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FURTHER STUDIES ON THE SPOROZOITE TRANSMISSION OF THE SALVADOR I STRAIN OF *PLASMODIUM VIVAX*

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ABSTRACT: Different species of *Saimiri* and *Aotus* monkeys were inoculated with sporozoites of the Salvador I strain of *Plasmodium vivax*. Of 58 *Saimiri* inoculated, 45 developed parasitemia (4 following bites and 41 following intravenous inoculation). Prepatent periods ranged from 10 to 63 days. Twelve of 19 monkeys inoculated with sporozoites that had been stored frozen developed patent parasitemia after 16–53 days. Of 41 *Aotus* monkeys inoculated, only 10 (2 via bites and 8 via intravenous inoculation) developed parasitemia. One of 7 *Aotus* inoculated with sporozoites that had been frozen developed parasitemia with a prepatent period of 26 days. Mosquitoes were infected by feeding on gametocytes from *Aotus* and *Saimiri* monkeys, chimpanzees, and a human. Sporozoites from *Anopheles stephensi, Anopheles freeborni, Anopheles dirus,* and *Anopheles gambiae* induced infection.

Previously, Collins et al. (1988) reported the susceptibility of splenectomized *Saimiri sciureus boliviensis* monkeys to infection with sporozoites of the Salvador I strain of *Plasmodium vivax*. Subsequently, 2 separate anti-sporozoite vaccine trials were conducted using this model system. In the first (Collins et al., 1989), 48 monkeys were challenged by intravenous inoculation of 10,000 sporozoites; 18 of 18 control animals developed patent parasitemia with prepatent periods of 14–39 days (median 18 days). In the second trial (Collins et al., 1990), 35 animals were inoculated with 10,000 sporozoites; 16 of 17 control animals developed patent parasitemia after prepatent periods of 16–37 days (median 19 days). In summary, each of 40 control monkeys was inoculated with 10,000 sporozoites; 39 developed patent parasitemia after prepatent periods of 14–39 days.

In attempts to determine the parameters of sporozoite-induced infection in Bolivian *Saimiri* monkeys further and to establish the potential usefulness of other species of South American monkeys for vaccine and immunologic studies, additional animals have been inoculated with sporozoites of the Salvador I strain of *P. vivax* whenever animals and infected mosquitoes were available. Attempts to induce infection in monkeys also were made using sporozoites that had been stored frozen for different periods. The results of these studies are reported here.

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MATERIALS AND METHODS

The Salvador I strain of *P. vivax* was originally isolated from an infected human in the area of Congrejera in the Department of La Paz, El Salvador (Collins et al., 1972). This strain has been maintained by passage in *Aotus* and *Saimiri* monkeys and chimpanzees.

In addition to feral and laboratory-born *S. boliviensis* animals, feral *Saimiri peruviensis* (both green-headed and black-headed) and *Saimiri guyanensis, Aotus nancymai, Aotus vociferans, Aotus azarae boliviensis, Aotus lemurinus griseimembra,* and hybrid *Aotus* monkeys were available. Black-headed *Saimiri* imported from Peru are similar yet distinct from the *S. boliviensis* animals imported from Bolivia. We have elected to designate them as black-headed *S. peruviensis* because of their geographic origin, although they are probably more closely related to the black-headed animals from Bolivia. All monkeys were splenectomized either before or within 7 days after sporozoite inoculation. Many of the animals had been infected with different species of *Plasmodium* and cured before their current infection.

Monkeys were infected either by the intravenous inoculation of sporozoites from dissected salivary glands or the bites of infected mosquitoes. For sporozoite inoculation, the salivary glands were dissected into 20% fetal bovine serum/saline and crushed under a coverslip. The sporozoites were then washed from the slide, counted in a hemocytometer, and injected into the femoral vein of the monkeys. For bite transmissions, the mosquitoes were allowed to feed directly on the abdomen of tranquilized monkeys through the screen mesh of the cage. After engorgement, the salivary glands were dissected and graded 1+ (1–10 sporozoites), 2+ (11–100 sporozoites), 3+ (101–1,000 sporozoites), or 4+ (>1,000 sporozoites). The ratings were totaled for all the mosquitoes feeding on the monkey.

