

SUSCEPTIBILITY OF MOSQUITOES TO HUMAN
MALARIA INDUCED IN PANAMANIAN
MONKEYS

BY

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Introduction

ADAPTATION of the four species of human malaria to simian hosts through blood passage has been followed by attempts to verify mosquito susceptibility to the parasites in these systems. *Plasmodium ovale* and *P. malariae* when inoculated into the chimpanzee, *Pan satyrus*, were found to produce mature gametocytes capable of infecting mosquitoes.^{3,4} Falciparum malaria, although induced in several species of apes and monkeys, has been transmitted by a vector only from the night monkey, *Aotus trivirgatus*.⁶ *P. vivax* also has been widely studied, and has been shown to be infective to mosquitoes when grown in the chimpanzee,^{2,7} night monkey,^{8,10} and recently, the Panamanian marmoset, *Saguinus Geoffroyi*, and black spider monkey, *Ateles fusciceps*.¹

This paper is a report of the comparative susceptibility of mosquitoes to strains of *P. vivax* induced in several species of Panamanian monkeys. Included is a summary of trial mosquito feedings upon monkeys infected with *P. falciparum*.

Materials and Methods

Anopheles albimanus mosquitoes (A-1 strain), maintained in colony in the Rand Insectary at the Gorgas Memorial Laboratory, were pooled in lots averaging 200 females each for feeding upon malarious monkeys and patients. Fewer numbers of four other anophelines reared in colony or from field-collected immatures were applied during these studies. Techniques for feeding and sources of donor monkeys and patients have been described

elsewhere.^{1,8} Parasite development was followed at an incubation temperature of 76°F and in a series of companion lots at 72°F and 80°F. Usually 20 mosquitoes were dissected per fed lot. Midguts and salivary glands routinely were examined at odd-day intervals from 7-11 and 11-14 days, respectively, after feeding. Sporozoite concentrations were recorded by the plus-grouping method.¹¹

All strains of *P. vivax* used were Panamanian origin. The *P. falciparum* strains were from Panama, Malaya, and Uganda.

The infections had been induced in monkeys by intraperitoneal injection of parasitized blood, and were maintained by serial passages from monkey to monkey.^{1,8,10}

Results

Infections of eight strains of human *P. vivax* were established in 153 monkeys. Over 21,000 mosquitoes were fed upon the monkeys and subsequently examined. As shown in Table 1, six strains in *Aotus*, *Saguinus*, and *A. fusciceps* hosts were infective to, and completed the sporogonous cycle in *A. albimanus*. Greatest numbers of mosquitoes were fed upon *Aotus* and *A. fusciceps* over the longest periods and it was from these species of monkeys that the highest levels of mosquito infection resulted. Principally two strains, the Achote and Santa Rosa, were involved. The Achote strain appeared to infect mosquitoes at a similar rate whether in *Aotus* or *A. fusciceps*. Additionally, there was no significant difference in overall infectivity of the Santa Rosa strain between the hosts.

Some mosquitoes were infected when the male gametocytemias were lower than 10 per mm³ (Table 2), although higher numbers of male gametocytes produced more oocysts on the midguts. The Achote strain in *Aotus* and *A. fusciceps* produced heavier infections than did the Santa Rosa strain. Mosquitoes fed on *Aotus* with the Achote strain had more

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TABLE 1

OOCYST AND SPOOROZITE DEVELOPMENT IN *Anopheles albimanus* FED UPON FIVE SPECIES OF PANAMANIAN MONKEYS INFECTED WITH VARIOUS STRAINS OF *Plasmodium vivax* AND *P. falciparum*

