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Plasmodium malariae: Parasite and Disease

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INTRODUCTION

Plasmodium malariae is a malaria parasite that causes a disease that has been recognized since the Greek and Roman civilizations over 2,000 years ago. Quartan, tertian, and semitertian patterns of fever in patients were described by the early Greeks. After the discovery by Alphonse Laveran in 1880 (75) that the causative agent for malaria was a parasite, detailed studies on these organisms commenced. The early detailed work of Golgi in 1886 demonstrated that in some patients there was a relationship between the 72-hour life cycle of development of the parasites and a similar periodicity of the paroxysm (chill and fever pattern in the patient), whereas in other patients there were 48-hour cycles of development (54). He came to the conclusion that there must be more than one species of malaria parasite responsible for these different patterns of cyclical infection.

Eventually, the different parasites were separated and given the names that they carry at the present time. In 1890, Grassi and Feletti (58) reviewed the available information and named *P. malariae* and *P. vivax* with the following statement: "C'est pour cela que nous distinguons, dans le genre Haemamoeba, trois espèces (*H. malariae* de la fièvre quarte, *H. vivax* de la fièvre tierce et *H. praecox* de la fièvre quotidienne avec coutres intermittences etc.)." The current name for the parasite that we discuss here is *Plasmodium malariae* (Grassi and Feletti 1890).

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LIFE HISTORY

Plasmodium malariae has developmental cycles in the mosquito and in the primate host (20). When gametocytes are ingested during mosquito feeding, a process called exflagellation of the microgametocyte occurs, resulting in the formation of up to eight mobile microgametes. Following fertilization of the macrogamete, a mobile ookinete is formed, which penetrates the peritropic membrane surrounding the blood meal and travels to the outer wall of the midgut of the *Anopheles* mosquito. There, under the basal membrane, the oocyst develops. After a period of 2 to 3 weeks, depending on the temperature, many hundreds to a few thousand sporozoites are produced within each oocyst. The oocyst ruptures and the sporozoites are released into the hemocoel of the mosquito. The sporozoites are carried by the circulation of the hemolymph to the salivary glands, where they become concentrated in the acinal cells. During feeding, a small number of sporozoites (<100) are introduced into the salivary duct and injected into the venules of the bitten human, to initiate the cycle in the liver.

In the human, following introduction into the bloodstream, the sporozoites rapidly invade the liver within an hour, where, within a parenchymal cell, the parasite matures in approximately 15 days. Eventually many thousands of merozoites are produced in each schizont. Upon release, these merozoites invade erythrocytes and initiate the erythrocytic cycle. There is no evidence of quiescent liver stage forms (hypnozoites) such as are found in *P. vivax* and *P. ovale* infections in humans. However, not all liver stage forms will mature on the same day; biopsies indicate that these forms may rupture and release parasites over a number of days. Following a developmental

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TABLE 1. Parasite counts and fever for 69 patients infected with *Plasmodium malariae* for 60 days or more[a]

Patient	Days with parasitemia	Maximum parasitemia (/µl)	Days with parasitemia of >1,000/µl	Days with fever of ≥101°F	Maximum fever (°F)	Days with fever of ≥104°F
G-0081	517	1,648	14	6	102.4	0
S-1037	94	2,000	9	6	104.0	1
S-1282	134	2,300	22	26	105.6	9
S-0619	63	2,620	23	17	105.0	13
S-1103	119	2940	53	29	106.0	14
S-1007	107	2,976	34	35	105.4	1
S-1109	114	3,208	40	8	103.0	0
S-0623	130	3,240	8	8	103.4	0
S-1093	263	3,350	11	3	105.4	1
S-0398	84	3,400	48	22	105.6	21
S-1121	170	3,640	9	16	105.2	1
S-1112	100	4,100	25	0		0
G-0007	61	4,240	15	15	105.0	5
S-0489	65	4,350	51	24	106.0	21
S-1101	120	4,950	55	28	105.8	12
S-0647	132	5,108	82	41	105.4	5
S-1119	73	5,434	26	17	104.4	7
S-1061	98	5,618	66	34	105.8	19
S-1039	67	5,804	53	24	106.0	20
S-1090	102	5,950	87	42	106.0	16
S-1137	81	6,080	63	30	106.2	19
S-0408	66	6,100	44	21	106.6	17
S-0508	272	6,250	85	19	105.4	4
S-0488	65	6,350	52	26	105.6	15
S-0734	65	6,350	38	17	105.6	4
S-1120	167	6,460	21	4	103.6	0
S-0651	127	6,725	93	41	106.0	20
S-0960	79	7,120	57	22	106.6	20
S-0468	71	7,700	49	22	106.0	21
S-1068	72	7,840	46	20	105.8	9
S-1303	88	8,413	64	27	105.4	14
S-0532	62	8,450	49	28	106.0	17
S-0593	76	8,750	70	30	105.0	8
S-0788	85	8,850	66	31	106.2	24
S-1078	77	8,875	57	29	106.0	17
S-0495	72	9,350	59	31	106.0	15
G-0063	96	9,360	30	23	105.0	10
S-1042	163	9,650	61	27	106.0	10
S-0818	78	10,150	54	28	105.4	8
S-0396	92	10,400	66	24	106.2	21
S-1268	76	10,711	59	21	106.4	17
S-1105	67	10,752	51	28	106.0	15
G-0040	60	10,880	41	17	105.4	4
G-0013	65	11,100	48	32	105.0	9
S-1085	112	11,450	71	13	104.2	2
S-0984	69	11,750	36	25	105.0	11
S-1096	160	11,850	90	16	106.0	9
G-0080	726	12,120	75	19	105.6	7
G-0062	101	12,330	72	18	103.6	0
S-1071	62	12,544	48	25	106.4	12
G-0077	111	12,600	36	11	104.6	3
S-1041	120	12,608	20	7	106.0	5
S-0799	78	13,550	55	20	105.6	8
S-1151	78	14,000	48	23	106.6	18
G-0042	76	14,160	64	25	104.6	4
S-0600	69	14,650	53	34	105.6	17
S-0740	64	14,776	42	21	106.0	5
S-0752	63	14,800	47	22	106.4	18
S-1081	132	14,850	101	5	104.4	1
G-0026	85	15,240	70	23	105.0	4
S-0985	93	16,100	55	20	106.4	13
S-1072	60	16,100	51	19	105.6	12
S-0218	77	16,160	59	29	105.2	21
S-1289	72	17,034	28	14	106.0	12
G-0029	74	20,320	55	17	105.0	5
S-1015	92	29,850	34	20	105.6	7
G-0025	74	33,920	59	31	106.0	16
G-0110	218	34,660	62	20	104.6	7
G-0016	68	49,680	49	20	104.0	1
Range, median, or mean[b]	60–726	8,875	50.5	21.9	105.6	10.2

[a] Data are from reference 83.
[b] Range and median are shown for days with parasitemia and maximum parasitemia; means are shown for the other parameters.

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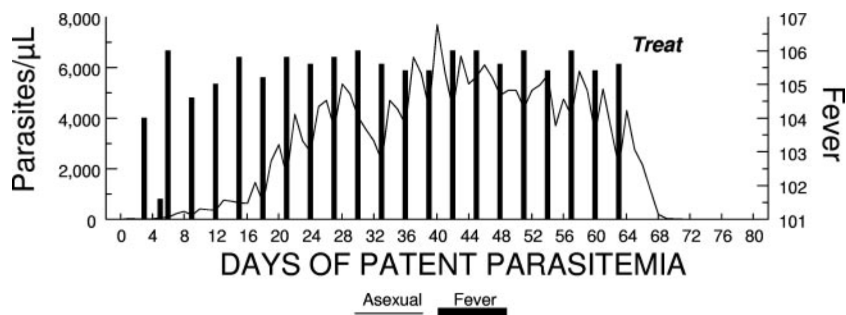

FIG. 1. Daily peak parasite counts and fever peaks in a patient infected with *Plasmodium malariae*, showing the synchronous quartan pattern of fever and peak parasite count.

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cycle in the erythrocyte that lasts, on average, for 72 h, from 6 to 14 (average, 8) merozoites are released to reinvade other erythrocytes. Some of the merozoites develop into the two forms of gametocytes (micro- and macrogametocytes). When they are taken into the mosquito during feeding, the cycle is repeated.

Human Host

Prepatent period. There are only a limited number of reports on the transmission of *P. malariae* to humans to determine prepatent periods. The prepatent period is defined as the time until the first day that parasites are detected on a thick blood film. Shute and Maryon (104) reported the shortest prepatent period of 16 days for a West African strain. Boyd and Stratman-Thomas (10) reported prepatent periods of 27, 32, and 37 days for two different strains, and Mer (86) transmitted a Palestinian strain to three patients, in whom the periods were 26, 28, and 31 days. Prepatent periods of 23 and 26 days were reported by de Buck (42) for four patients infected with a Vienna strain, and Boyd and Stratman-Thomas (12) reported 28- and 40-day prepatent periods. Marotta and Sandicchi (81) reported incubation periods (days until symptoms first appeared) of 23 and 29 days in two patients. Boyd (9) reported on three different strains for which prepatent periods ranged from 28 to 37 days. Siddons (107) reported a prepatent period of 30 days, and Young and Burgess (120) reported prepatent periods of 29 and 59 days. Mackerras and Ercole (79) reported a 24-day period for a Melanesian strain, and Kitchen (72) reported a mean prepatent period of 32.2 days (range, 27 to 37 days) for American strains of *P. malariae*. Young and Burgess (121) transmitted the USPHS strain of *P. malariae* to patients, and the prepatent periods were 33 and 36 days. Ciuca et al. (18) reported prepatent periods for the Romanian VS strain ranging from 18 to 25 days. Lupascu et al. (78) reported incubation periods of 18 to 19 days for the VS strain; in four additional patients the prepatent period ranged from 21 to 30 days (48). In transmission studies with a Nigerian strain involving four volunteers, the prepatent periods ranged from 24 to 33 days (40). Thus, as these data show, there is a wide range in the length in the prepatent period in mosquito-transmitted *P. malariae* (16 to 59 days).

