SPOROZOITE TRANSMISSION OF *PLASMODIUM VIVAX* TO PANAMANIAN PRIMATES

DAVID C. BAERG, JAMES A. PORTER, JR., AND MARTIN D. YOUNG

Reprinted from THE AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE
Vol. 18, No. 3, May 1969
p. 346–350
Printed in United States of America

SPOROZOITE TRANSMISSION OF *PLASMODIUM VIVAX* TO PANAMANIAN PRIMATES*

DAVID C. BAERG, JAMES A. PORTER, JR.,† AND MARTIN D. YOUNG
Gorgas Memorial Laboratory, P. O. Box 2016, Balboa Heights, Canal Zone

ABSTRACT: *Anopheles albimanus* mosquitoes transmitted blood-induced *Plasmodium vivax* infection from three night monkeys, *Aotus trivirgatus*, to three other night monkeys and to a Panamanian marmoset, *Saguinus geoffroyi*. Mosquito-transmissions of the malaria also were successful from one black spider monkey, *Ateles fusciceps*, to two other black spider monkeys. Prepatent periods ranged from 14 to 29 days and patent periods from 19 to 37 days. *Plasmodium vivax* in *S. geoffroyi* developed less than 10 parasites per cmm in contrast to maxima of 42,850 and 8,390 per cmm in *A. trivirgatus* and *A. fusciceps* on primary infection. Relapse occurred in one *A. trivirgatus* and in one *A. fusciceps* after subpatent intervals of 27 and 4 days. The relapse infections were patent for 29 and 24 days and reached maxima of 15,420 and 15,470 parasites per cmm. Mosquitoes were infected from feeding upon each *A. trivirgatus* and one *A. fusciceps*. This report of mosquito-transmissions of *P. vivax* infection to Panamanian primates appears to be the first of sporozoite-induced human malaria in monkeys.

We have previously reported infections of night monkeys, *Aotus trivirgatus*, and Panamanian marmosets, *Saguinus geoffroyi*, with erythrocytic forms of *Plasmodium vivax*.[1] In one experiment, the malaria was transmitted by mosquitoes from a night monkey to two human volunteers, but not to another night monkey. This paper deals with later successful sporozoite-induced infections of *P. vivax* in Panamanian primates.

MATERIALS AND METHODS

Methods of handling primates and mosquitoes for this study are detailed elsewhere.[1,2] Over a 3-year period (1 October 1965 to 31 July 1968), *Anopheles albimanus* mosquitoes were fed on man and monkeys infected with indigenous strains of *P. vivax*. Mosquito-transmission attempts with infected lots were to one or more primate hosts, including night monkeys, Panamanian marmosets, and black spider monkeys, *Ateles fusciceps*. Recipients were either intact and untreated, splenectomized, treated with azathioprine (Imuran®), or both splenectomized and treated with azathioprine. Azathioprine was given one or more days, and was initiated before or at the time of transmission. The drug was administered orally by gastric tube at a dose of 2.5 to 5 mg per kg of body weight. Sporozoites were introduced by interrupted-biting of mosquitoes, or by intravenous (iv) or intraperitoneal (ip) injection of their salivary glands in normal saline solution and 5 or 10% serum of the recipient monkey species, or by both methods. Mosquitoes used in each experiment ranged in number from eight to 106, and were graded at least 2+ for sporozoite concentration according to the method of Young *et al.*[3]

RESULTS

A total of 780 lots of *A. albimanus* were applied to donors on consecutive days and over a wide range of vivax parasitemia. In these trials mosquito-infections resulted from feedings on man, *Aotus*, *Saguinus*, and *Ateles fusciceps* (Table 1). The parasites in monkey hosts were infectious to mosquitoes through the 40th patent day; however, the best rates of lot-infection occurred during the initial 15 days of parasitemia. Higher average rates were obtained from man than monkeys, although oöcysts and sporozoites in mosquitoes fed on the latter developed normally. Sporozoites appeared in the salivary glands after 12 and 15 days at incubation temperatures of 76 ± 2° F and 72 ± 1° F.

Cyclic transmission of the parasite to monkeys through *A. albimanus* was attempted 72 times (Table 2). Fifty-six monkeys received sporozoites of the Achiote strain, and 16 received sporozoites of the Emperador, Polo, Santa Rosa,

* Research sponsored by the U. S. Army Medical Research and Development Command, under Grant No. DADA 17-68-G-9264. This is paper No. 567 from the Army Research Program on Malaria.

† Present address: Veterans Administration, Edward Hines Jr. Hospital, RILAMSAT, Bldg. 189a, Hines, Illinois 60141.

