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Characterization of *Plasmodium vivax* Infections in *Saimiri sciureus* (Squirrel Monkeys)

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ABSTRACT: Infections with a monkey-adapted strain of human *Plasmodium vivax* (Achiote) were established in *Saimiri sciureus* and were serially transferred 26 times in this host species by trophozoite inoculation. Patent infections were produced in all of the 42 unaltered and 1 splenectomized recipients.

Primary infections persisted for as long as 72 days and maximum parasite concentrations reached more than 100,000 per cmm. Relapses occurred, and were usually of shorter duration with parasite densities lower than in the primary attack.

Eleven unaltered *Saimiri* were infected by the sporozoite stages in *Anopheles albimanus* derived from *Aotus trivirgatus* carrying this vivax strain. Although prepatent periods were longer than for trophozoite induced infections, most of the characteristics of the parasitemias were similar.

Oocysts were demonstrated in *Anopheles albimanus* and *A. aztecus* following feeding upon several *Saimiri* with trophozoite induced infections.

It has been shown that infections with *Plasmodium vivax* can be induced in 5 species of New World monkeys by the trophozoite and sporozoite stages of the parasite. The most widely used experimental host is *Aotus trivirgatus* (the night monkey); vivax strains are easily adapted and maintained in this model (Young et al. 1966; Porter and Young 1966; Hickman 1969; Baerg et al. 1969; and Ward et al. 1969). Vivax malaria also has been transferred from *Aotus* by trophozoites and sporozoites to *Saguinus geoffroyi* (the Panamanian marmoset) (Porter and Young 1966; Baerg et al. 1969; Baerg et al. —unpublished; and Porter 1970) and to *Ateles fusciceps* and *A. geoffroyi* (spider monkeys) (Baerg et al. 1969; Baerg et al.—unpublished; Young and

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Table 1. Serial trophozoite passages of *Plasmodium vivax* in *Saimiri sciureus*—primary parasitemia.

No. of monkeys	Inoculum $\times 10^6$ range	Prepatent period —days Mean (Range)	Patent period —days* Mean (Range)	Parasitemia 1,000 per cmm No. of monkeys	Patent day Mean (Range)	Maximum per cmm Mean (Range)	Patent day Mean (Range)	Remarks
Passage Nos. 1–5								
12	1.1–12.5	7 (1–18)	32 (23–72)	11	8 (4–13)	30,455 (910–86,290)	15 (11–18)	2 died patent days 21, 38
Passage Nos. 6–10								
11	<1–12.5	6 (1–21)	27 (24–28)	11	6 (3–15)	16,841 (1,720–39,940)	13 (3–20)	3 died patent days 14, 29, 59. 4 sacrificed when parasitemia approximated 40,000 per cmm
Passage Nos. 11–15								
5	1.3–2.1	8 (4–19)	28 (25–34)	5	7 (5–8)	48,138 (16,570–111,070)	14 (10–16)	2 died patent days 17, 19
Passage Nos. 16–20								
6	<1–9.9	6 (2–11)	22 (18–27)	5	9 (3–19)	43,360 (6,030–87,130)	14 (6–29)	2 died patent days 3, 39
Passage Nos. 21–26								
9	<1–10.9	4 (1–7)	35 (20–55)	9	6 (4–8)	48,955 (14,700–93,210)	14 (11–18)	2 died patent day 34

* Monkeys dying during patency are not included in tabulation of length of patent period.

Porter 1969; and Porter and Young 1970). The latter 2 hosts require splenectomy to produce significant infections.

Deane et al. (1966) found that a splenectomized *Saimiri sciureus* (squirrel monkey) would support an infection of vivax malaria derived directly from man. We since have determined that intact *Saimiri* will sustain infections of a monkey adapted vivax malaria introduced by blood stages or mosquitoes (Young et al. 1971). Subsequent to our preliminary report, we continued our investigations on the characteristics of the infections in *Saimiri* monkeys. The results are reported here.

Materials and Methods

Procedures concerning the procurement, handling and husbandry of *Saimiri* monkeys at Gorgas Memorial Laboratory were given by Rossan et al. (1972).

The human malaria strain, Achiote, was isolated in Panama and has been serially transferred by blood or sporozoites in monkeys since 1966 (Porter and Young 1966). Several passage lines in *Aotus* and *Saimiri* were maintained to achieve further parasite adaptation and to study the course of infection in these primates. In most cases unaltered *Aotus* and *Saimiri* served as recipients. Inoculation procedures, staining of blood films and counting of parasites were as described previously, with some modifications (Porter and Young 1966).