Fever. The most detailed study of the paroxysm of *P. malariae* is probably that by Young et al. (123) in which they examined 420 paroxysms. The average fever peak was 104.1°F (rectally), with the highest recorded being 106.4°F. Fevers (≥101°F) ranged in duration from 5 to 32 h, with an average of 10 h 58 min. Some fevers were introduced by chills, while others were not.

A retrospective examination of induced infections with *P. malariae* was made by McKenzie et al. (83). These data were extracted from the records of patients who were given malaria therapy for the treatment of neurosyphilis between 1940 and 1963. Prior to the introduction of penicillin for the treatment of syphilis, malaria was one of the most effective treatments for the disease (118). It was estimated that perhaps 20% of patients in U.S. mental hospitals had neurosyphilis (62), and infection with *P. vivax* or *P. malariae* was standard practice in the treatment of the disease. *Plasmodium falciparum* was less commonly used because of the difficulty of controlling infections with this species of parasite. It was believed that a combination of repeated episodes of high-intensity fever combined with a nonspecific stimulation of the immune system induced by the malaria parasite combined to destroy the spirochete. Because most African American patients were resistant to infection with *P. vivax* (due to the Duffy negative blood grouping), they were most often treated with *P. malariae*.

A listing of the days of fever of ≥101°F and ≥104°F and the maximum fevers for 69 of the patients examined by McKenzie et al. (83) with no known previous malaria infection who were allowed to have parasitemia of *P. malariae* for 60 or more days is presented in Table 1. For these patients, the median number of days of fever of ≥101°F was 21.9 and the median number of days of fever of ≥104°F was 10.2. The median maximum fever for the 69 patients was 105.6°F. One patient (S-1112) failed to exhibit fever of ≥101°F in spite of a maximum parasite count of 4,100/µl. Fever often occurred on an every-third-day pattern, as shown in Fig. 1. It is also apparent that the fever occurred just prior to an increased parasite count associated with release of a new population of parasites. Because fever regularly occurs again on the fourth day in many patients, *P. malariae* infections are often referred to as being "quartan" malaria.

Parasitemia. Maximum parasite counts are usually low compared to those in patients infected with *P. falciparum* or *P. vivax*. This is due to several factors: (i) the lower number of merozoites produced per erythrocytic cycle, (ii) the extended 72-hour developmental cycle versus the 48-hour cycle of *P. vivax* and *P. falciparum*, (iii) the preference of the parasite to developed in older erythrocytes, and (iv) the combination of

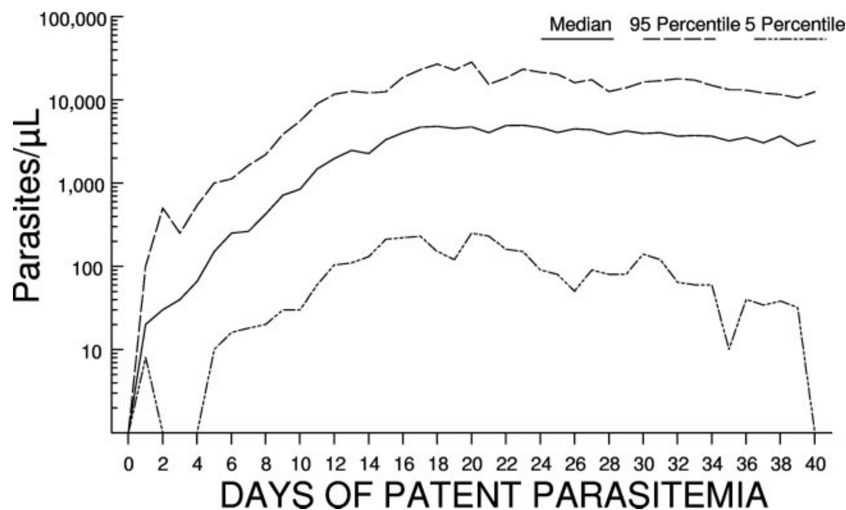

FIG. 2. Median parasite counts during the first 40 days of patent parasitemia for 69 patients infected with *Plasmodium malariae*. Maximum parasite counts are limited in infections with *P. malariae* due to the low number of merozoites produced, 72-hour developmental cycle, and preference for older erythrocytes.

these factors that allows for the earlier development of immunity by the human host. In the 69 patients (Table 1), the maximum parasite count ranged from 1,648/µl to 49,680/µl, with a median count of 8,875/µl (10,000/µl = 0.25% of erythrocytes infected). Some patients had long periods of parasitemia and extended periods when parasite counts were >1,000/µl. These patients averaged 50.5 days with parasite counts of >1,000/µl. When the parasite counts for these patients were averaged for the first 40 days of patent parasitemia (Fig. 2), it was apparent that the parasite count peaked at approximately 2 weeks and then remained relatively stable. The median parasite count actually did not begin to decline until 60 days or more of patent parasitemia.

Other patients in the data studied by McKenzie et al. (83) had been infected with *P. malariae* following previous infection with other species of malaria parasites. Forty-six patients were infected following infection with *P. falciparum* (Table 2). The maximum parasite counts ranged from 312/µl to 29,825/µl, with the median being 6,608/µl. The length of parasitemia was shorter, and there were fewer days with parasite counts of >1,000/µl. The ratio between the number of days of fever of ≥101°F to ≥104°F was almost identical to that for patients with no previous infection. In addition, 39 patients had been infected with *P. malariae* following infection with *P. vivax* (Table 3). The maximum parasite counts ranged from 424/µl to 19,624/µl, with a median of 9,250/µl. Only eight patients were infected with *P. malariae* following infection with *P. ovale* (Table 4). The median maximum parasite count was 13,575/µl.

Recrudescence. *Plasmodium malariae* does not relapse from persistent liver stage parasites. However, the blood stage parasites persist for extremely long periods, often, it is believed, for the life of the human host. There have been many reports of people who have left zones of endemicity and, either following donation of blood in which the recipient developed an infection or under stress, whose infections have recrudesced after many years of dormancy. For example, Collins et al. (33) reported on a transfusion case in which the donor had probably acquired infection with *P. malariae* in China 50 years previously. Vinetz et al. (116) report a case of an infection acquired in Greece over 40 years (and possibly up to 70 years) previous to splenectomy and subsequent diagnosis. Because almost all of these long-term infections have been detected following transfusion donations, it is believed that the parasites have persisted in the blood at very low densities.

Preerythrocytic Stages

The preerythrocytic tissue stages develop in the liver following the introduction of sporozoites. The time required for maturation and release of merozoites from the mature schizonts to invasion of erythrocytes is approximately 15 days. The tissue stages of *P. malariae* were first described by Bray (13, 14) in liver biopsy specimens from sporozoite-inoculated chimpanzees. The host cell nucleus was enlarged and pushed to one side. In over 50% of the parasitized parenchymal cells, two or more nuclei were present. He was able to describe the 8-, 9-, 10-, 11-, 12-, and 12.5-day-old forms. The nuclei were always randomly distributed; there were no pseudocytomeres, no evidence of septum formation or plasmotomy, and no mature schizonts at these time points.

Lupascu et al. (77) obtained biopsy material from a chimpanzee at 12, 13, 14, and 15 days after introduction of sporozoites of *P. malariae*. The schizonts were considered mature at 15 days. The main characteristics were enlargement of the host cell nucleus, many peripheral and internal vacuoles, no cytomeres, large clefts, red-staining strands, and plaques in the mature schizonts.

Millet et al. (87) reported the development of preerythrocytic stages of *P. malariae* in cultures of hepatocytes from chimpanzees and *Aotus lemurinus griseimembra* monkeys. Schizonts were observed in chimpanzee hepatocytes at 8, 11, and 13 days after inoculation of sporozoites. Only one schizont was seen in *Aotus* hepatocytes at day 13.

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TABLE 2. Parasite counts and fever for 46 patients infected with *Plasmodium malariae* following previous infection with *P. falciparum*[a]

Patient	Days with parasitemia	Maximum parasitemia (/μl)	Days with parasitemia of >1,000/μl	Days with fever of ≥101°F	Maximum fever (°F)	Days with fever of ≥104°F
S-1102	47	312	0	0		0
S-0679	199	430	0	1	101.0	0
S-1032	120	540	0	0		0
S-1333	52	580	0	0		0
S-1336	72	1,340	6	15	105.6	7
S-0403	192	1,350	5	0		0
S-0457	195	1,350	7	0		0
S-1295	477	1,586	20	20	106.0	2
S-1263	206	2,856	43	19	105.2	10
S-0937	90	2,864	30	10	104.0	2
S-0774	74	3,270	17	9	105.0	1
S-0678	76	3,550	35	15	105.2	7
S-1008	87	3,752	8	7	104.4	1
S-0463	58	3,850	18	4	102.4	0
S-1022	128	3,910	79	12	104.2	4
S-1321	33	4,080	12	9	103.6	0
S-0965	68	4,650	21	17	105.8	5
S-0782	40	4,785	20	10	105.6	7
S-0953	73	5,120	52	23	106.0	18
S-1313	42	5,335	30	13	106.0	8
S-0713	129	5,520	77	31	105.8	16
S-0791	57	6,050	41	18	105.4	12
S-1302	74	6,591	56	21	105.8	16
S-1018	39	6,625	30	8	104.2	3
S-1285	141	6,800	21	9	105.8	3
S-0995	47	6,875	27	17	105.6	9
S-0980	96	6,890	41	21	105.4	7
S-0760	97	7,120	75	35	106.4	19
S-1058	20	7,655	12	6	105.0	4
S-0775	34	7,722	24	13	106.4	12
S-1304	55	8,210	38	28	105.4	12
S-0991	94	8,424	28	14	104.6	5
S-1067	78	9,950	51	18	105.4	7
S-0778	66	10,224	55	35	104.4	7
S-0789	30	10,450	22	18	106.0	6
S-1065	51	10,550	39	19	106.2	13
S-1088	41	11,800	28	14	106.0	3
S-1002	57	13,472	42	25	105.6	13
S-0951	24	14,250	10	5	105.2	3
S-1050	85	15,840	35	21	106.2	7
S-0986	50	16,850	41	13	105.2	6
S-1338	62	19,094	46	26	106.4	13
S-0966	19	19,200	10	6	106.6	3
S-1079	46	19,744	36	12	105.6	7
S-0787	70	27,250	44	17	106.0	8
S-1004	42	29,825	26	10	105.8	6
Range, median, or mean[b]	20–477	6,608	29.5	14.0	105.4	6.3

[a] Data are from reference 83.
[b] Range and median are shown for days with parasitemia and maximum parasitemia; means are shown for the other parameters.