Host Species Malaria Strain	No. Monkeys	Lots		Mosquitoes		No. Oocysts		Sporozoite Conc. ¹	
		No. Fed.	% Pos.	No. Diss.	% Pos.	Range	Mean	Range (+)	Mean (+)
<i>P. vivax</i>									
<i>Aotus trivirgatus</i>									
Achiote	81	629	24.2	11,777	6.7	1-160	5.7	1-4	2.3
Santa Rosa	33	182	20.0	3,734	6.3	1-20	2.8	1-3	2.0
Fort Clayton	4	31	9.7	574	2.4	1-2	1.1	1-4	2.4
Emperador	4	16	56.3	425	30.8	1-13	2.8	1-3	2.9
Polo	3	9	44.4	195	22.6	1-21	3.6	1-3	1.3
Chivo Chivo-2	2	9	77.8	182	8.8	1-4	1.7	3	3.0
Pavarando	1	12	8.3	245	2.4	1-2	1.3	0	0
Chivo Chivo	1	4	0	13	0	0	0	0	0
<i>Saguinus geoffroyi</i>									
Achiote	2	5	0	94	0	0	0	0	0
Santa Rosa	1	2	0	40	0	0	0	0	0
Emperador	5	27	3.7	442	0.2	1	1.0	0	0
Chivo Chivo-2	1	2	50.0	60	5.0	1-3	1.7	0	0
Chivo Chivo	1	2	0	9	0	0	0	—	—
<i>Ateles fusciceps</i>									
Achiote	10	193	11.9	3,620	2.6	1-15	2.7	1-3	2.1
Santa Rosa	2	15	40.0	304	11.2	1-7	2.2	1-3	1.7
<i>Ateles geoffroyi</i>									
Achiote	2	6	0	100	0	0	0	0	0
Total	153	1,144	21.5	21,814	6.4	1-160	4.6	1-4	2.1
<i>P. falciparum</i>									
<i>Aotus trivirgatus</i>									
Uganda Palo Alto	11	46	0	902	0	0	0	0	0
Malayan Camp	6	46	0	779	0	0	0	0	0
<i>Cebus capucinus</i>									
Uganda Palo Alto	3	34	0	569	0	0	0	0	0
<i>Saguinus geoffroyi</i>									
Majé	1	5	0	196	0	0	0	0	0
Total	21	131	0	2,446	0	0	0	0	0

¹ Sporozoite grouping: 1+, 1-9; 2+, 10-99; 3+, 100-999; 4+, >999.

oocysts than those fed on *A. fusciceps* with the same strain.

The highest mean percentage of infected *A. albimanus* occurred when the male gametocyte density of Achiote vivax was above 500 per mm³ in both *Aotus* and *A. fusciceps* donors

(Tables 3 and 4). The former gave the higher rates of lot infection. In general, mosquitoes in lots with high infection rates tended to have more oocysts per midgut. No vector infections were produced in the few trials with *A. geoffroyi*.

TABLE 2

OOCYST DEVELOPMENT OF THE ACHIOTE AND SANTA ROSA STRAINS OF *P. vivax* IN RELATION TO MALE GAMETOCYTE CONCENTRATION

Male Gametocytes per mm ³	No. Lots Infected	Guts		No. Oocysts per Pos. Gut	
		No. Examined	% Pos.	Range	Mean
Achiote Strain					
<i>Aotus trivirgatus</i>					
<10	13	266	12.4	1-13	2.1
10-99	32	594	21.2	1-38	2.9
100-499	60	1,157	23.2	1-160	6.6
500-999	22	515	16.1	1-137	8.7
>999	15	283	24.0	1-80	6.9
<i>Ateles fusciceps</i>					
<10	3	50	12.0	1-6	1.8
10-99	6	119	19.3	1-7	2.4
100-499	10	214	16.8	1-12	3.1
500-999	2	45	28.9	1-15	2.7
>999	1	15	6.7	1	1.0
Santa Rosa strain					
<i>Aotus trivirgatus</i>					
<10	2	34	29.4	1-3	1.6
10-99	10	172	22.1	1-14	2.8
100-499	19	323	17.6	1-11	2.7
500-999	2	41	29.3	1-20	1.2
>999	4	84	7.1	1-7	3.3
<i>Ateles fusciceps</i>					
10-99	3	56	21.4	1-5	2.0
100-499	3	49	16.3	1-7	2.4

The period of parasitemia patency appeared to have no major influence on the infectivity of the Achiote strain to the mosquitoes. Infections could result during any week of patency (Table 5). A tabulation of all malaria strains gave the same results (Table 6). The second week of patency in *Aotus* produced infections most consistently. In *A. fusciceps* the third week appeared to be the best. These intervals corresponded with periods of maximum gametocyte development in the respective host species.

A. albimanus normally were maintained on a 5-10 per cent sugar solution, and a change in this diet to raisins or molasses subsequent to feeding upon several donor monkeys did not appreciably alter their susceptibility to malaria or their life span. In other experiments the mosquitoes were allowed a second

blood meal on a clean animal (guinea pig) without apparent effect.

A separate *A. albimanus* colony was established from a local area and compared with the laboratory strain, which had been maintained for many years; no meaningful differences in rates of infection were evident.