Infected *Aotus* served as donors for sources of mosquito infections as given in previous reports (Baerg et al. 1969). Only *Anopheles albimanus* was used as the vector for sporozoite transmissions. Sporozoites were introduced either by the interrupted bite technique or by intravenous inoculation of a sporozoite suspension. Animals inoculated intravenously were administered a single dose of penicillin (400,000 units). *Anopheles aztecus*, *A. pseudopunctipennis*, and *A. punctimacula* were utilized for their comparative susceptibilities to the induced malaria in the monkeys.

Results

Trophozoite Induced Infections

After 78 consecutive blood passages in *Aotus* monkeys, infections were established in *Saimiri* by subinoculation into 1 splenectomized and 2 unaltered animals. Passage lines were continued in intact *Saimiri* from the splenectomized recipient and from 1 unaltered recipient; these were carried through a total of 8 and 26 passages, respectively. Data on the primary parasitemias of the infections in the monkeys comprising the 2 lines are grouped and summarized in Table 1 according

Table 2. Characteristics of relapses in *Saimiri sciureus* with trophozoite induced infections of *Plasmodium vivax*.

No. of monkeys	Subpatent period —days Mean (Range)	Patent period —days Mean (Range)	Maximum parasitemia Per cmm Mean (Range)	Maximum parasitemia Patent day Mean (Range)
1st Relapse				
19	29 (5–66)	14 (3–24)	915 (<10–10,910)	9 (4–14)
2nd Relapse				
9	29 (5–51)	17 (12–21)	992 (<10–5,430)	10 (8–14)
3rd Relapse				
5	31 (10–77)	18 (3–28)	1,816 (<10–6,240)	13 (4–21)
4th Relapse				
1	24	15	390	10

to the number of serial transfers. Parasitemias were produced in all of 43 monkeys inoculated (40 intraperitoneally and 3 intravenously).

Overall prepatient periods ranged from 1 to 21 days, and were as short as 1 day in both the early and late passages. There was no apparent correlation between the prepatent period and numbers of parasites inoculated. Generally, there was a relatively rapid ascent of parasite numbers. In 41 of the 43 recipients parasitemias reached 1,000 per cmm between the 6th and 9th day of patency with the maximum concentration about 1 week later.

In the initial passage into 3 *Saimiri*, the splenectomized recipient experienced a maximum parasitemia of 86,290 per cmm, whereas maxima of 910 and 2,360 per cmm were achieved in the unaltered, companion subjects. However, within a few subsequent passages, the infections in unaltered recipients reached as high as 84,360 per cmm. The average maximum parasitemias, with a peak in one animal of 111,070 during the 15th passage, were consistently higher after 10 serial transfers.

The initial patent periods in 28 monkeys (all unaltered) were as short as 18 days and as long as 72 days, averaging 30 days. Eleven other monkeys died while showing parasites, but 9 of these subjects succumbed during a descending phase of the infection. An additional 4 monkeys were sacrificed for tissue studies when parasitemias approximated 40,000 per cmm.

Blood films were examined daily from the 28 monkeys following termination of the primary attack. No parasites were detected in 6 of these animals which died from the 15th to the 87th day post patency and in 3 surviving for more than 500 days. However, the infections in 19 monkeys (68%) relapsed 1 or more times after subpatent periods ranging from 5 to 77 days (Table 2). One relapse only was recorded in 10 monkeys, while as many as 4 were observed in the remaining animals. The mean duration of the subpatent periods between the relapses were similar. In all cases, the maximum parasitemia achieved during relapse was markedly lower than in the primary attack. Additionally, the patent periods in most instances were shorter for relapses than in the primary attack.

Table 3. *Plasmodium vivax* infections in *Saimiri sciureus*. Gametocyte infectivity to mosquitoes.

Mosquito species	Hosts Yielding pos. Mosq./Total	Lots Pos./ Tot.	Lots Percent infected
Trophozoite Induced Infections			
Anopheles albimanus	1/20	2/141	4–9
Anopheles aztecus	2/11	4/49	9–71
Anopheles pseudopunctipennis	0/5	0/12	0
Sporozoite Induced Infections			
Anopheles albimanus	0/7	0/50	0
Anopheles aztecus	0/3	0/23	0
Anopheles punctimacula	0/1	0/3	0

Three species of mosquitoes were fed upon *Saimiri* hosts harboring trophozoite induced infections. As indicated in Table 3, although some *A. albimanus* and *A. aztecus* showed oocyst development, the gametocytes in *Saimiri* were poorly infective for these vectors. No sporozoites were seen in the salivary glands of the infected mosquitoes, however mature oocytes, containing sporozoites, were observed on the stomach wall of *A. aztecus*.