Some monkeys were inoculated with sporozoites that had been stored frozen. Sporozoites were harvested from dissected salivary glands (as described above) into 50% fetal bovine serum/saline, distributed into vials, and then frozen and stored in the vapor phase of a liquid N_2 freezer. No cryopreservative was used. Prior

TABLE I. Transmission attempts with the Salvador I strain of *Plasmodium vivax* to 2 species of *Saimiri* monkeys via the bites of infected anopheline mosquitoes or the intravenous inoculation of dissected sporozoites.

Animal	Species*	Prevalence mal.†	Bites or inoculation	Number‡	Mosquito	Source of infection	Prepatent period	Maximum parasitemia	
								Day	Parasites/μl
SS-77	Sb	fal, sim	Bites	45+	*An. stephensi*	*Aotus*	24 days	48	150
SI-274	Sb	kno	Bites	14+	*An. gambiae*	Chimp	35 days	81	5,580
SI-33	Sb	—	Inoculation	10,000	*An. stephensi*	Chimp	26 days	49	100,899
SI-69	Sb	—	Inoculation	10,000	*An. stephensi*	Chimp	26 days	48	42,723
SI-195	Sb	fal, viv(h)	Inoculation	10,000	*An. dirus*	Chimp	43 days	43	31
SI-220	Sb	fal, viv(h)	Inoculation	10,000	*An. dirus*	Chimp	No infection	—	—
SI-247	Sb	—	Inoculation	10,000	*An. dirus*	Chimp	34 days	55	19,530
SI-75	Sb	—	Inoculation	25,000	*An. stephensi*	Chimp	46 days	74	74,586
SI-69	Sb	viv(h)	Inoculation	44,000	*An. stephensi*	Chimp	53 days	56	59
SI-81	Sb	viv(h)	Inoculation	50,000	*An. stephensi*	Chimp	63 days	96	93
SI-37	Sb	—	Inoculation	60,200	*An. stephensi*	Human	18 days	68	64,728
SI-58	Sb	—	Inoculation	255,000	*An. stephensi*	Human	17 days	31	4,278
SI-291	Sb	—	Inoculation	2,000,000	*An. dirus*	Chimp	19 days	47	4,140
SI-308	Sb	—	Inoculation	2,000,000	*An. dirus*	Chimp	10 days	47	14,580
SI-277	Sb	—	Inoculation	25,000§	*An. stephensi*	Chimp	41 days	62	78,660
SI-260	Sb	—	Inoculation	25,000§	*An. stephensi*	Chimp	32 days	72	180,000
SI-263	Sb	—	Inoculation	25,000§	*An. stephensi*	Chimp	32 days	64	47,340
SI-266	Sb	—	Inoculation	25,000§	*An. stephensi*	Chimp	47 days	92	48,000
SI-192	Sb	—	Inoculation	30,000‖	*An. stephensi*	Chimp	No infection	—	—
SI-80	Sb	—	Inoculation	30,000‖	*An. stephensi*	Chimp	19 days	49	24,480
SI-91	Sb	—	Inoculation	30,000‖	*An. stephensi*	Chimp	No infection	—	—
SI-207	Sb	—	Inoculation	30,000‖	*An. stephensi*	Chimp	16 days	55	15,300
SI-258	Sb	—	Inoculation	130,000#	*An. stephensi*	Chimp	No infection	—	—
SI-276	Sb	—	Inoculation	130,000#	*An. stephensi*	Chimp	53 days	96	17,271
SS-1	Sg	fal, mal	Bites	60+	*An. stephensi*	Chimp	17 days	33	2,976
SS-2	Sg	fal, mal	Bites	44+	*An. stephensi*	Chimp	31 days	41	186

* Sb = *Saimiri boliviensis*; Sg = *Saimiri guyanensis.*

† viv = *Plasmodium vivax*; fal = *Plasmodium falciparum*; mal = *Plasmodium malariae*; sim = *Plasmodium simium*; kno = *Plasmodium knowlesi*; viv(h) = *P. vivax* (homologous strain).