Temperatures at which the mosquitoes were incubated after a blood meal are compared in Table 7. The percentages positive for vivax malaria in lots retained at 72°F or 80°F did not vary significantly from their controls at 76°F. An advantage was observed at 72°F in that a larger portion of the females lived over a longer period, although the parasites developed more slowly. As previously reported,¹ the length of the sporogonous cycle at 76°F and 72°F was 12 and 15 days, respectively. No sporozoites appeared in salivary

glands of the few infected mosquitoes at 80°F.

Preliminary experiments on the susceptibility of four other vector anophelines of Panama to vivax and falciparum malarias were conducted as listed in Table 8. Besides *A. albimanus*, *A. pseudopunctipennis* was the only mosquito that was infected. However, in most cases not enough individuals of other species were used for a valid test.

No oocyst development was recorded in a total of 131 *A. albimanus* lots applied to *Aotus*, *Cebus*, and *Saguinus* monkeys infected with three strains of *P. falciparum* (Tables 1 and 8). These mosquitoes were exposed to infection on consecutive days and throughout periods of gametocytemia. During feedings on the 4th to the 35th patent day gametocyte concentrations ranged from less than 10 to 2,310 per mm³.

Discussion and Conclusions

We previously showed that *A. albimanus* mosquitoes generally were less susceptible to

blood-induced *P. vivax* infections in Panamanian primates than to the natural infections in man.¹ Some strains adapted to monkeys (Fort Clayton, Pavarando, and Chivo Chivo) were poorly infective to *A. albimanus*. Others (Emperador, Polo, and Chivo Chivo-2) produced relatively good infections in the mosquitoes. Serial transfer of several strains among the species of monkeys apparently did not further alter their vector infectivity. Achiote and Santa Rosa vivax were subinoculated through *Aotus* hosts 50 and 80 times, respectively, yet frequencies of positive lots and rates of infections continued as in earlier passages.

The results of the many feedings upon *Aotus* and *A. fusciceps* were most meaningful. Overall, the percentages of *A. albimanus* infected with the Achiote and Santa Rosa strains from the two host species were similar. A wider range of infection rates was obtained from *Aotus*, but more donor animals and mosquitoes were used. Additional trials with the various strains are necessary, especially to de-

TABLE 3
INFECTIVITY OF THE ACHIOTE STRAIN OF *P. vivax* TO *A. albimanus* IN RELATION TO HOST
AND MALE GAMETOCYTE CONCENTRATION

No. Male Gametocytes per mm ³	Monkeys		Lots		% Infection in Pos. Lots	
	No.	% Infective	No.	% Pos.	Range	Mean
<i>Aotus trichirgatus</i>						
<10	33	27	107	12.1	2.0-64.7	13.3
10-99	44	48	143	23.1	5.0-78.9	23.2
100-499	60	53	263	24.7	4.0-100	27.1
500-999	38	50	83	28.9	4.0-80.0	32.8
>999	21	57	33	51.6	3.8-75.0	32.9
<i>Ateles fusciceps</i>						
<10	7	14	38	5.3	11.1-13.0	12.1
10-99	9	33	56	10.7	2.5-32.0	20.5
100-499	9	22	67	14.9	3.3-56.7	17.9
500-999	5	20	21	9.6	24.0-28.0	26.0
>999	3	33	11	18.2	15.4-28.0	21.7
<i>Ateles geoffroyi</i>						
10-99	2	0	2	0	0	0
100-499	2	0	3	0	0	0
500-999	1	0	1	0	0	0
<i>Saguinus geoffroyi</i>						
10-99	1	0	1	0	0	0
100-499	1	0	4	0	0	0

TABLE 4
INFECTIVITY OF *P. vivax* TO *A. albimanus* IN RELATION TO HOST AND MALE GAMETOCYTE CONCENTRATION—
TOTALS OF ALL STRAINS