Sporozoite Induced Infections

A total of 49 normal *Saimiri* in 10 experiments, and 2 splenectomized *Saimiri* in 1 experiment, received sporozoites from *A. albimanus* mosquitoes which had been infected on malarious *Aotus* monkeys. Patent infections have developed in 11 unaltered recipients from 7 of these trials. The remaining 40 monkeys were followed 30 or more days and 6 of these are continuing to be examined. Trans-

Table 4. Sporozoite induced infections of *Plasmodium vivax* in *Saimiri sciureus*.

Monkey	Prepatent and (Subpatent) periods—Days	Parasitemia			Remarks
		Maxima per cmm	Patent day	Duration	
5984	20	17,620	20	30	
	(14)	540 R	12	19	
	(17)	290 R	22	34	
	(33)	330*R	9	>20	*Parasitemia continuing
4792	37	260	12	30	
	(7)	80 R	7	15	Alive 10 days post patency
5918	28	37,410	17	20*	*At death
6295	15	<10	–	3	Died 39 days post patency
5915	15	1,030*	10	14	
5912	26	1,010*	11	16	*Treated with chloroquine for chemotherapy studies
6298	29	1,870*	13	14	
6270	48	1,260*	19	>31	
6296	29	7,620*	15	17	
4806	43	<10*	–	>10	*Parasitemia continuing
5988	26	40*	3	>4	

R Relapse.

missions were achieved by both mosquito bite and by intravenous inoculation of sporozoites.

Some of the infection characteristics are shown in Table 4. Prepatent periods were variable, ranging from 15 to 48 days. Parasitemias as high as 37,410 per cmm were recorded. Infections in 5 *Saimiri* were treated with chloroquine in the early stages of the parasitemia for use in another study; patency is continuing in 2 additional recipients.

The infections relapsed in 2 of 3 untreated subjects, 7 and 14 days after the primary attack. A total of 4 relapses has been experienced by one of these hosts. As in relapses observed for trophozoite induced infections, the primary parasitemias were greater than in subsequent attacks.

No oocysts were seen in 3 species of anophelines fed upon *Saimiri* with sporozoite induced infections (Table 3).

Discussion

The Achiote strain of *P. vivax* had been maintained for 6 years, by serial transfer in *Aotus* monkeys, before inoculation into Panamanian *Saimiri*. After the initial passage the parasites were readily adapted to the *Saimiri* model and produced, in some cases, very high parasitemias in unaltered subjects. No infection failures resulted by trophozoite passage to 43 *Saimiri* monkeys in a total of 26 serial passages. The inoculum size used was within the normal range of that for routine transfer in *Aotus*. There appeared to be no appreciable change in most of the characteristics of the infections after adaptation to *Saimiri*, although the highest maximum parasitemias in normal recipients developed after the 10th passage.

In an initial report (Young et al. 1971) we showed that *P. vivax* could be transmitted to *Saimiri* by sporozoites from *Aotus* donors. Infections now have been produced in 11 more subjects by this method. It was indicated that *Saimiri* monkeys were poor hosts for infecting experimental vectors. Further, no positive mosquito lots were obtained from *Saimiri* harboring sporozoite induced infections. These results contrast with our findings that the majority of *Aotus* hosts, with sporozoite induced infections, will infect mosquitoes.

Relapses occurred in both trophozoite and sporozoite induced infections. It was not determined if the relapses in the latter infections were initiated by persisting exoerythrocytic stages or from subpatent erythrocytic forms. The maximum parasite concentrations achieved during these relapses were lower than during the corresponding primary infection, which indicated that the hosts had acquired some degree of immunity. Also, in

most instances the patent periods during relapses were shorter. These animals were not rechallenged.

During the course of the experiments with vivax infections in *Saimiri*, 12 of 54 (22%) succumbed during patency. This mortality rate is much lower than corresponding data which showed that 67% of 387 *Aotus* hosts died during patency (unpublished data). In *Saimiri*, although in some cases the deaths could have been attributed to high parasite concentrations, other factors such as stress from handling and the presence of naturally occurring parasites (esp. *Acanthocephala* sp.) may have contributed to this mortality.

The above findings demonstrate that *Saimiri* will support development of vivax malaria, either through serial transfer or by sporozoite transmission. This New World primate represents another experimental model for the study of human malaria.

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