‡ Accumulated + values for salivary glands where 1+ = 1–10 sporozoites, 2+ = 11–100 sporozoites, 3+ = 101–1,000 sporozoites, and 4+ = >1,000 sporozoites.

§ Sporozoites had been stored frozen for 11 days.

‖ Sporozoites had been stored frozen for 158 days.

Sporozoites had been stored frozen for 402 days.

to inoculation, the sporozoites were thawed rapidly and injected into the femoral vein of the monkeys.

Beginning 10–12 days after inoculation, thick and thin blood films were made daily according to the technique of Earle and Perez (1932), stained with Giemsa, and the parasite counts recorded per μl of blood.

The *Anopheles freeborni* mosquitoes were originally from Marysville, California, and have been maintained in the laboratory since 1944. The *Anopheles dirus,* originally from Thailand, were obtained from the Walter Reed Army Institute of Research, Washington, D.C. The *Anopheles stephensi,* from Delhi India, were obtained from the National Institutes of Health, Bethesda, Maryland. The *Anopheles gambiae* (G-3 strain), originally from The Gambia, were obtained from the London School of Hygiene and Tropical Medicine, London, U.K. All colonies had been maintained in our laboratory for many years.

RESULTS

Saimiri monkeys

Two *S. boliviensis* were fed upon by infected mosquitoes (Table I). Both developed parasitemia after 24 and 35 days. Twelve monkeys were inoculated with sporozoites from dissected salivary glands. Eleven of the 12 developed parasitemia after prepatent periods of 10–63 days. Four monkeys (SI-195, SI-220, SI-69, and SI-81), which were previously infected with the homologous strain of *P. vivax* and cured, had prepatent periods of 43, 53, and 63 days (monkey SI-220 failed to develop parasitemia). The median prepatent period for the remaining 8 monkeys was 24 days. Ten monkeys were inoculated with sporozoites that had been frozen for 11–402 days. Seven monkeys developed parasitemia with prepatent periods ranging from 16 to 53 days (median 32 days). Transmission was obtained with sporozoites developed in *An. stephensi, An. dirus,* and *An. gambiae* mosquitoes.

Two *S. guyanensis* (from Guyana) were infected via the bites of infected *An. stephensi* mosquitoes. The prepatent periods were 17 and 31 days.

TABLE II. Transmission attempts with the Salvador I strain of *Plasmodium vivax* to 2 types of *Saimiri* monkeys from Peru via the bites of infected anopheline mosquitoes or the intravenous inoculation of dissected sporozoites.