TABLE 1

Susceptibility of A. albimanus *to* P. vivax *from natural infections in man and blood-induced infections in Panamanian primates 1 October 1965–31 July 1968*

Host species	Mosquito feeding lots		Midgut dissections		Salivary-gland dissections		% infection per positive lot		Host infectivity to mosquitoes		Infectious male gamet. per cmm	Infective days of patency
	No.	% pos.	No.	% pos.	No.	% pos.	Range	Avg.	No.	% inf.	Range	Range
Man	23	60.9	484	17.4	208	13.9	3.3–91.7	39.9	11	63.6	<10–147	Unknown
Aotus trivirgatus (night monkey)	642	21.3	11,494	5.2	4,371	8.9	2.0–100	25.8	109	51.4	<10–2,426	5–40
Ateles fusciceps (black spider monkey)	75	30.7	1,310	6.7	495	7.9	3.3–56.7	24.1	7	57.1	<10–886	9–30
Ateles geoffroyi (red spider monkey)	6	0	85	0	40	0	—	—	2	0	—	—
Saguinus geoffroyi (Panamanian marmoset)	34	5.9	560	0.7	222	0	5.0–8.6	6.8	11	18.2	<10	5–9

or Santa Rosa-6 strains. Six transmissions were successful with the Achiote strain of *P. vivax* only. Two night monkeys (255B and 375B) and a marmoset (802A) were infected by bites of 35, 33, and 53 mosquitoes, respectively. A third night monkey (769B) and two black spider monkeys (534B and 736B) were infected by combined bite and iv injection of 10, 15, and 29 mosquitoes. Patent parasitemia did not develop in one marmoset and five night-monkey companion recipients.

The sporozoite-induced infections became well-

TABLE 2

Attempts at sporozoite transmission of P. vivax *to Panamanian primates*

Source of infection	Primate		Transmission†				
	Recipient	Treatment	Mosquito bite	Injection (*iv*)	Injection (*ip*)	Bite & iv	Bite & ip
Man	*A. trivirgatus*	Splenectomy	0/1(45)				
		Splenectomy & Imuran®*	0/3(33)	0/3(34)			
	Saguinus geoffroyi	Splenectomy & Imuran®	0/2(31)				
Aotus trivirgatus	*A. trivirgatus*	None	0/1(106)‡				
		Imuran®	0/1(25)				
		Splenectomy	0/2(19)	0/2(22)			
		Splenectomy & Imuran®	2/18(40)	0/11(37)	0/4(55)	1/5(9)	
	S. geoffroyi	Splenectomy	0/1(24)				
		Splenectomy & Imuran®	1/4(37)	0/1(17)	0/2(63)		
Ateles fusciceps	*A. fusciceps*	Splenectomy & Imuran®	0/1(48)			2/2(22)	
	A. trivirgatus	Splenectomy & Imuran®	0/2(29)	0/1(35)			
	S. geoffroyi	Splenectomy & Imuran®	0/1(47)	0/2(40)		0/1(8)	0/1(9)
		Totals	3/37(38)	0/20(34)	0/6(58)	3/8(12)	0/1(9)

* Azathioprine; Burroughs, Wellcome and Co., Inc.
† Successes/Attempts (Avg. number of infected mosquitoes).
‡ Malaria developed in two human companion recipients (Young *et al.*, 1966).

TABLE 3

Sporozoite-induced infections of P. vivax *in Panamanian primates*

Primate	No.	Primary infection: Prepatent period (*days*)	Primary infection: Patent period (*days*)	Primary infection: Parasitemia maximum/cmm	Relapse: Subpatent period to relapse (*days*)	Relapse: Patent period (*days*)	Relapse: Parasitemia maximum/cmm
Aotus trivirgatus	375B	18	21	14,310	27	29	15,420
	255B	26	37	20,920			
	769B	19	18*	42,850			
Ateles fusciceps	736B	29	19	6,760	4	24	15,470
	534B	24	30	8,390			
Saguinus geoffroyi	802A	14	20	<10			

* Died on 18th day of patency.

established in the night monkeys and black spider monkeys (Table 3). Primary parasitemia reached maximum concentrations in 255B and 375B on the 14th and 15th days, and in 534B and 736B on the 21st and 9th days of their respective patent periods. Relapses in 375B and 736B produced greatest parasite concentrations on the 14th and 11th days. Night monkey 769B died on the 18th day of patency, when the parasitemia was at the recorded high of 42,850 per cmm. There was no discernible peak parasitemia in the marmoset, with fewer than 10 per cmm seen in blood films taken daily.

