

## Development of Sporogonic Cycle of *Plasmodium vivax* in Experimentally Infected *Anopheles albimanus* Mosquitoes

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*The sporogonic cycle of Plasmodium vivax was established and maintained under laboratory conditions in two different strains of Anopheles albimanus mosquitoes using as a parasite source blood from human patients or from Aotus monkeys infected with the VCC-2 P.vivax colombian isolate. Both the Tecojate strain isolate from Guatemala and the Cartagena strain from the colombian Pacific coast were susceptible to infections with P.vivax. A higher percentage of Cartagena mosquitoes was infected per trial, however the Tecojate strain developed higher sporozoite loads. Intravenous inoculation of Aotus monkeys with sporozoites obtained from both anopheline strains resulted in successful blood infections. Animals infected with sporozoites from the Tecojate strain presented a patent period of 21-32 days whereas parasitemia appeared between days 19-53 in monkeys infected with sporozites from Cartagena strain.*

Key words: malaria - *Plasmodium* - sporozoites - *Anopheles albimanus*

The development and regular availability of the malaria sporogonic cycle under laboratory conditions is of utmost importance for the study of the parasite biology, particularly for the human malaria species.

This part of the parasite cycle is initiated by the ingestion of mature viable sexual stages (gametocytes/gametes) by a anopheline species. After fertilization of macrogametes, the resultant zygotes quickly become motile ookinete that invade the wall of the mosquito midgut where parasites differentiate and multiply leading to the formation of oocyst. The burst of oocyst into host cell allows the distribution of the sporozoites through the whole body cavity of the mosquito or hemocel including the salivary glands. Mature sporozoites are injected into the human host during mosquito blood feeding and start a new infection.

The availability of such cycle allows the study of numerous important features on the biology of the parasite. First, during malaria infection the human host produces antibodies specific to components of

the sexual blood stages which are capable to block the fertilization, parasite transmission and further development into the mosquito midgut. Only a few parasite components involved in such a process in a limited number of parasite species are currently known (Carter et al. 1988). Second, the differentiation of the parasite into the mosquito midgut and further migration to the salivary gland is another poorly understood process that would profit from the availability of this model. Third, the maturation process of sporozoites the liver invasion mechanism and the sporozoite-host interaction although being intensively studied (Druilhe et al. 1989) require further analyses.

*P. vivax* is the prevalent human malaria species in Latin America and Asia. Although it does not cause a significant mortality, it produces a very incapacitating disease that leads to a great economic impact due to morbidity in endemic areas. Because of the lack of continuous *in vitro* cultures and the low parasitemia developed by infected patient, limited amounts of parasite material is available for research and therefore much less is known about this plasmodium species than regarding *P. falciparum*. In the present study we have focused on the establishment and maintenance of the sporogonic cycle of *P. vivax* by using blood from both human patients

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and experimentally infected *Aotus* monkeys. For that purpose the susceptibility of two anopheline strains from different regions of Latin America was evaluated. Sporozoites were obtained in these experiments and were used to infect *Aotus* monkeys indicating not only the potential of this model for the study of the parasite biology but also as a source of infective sporozoites to be used in malaria vaccine trials.

### MATERIALS AND METHODS

**Mosquitoes** - Two strains of *Anopheles albimanus* were used in the course of the present experiments. The Cartagena strain from Colombian origin was isolated in 1977 in the Atlantic coast (Carrillo et al. 1981) and was kindly provided by Dr A Morales from the Instituto Nacional de Salud of Colombia. The Tecojate strain from Central America was obtained through the Malaria National Control Program of Guatemala.

Mosquitoes were adapted to grow in our insectary maintained in batches of 100-500 females per cage at 26±1°C and 72% relative humidity. The procedures used for the feeding, handling and dissection of the mosquitoes have been previously described (Collins et al. 1966).

**Experimental infection** - Mosquitoes were infected by feeding on blood from malaria patients carrying gametocytes or *Aotus* monkeys experimentally infected with a *P. vivax* colombian isolate (VCC-2). Human volunteers were attending to the Malaria Control Service (SEM) in Cali and primates were *Aotus lemurinus griseimembra* from the Primate Center of Universidad del Valle, Cali-Colombia. Artificial feeding was routinely done by using parafilm covered pre-warmed glass feeders or by direct exposure to the *Aotus* monkeys. Human blood samples were obtained by venipuncture after patients informed consent. Samples were collected in tubes containing heparin and immediately transported to the laboratory for mosquito feeding. Mosquitoes were maintained in a fasting period of 24 hours previous to the blood feeding which was usually completed within a period of 15-20 min. A mosquito sample was dissected on day 6 of the experiment to determine the presence of oocyst in the midgut and infected batches were further dissected on day 14-15 to recover the sporozoites from salivary glands.