Animal	Species*	Prevalence mal.†	Bites or inoculation	Number‡	Mosquito	Source of infection	Prepatent period	Maximum parasitemia	
								Day	Parasites/μl
SI-944	Sp (G)	—	Bites	63+	*An. stephensi*	*Aotus*	No infection	—	—
SI-954	Sp (G)	—	Bites	47+	*An. stephensi*	*Saimiri*	No infection	—	—
SI-19	Sp (G)	—	Inoculation	10,000	*An. dirus*	Chimp	46 days	60	2,139
SI-22	Sp (G)	bra	Inoculation	10,000	*An. dirus*	Chimp	12 days	31	8,928
SI-21	Sp (G)	—	Inoculation	10,000	*An. dirus*	Chimp	No infection	—	—
SI-8	Sp (G)	—	Inoculation	10,000	*An. dirus*	Chimp	No infection	—	—
SI-15	Sp (G)	bra	Inoculation	10,000	*An. dirus*	Chimp	12 days	37	68,000
SI-949	Sp (G)	—	Inoculation	18,000	*An. freeborni*	*Saimiri*	19 days	25	1,116
SI-951	Sp (G)	—	Inoculation	18,000	*An. freeborni*	*Saimiri*	16 days	18	121
SI-953	Sp (G)	—	Inoculation	19,500	*An. freeborni*	*Saimiri*	19 days	54	27,270
SI-954	Sp (G)	—	Inoculation	19,500	*An. freeborni*	*Saimiri*	No infection	—	—
SI-948	Sp (G)	—	Inoculation	22,400	*An. freeborni*	*Saimiri*	20 days	54	3,636
SI-961	Sp (G)	fra	Inoculation	90,000§	*An. stephensi*	Chimp	No infection	—	—
SI-968	Sp (G)	fra, fal	Inoculation	90,000§	*An. stephensi*	Chimp	43 days	69	35,460
SI-971	Sp (G)	fra	Inoculation	90,000§	*An. stephensi*	Chimp	50 days	50	120
SI-972	Sp (G)	fra	Inoculation	90,000§	*An. stephensi*	Chimp	No infection	—	—
SI-965	Sp (B)	viv, sim	Bites	38+	*An. stephensi*	*Aotus*	No infection	—	—
SI-26	Sp (B)	bra	Inoculation	10,000	*An. dirus*	Chimp	35 days	70	18,414
SI-3	Sp (B)	—	Inoculation	10,000	*An. dirus*	Chimp	32 days	62	3,162
SI-25	Sp (B)	bra	Inoculation	10,000	*An. dirus*	Chimp	12 days	57	112,000
SI-24	Sp (B)	bra	Inoculation	10,000	*An. dirus*	Chimp	19 days	49	12,834
SI-958	Sp (B)	—	Inoculation	17,000	*An. freeborni*	*Saimiri*	16 days	35	27,714
SI-959	Sp (B)	—	Inoculation	17,000	*An. freeborni*	*Saimiri*	17 days	71	77,676
SI-955	Sp (B)	—	Inoculation	20,000	*An. freeborni*	*Saimiri*	18 days	52	18,725
SI-956	Sp (B)	—	Inoculation	20,000	*An. freeborni*	*Saimiri*	No infection	—	—
SI-1	Sp (B)	—	Inoculation	500,000	*An. dirus*	Chimp	25 days	53	1,860
SI-27	Sp (B)	—	Inoculation	2,400,000	*An. dirus*	Chimp	21 days	58	5,022
SI-960	Sp (B)	fra	Inoculation	90,000§	*An. stephensi*	Chimp	No infection	—	—
SI-964	Sp (B)	fra	Inoculation	90,000§	*An. stephensi*	Chimp	No infection	—	—
SI-966	Sp (B)	fra, fal	Inoculation	90,000§	*An. stephensi*	Chimp	43 days	67	11,160
SI-967	Sp (B)	fra	Inoculation	90,000§	*An. stephensi*	Chimp	39 days	49	9,900
SI-975	Sp (B)	fra	Inoculation	90,000§	*An. stephensi*	Chimp	45 days	54	60

* Sp (G) = *Saimiri peruviensis* (green-headed); Sp (B) = *Saimiri peruviensis* (black-headed).
† viv = *Plasmodium vivax*; fal = *Plasmodium falciparum*; fra =*Plasmodium fragile*; sim = *Plasmodium simium*; bra = *Plasmodium brasilianum.*
‡ Accumulated + values for salivary glands where 1+ = 1–10 sporozoites, 2+ = 11–100 sporozoites, 3+ = 101–1,000 sporozoites, and 4+ = >1,000 sporozoites.
§ Sporozoites had been stored frozen for 312 days.

Two types of *Saimiri* were obtained from Peru (Table II). Two of the green-headed *S. peruviensis* were fed upon by infected mosquitoes. No infections were obtained. Seven of 10 monkeys inoculated intravenously with sporozoites developed parasitemia with prepatent periods ranging from 12 to 46 days. Four monkeys were inoculated with sporozoites that had been frozen for 312 days. Two monkeys developed parasitemia after 43 and 50 days.