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Mosquito Host

Many different vectors have been shown to be capable, at least experimentally, of infection with this parasite. These are listed in Table 5. Those that have been proven to be capable of transmitting *P. malariae* to humans experimentally are also indicated. The development of *P. malariae* in mosquitoes has been described by a number of workers; the first definitive studies were carried out by Shute and Maryon (105) on its development in *Anopheles atroparvus* mosquitoes. In the studies of Collins et al. (38) with *Anopheles freeborni*, when incubated at a temperature of 25°C, sporozoites were present in the salivary glands in 17 days. At day 6, the mean oocyst diameter was 12 μm, with a range of 9 to 14 μm. The oocysts continued to grow so that by day 14 they ranged from 20 to 65 μm, with a mean of 38 μm. Early differentiation and formation of sporozoites were apparent by day 14 (Fig. 3).

DISTRIBUTION

In general, the distribution of *P. malariae* coincides with that of *P. falciparum*. In areas of endemicity in Africa, infections of *P. malariae* are mixed with *P. falciparum* infec-

TABLE 3. Parasite counts and fever for 39 patients infected with *Plasmodium malariae* following previous infection with *P. vivax*[a]

Patient	Days with parasitemia	Maximum parasitemia (/μl)	Days with parasitemia of >1,000/μl	Days with fever of ≥101°F	Maximum fever (°F)	Days with fever of ≥104°F
S-0749	39	424	0	1	101.1	0
S-0838	35	495	0	13	105.4	8
S-0325	50	850	0	5	104.0	1
S-0624	31	1,510	5	11	105.4	2
S-1267	95	1,840	11	27	105.0	9
S-0921	61	2,024	10	11	105.0	2
S-1283	192	2,160	32	7	104.6	1
S-0437	63	2,300	33	18	105.8	8
S-0874	54	3043	22	19	105.6	15
S-0490	29	3,250	15	13	105.6	8
S-1082	30	4,864	14	9	106.0	6
S-0718	65	4,960	55	21	107.6	16
S-0866	67	5,448	49	22	106.6	19
S-1314	47	6,170	25	23	106.2	15
S-0518	72	6,450	64	20	105.6	11
S-0414	30	6,700	17	11	106.0	11
S-0586	49	6,750	47	12	105.2	2
S-0543	33	8,350	25	19	105.8	8
S-0916	31	9,040	22	11	106.0	7
S-0534	51	9,250	39	23	106.0	13
S-0592	119	9,521	44	23	105.8	15
S-0911	65	9,888	51	22	106.2	19
S-0504	74	10,350	49	21	106.0	13
S-0559	35	10,900	18	11	106.0	7
S-0560	55	11,100	48	26	105.4	17
S-0846	41	11,418	29	13	106.2	10
S-0790	30	11,727	11	11	104.6	5
S-0611	62	12,150	47	18	105.8	8
S-0571	49	12,450	37	10	106.0	6
S-0970	23	12,500	14	10	106.0	6
S-1019	33	12,640	18	10	106.0	6
S-0920	30	13,050	21	15	106.8	9
S-1046	33	13,350	24	12	106.0	8
S-0786	46	13,420	32	19	106.0	10
S-0675	31	13,750	15	16	106.4	7
S-1026	47	14,976	34	23	106.4	13
S-0610	50	15,510	36	18	105.4	15
S-0506	48	16,776	33	20	106.4	14
S-0939	31	19,624	18	10	106.0	6
Range, median, or mean[b]	23–191	9,250	27.3	15.5	106.0	9.4

[a] Data are from reference 83.
[b] Range and median are shown for days with parasitemia and maximum parasitemia; means are shown for the other parameters.

tions. In many instances, the presence of *P. malariae* infections is unapparent unless PCR techniques are used to reveal low-level or subpatent infections. *Plasmodium malariae* is wide spread throughout sub-Saharan Africa, much of southeast Asia, into Indonesia, and on many of the islands of the western Pacific. It is also reported in areas of the Amazon Basin of South America, along with *Plasmodium brasilianum*, a parasite commonly found in New World monkeys. This parasite is apparently the same species as *P. malariae* that has naturally adapted to grow in monkeys following human settlement of South America within the last 500 years. In the recent past, *P. malariae* was prevalent in Europe and in southern parts of the United States.

LABORATORY DIAGNOSIS

Diagnosis of *P. malariae* infection is preferentially made by the examination of peripheral blood films stained with Giemsa stain. PCR techniques are now routinely used in many laboratories to confirm diagnoses and to separate mixed infections. Recently, in southeast Asia it has been shown that infections with the monkey malaria parasite *Plasmodium knowlesi* in humans have been misdiagnosed as being infections with *P. malariae* (68, 108). Identification was confirmed by PCR. Thus, careful microscopic examination may not be sufficient for positive confirmation in certain situations where monkey malaria parasites such as *P. knowlesi* or *P. inui* may be transmitted to humans. In areas of South America where humans and monkeys coexist, it is impossible to differentiate infections of *P. malariae* from infections of *P. brasilianum* because they may, in fact, be one and the same.

The first stages that appear in the blood are the ring stages that are formed by the invasion of merozoites released by rupturing liver stage schizonts. As described by Coatney et al. (Fig. 4) (20), these grow slowly but soon occupy one-fourth to

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TABLE 4. Parasite counts and fever for eight patients infected with *Plasmodium malariae* following previous infection with *P. ovale*[a]

Patient	Days with parasitemia	Maximum parasitemia (/μl)	Days with parasitemia of >1,000/μl	Days with fever of ≥101°F	Maximum fever (°F)	Days with fever of ≥104°F
S-1293	485	1,477	6	5	102.41	0
S-1278	134	5,040	56	14	104.4	3
S-1106	97	6,350	63	29	105.60	11
S-1271	96	11,100	26	17	105.6	5
S-1092	14	16,050	11	6	105.0	1
S-1305	21	18,166	11	7	105.8	4
S-1273	196	18,960	57	21	105.8	12
S-1264	48	23,486	25	13	106.2	7
Range, median, or mean[b]	14–485	13,575	31.9	14.0	105.6	5.4

[a] Data are from reference 83.
[b] Range and median are shown for days with parasitemia and maximum parasitemia; means are shown for the other parameters.

one-third of the parasitized cell. Pigment increases rapidly, and the half-grown parasite may have from 30 to 50 jet-black granules. As the parasite grows, it assumes various shapes, and it often stretches across the host cell to form what is known as the band form. These are often considered diagnostic, although they are sometimes seen in other species. The host cell is not enlarged as the parasite grows to fill the infected erythrocyte.

At about the 54th hour, segmentation begins, and by the 65th hour, the host cell is nearly filled and the parasite contains five or six chromatin masses; pigment is scattered. The nuclei and cytoplasm begin to separate, and the pigment becomes segregated and clumped in a loose mass in the center of the cell surrounded by the more or less symmetrically arranged merozoites. The number of merozoites may be from 6 to 14, but the average number is 8.

The mature macrogametocyte has a dense, deeply staining blue cytoplasm with a small red-staining nucleus. The pigment is scattered. The parasite completely fills the host cell. The

TABLE 5. Species of *Anopheles* mosquitoes that have been infected with *Plasmodium malariae*

Geographic region	Species of *Anopheles*	References(s)
Southeast Asia	*A. aconitus*	99
Australia	*A. annulipes*	47[a]
Africa	*A. arabiensis*	29
Europe	*A. atroparvus*[b]	17, 18[b], 25, 39, 42[b], 65, 66, 106[b]
Mexico	*A. aztecus*	121
Europe, Middle East	*A. claviger*	57
India, Burma, Sri Lanka	*A. culicifacies*	29, 64, 107, 111
South America	*A. darlingi*	47[a]
Thailand	*A. dirus*	25, 29, 32, 33
Southwest Pacific	*A. farauti*	37, 89
Middle East, India	*A. fluviatilis*	47[a]
Western United States	*A. freeborni*[b]	24, 25, 29, 31, 32, 33, 34, 40[b], 121[b], 122[b]
Southeast Asia	*A. fuliginosus*[c]	3, 6, 113
Africa	*A. funestus*	47[a]
Africa	*A. gambiae*	30, 33, 34, 56, 88
Southeast Asia, Indonesia	*A. hyrcanus sinensis*	3, 69, 114
Bangladesh, Myanmar	*A. jeyporiensis*	47[a]
India, Southeast Asia	*A. lindesayi*	3
Malaysia	*A. maculatus*	3, 25, 29, 32, 59, 112
Africa	*A. melas*	47[a]
Europe	*A. messeae*[b]	81[b]
Southeast Asia, Indonesia	*A. minimus*	3
Africa	*A. moucheti*	47[a]
Europe	*A. plumbeus*	50
Australasia	*A. punctulatus*[b]	79, 80[b]
Southeastern United States	*A. punctipennis*	82, 121
Southeastern United States	*A. quadrimaculatus*[b]	10[b], 11[b], 12[b], 29, 32, 33, 120[b], 121[b], 122
Europe	*A. saccharovi*[b]	5[b], 70, 86[b]
Southeast Asia	*A. splendidus*	3
Middle East, Asia,	*A. stephensi*[b]	25, 29, 32, 33, 64, 73, 98, 103[b], 104, 113
Southeast Asia, Indonesia	*A. sundiacus*[b]	3, 16[b], 63, 64, 114
India	*A. varuna*	64, 113

[a] Listed by Garnham (47); reference not given.
[b] Transmission to human reported.
[c] *A. fuligenosis* = *A. annularis*.