**Monkey experiments** - Animals were infected by intravenous injection of blood stages of the *P. vivax*

VCC-2 isolate in order to produce circulating gametocyte to infect the mosquitoes. In addition, monkeys were used to determine the infectivity of these sporozoites. *Aotus* monkeys were intravenously inoculated with freshly dissected sporozoites with doses ranging between 2.500 and 5.000 sporozoites/monkey. Animals were later splenectomized and patent parasitemia followed for several weeks. Some of these sporozoite infected monkeys were gametocyte donors in order to reinfect mosquitos and complete the malaria cycle.

A total of 61 artificial attempts were made with human infected blood samples, whereas 54 infection trials were made using the *Aotus* model.

### RESULTS

The Tecojate strain from Guatemala was better adapted to laboratory conditions and produced a larger number of mosquitoes than the Cartagena strain from Colombia. The susceptibility of these two anopheline strains to the infection with *P. vivax* parasites is presented in Table I. Due to the more abundant availability of Tecojate mosquitoes a major number of infection trials was performed with this mosquito strain. Fifty attempts to infect the Tecojate strain were done using human blood and 40 using infected *Aotus* blood, whereas only 12 trials could be done with human blood and 14 with monkey blood using the Cartagena strain.

Early mosquito infections determined on day 6 after blood meal were more frequent in the Tecojate

TABLE I

Rates of *Anopheles albimanus* infected with *Plasmodium vivax* during 1992 - 1993

Parasite source	<i>Anopheles</i> strains	Oocyst / gut		Sporozoite	
		Pos / Dis*	% Inf	Pos / Dis <sup>a</sup>	% Inf
Human	Tejocate	331/1,324	25	22/337	9.5
	Cartagena	23/209	11	14/42	33
<i>Aotus</i>	Tejocate	28/933	3	26/1,300	2
	Cartagena	4/400	1	5/20	2

<sup>a</sup> Pos / Dis: Rate of positive Vs. dissected mosquitoes midguts.

mosquitoes exposed to infected human blood. Twenty five percent of them developed oocyst in the midgut in contrast to Cartagena strain that presented a lower percentage (11%) on the same day. The sporozoite production as determined by the percent

of mosquitoes harboring infected salivary glands was however higher in the Cartagena (33%) than in the Tecojate (9.5%). When mosquitoes were fed on *Aotus* infected with the VCC-2 strain, equal percentage (2%) of sporozoite infections was obtained.

Although, in *A. albimanus* Cartagena strain mosquitoes appeared more susceptible to *P. vivax* infection and a larger group became infected, when the sporozoite loads in the salivary glands were analyzed, the Tecojate strain produced a greater number of sporozoites than Cartagena when human infected blood was used. No significant difference was seen between the two strains when mosquitoes were fed on *Aotus* infected blood (Table II).

TABLE II

Rates of *Anopheles albimanus* infectedes with *Plasmodium vivax* during 1992 - 1993

Parasite source	<i>Anopheles</i> strains	x 10 <sup>5</sup> Spz/ fem.dis <sup>a</sup>	Spz/ female
Human	Tejocate	12 / 3833	313
	Cartagena	0.2 / 204	107
<i>Aotus</i>	Tejocate	0.1 / 56	178
	Cartagena	0.04 / 20	220

<sup>a</sup>: total number of sporozoite / female dissected.

Seven *Aotus* monkeys were selected and infected with sporozoites obtained by salivary glands dissection in the previous experiments. The course of *Aotus* infection in terms of sporozoite inoculum,

prepatent period, malaria previous experience and peak of parasitemia are presented in Table III. Five monkeys had previously experienced malaria infections or had been immunized with malarial antigens whereas the other two were naive animals. *Aotus* inoculated intravenously with sporozoites were splenectomized 10 to 25 days after inoculation. Patent parasitemia appeared on days 21-32 in animals infected with sporozoites obtained from the Tecojate strain whereas it appeared between days 19-53 in those infected with parasites from the Cartagena strain. Primate M43 inoculated with 3000 sporozoites from the latter mosquito strain did not develop patent parasitemia. Except for this *Aotus* the remaining animals developed parasitemias similar to those present in human (0.1-0.8%). Previous exposure to *P. falciparum* infections did not appear to prevent the infection with *P. vivax* sporozoites.

