

Trophozoite Induced Infections of *Plasmodium falciparum* in *Saimiri sciureus* (Squirrel Monkeys)

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ABSTRACT: Unaltered *Saimiri sciureus* were susceptible to the trophozoite stages of chloroquine sensitive (Uganda-Palo Alto) and chloroquine resistant (Vietnam-Oak Knoll) strains of *Plasmodium falciparum*. The infections were serially transferred in these hosts.

High parasitemias, reaching more than 400,000 asexual parasites per cmm of the Uganda-Palo Alto strain, were sustained.

Gametocytémias were produced in 2 hosts but failed to infect 3 species of anophelines.

The monkeys adapted well to captivity and survived long periods following the infections.

The monkeys of Panama have failed generally to be good hosts for indigenous strains of *Plasmodium falciparum*. The Taliaferros (1934) reported only short periods of parasitemias in *Alouatta villosa* (black howler monkeys). Porter and Young (1967) were able to produce moderate infections of *P. falciparum* in *Saguinus geoffroyi* (the marmoset), which persisted for as long as 15 days, but serial passages were not achieved. Many other attempts in this laboratory to infect different species of Panamanian monkeys with parasitized human blood have not resulted in establishing infections which could be maintained serially.

Geiman and Meagher (1967), using an African strain of *falciparum* (Uganda-Palo Alto) directly from man, adapted this parasite to *Aotus trivirgatus* (night monkey) and established serial passages. Using this adapted strain, we found that it would grow well and could be passed serially in *Cebus capucinus* (the white-faced capuchin) (Young and Baerg 1969). Only transitory parasitemias were produced in *Ateles fusciceps* (the black spider monkey) and *A. villosa* (Baerg and Young 1970). Subsequently, a preliminary report indicated that this strain would grow in *Saimiri sciureus* (the squirrel monkey) (Young and Rossan 1969). The potential of *Saimiri* as a model for *P. falciparum* infections has been investigated further using the African strain (Uganda-Palo Alto) as well as a strain from

Vietnam (Vietnam-Oak Knoll). The results are presented in this report.

Material and Methods

Panamanian *Saimiri* monkeys used in this study were collected in the Chiriqui Province, Panama, from an area about 35 km east of the Costa Rican border. None of these animals were found to be harboring naturally acquired plasmodial infections.

Initially, the monkeys were housed in groups of 20 to 30 in out-door gang cages. After inoculation, the animals were moved into individual cages located in indoor laboratory rooms. As *Saimiri* monkeys are fastidious eaters, diverse foods were offered. The diet consisted of bananas, canned fruit cocktail, cottage cheese, raw peanuts, monkey chow (Wayne Monkey Diet) and a canned preparation (Science Diet®). This ration was supplemented occasionally with live, neonatal mice and weekly with a vitamin mixture (Octavitamin). Water was available ad libitum. The monkeys adjusted well to laboratory conditions.

All *Saimiri* were healthy adults and subadults, of either sex, and weighed approximately 300 to 500 gms. The monkeys initially were inoculated with the Uganda-Palo Alto strain of *P. falciparum* from *Aotus* bearing the 32nd and 33rd passages at Gorgas Memorial Laboratory (Young and Baerg 1969). Subsequent serial trophozoite transfers then were continued between *Saimiri*. The Vietnam-Oak Knoll strain, which had been adapted to *Aotus* monkeys, was provided kindly by Dr. W. Siddiqui of the University of Hawaii. *Saimiri*

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Table 1. Trophozoite induced infections of *Plasmodium falciparum* (Uganda-Palo Alto) in squirrel monkeys (*Saimiri sciureus*)—initial challenge.

Passage no.	Monkey no.	No. parasites inoc. $\times 10^4$ and method	Prepatent and (sub-patent) periods—days	Patent period—days	Maximum observed parasitemia		Comments
					Per cmm	Patent day	
1	4687 ^a D	94 ip	2	40	163,060	14	Amodiaquine 10 mg base/kg on patent day 13 Rechallenged—(Table 2)
2	4792 D	10 ip	13	29	79,720	14	Rechallenged—(Table 2)
3	4805 D	10 ip	5	31	404,050	15	Rechallenged—(Table 2)
4	4803 D	10 ip	5	21	417,930	14	Negative 526 days
5	5214	16 iv	1	11*	25,060	9	
5	5249	16 iv	7	9*	48,870	7	
5	4688 D	16 ip	7	9†	130,980	9	
6	5199	17 iv	4	11*	50,630	11	
6	5193 D	13 ip	24	15†	421,810	15	
7	5192 ^b	5 ip	4	14†	389,190	14	
1	4800	112 ip	16	12	8,230	6	
1	4800		(11)	13	750	8	Rechallenged—(Table 2)

^a Splenectomized prior to inoculation.

^b Served as control for rechallenge study.

† Died.

* Sacrificed.

D Donor for next serial transfer.

ip Intraperitoneally.

iv Intravenously.

hosts received this strain after the 7th *Aotus* passage at our laboratory.

Procedures for preparation and staining of blood films and enumeration of parasites were summarized by Young and Baerg (1969).

Results

A splenectomized *Saimiri* (4687) was inoculated with the Uganda-Palo Alto strain from the 32nd *Aotus* passage; this line then was passaged without failure through 7 serial transfers in intact, unaltered recipients. One recipient (4800) from the 33rd *Aotus* passage also developed an infection. All 8 subjects were inoculated intraperitoneally (see Table 1).