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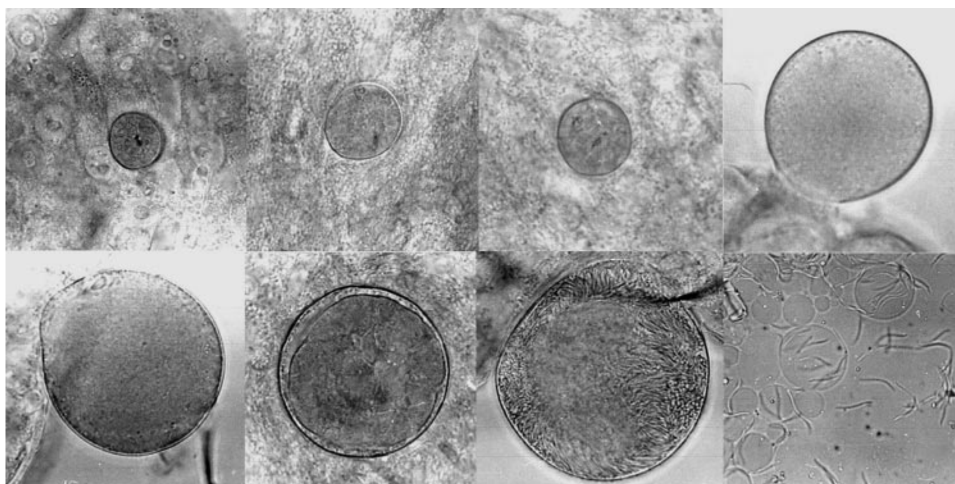

FIG. 3. Development of oocysts of *Plasmodium malariae* in *Anopheles freeborni* mosquitoes. Top row, 10-, 11-, 12-, and 13-day oocysts; bottom row, 14-, 15-, and 17-day oocysts and sporozoites.

cytoplasm of the adult microgametocyte has a light bluish pink stain. The pigment is limited to the cytoplasm of the parasite. The nucleus is diffuse, takes a pinkish-blue stain, and may occupy half the infected cell. The parasite appears to occupy the entire host cell. Ordinarily, microgametocytes outnumber the macrogametocytes.

Snounou et al. (109) applied the nested PCR technique to the diagnostic identification of all four human-infecting species of *Plasmodium*, using genus- and species-specific primers targeting the 18S rRNA gene. Failure to detect some *P. malariae* infections has prompted alteration of the species-specific primers for this parasite.

Recent efforts have been directed towards the development of real-time PCR assays. Rougemonet et al. (97) used a set of generic primers targeting a highly conserved region of the 18S rRNA genes of the four human-infecting species of *Plasmodium* to develop such an assay, which was highly specific and sensitive.

McNamara et al. (85) described a PCR/ligase detection reaction fluorescent-microsphere assay for the diagnosis of infection levels with all four species of human malaria, which shows promise for the detection of minority species in infections with mixed *Plasmodium* species.

Preservation

The preservation of viable malaria parasites by freezing made possible the study of these organisms without continuous cyclical passage. In 1955, Jeffery and Rendtorff (67) reported the frozen preservation of blood stages of *P. malariae*. Blood stages were stored for 20 and 60 days at a temperature of −70°C. The frozen preservation of *P. malariae*-infected erythrocytes has now become routine. Once the infections were established in chimpanzees and New World monkeys, subsequent infections were most frequently induced by the injection of parasitized erythrocytes that had been stored frozen over liquid nitrogen, often after many years of storage. Parasites are usually stored in Glycerolyte (Baxter Healthcare Corp., Fenwal Div., Deerfield, IL) and are expected to be viable for decades when held at extremely low temperatures over liquid nitrogen. Thick and thin blood films for immunofluorescence studies and teaching can be stored unfixed and frozen for extended periods. However, frozen blood is unsuitable for the preparation of blood films for microscopic diagnosis.

SEROLOGIC STUDIES

Serologic tests are not specific enough for diagnostic purposes but are basic epidemiologic tools. They allow for the measurement of past exposure to infection. The immunofluorescent-antibody (IFA) technique has been used to measure the presence of antibodies to *P. malariae*. It was shown that when an infection was of short duration, the response soon declined. However, if the parasite count recrudesced or reinfection occurred, the IFA response rose to a higher lever and persisted for many months or years, as shown in Fig. 5 (27). Cross-reaction studies indicated that *P. brasilianum*, the monkey malaria parasite from South American monkeys that appears to be identical to *P. malariae*, could be used in serologic testing (26). *Plasmodium fieldi*, a parasite of macaques from southeast Asia, also cross-reacted strongly with *P. malariae* (26). In a serologic study of 498 sera collected from Nigerians, 43.2% had positive responses to *P. brasilianum* (35). The response was low in children but was equal to that to *P. falciparum* with sera from individuals 13 years of age and older. In a study of a jungle aboriginal area in Malaysia, there was an almost complete absence of *P. malariae* infection during a parasitologic survey, whereas historically the incidence was known to be quite high (38). The high incidence of maximum IFA responses (51%) to *P. malariae*, however, was probably more indicative of the malarial experience or of subpatent parasitemia than the slide survey because of recent drug interventions (38).

The structure of the circumsporozoite (CS) gene of *P. malariae* was first described by Lal et al. (74). Serologic studies were subsequently conducted for responses to CS proteins of *P. malariae* by using the CS repeat $(NAAG)_5$ in an enzyme-linked immunosorbent assay (ELISA). In a study in Asembo

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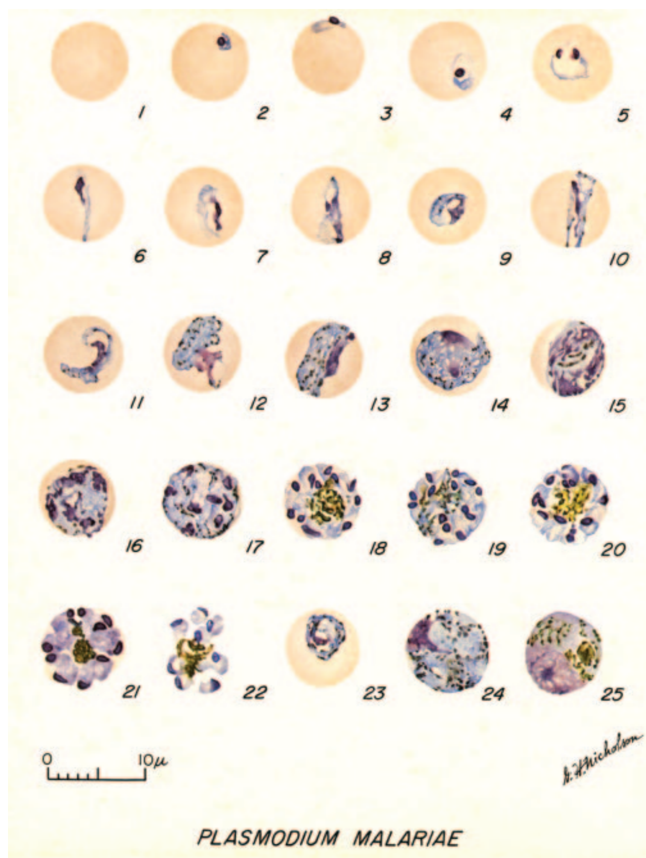

FIG. 4. Development of the erythrocytic stages of *Plasmodium malariae*. 1, normal red cell; 2 to 5, young trophozoites; 6 to 11, growing trophozoites; 12 and 13, nearly mature and mature trophozoites, respectively; 14 to 20, developing schizonts; 21 and 22, mature schizonts; 23, developing gametocyte; 24, mature macrogametocyte; 25, mature microgametocyte. (Reprinted from reference 20.)

Bay, Kenya, 59% of persons had antibodies to the peptide; all positivity rates increased with age (43). In a seroepidemiologic study conducted on Indian tribes in the Amazon Basin of northern Brazil, Arruda et al. (41) found that almost all Metuktire and almost 90% of the Asurini adults had antisporozoite antibodies against *P. brasilianum*/*P. malariae*. A monoclonal antibody specific for the repeat epitope of the CS protein of *P. malariae* was developed to detect sporozoites in infected mosquitoes (22). The $(NAAG)_5$ ELISA has also been used extensively. Beier et al. (7), for example, identified 3.2% of infected *Anopheles gambiae* sensu lato and *A. funestus* mosquitoes collected in western Kenya as being infected with *P. malariae*. This has proven to be a valuable epidemiologic tool in identifying potential vectors of *P. malariae*.