## DISCUSSION

The aim of the present study was the development of the sporogonic cycle and regular production of sporozoites in laboratory conditions. This parasite cycle recovers importance given the availability of new tools for the study of the molecular interaction between the parasite and both the vertebrate and the mosquito hosts.

In order to establish such a cycle we initiated a mosquito colony using different strains of *A. albimanus*. This mosquito species is widely spread in malaria endemic areas from different regions of Latin America from Mexico to Perú and is con-

TABLE III

Rates of *Anopheles albimanus* infectedes with *P. vivax* during 1992 - 1993

Mosquito strain	Monkey code	Sporozoite inoculum	Prepatent period	Prior malaria <sup>a</sup>	Peak of parasitemia	Day <sup>b</sup>
Tejocate	M-25	2500	32	pf, im	0.1	39
	C-15	1500	34	pf, im	0.1	43
	M-27	3000	23	pf, im	0.1	38
	D-67	5000	21	pf, im	0.1	32
Cartagena	M-17	5000	53	pf, im	0.2	60
	M-43	3000	-	pf, im	0.0	-
	A-101	3200	19	none	0.8	44

<sup>a</sup>: pf: *Plasmodium falciparum*; im: immunization with synthetic peptides.

<sup>b</sup>: Day after sporozoite inoculation.

sidered a major malaria vector in Colombia. We therefore selected this species for colonization and artificial infection in these studies. In addition, a number of *P. vivax* wild isolates from infected humans or parasite strains adapted to *Aotus* monkeys were used.

Our results indicate that both anopheline strains studied here are susceptible to *P. vivax* infection. Although the Cartagena strain presented a higher percent of sporozoite infected mosquitoes per trial, the sporozoite production per female was greater in the Tecojate strain when it was exposed to infected human blood. This results support previous findings in which *A. albimanus* collected in different endemic regions of Latin America had shown sporozoite rates significantly different (Herrera et al. 1987, Warren et al. 1975, Olano et al. 1985). This may indicate selective advantages for individuals or populations within this mosquito species. The low sporozoite rates and loads obtained in these experiments would support the fact that although widely spread and considered a major malaria vector in Latin America under natural conditions *A. albimanus* is a poor malaria vector. This seems to be in agreement with the low malaria transmission in areas where this anopheline species is prevalent.

The susceptibility of different anopheline species including *A. albimanus* to the infection by *P. vivax* parasites under laboratory conditions has been previously studied (Warren et al. 1980, Olano et al. 1985, Ramsey et al. 1994). In the present study we were able to reproducibly transmit our *P. vivax* isolate VCC-2 through *Aotus lemurinus* using both *A. albimanus* strains. Our results are similar to those from Collins et al. (1980) who had tested the Chesson strain of *P. vivax* from New Guinea in *Aotus trivirgatus* monkeys. In both studies *P. vivax* was easily transmitted through the primate models indicating the adaptability of this parasite species to the laboratory conditions.

Infectivity of *A. albimanus* had been previously shown to be less efficient under lab conditions than other anopheline species (Collins et al. 1977). The quantity of sporozoites obtained in our experimental infections was very low as compared to other mosquito species (Ponnudurai et al. 1990., Collins et al. 1986), however it allowed experiments such as *P. vivax* cyclical transmission through *Aotus* monkeys, immunogenicity studies and antigenical analysis of sporozoites.

In a recent study Ramsey et al. (1994) using two strains *A. albimanus* in Mexico demonstrated a great reduction in infectivity of gametocyte containing blood if it were used with its autologous serum probably due to the presence of host immune factors. This may explain our low infection rates as mosquitoes were always directly exposed to infected blood that may have contained anti-gamete antibodies.

The reproducible cyclical transmission of both wild and adapted parasites through *Aotus* monkeys gives to this model a great potential in the study of the biology of the complete cycle of *P. vivax*, particularly for the liver stages that hardly could be studied otherwise. Sporozoites obtained in this way are being used for biological studies as well as for the challenge of immunized monkeys in malaria vaccine phase 0 trials and would be of great potential in the controlled challenge of malaria vaccinated human volunteers.

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