Gametocytes developed in the night monkeys and black spider monkeys, and were infectious to *A. albimanus* (Table 4). The best mosquito-infection rates from 255B and 769B were 43 and 24% on the 14th and 16th patent days. Respective male gametocytemia were 628 and 742 per cmm. Maximum rates of 88 and 61% from 375B resulted on the 10th day of primary parasitemia and 12th day of relapse parasitemia at respective gametocyte counts of 143 and 116 per cmm. The infective feeding on 736B occurred the 10th day of relapse patency. No positive mosquitoes were obtained from the two black spider-monkey hosts when fed during primary parasitemia. Salivary-gland sporozoite concentrations ranged to 2+, 2+, 3+, and 4+ from 769B, 736B, 255B, and 375B, respectively. Two attempts at sporozoite-transmission from 255B and 14 from 375B to other night monkeys failed.

Subinoculations of parasitized blood from night monkeys 255B, 375B, and 769B, and black spider monkey 534B to recipients of the same species produced parasitemia that in turn were infectious to *A. albimanus*.

DISCUSSION

The present work extends our previous findings on the ability of *P. vivax* infection, induced in monkeys, to infect mosquitoes. Successful transmissions to other monkeys proved the infectiousness of the sporozoites. These transmissions were with mosquitoes infected after a maximum of 58 serial monkey-to-monkey blood subinoculations of the Achiote strain of *P. vivax*.

In the first attempt to transmit vivax malaria by sporozites from monkey to monkey, parasitemia did not develop in the recipient night

TABLE 4

Infectiousness of sporozoite-induced P. vivax *in Panamanian primates to* A. albimanus *mosquitoes*

Primate	No.	Mosquito feeding lots: No.	Mosquito feeding lots: % pos.	Midgut dissections: No.	Midgut dissections: % pos.	Salivary-gland dissections: No.	Salivary-gland dissections: % pos.	% infection per positive lot: Range	% infection per positive lot: Avg.	Infectious male gamet. per cmm: Range	Infective days of patency: Range
Aotus trivirgatus	375B	32	40.6	533	9.9	188	18.6	5.0–87.5	36.5	32–254	8–22
	255B	11	54.5	200	5.5	98	14.3	5.0–43.3	15.7	54–628	10–17
	769B	12	33.3	257	4.3	107	1.9	3.8–24.0	11.8	240–1,100	10–16
Ateles fusciceps	736B	22	4.5	441	0.7	131	0.8	16.0	16.0	221	10
	534B	23	0	458	0	128	0	—	—	—	—

monkey, but two volunteers bitten by the same mosquitoes were infected.[11] The recipient monkey was not splenectomized, nor did it receive an immunosuppressant drug. It was bitten by 106 sporozoite-positive mosquitoes. The present results show that it is possible to transmit *P. vivax* by sporozoites from night monkey to night monkey with as few as 10 mosquitoes. The only obvious difference between the first failure and the later successes is that in the latter, the recipients were splenectomized and received an immunosuppressant drug. However, as 20 other night monkeys, splenectomized and given azathioprine, had no parasitemia after receiving sporozoites by similar methods of transmission, further investigation is needed.

The infection in the marmoset, splenectomized and treated with drug, also originated from mosquitoes fed upon a blood-induced infection in a night monkey. As with night-monkey recipients, no transmissions were successful when sporozoites were introduced by iv or ip injection alone.

The black spider monkey appears to be a favorable primate species for future sporozoite transmission. It is a natural host of *Plasmodium brasilianum*, whereas malaria has not been reported in populations of night monkeys or marmosets.[4, 5] Patent vivax parasitemia developed in two of three black spider monkey recipients. The infections originated in a black spider monkey donor and were transmitted by combined mosquito bite and iv injection of sporozoites. At present, it is unknown if sporozoite-induced *P. vivax* infection is possible in unaltered and untreated hosts of this species.

A. albimanus were similarly susceptible to blood- or sporozoite-induced *P. vivax* in monkeys, and maxima ranging to 100% infection of the lot were obtained. However, the parasitemia infective to mosquitoes varied considerably from one donor to another and, in the same individual, from one patent day to another. So far, we have infected mosquitoes with *P. vivax* by their feeding upon four different malarious hosts including man, *A. tarvirgatus*, *S. geoffroyi*, and *A. fusciceps*. Yet sporozoite transmission has been successful only when the donor was a night monkey or black spider monkey; these transmissions were to the same four hosts listed as infective to mosquitoes. Future studies should help clarify the relation of donor hosts and recipients to successful sporozoite transmissions.