MOLECULAR STUDIES

Cochrane et al. (21) produced a hybridoma secreting a monoclonal antibody against the CS protein of *P. malariae* (Uganda I/CDC strain). A two-dimensional electrophoretic assay showed that the CS protein recognized by the monoclonal antibody contains a repetitive epitope. The antibody also reacted strongly with sporozoites of the simian parasite *P. brasilianum* but did not bind to sporozoites of *P. falciparum*, *P. vivax*, and *P. ovale*. Monoclonal antibodies specific for a repeat epitope of the CS protein of *P. malariae* sporozoites were then used to develop a two-site, single-antibody-based ELISA to detect sporozoites in mosquitoes (22). The major repeat was determined to be Asn Ala Ala Gly (NAAG), with two different minor repeats, Asn Asp Ala Gly (NDAG) and Asn Asp Gln Gly (NDEG). In a study in Cameroon, the length of the CS protein gene varied due to the number of tandem repeat units (115).

A gene encoding the small-subunit rRNA of *P. malariae* was sequenced and shown to contain unique regions that could be used as diagnostic probes (55). Studies indicated a variant form in the small-subunit rRNA gene sequence in the Sichuan province of China and along the Thai-Myanmar border, by deletion of 19 bp and seven substitutions of base pairs in the target sequence (76). Thus, there appear to be two different types or potentially two subspecies of *P. malariae*, based on molecular differences in Asian parasites.

There are few genomic data on this parasite. Studies on the gene encoding cytochrome *b* from the linear mitochondrial genome indicated that *P. malariae* was separate from other members of the primate-infecting *Plasmodium* species (46). *Plasmodium inui* and *P. malariae* do not form a monophyletic group, demonstrating that periodicity is convergent in the evolution of the genus.

INFECTIONS IN CHIMPANZEES AND MONKEYS

Attempts to infect Old World monkeys have been unsuccessful. The first adaptation of *P. malariae* to New World

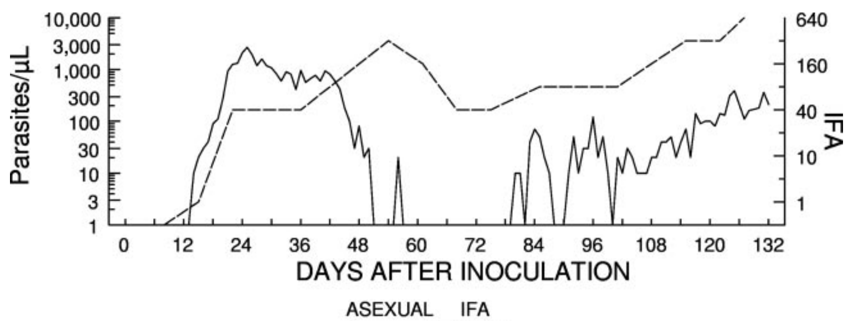

FIG. 5. Development of the IFA response in a patient following infection and recrudescence of an infection with *Plasmodium malariae*. Recrudescence of infection began at day 84.

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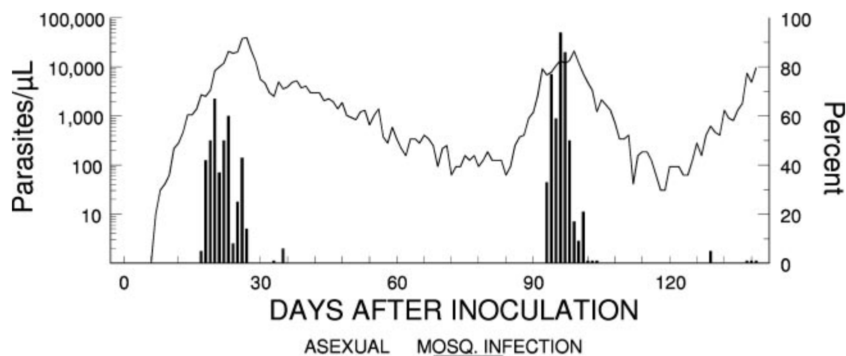

FIG. 6. Daily parasite counts and percent infection of *Anopheles freeborni* mosquitoes when fed on a splenectomized *Aotus lemurinus griseimembra* monkey infected with the Uganda I strain of *Plasmodium malariae*.

monkeys was reported by Geiman and Siddiqui (49). Additional studies were made with different species of *Aotus* and *Saimiri* monkeys (23, 28, 32, 33, 34, 36). In splenectomized *Aotus* monkeys, maximum parasite counts with *P. malariae* varied markedly, from 10 to 56,800/µl. Parasitemia often persisted for many weeks; recrudescence occurred, and mosquito infection was readily obtained (Fig. 6). The median maximum parasite count depended on the previous heterologous malarial experience of the animals. When 18 *Aotus* monkeys with no previous history of infection were infected with *P. malariae*, the median maximum parasite count was 13,760/µl. In 29 monkeys that had been previously infected with *P. falciparum*, the median maximum parasite count was 6,270/µl. In 46 monkeys that had been previously infected with *P. vivax*, the maximum parasite count with *P. malariae* was 1,488/µl. Following the infection of 49 animals that had been previously infected with both *P. vivax* and *P. falciparum*, the median maximum parasite count with *P. malariae* was only 899/µl. Splenectomized *Saimiri boliviensis* monkeys had maximum parasite counts that varied from 62/µl to 22,134/µl.

Splenectomized chimpanzees were shown by Rodhain (95) and Garnham et al. (48) to be readily infected. Bray (14) observed parasite counts in splenectomized animals of between 25,000 and 50,000 per µl, and Garnham et al. (48) observed a maximum parasite count of 160,000 per µl. In a study with 31 splenectomized chimpanzees with various previous histories of infection with *P. vivax* and *P. ovale*, maximum parasite counts following inoculation with a strain of *P. malariae* from Uganda ranged from 930 to 75,700 per µl (29). Infections were infective to a variety of mosquito species on more than 50% of the days on which they were fed. In most instances, infection was obtained when the parasite count was rising and diminished as soon as the count peaked.

In 1920, Reichenow (91) studied malarias in chimpanzees and gorillas in the Cameroons and found *P. malariae*. Blacklock and Adler (8) in 1922 in Sierra Leone and Schwetz (100, 101, 102) in the Belgian Congo also saw *P. malariae* in these animals. In 1939, Brumpt (15) gave the name *P. rodhaini* to the quartan parasite that infected chimpanzees and gorillas. However, subsequent transmission studies with quartan parasites isolated from chimpanzees convinced investigators that this parasite was actually *P. malariae* (92, 93, 94, 96).

PATHOLOGY

Watson in 1905 (117) noted the presence of edema in a patient with malaria in Malaysia, and subsequently the relationship between *P. malariae* infection and the nephrotic syndrome has been well documented. Many investigators (9, 51, 52, 53, 110) indicated a close relationship between quartan malaria and renal disease. Hendrikse and Adeniyi (60) described the clinico-pathological features associated with infection with *P. malariae* and suggested that immune complexes may cause structural glomerular damage. Dixon (44) demonstrated immune complexes in the kidneys of patients with nephrotic syndrome associated with quartan malaria.

The essential lesions are a thickening of the glomerular basement membrane and endocapillary cell proliferation (61, 71). This gives rise to a double-contour or plexiform arrangement of periodic acid-Schiff stain-positive, argyrophilic fibrils (45, 61, 119). As the disease progresses, more capillaries become affected, and the lesions extend to cause progressive narrowing and eventually complete obliteration of capillary lumens.

Electron microscopy shows thickening of the glomerular basement membrane with an increase in the basement membrane-like material of varying density in the subendotherial zone (2). Hendrickse et al. (61) graded the severity of pathological changes based on the percentage of glomeruli showing lesions. If patients had up to 30% of glomeruli showing lesions, they responded to therapy. However, if they had greater than that, they did not show a response to therapy. The renal disease tended to become chronic and nonresponsive to treatment with antimalarial and immunosuppressive drugs.

Aikawa et al. (1) examined the kidneys of *Aotus* monkeys infected with *P. malariae* and demonstrated that the nephrotic syndrome seen in monkeys was consistent with that seen in humans. Histologically, glomeruli of these monkeys infected with *P. malariae* showed thickening and reduplication of the basement membrane and endocapillary cell proliferation. Electron microscopy revealed electron-dense deposits in the subendothelial and mesangial areas. The changes were consistent with membranoproliferative glomerulonephritis, similar to that of humans infected with *P. malariae*.

