD]) (15). All tests were carried out at a 95% significance level ($\alpha = 0.05$) by using Stata statistical software (24).

Nine of 12 simultaneous infected-blood feedings produced 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 ($\alpha = 0.05$ 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

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TABLE 1. Infection rates and infection intensity in colonized *A. albimanus* and *A. pseudopunctipennis* infected with coindigenous *P. vivax* VK210 and VK247[a]

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

[a] Results of nine experiments in which the two mosquito colonies were fed with the same infected blood.
[b] Number of mosquitoes examined.

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 $\alpha = 0.05$). Also, the proportion of *A. albimanus* mosquitoes infected with VK247 was significantly lower than that of those infected with VK210 ($P < 0.0001$ and $\alpha = 0.05$). 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 $\alpha = 0.05$) and 2.24 and 29.85 oocysts per mosquito, respectively, for VK247 infections ($P < 0.0001$ and $\alpha = 0.05$).

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 antibodiies 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 (F_1) 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

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.

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