The species of *Plasmodium* infecting man have been transmitted by mosquitoes from man to the chimpanzee, *Pan satyrus*.[1, 10] *Plasmodium ovale* and *P. vivax* additionally were transmitted by mosquitoes between chimpanzees in the experiments. The white-handed gibbon, *Hylobates lar*, also is susceptible to sporozoite infection of *P. falciparum* from man.[13] Falciparum parasites developing in both chimpanzees and gibbons were found not to be infectious to mosquitoes. However, recently this malaria induced by blood in a night monkey was transmitted to mosquitoes, and subsequently to man.[12]

The present report of mosquito-transmissions of *P. vivax* appears to be the first sporozoite-induced malaria of man in monkeys. There have been no consecutive transmissions by sporozoites. However, a blood subinoculation from sporozoite-infected 255B produced an infection in a recipient monkey, and mosquitoes fed on this recipient transmitted the infection to monkey 375B. This indicates that it may be possible to develop a standard procedure for serial transmissions by mosquitoes between monkeys.

SUMMARY

Plasmodium vivax of human origin induced by blood inoculation into the Panamanian primates, *Aotus trivirgatus*, *Ateles fusciceps*, and *Saguinus geoffroyi*, was infectious to *Anopheles albimanus* mosquitoes. The sporogonous cycle was 12 days at 76°F and 15 days at 72°F. Mosquitoes transmitted the infections from *A. trivirgatus* to *A. trivirgatus* on three occasions, from *A. trivirgatus* to *S. geoffroyi* on one occasion, and from *A. fusciceps* to *A. fusciceps* on two occasions. Fifty-seven other attempts to transmit *P. vivax* from primate to primate by infected mosquitoes failed, as did nine from man to primate.

The resulting sporozoite-induced infections showed significant rates of parasitemia in *A. trivirgatus* and *A. fusciceps*. Mosquitoes were infected from these hosts; however 16 subsequent transmission attempts to other monkeys were unsuccessful. Work is in progress to develop sporozoite-induced vivax malaria in monkeys as a dependable procedure.

ACKNOWLEDGMENTS

We are especially grateful to Lionel De Sousa, Lionel Martinez, and Alberto Kant for technical assistance in preparing and examining blood slides, and to Belisario Polo, Jr., Luis Chandeck, Jorge Aguilar, and Luis Vega for their service in insectary operations.

The authors also wish to thank Dr. George Hitchings, of Burroughs, Wellcome and Co., Tuckahoe, New York, for supplying Imuran[30] used in these studies.

REFERENCES

1. Young, M. D., Porter, J. A., Jr., and Johnson, C. M., 1966. *Plasmodium vivax* transmitted from man to monkey to man. *Science, 153:* 1006–1007.
2. Porter, J. A., Jr., and Young, M. D., 1966. Susceptibility of Panamanian primates to *Plasmodium vivax*. *Mil. Med., 131:* 952–958.
3. Young, M. D., Stubbs, T. H., Ellis, J. M., Burgess, R. W., and Eyles, D. E., 1946. Studies on imported malarias. 4. The infectivity of malarias of foreign origin to anophelines of the southern United States. *Am. J. Hyg., 43:* 326–341.
4. Dunn, F. L., and Lambrecht, F. L., 1963. The hosts of *Plasmodium brasilianum* Gonder and von Berenberg-Gossler, 1908. *J. Parasitol., 49:* 316–319.
5. Porter, J. A., Jr., Johnson, C. M., and De Sousa, Lionel, 1966. Prevalence of malaria in Panamanian primates. *J. Parasitol., 52:* 669–670.
6. Rodhain, J., 1956. Paradoxical behaviour of *Plasmodium vivax* in the chimpanzee. *Trans. Roy. Soc. Trop. Med. & Hyg., 50:* 287–293.
7. Bray, R. S., 1957. Studies on malaria in chimpanzees. II. *Plasmodium vivax*. *Am. J. Trop. Med. & Hyg., 6:* 514–520.
8. Bray, R. S., 1957. Studies on malaria in chimpanzees. IV. *Plasmodium ovale*. *Am. J. Trop. Med. & Hyg., 6:* 638–645.
9. Bray, R. S., 1958. Studies on malaria in chimpanzees. VI. *Laverania falciparum*. *Am. J. Trop. Med. & Hyg., 7:* 20–24.
10. Bray, R. S., 1960. Studies on malaria in chimpanzees. VIII. The experimental transmission and pre-erythrocytic phase of *Plasmodium malariae*, with a note on the host-range of the parasite. *Am. J. Trop. Med. & Hyg., 9:* 455–465.
11. Gould, D. J., Cadigan, F. C., and Ward, R. A., 1966. Falciparum malaria: Transmission to the gibbon by *Anopheles balabacensis*. *Science, 153:* 1384.
12. Contacos, P. G., and Collins, W. E., 1968. Falciparum malaria transmissible from monkey to man by mosquito bite. *Science, 161:* 56.