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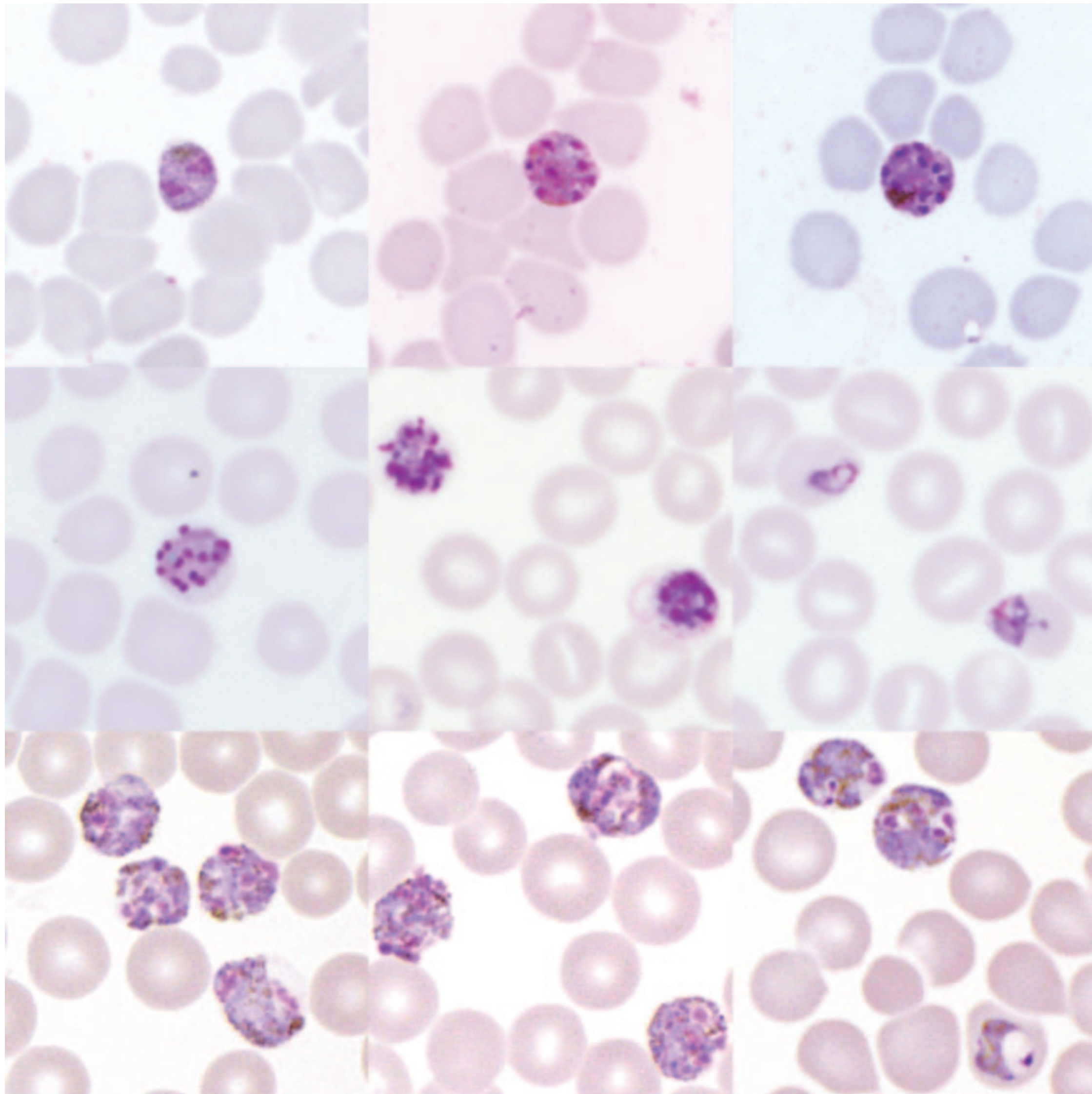

FIG. 7. Top row, trophozoite stages of *Plasmodium malariae*; middle row, trophozoite stages of *Plasmodium knowlesi*; bottom row, trophozoite stages of *Plasmodium inui*.

ULTRASTRUCTURE

Ultrastructural studies have also been made on the erythrocytic stages of *P. malariae* and on the oocyst and sporozoite stages in the mosquito. Atkinson et al. (4) indicated that *P. malariae* was morphologically indistinguishable and structurally similar to other primate malaria species. There were highly structured arrays of merozoite surface coat proteins in the cytoplasm of early schizonts and on the surface of budding merozoites. Knobs were present in the membranes of Maurer's clefts. Morphological evidence suggested that proteins are transported between the erythrocyte surface and intracellular parasites via two routes: one associated with Maurer's clefts for transport of membrane-associated knob material and a second associated with caveolae in the host cell membrane for the import or export of host- or parasite-derived substances through the erythrocyte cytoplasm.

Nagasawa et al. (90) used immunoelectron microscopy and a monoclonal antibody for the CS protein of *P. malariae* to determine ultrastructural localization of this protein in midgut oocysts and salivary gland sporozoites. The CS protein was found along the capsule of immature oocysts but rarely within the cytoplasm. It was detected on the inner surface of peripheral vacuoles during oocyst maturation and on the plasma membrane of the sporoblast. Salivary gland sporozoites and sporozoites in mature oocysts were labeled uniformly on the outer surfaces of their plasma membranes. Antibodies against *P. brasilianum* CS protein reacted with *P. malariae* sporozoites.

RELATIONSHIP TO OTHER SPECIES

Malaria parasites of primates are organized based on biologic characteristics. *Plasmodium brasilianum*, the monkey-infecting malaria parasite of South America, probably is an adaptation of *P. malariae* to New World primates with the

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introduction of Old World humans to the New World. The adaptation probably occurred within the last 500 years with the introduction of large numbers of people from Africa, where *P. malariae* was prevalent. The chronic nature of the parasite easily allowed for its survival in the human host during transit to the New World. The ready passage of *P. brasilianum* to humans and the passage of *P. malariae* to New World monkeys indicated that such interspecies transmission between primates and humans is both feasible and probable.

In southeast Asia, the other complex of parasites with a 72-hour developmental cycle in the blood of the primate host is *Plasmodium inui*. This parasite is also experimentally transmissible to humans (19). Whether or not such transmission occurs in nature has not been demonstrated. However, monkeys are commonly found to be infected with *P. inui* in close proximity to humans, and many different mosquito vectors are capable of transmitting the parasite to humans. Morphologically, it would be difficult to separate infections with monkey malaria parasites such as *P. knowlesi* and *P. inui* from those with *P. malariae*, particularly if reliance on a thick blood film diagnosis was made. This is illustrated in Fig. 7, which shows the blood stages of *P. malariae*, *P. inui*, and *P. knowlesi*. Neither of the erythrocytes that are infected with trophozoites of these parasites shows cellular enlargement or prominent stippling. Initial examination of the blood film, if from a human, would have immediately ruled out infection with *P. vivax* and *P. ovale*, both of which result in enlargement of the host cell erythrocyte and prominent stippling, or with *P. falciparum*, which rarely exhibits mature forms in the peripheral blood. Thus, the diagnostician would be left with *P. malariae* as the probable diagnosis. Only the proximity of monkeys would have suggested a secondary examination by PCR or subpassage to susceptible monkeys to confirm infection with a *Plasmodium* species other than *P. malariae*.

There is no molecular evidence suggesting that *P. malariae* is closely related to any of the other primate malaria parasites that have been thus far examined (except *P. brasilianum*). *Plasmodium malariae* and *P. brasilianum* are either the same species or variants of the same species. *Plasmodium malariae* appears to represent an independent colonization of humans by malaria parasites (46). However, there is no indication of a close relationship to other primate-infecting species of *Plasmodium*, and the evolutionary origin of the species is unclear.