One black-headed *S. peruviensis* was exposed to the bites of infected mosquitoes (Table II); no infection was obtained. Ten monkeys were inoculated intravenously with sporozoites; 9 monkeys developed parasitemia after prepatent periods of 12–35 days (median = 19 days). Five monkeys were inoculated with sporozoites that had been stored frozen for 312 days. Three of the 5 developed parasitemia after 39, 43, and 45 days.

Of 58 *Saimiri* inoculated, 45 developed parasitemia (4 following bites and 41 following intravenous inoculation). Infections were obtained with sporozoites that developed in each of the 4 species of mosquitoes. Mosquitoes had been infected by feeding on gametocytes in blood from 1 *Aotus* monkey, 1 *Saimiri* monkey, 8 chimpanzees, and a human with a laboratory-acquired infection. Twelve of 19 monkeys inoculated with sporozoites that had been stored frozen developed patent parasitemia.

Aotus monkeys

Six *A. nancymai* were fed upon by infected mosquitoes (Table III). Only monkey AI-927 (previously infected with *P. falciparum*) devel-

TABLE III. Transmission attempts with the Salvador I strain of *Plasmodium vivax* to different species of *Aotus* monkeys via the bites of infected anopheline mosquitoes or the intravenous inoculation of dissected sporozoites.

Animal	Species*	Prevalence mal.†	Bites or inoculation	Number‡	Mosquito	Source of infection	Prepatent period	Maximum parasitemia	
								Day	Parasites/μl
AI-188	An	fal, mal	Bites	20+	*An. gambiae*	Chimp	No infection	—	—
AI-228	An	fal, mal, viv(h)	Bites	23+	*An. stephensi*	Chimp	No infection	—	—
AO-407	An	fal, mal	Bites	54+	*An. stephensi*	Chimp	No infection	—	—
AI-927	An	fal	Bites	122+	*An. stephensi*	Chimp	27 days	33	11,817
AI-943	An	fal	Bites	150+	*An. stephensi*	Chimp	No infection	—	—
AI-663	An	viv	Bites	252+	*An. stephensi*	Chimp	No infection	—	—
AI-914	An	—	Inoculation	4,000	*An. stephensi*	*Aotus*	No infection	—	—
AI-905	An	—	Inoculation	10,800	*An. freeborni*	*Aotus*	No infection	—	—
AI-943	An	fal	Inoculation	11,200	*An. stephensi*	Chimp	No infection	—	—
AI-663	An	viv	Inoculation	20,000	*An. stephensi*	Chimp	No infection	—	—
AI-943	An	fal	Inoculation	20,000	*An. stephensi*	Chimp	No infection	—	—
AI-698	An	—	Inoculation	50,000	*An. freeborni*	*Aotus*	22 days	44	4,272
AI-231	An	sim	Inoculation	65,000	*An. dirus*	Chimp	No infection	—	—
AI-556	An	fal	Inoculation	450,000	*An. dirus*	Chimp	23 days	37	35,526
AI-557	An	fal	Inoculation	450,000	*An. dirus*	Chimp	26 days	43	35,712
AI-558	An	fal	Inoculation	450,000	*An. dirus*	Chimp	33 days	41	1,581
AI-559	An	fal	Inoculation	450,000	*An. dirus*	Chimp	No infection	—	—
AI-1041	An	fal	Inoculation	90,000§	*An. stephensi*	Chimp	No infection	—	—
AI-1153	An	fal	Inoculation	90,000§	*An. stephensi*	Chimp	No infection	—	—
AI-1136	An	fal	Inoculation	90,000§	*An. gambiae*	Chimp	26 days	38	39,420
AI-1412	An	fal	Inoculation	90,000§	*An. gambiae*	Chimp	No infection	—	—
AI-1504	Av	fal	Bites	14+	*An. gambiae*	Chimp	No infection	—	—
AO-495	Hyb	fal, mal	Bites	18+	*An. gambiae*	Chimp	No infection	—	—
AI-1702	Hyb	fal, mal	Bites	20+	*An. gambiae*	Chimp	No infection	—	—
AO-487	Hyb	viv, fal, mal	Inoculation	4,100	*An. stephensi*	Chimp	No infection	—	—
AO-489	Hyb	—	Inoculation	47,500	*An. stephensi*	*Aotus*	20 days	34	51,708
AO-494	Aab	fal, mal	Bites	16+	*An. gambiae*	Chimp	No infection	—	—
AO-519	Aab	—	Bites	19+	*An. gambiae*	Chimp	No infection	—	—
AI-284	Aab	—	Bites	23+	*An. freeborni*	Chimp	No infection	—	—
AI-284	Aab	—	Inoculation	19,400	*An. stephensi*	Chimp	No infection	—	—
AI-284	Aab	—	Inoculation	20,000	*An. stephensi*	Chimp	No infection	—	—
AI-283	Aab	—	Inoculation	50,000	*An. freeborni*	*Aotus*	24 days	37	7,817
AO-471	Aab	mal, fal	Inoculation	65,000	*An. dirus*	Chimp	No infection	—	—
AI-290	Aab	mal	Inoculation	75,000‖	*An. gambiae*	Chimp	No infection	—	—
AI-291	Aab	mal	Inoculation	75,000‖	*An. gambiae*	Chimp	No infection	—	—
AI-285	Aab	—	Inoculation	75,000‖	*An. gambiae*	Chimp	No infection	—	—
AI-43	Alg	fal, mal	Bites	45+	*An. stephensi*	*Aotus*	17 days	29	11,904
AI-51	Alg	fal, mal, viv	Bites	68+	*An. stephensi*	Chimp	No infection	—	—
AI-363	Alg	viv, fal, mal	Inoculation	26,000	*An. stephensi*	Chimp	No infection	—	—
AI-56	Alg	—	Inoculation	30,000	*An. dirus*	*Aotus*	No infection	—	—
AI-51	Alg	fal, mal	Inoculation	65,000	*An. dirus*	Chimp	57 days	70	4,650