Four normal *Saimiri* were inoculated intravenously from the 4th *Saimiri* passage (4803). Infections were established in 2 animals (5214 and 5249). The other 2 recipients died (4 and 8 days after inoculation), before it was possible to determine if a consistent parasitemia had developed. An infection was produced in 5199 by a consecutive intravenous passage from 5249.

Prepatent periods ranged from 1 to 4 days and from 2 to 24 days in recipients inoculated intravenously and intraperitoneally, respectively. Once established, the infections developed rapidly and all parasitemias reached at

least 1,000 per cmm from the 2nd to the 7th day of patency. Parasitemias increased as much as 60-fold in a 24 hour period.

In 4 of 7 cases, the infections were self-limiting with the monkeys surviving maximum parasitemias of 8,230 to 417,930 per cmm. One other surviving monkey (4687) was administered a subcurative dose of amodiaquine (10 mg base per kg) when the parasitemia had reached 162,530 parasites per cmm. Of the 6 monkeys in the 5th through 7th passages, 3 were sacrificed during ascending parasitemia, while the remaining 3 monkeys succumbed during fulminating parasitemias.

While all stages of the asexual parasites were seen in the peripheral blood, the parasitemias consisted primarily of rings and trophozoites. Occasionally, schizonts were observed and sometimes were the first forms detected in the patent period.

Primary patent periods in the 4 untreated, surviving *Saimiri* persisted from 12 to 31 days, averaging 23 days. In 1 of these monkeys the infection relapsed after 11 negative days. A low grade parasitemia subsequently was seen over a 13 day period.

Four monkeys were rechallenged by inoculation of trophozoites as early as the 42nd day or as late as the 72nd day after the primary attack (Table 2). Prior to rechallenge, 3 monkeys had received a curative course of amodi-

Table 2. *Plasmodium falciparum* infections in squirrel monkeys—after intraperitoneal rechallenge with 5×10^6 trophozoites of the Uganda-Palo Alto strain.

Monkey no.	Subpatent period prior to rechallenge—days	Prepatent and (Subpatent) periods—days	Patent period—days	Maximum observed parasitemia		Comments
				Per cmm	Patent day	
4792 ^a	61	—	—	—	—	Negative 263 days
4687 ^a	72	3	3	40	2	
"	—	(18)	26	1,140	4	
"	—	(47)	11	5,440	7	Negative 383 days
4800 ^a	62	4	8	1,840	6	
"	—	(34)	3†	30	3	
4805	42	6	17†	148,860	17	

^a Amodiaquine (10 mg base/kg) administered days 17, 16, 15 prior to rechallenge.

† Died.

aquine (a total of 30 mg base per kg over a 3 day period). One of these subjects (4792) did not develop an infection during a 263 day observation period. Parasitemias appeared in the remaining 3 *Saimiri* after prepatent period of 3 to 6 days. Although a high parasitemia resulted in 1 *Saimiri*, relatively low grade infections persisted for 3 and 18 days, respectively, developed in the other 2 recipients. In the latter 2 monkeys, relapses occurred after subpatent periods of 18, 47 and 34 days.

Two monkeys, 4805 and 4697, evidenced gametocytemias during the 26th to 28th day and the 7th to 39th day (intermittently) of the primary attack. Three species of colonized mosquitoes, *Anopheles albimanus*, *A. aztecus* and *A. pseudopunctipennis*, were fed upon these hosts at gametocyte concentrations of 10 to 30 per cmm. Dissections of anophelines from a total of 25 fed lots were negative for oocysts and sporozoites.

The Vietnam-Oak Knoll strain of *P. falciparum* inoculated intraperitoneally (90×10^6 parasites) into 2 normal *Saimiri* produced patent infections 5 and 6 days later, respectively. The parasitemias were patent for 11 and 17 days, and maximum parasitemias were 210 and 5,610 per cmm. There were no gametocytemias, and no relapses were recorded during subsequent observation periods of 50 and 57 days. Subinoculation was not attempted from these *Saimiri*.

Discussion

Both the Uganda-Palo Alto and Vietnam Oak Knoll strains had been adapted to normal *Aotus* monkeys prior to inoculation into *Sai-*

miri hosts. There was no difficulty in initially establishing significant infections in the unaltered *Saimiri* recipients, and in continuing a passage line with the Uganda-Palo Alto strain. In these passages, fulminating parasitemias were achieved in unaltered recipients. However, some of the infections in the *Saimiri* were self-limiting, while the *Aotus* monkeys required chemotherapeutic intervention to sustain the life of the host. Infections of the Uganda-Palo Alto strain in intact or splenectomized *Cebus* (Young and Baerg 1969), in contrast, were generally lower than in *Saimiri*.

Although patent periods as short as 12 days were observed, parasites persisted in several *Saimiri* at high levels for more than 4 weeks during the primary attack, signifying a tolerance of this host species for *P. falciparum*. Further, persistence of the parasites at subpatent levels was shown by the fact that relapses did occur.

The results obtained after rechallenge of a limited number of subjects were variable, but indicated that 3 monkeys were protected as evidenced by either complete absence of parasitemia or by low maximum parasitemias and patent periods of short duration. This host-parasite combination may be useful in further studies of immunity acquired against the erythrocytic phase of *P. falciparum*.

Limited trials with 3 species of anophelines, fed upon 2 monkeys that showed low gametocyte concentrations of the Uganda-Palo Alto strain, did not yield infected mosquitoes. These results are in accord with the failure to infect experimental vectors from *Aotus* and *Cebus* monkeys bearing this strain (Baerg and Young 1969).

The small size and availability of *Saimiri* and its relative ease of adaptation to the laboratory environment may offer further advantages for this species as an experimental model.

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