REFERENCES

1. **Aikawa, M., J. R. Broderson, I. Igarashi, G. Jacobs, M. Pappaioanou, W. E. Collins, and C. C. Campbell.** 1988. An atlas of renal disease in *Aotus* monkeys with experimental plasmodial infection, p. 1–97. American Institute of Biological Sciences, Washington, DC.
2. **Allison, A. C., V. Houba, R. G. Hendrickse, S. De Petrise, G. M. Edington, and A. Adeniyi.** 1969. Immune complexes in the nephritic syndrome of African children. Lancet **1:**1232–1238.
3. **Anazawa, K.** 1931. Experimental studies on the infectibility of anopheline mosquitoes of Formosa. J. Med. Assoc. Formosa **30:**609–632.
4. **Atkinson, C. T., M. Aikawa, A. P. Rock, K. Marsh, P. M. Andrysiak, G. H. Campbell, W. E. Collins, and R. J. Howard.** 1987. Ultrastructure of the erythrocytic stages of *Plasmodium malariae*. J. Protozool. **34:**267–274.
5. **Averbouch, I.** 1930. Expériences sur la transmission á l'homme de la fiévre quarte par les piqûres d'*Anopheles maculipennis* var *saccharovi*. Rev. Microbiol. Epid. Parasit. **9:**379–381.
6. **Basu, B. C.** 1943. Laboratory studies on the infectivity of *Anopheles annularis*. J. Malaria Inst. India **5:**31–51.
7. **Beier, M. S., I. K. Schwartz, J. C. Beier, P. V. Perkins, F. Onyango, J. K. Koros, G. H. Campbell, P. M. Andrysiak, and A. D. Brandling-Bennett.** 1988. Identification of malaria species by ELISA in sporozoite and oocyst infected *Anopheles* from western Kenya. Am. J. Trop. Med. Hyg. **39:**323–327.
8. **Blacklock, B., and S. Adler.** 1922. A parasite resembling *Plasmodium falciparum*in a chimpanzee. Ann. Trop. Med. Parasitol. **16:**99–106.
9. **Boyd, M. F.** 1940. Observations on naturally and artificially induced quartan malaria. Am. J. Trop. Med. **20:**749–798.
10. **Boyd, M. F., and W. K. Stratman-Thomas.** 1933. A note on the transmission of quartan malaria by *Anopheles quadrimaculatus*. Am. J. Trop. Med. **13:** 265–271.
11. **Boyd, M. F., and W. K. Stratman-Thomas.** 1934. The comparative susceptibility of *Anopheles quadrimaculatus* Say, and of *Anopheles crucians* Wied. (inland variety) to the parasites of human malaria. Am. J. Hyg. **20:**247–257.
12. **Boyd, M. F., and W. K. Stratman-Thomas.** 1936. The transmission of quartan malaria through two consecutive human-anopheline passages. Am. J. Trop. Med. **16:**63–65.
13. **Bray, R. S.** 1959. Pre-erythrocytic stages of human malaria parasites: *Plasmodium malariae*. Br. Med. J. **2:**679–680.
14. **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.
15. **Brumpt, E.** 1939. Les parasites du paludisme des chimpanzés. C. R. Soc. Biol. **130:**837–840.
16. **Brumpt, E.** 1949. The human parasites of the genus *Plasmodium*, p. 65–121. *In* M. F. Boyd (ed.), Malariology, vol. I. W. B. Saunders Co., Philadelphia, PA.
17. **Cambournac, F. J. C.** 1942. Sôbre a epidemiologia do sezonismo em Portugal. Sociedade Industrial de Tipografia, Lda., Lisbon, Portugal.
18. **Ciuca, M., G. Lupascu, E. Negulici, and P. Constantinescu.** 1964. Recherches sur la transmission expérimentale de *P. malariae* à l'homme. Arch. Roum. Pathol. Exp. Microbiol. **23:**763–776.
19. **Coatney, G. R., W. Chin, P. G. Contacos, and H. K. King.** 1966. *Plasmodium inui*, a quartan-type malaria parasite of Old World monkeys transmissible to man. J. Parasitol. **52:**660–663.
20. **Coatney, G. R., W. E. Collins, M. Warren, and P. G. Contacos.** 1971. The primate malarias. National Institutes of Health, Bethesda, MD.
21. **Cochrane, A. D., W. E. Collins, and R. S. Nussenzweig.** 1984. Monoclonal antibodies identify the circumsporozoite protein of *Plasmodium malariae* and detect a common epitope on *P. brasilianum* sporozoites. Infect. Immun. **45:**592–595.
22. **Collins, F. H., P. M. Procell, G. H. Campbell, and W. E. Collins.** 1988. Monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA) for detection of *Plasmodium malariae* sporozoites in mosquitoes. Am. J. Trop. Med. Hyg. **38:**283–288.
23. **Collins, W. E., and P. G. Contacos.** 1969. Infectivity of *Plasmodium malariae* in the *Aotus trivirgatus* monkey to *Anopheles freeborni* mosquitoes. J. Parasitol. **55:**1253–1257.
24. **Collins, W. E., P. G. Contacos, and W. Chin.** 1973. Experimental infection in man with *Plasmodium malariae*. Am. J. Trop. Med. Hyg. **22:**685–692.
25. **Collins, W. E., P. G. Contacos, and B. B. Richardson.** 1975. Studies on human malaria in *Aotus* monkeys. VI. Infectivity of *Plasmodium malariae* to different anophelines. J. Parasitol. **61:**941–943.
26. **Collins, W. E., G. M. Jeffery, E. Guinn, and J. C. Skinner.** 1966. Fluorescent antibody studies in human malaria. IV. Cross-reactions between human and simian malaria. Am. J. Trop. Med. Hyg. **15:**11–15.
27. **Collins, W. E., G. M. Jeffery, and J. C. Skinner.** 1964. Fluorescent antibody studies in human malaria. I. Development of antibodies to *Plasmodium malariae*. Am. J. Trop. Med. Hyg. **13:**l–5.
28. **Collins, W. E., H. O. Lobel, H. McClure, E. Strobert, G. G. Galland, F. Taylor, A. L. Baretto. R. R. Roberto, J. C. Skinner, S. Adams, C. L. Morris, and J. Sullivan.** 1994. The China I/CDC strain of *Plasmodium malariae* in *Aotus* monkeys and chimpanzees. Am. J. Trop. Med. Hyg. **50:**28–32.
29. **Collins, W. E., H. M. McClure, E. Strobert, V. K. Flipski, J. C. Skinner, P. S. Stanfill, B. B. Richardson, and C. Morris.** 1990. Infection of chimpanzees with the Uganda I/CDC strain of *Plasmodium malariae*. Am. J. Trop. Med. Hyg. **42:**99–103.
30. **Collins, W. E., H. McClure, E. Strobert, B. B. Richardson, B. B. Richardson, J. M. Roberts, G. G. Galland, J. Sullivan, C. L. Morris, and S. R. Adams.** 1993. Experimental infection of *Anophele gambiae* s. s. with *Plasmodium malariae* and *P. brasilianum*. J. Am. Mosq. Control Assoc. **9:**68–71.
31. **Collins, W. E., B. B. Richardson, J. S. Sullivan, C. L. Morris, and G. G. Galland.** 1997. Infection of *Anopheles freeborni* mosquitoes on New World monkeys infected with the Uganda I/CDC strain of *Plasmodium malariae*. J. Parasitol. **83:**1099–1103.
32. **Collins, W. E., I. K. Schwartz, J. C. Skinner, and J. R. Broderson.** 1984. Studies on the Uganda I/CDC strain of *Plasmodium malariae* in Bolivian *Aotus* monkeys and different anophelines. J. Parasitol. **70:**677–681.
33. **Collins, W. E., J. C. Skinner, J. R. Broderson, M. Pappaioanou, V. Filipski, B. B. Sutton, P. S. Stanfill, A. Y. Huong, J. Roberts, and C. Wilson.** 1989. The Uganda I/CDC strain of *Plasmodium malariae* in *Aotus lemurinus griseimembra* monkeys. J. Parasitol. **75:**61–65.
34. **Collins, W. E., J. C. Skinner, J. R. Broderson, B. B. Richardson, and P. S.**