Host Species	Monkeys		Lots		% Infection in Pos. Lots	
	No.	% Infective	No.	% Pos.	Range	Mean
<i>Aotus trivirgatus</i>						
< 10	57	21	162	10.5	2.0-64.7	14.7
10-99	73	47	215	26.0	3.4-84.8	23.4
100-499	93	50	364	23.9	4.0-100	25.1
500-999	50	42	100	28.0	4.0-80.0	33.6
> 999	31	61	51	47.1	3.8-75.0	28.4
<i>Ates fusciceps</i>						
< 10	8	12	39	5.1	11.1-13.0	12.1
10-99	11	45	62	14.5	2.5-56.3	21.9
100-499	11	36	75	17.3	3.3-56.7	18.7
500-999	5	20	21	9.5	24.0-28.0	26.0
> 999	3	33	11	18.2	15.4-28.0	21.7
<i>Saguinus geoffroyi</i>						
< 10	4	25	14	14.3	5.0-8.6	6.8
10-99	3	0	2	0	0	0
10-99	3	0	2	0	0	0
100-499	5	0	12	0	0	0
500-999	2	0	2	0	0	0
> 999	3	0	8	0	0	0

termine their responses in *Saguinus* and *A. geoffroyi*. *Saguinus* hosts may be favorable for these studies, as relatively few feedings gave mosquito infection.

The highest gametocytemias during primary or recrudescence parasitemia in the donor animals tended to produce the best rates of lot infection and oocyst concentrations. Usually in lots applied during the optimal periods less than one-third of the mosquitoes were infected and oocysts numbered fewer than 10 per midgut. In some cases up to 100 per cent of the mosquitoes were infected, and oocyst concentrations ranged to 160 per midgut. Some hosts produced more infected mosquitoes than others.

The inability of *A. albimanus* to develop infections of Malayan Camp and Uganda Palo Alto falciparum malaras induced in *Aotus* is consistent with the results of others.⁹ Although the African strain produced good ga-

metocytemias when subinoculated to the Panamanian *Cebus*, again mosquitoes failed to become infected. Vector susceptibility to one Panamanian strain of *P. falciparum*, Majé, subinoculated to *Saguinus*,⁹ could not be evaluated on the basis of five negative feedings. Reports of mosquito infection of the Malayan IV and Cambodian I strains in *Aotus*^{5,6} are encouraging, and indicate that other falciparum strains adapted to these monkeys also may prove infective to mosquitoes.

The susceptibility of the strain of *A. albimanus* to human malaria essentially had not changed through 30 years of laboratory culture. Various modifications of holding conditions following feedings on malarious monkeys did not improve infection rates. The Panamanian strain of *A. pseudopunctipennis* was infected with *P. vivax* from human and monkey hosts, but not as well as *A. albimanus*. Additional investigations will be necessary to

determine the relative susceptibility of other anopheline species. *A. albimanus* would appear to be a mosquito of choice for these studies, as it is a primary vector of vivax and falciparum malarias in Panama.

Summary

Seven of eight strains of *P. vivax* grown in *Aotus trivirgatus*, *Ateles fusciceps*, and *Saguinus geoffroyi* were infective to *Anopheles albimanus*.

The rate of infected mosquitoes was similar with *Aotus* and *A. fusciceps* hosts. However, greater numbers of oocysts and sporozoites resulted from feedings upon *Aotus*.

A. albimanus were infected when male gametocytes were lower than 10 per mm³, although higher gametocytemias gave higher rates of lot infection and greater oocyst concentrations. These relationships were apparent

throughout periods of parasite patency.

Three strains of *P. falciparum* induced in *Aotus*, *Saguinus*, and *Cebus capucinus* were not infective to *A. albimanus*.

Susceptibility of *A. albimanus* was not improved by change of diet or incubation temperature. *A. pseudopunctipennis* was less susceptible than *A. albimanus*.

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TABLE 5
INFECTIVITY OF THE ACHIOTE STRAIN OF *P. vivax* TO *A. albimanus* IN RELATION HOST AND WEEK OF PATENT PERIOD

Host Species	Monkeys		Lots		% Infection in Pos. Lots	
	No.	% Infective	No.	% Pos.	Range	Mean
<i>Aotus trivirgatus</i>						
1	36	47	90	28.9	5.0-53.3	19.5
2	59	44	287	24.7	3.8-100	31.0
3	41	41	164	22.7	2.8-72.5	25.1
4	6	50	69	23.2	5.0-63.3	19.7
5	5	20	12	8.3	2.0	2.0
6	5	40	7	42.8	8.3-77.8	42.3
<i>Ateles fusciceps</i>						
1	5	20	12	9.1	13.3	13.3
2	9	44	71	8.3	15.4-28.0	21.1
3	10	10	71	9.8	3.3-56.7	23.6
4	4	25	32	25.0	6.7-27.3	14.7
5	2	0	7	0	0	0
<i>Ateles geoffroyi</i>						
3	11	0	2	0	0	0
4	1	0	1	0	0	0
5	1	0	3	0	0	0
<i>Saguinus geoffroyi</i>						
2	1	0	1	0	0	0
3	1	0	3	0	0	0
5	1	0	1	0	0	0