* An = *Aotus nancymai*; Av = *Aotus vociferans*; Aab = *Aotus azarae boliviensis*; Alg = *Aotus lemurinus griseimembra*; Hyb = hybrid (AO-495, AO-487, and AO-489 = Alg × Aab; AI-1702 = Alg × [Alg × Aab]).

† viv = *Plasmodium vivax*; fal = *Plasmodium falciparum*; mal = *Plasmodium malariae*; sim = *Plasmodium simium*; kno = *Plasmodium knowlesi*; viv(h) = *P. vivax* (homologous strain).

‡ Accumulated + values for salivary glands where 1+ = 1–10 sporozoites, 2+ = 11–100 sporozoites, 3+ = 101–1,000 sporozoites, and 4+ = >1,000 sporozoites.

§ Sporozoites had been stored frozen for 137 days.

‖ Sporozoites had been stored frozen for 416 days.

oped parasitemia after a prepatent period of 27 days. Eleven *A. nancymai* were inoculated with dissected sporozoites (Table III). Eight of the monkeys had been infected previously. None of the monkeys challenged with ≤20,000 sporozoites developed patent parasitemia. Four of 6 monkeys inoculated with ≥50,000 sporozoites developed parasitemia with prepatent periods ranging from 22 to 33 days. One of 4 monkeys inoculated with 90,000 sporozoites that had been stored frozen for 137 days had a prepatent period of 26 days; all of these animals had been infected previously with *P. falciparum*.

Three *A. azarae boliviensis* monkeys were fed upon by infected mosquitoes (Table III). No infections developed. Four monkeys were inoculated intravenously with sporozoites; one monkey (AI-283) developed parasitemia with a

prepatent period of 24 days. Three monkeys were inoculated with sporozoites that had been frozen for 416 days. No infections were obtained.

Two *A. lemurinus griseimembra* monkeys were challenged via mosquito bite and 3 by intravenous inoculation. One animal from each group (AI-43 and AI-51) developed patent parasitemia after 17 and 57 days, respectively. Four hybrid animals (3 *A. azarae boliviensis* × *A. lemurinus griseimembra*; 1 *A. lemurinus griseimembra* × [*A. lemurinus griseimembra* × *A. azarae boliviensis*]) were inoculated, 2 via mosquito bites and 2 via intravenous inoculation. One monkey (AO-489) developed detectable parasitemia after 20 days. One *A. vociferans* was fed upon by infected mosquitoes. No infection was obtained.

Of 41 *Aotus* monkeys inoculated, only 10 (2 via bites and 8 via intravenous inoculation) developed parasitemia. Sporozoites from *An. stephensi, An. freeborni, An. dirus,* and *An. gambiae* resulted in infection. Mosquitoes had been infected by feeding on gametocytes from 5 *Aotus* monkeys and 7 chimpanzees.

DISCUSSION

Although transmission was obtained by the bite of *An. stephensi* or *An. gambiae* mosquitoes to *S. boliviensis, S. guyanensis, A. nancymai,* and *A. l. griseimembra* monkeys, the rate of transmission (4 of 7 to *Saimiri* and 2 of 14 to *Aotus*) was low. The successful induction of infection following intravenous inoculation of sporozoites (7 of 20 to *Aotus* and 27 of 32 to *Saimiri*) indicated that sporozoites produced in all 4 mosquito species were capable of inducing infection and that the rate of transmission was increased when larger numbers of sporozoites were introduced intravenously. It is apparent from these studies that the injection of large numbers of sporozoites is needed to increase the rate of transmission of the Salvador I strain of *P. vivax* to monkeys. It suggests that sporozoites of the Salvador I strain are not highly infectious to monkeys. However, a similar result might occur if merozoites produced by the exoerythrocytic stage in monkeys were rarely infectious to monkey erythrocytes.

As in the previously reported studies, prepatent periods varied greatly. However, shorter prepatent periods appeared to be related to the injection of larger numbers of sporozoites.

Of particular interest was the induction of infection using sporozoites that had been stored frozen. Jeffery and Rendtorff (1955) reported on the successful infection of human patients with sporozoites of *P. vivax* that had been stored at −70 C in plasma or serum-saline for up to 375 days. Presented here is the first report of the successful transmission of *P. vivax* to monkeys using sporozoites that had been frozen. One of 7 attempts to infect *Aotus* and 12 of 19 attempts to infect *Saimiri* were successful. Because the prepatent periods for those animals inoculated with sporozoites that had been stored frozen were much longer than obtained with unfrozen sporozoites, it is assumed that a high percentage of sporozoites failed to survive the freezing and thawing process. An improved method for storing viable sporozoites is needed.

In the quest for suitable models for immunologic and biologic studies with the human malarias, most of the infections in monkeys have been induced via the inoculation of parasitized erythrocytes. We report here that the Salvador I strain of *P. vivax* may be suitable for many studies requiring sporozoite-induced infections. These observations suggest that: (1) *Saimiri* are more susceptible than *Aotus* monkeys; (2) intravenous inoculation of sporozoites is more apt to result in infection than mosquito bite (this may be related to the number of sporozoites available); (3) sporozoites from any of the 4 species of mosquito examined are infectous; and (4) sporozoites that have been stored frozen were infectious to monkeys. Based on these observations, the vaccine-testing model of the Salvador I strain of *P. vivax* using *S. boliviensis* monkeys and intravenous challenge with large numbers of sporozoites is the most predictable. Whether or not additional strains of *P. vivax* are more infectious to other species of *Aotus* and *Saimiri* monkeys remains to be demonstrated.

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