TABLE 6
INFECTIVITY OF *P. vivax* TO *A. albimanus* IN RELATION TO HOST AND WEEK OF PATENT PERIOD—
TOTALS OF ALL STRAINS

Host Species	Monkeys		Lots		% Infection in Pos. Lots		
	Week of Patency	No.	% Infective	No.	% Pos.	Range	Mean
<i>Aotus trivirgatus</i>							
1	48	40	117	24.8	3.4-53.3	18.8	
2	91	41	409	23.1	3.8-100	28.9	
3	64	45	250	22.0	2.8-75.1	22.3	
4	14	50	85	27.1	5.0-63.3	21.8	
5	10	50	20	30.0	2.0-60.0	36.0	
6	6	50	11	45.4	8.3-77.8	31.7	
<i>Ateles fusciceps</i>							
1	5	20	12	8.3	13.3	13.3	
2	10	50	74	10.8	15.4-56.3	22.2	
3	11	18	77	16.2	3.3-56.7	25.5	
4	6	33	37	24.3	2.5-27.3	17.7	
5	6	33	8	12.5	20.0	20.0	
<i>Saguinus geoffroyi</i>							
1	3	33	5	20.0	8.6	8.6	
2	3	33	5	20.0	5.0	5.0	
3	3	0	9	0	0	0	
4	4	0	7	0	0	0	
5	4	0	9	0	0	0	
6	2	0	3	0	0	0	

TABLE 7
EFFECT OF INCUBATION TEMPERATURES UPON *P. vivax* INFECTION IN *A. albimanus*¹

Host Species	Lots Fed	Control Temperature			Test Temperature		
		Lots Infect.	Mosq. Dissect.	% Mosq. Infect.	Lots Infect.	Mosq. Dissect.	% Mosq. Infect.
		76°F			72°F		
<i>Aotus trivirgatus</i>	46	5	811	3.2	5	795	2.5
<i>Ateles fusciceps</i>	11	7	219	11.4	8	261	14.2
		76°F			80°F		
<i>Aotus trivirgatus</i>	20	2	274	1.1	1	296	0.7
<i>Ateles fusciceps</i>	43	1	723	0.3	2	718	0.7

¹ Mosquitoes incubated at control and test temperatures were fed in companion lots on the same hosts.

TABLE 8

COMPARATIVE SUSCEPTIBILITY OF VARIOUS ANOPHELINE SPECIES TO *P. vivax* AND *P. falciparum* FROM PANAMANIAN MONKEYS AND MAN¹

Hosts	Lots Fed	Control Anopheline			Test Anopheline		
		Lots Infect.	Mosq. Dissect.	% Mosq. Infect.	Lots Infect.	Mosq. Dissect.	% Mosq. Infect.
		<i>P. vivax</i>			<i>A. pseudopunctipennis</i>		
		<i>A. albimanus</i>			<i>A. oswaldoi</i>		
<i>Aotus trivirgatus</i>	56	11	954	2.5	1	853	0.1
<i>Ateles fusciceps</i>	23	1	395	0.5	0	363	0
Man	3	2	59	6.8	1	55	0.2
		<i>A. albimanus</i>			<i>A. apicimacula</i>		
<i>Aotus trivirgatus</i>	9	1	185	0.5	0	43	0
		<i>A. albimanus</i>			<i>A. punctimacula</i>		
<i>Aotus trivirgatus</i>	3	2	60	3.3	0	15	0
		<i>A. albimanus</i>			<i>A. pseudopunctipennis</i>		
<i>Aotus trivirgatus</i>	2	1	25	4.0	0	21	0
		<i>P. falciparum</i>			<i>A. pseudopunctipennis</i>		
		<i>A. albimanus</i>			<i>A. pseudopunctipennis</i>		
<i>Aotus trivirgatus</i>	16	0	269	0	0	299	0
<i>Cebus capucinus</i>	4	0	80	0	0	66	0
Man	7	4	120	20.0	0	112	0

¹ Control and test mosquitoes were fed in companion lots on the same hosts.

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