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Stanfill. 1989. The Uganda I/CDC strain of *Plasmodium malariae* in *Saimiri* sciureus boliviensis. J. Parasitol. **75:**310–313.
35. **Collins, W. E., J. C. Skinner, and R. E. Coifman.** 1967. Fluorescent antibody studies in human malaria. V. Response of sera from Nigerians to five *Plasmodium* antigens. Am. J. Trop. Med. Hyg. **16:**568–571.
36. **Collins, W. E., J. C. Skinner, M. Pappaioanou, J. R. Broderson, N. S. Ma, V. Filspski, P. S. Stanfill, and L. Rogers.** 1988. Infection of Peruvian *Aotus* nancymai monkeys with different strains of *Plasmodium falciparum*, *P. vivax*, and *P. malariae*. J. Parasitol. **74:**392–398.
37. **Collins, W. E., J. S. Sullivan, D. Nace, T. Williams, J. Kendall, J. J. Sullivan, G. G. Galland, K. K. Grady, and A. Bounngaseng.** 2002. Experimental infection of *Anopheles farauti* with different species of *Plasmodium*. J. Parasitol. **88:**295–298.
38. **Collins, W. E., M. Warren, J. C. Skinner, and H. J. Fredericks.** 1968. Studies on the relationship between fluorescent antibody response and ecology of malaria in Malaysia. Bull. W. H. O. **39:**451–463.
39. **Constantinescu, P., and E. Negulici.** 1967. The experimental transmission of *Plasmodium malariae* to *Anopheles labranchiae atroparvus*. Trans. R. Soc. Trop. Med. Hyg. **61:**182–188.
40. **Contacos, P. G., and W. E. Collins.** 1969. *Plasmodium malariae*: transmission from monkey to man by mosquito bite. Science **165:**918–919.
41. **de Arruda, M., E. H. Nardin, R. S. Nussenzweig, and A. H. Cochrane.** 1989. Sero-epidemiological studies of malaria in Indian tribes and monkeys of the Amazon Basin of Brazil. Am. J. Trop. Med. Hyg. **41:**379–385.
42. **de Buck, A.** 1935. Infection experiments with quartan malaria. Ann. Trop. Med. Parasit. **29:**171–175.
43. **Deloron P., G. H. Campbell, D. Brandling-Bennett, J. M. Roberts, I. K. Schwartz, J. S. Odera, A. A. Lal, C. O. Osanga, V. de la. Cruz, and T. M. McCutchan.** 1989. Antibodies to *Plasmodium falciparum* ring-infected erythrocyte surface antigen and *P. falciparum* and *P. malariae* circumsporozoite proteins: seasonal prevalence in Kenyan villages. Am. J. Trop. Med. Hyg. **41:**395–399.
44. **Dixon, F. J.** 1966. Comments on immuno-pathology. Mil. Med. **131**(Suppl.)**:** 1233–1234.
45. **Edington, G. M., and H. M. Gilles.** 1976. Pathology in the tropics, p. 616–652. Arnold, London, United Kingdom.
46. **Escalante, A. A., D. E. Freeland, W. E. Collins, and A. A. Lal.** 1998. The evolution of primate malaria parasites based on the gene encoding cytochrome *b* from the linear mitochrondrial genome. Proc. Natl. Acad. Sci. USA **95:**8124–8129.
47. **Garnham, P. C. C.** 1966. Malaria parasite and other haemosporidia, p. 1114. Blackwell Scientific Publications, Oxford, United Kingdom.
48. **Garnham, P. C. C., R. Lainson, and A. E. Gunders.** 1956. Some observations on malaria parasites in a chimpanzee, with particular reference to the persistence of *Plasmodium reichenowi* and *Plasmodium vivax*. Ann. Soc. Belge Méd. Trop. **36:**811–821.
49. **Geiman, Q. M., and W. A. Siddiqui.** 1969. Susceptibility of a New World monkey to *Plasmodium malariae* from man. Am. J. Trop. Med. Hyg. **18:** 351–354.
50. **Gendel'man, C. A.** 1936. Sull'infezione sperimentale dell' *Anopheles nigripes* con plasmodi. Trydii Tropitchkogo Inst. Narkomzdrava Assr. Abhhazii **2:**70.
51. **Giglioli, G.** 1930. Malarial nephritis: epidemiological and clinical notes on malaria. Blackwater fever, albuminuria and nephritis in the interior of British Guiana. Based on seven years' continual observation. Churchill Livingstone, London, United Kingdom.
52. **Gilles, H. M., and R. G. Hendrickse.** 1960. Possible aetiological role of *Plasmodium malariae* in "nephritic syndrome" in Nigerian children. Lancet **i:**806–807.
53. **Gilles, H. M., and R. G. Hendrickse.** 1963. Nephrosis in Nigerian children: role of *Plasmodium malariae* and effect of antimalarial treatment. Br. Med. J. **2:**29–31.
54. **Golgi, C.** 1886. Malarial infection. Arch. Sci. Med. **10:**109–135.
55. **Goman, M., B. Mons, and J. Scaife.** 1991. The complete sequence of a *Plasmodium malariae* SSUrRNA gene and its comparison to other plasmodial SSUrRNA genes. Mol. Biochem. Parasitol. **45:**281–288.
56. **Gordon, R. M., and T. H. Davey.** 1932. *P. malariae* in Freetown, Sierra Leone. Ann. Trop. Med. Parasitol. **26:**65–84.
57. **Grassi, B., A. Bignami, and G. Bastianelli.** 1899. Risoconto degli studi fatti sulla malaria durante il mese de Gennaio. Rend. Atti Accad. Lincei **8:**100–104.
58. **Grassi, B. and R. Feletti.** 1890. Parasites malariques chez les oiseaux. Arch. Ital. Biol. **13:**297–300.
59. **Green, R.** 1929. Observations on some factors influencing the infectivity of malarial gamete carriers in Malaya to *Anopheles maculates*. Bull. Inst. Med. Res. Fed. Malay States **5:**1–41.
60. **Hendrickse, R. G., and A. Adeniyi.** 1979. Quartan malaria nephritic syndrome in children. Kidney Int. **16:**64–74.
61. **Hendrickse, R. G., A. Adeniyi, G. M. Edington, E. F. Glascow, R. H. R. White, and V. Houba.** 1972. Quartan malaria nephritic syndrome: collaborative clinicopathological study in Nigerian children. Lancet **i:**1143–1148.
62. **Hook, E. W., and S. M. Marra.** 1992. Acquired syphilis in adults. N. Engl. J. Med. **326:**1060–1069.
63. **Hylkema, B.** 1920. The development of the parasites of quartan malaria in the *Myzomyia ludlowi* and their transmission to man. Meded. V.d. Burg. Geneesk. D **6:**51.
64. **Iyengar, M. O. T.** 1933. Experimental infection of anopheline mosquitoes. Indian J. Med. Res. **20:**841–861.
65. **Jamcsó, N.** 1921. Experimentelle Untersuchungen über die Malaria-infection des Anopheles und des Menschen beeininfussenden Umstände. Arch. F. Schiffsu. Trop. Hyg. **25:**5.
66. **James, S. P.** 1931. Some general results of a study of induced malaria in England. Trans. R. Soc. Trop. Med. Hyg. **24:**477–525.
67. **Jeffery, G. M., and R. C. Rendtorff.** 1955. Preservation of viable human malaria sporozoites by low-temperature freezing. Exp. Parasitol. **4:**445–454.
68. **Jongwutiwes, S., C. Putaporntip, T. Iwasaki, T. Sata, and H. Kanbara.** 2004. Naturally acquired *Plasmodium knowlesi* malaria in human, Thailand. Emerg. Infect. Dis. **10:**2211–2213.
69. **Khaw, O. K., and N. C. Kan.** 1934. Some observations on the prevalence of malaria in Nanking and its vicinity. Chinese Med. J. **48:**109–123.
70. **Khodukin, N. I., and A. I. Lisova.** 1930. Zur Frage über die Möglichkeit von Erkrankungen an Malaria im Winter. Meditz. Muisl' Uzbekitana **1:**76.
71. **Kibukamusoke, J. W., and M. S. R. Hutt.** 1967. Histological features of the nephritic syndrome of quartan malaria in Uganda. J. Clin. Pathol. **20:**117–123.
72. **Kitchen, S. F.** 1949. Quartan malaria, p. 1017–1026. *In* M. F. Boyd (ed.), Malariology, vol. II. W. B. Saunders Co., Philadelphia, PA.
73. **Knowles, R., and B. C. Basu.** 1934. Laboratory studies on the infectivity of *Anopheles stephensi*. J. Malaria Inst. India **5:**1–29.
74. **Lal, A. A., V. F. de la Cruz, G. H. Campbell, P. M. Procell, W. E. Collins, and T. F. McCutchan.** 1988. Structure of the circumsporozoite gene from the human malaria *Plasmodium malariae*. Mol. Biochem. Parasitol. **30:**291–294.
75. **Laveran, A.** 1880. Note sur un nouveau parasite trouvè dans le sang de plusieurs malades atteints de fièvre palustre. Bull. Acad. Med. **9:**1235–1236.
76. **Liu, Q., S. Zhu, S. Mizuno, M. Kimura, P. Liu, S. Isomura, X. Wang, and F. Kawamoto.** 1998. Sequence variation in the small-subunit rRNA gene of *Plasmodium malariae* and prevalence of isolates with the variant sequence in Sichuan, China. J. Clin. Microbiol. **36:**3378–3381.
77. **Lupascu, G., P. Constantinescu, E. Negulici, P. C. C. Garnham, R. S. Bray, R. Killick-Kendrick, P. G. Shute, and M. Maryon.** 1967. The late primary exoerythrocytic stages of *Plasmodium malariae*. Trans. R. Soc. Trop. Med. Hyg. **61:**482–489.
78. **Lupascu, G., P. Constantinescu, E. Negulici, P. G. Shute, and M. E. Maryon.** 1968. Parasitologic and clinical investigations on infections with the VS Roumanian strain of *Plasmodium malariae* transmitted by *Anopheles labranchiae atroparvus*. Bull. W. H. O. **38:**61–67.
79. **Mackerras, M. J., and Q. N. Ercole.** 1948. Observations on the life-cycle of *Plasmodium malariae*. Aust. J. Exp. Biol. Med. Sci. **26:**515–519.
80. **Mackerras, M. J., and F. H. S. Roberts.** 1947. Experimental malarial infections in Australasian anophelines. Ann. Trop. Med. Parasitol. **41:**329–356.
81. **Marotta, G., and G. Sandicchi.** 1939. Contributo all'infezione sperimentale della malattia di anofeli con *Plasmodium malariae* c inoculazione della malattia all'uomo. Riv. Malariol. **18:**89–94.
82. **Mayne, B.** 1932. Note on experimental infection of *Anopheles punctipennis* with quartan malaria. Public Health Rep. **47:**1771–1773.
83. **McKenzie, F. E., W. E. Collins, and G. M. Jeffery.** 2001. *Plasmodium malariae* blood-stage dynamics. J. Parasitol. **87:**626–638.
84. Reference deleted.
85. **McNamara, D. T., L. J. Kasehagen, B. T. Grimberg, J. Cole-Tobian, W. E. Collins, and P. A. Zimmerman.** 2006. Diagnosing infection levels of four human malaria parasite species by a polymerase chain reaction/ligase detection reaction fluorescent microsphere-based assay. Am. J. Trop. Med. Hyg. **74:**413–421.
86. **Mer, G.** 1933. Observations on the development of *Plasmodium malariae* Lav. in *Anopheles elutus* Edw. Ann. Trop. Med. Parasitol. **27:**483–488.
87. **Millet, P., W. E. Collins, T. L. Fisk, and P. Nguyen-dinh.** 1988. In vitro cultivation of exoerythrocytic stages of the human malaria parasite *Plasmodium malariae*. Am. J. Trop. Med. Hyg. **38:**470–473.
88. **Muirhead-Thomson, R. C.** 1957. Notes on the characters of *P. malariae* oocysts of possible value in mixed infections. Am. J. Trop. Med. Hyg. **6:**980–986.
89. **Nace, D., T. Williams, J. S. Sullivan, A. Williams, G. G. Galland, and W. E. Collins.** 2004. Susceptibility of *Anopheles farauti* to infection with different species of *Plasmodium*. J. Am. Mosquito Control Assoc. **20:**272–276.
90. **Nagasawa, H., M. Aikawa, P. M. Procell, G. H. Campbell, W. E. Collins, and C. C. Campbell.** 1988. *Plasmodium malariae*: distribution of circumsporozoite protein in midgut oocysts and salivary gland sporozoites. Exp. Parasitol. **66:**27–34.
91. **Reichenow, E.** 1920. Ueber das vorkommen der malariaparasiten des menshen bei den afrikanischen menschenaffen. Zentbl. Bakteriol. Abt. I Orig. **85:**207–216.

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92. **Rodhain, J.** 1940. Les plasmodiums des anthropoids de l'Afrique centrale et leurs relations avec les plasmodiums humains. Ann. Soc. Belge Med. Trop. **20:**489–505.
93. **Rodhain, J.** 1940. Les plasmodiums des anthropoids de l'Afrique centrale et leurs relations avec les plasmodiums humains. Récepticité de l'homme au *Plasmodium malariae*. (*Plasmodium rodhaini* Brumpt) du chimpanzé. C. R. Soc. Biol. **133:**276–277.
94. **Rodhain, J.** 1941. Les plasmodiums des anthropoids de l'Afrique centrale et leurs relations avec les plasmodiums humains. Bull. Acad. R. Med. Belgique **6:**21–60.
95. **Rodhain, J.** 1948. Contribution a l'etude des Plasmodiums des anthropoids Africaines. Ann. Soc. Belge Méd. Trop. **28:**39–49.
96. **Rodhain, J., and R. Dellaert.** 1943. L'infection á *Plasmodium malariae* du chimpanzé chez l'homme. Etude d'une premiére souche isolée de l'anthropoide *Pan satyrus verus*. Ann. Soc. Belge. Med. Trop. **23:**19–46.
97. **Rougemont, M., M. Van Saanen, R. Sahli, H. P. Hinrikson, J. Bille, and K. Jaton.** 2004. Detection of four *Plasmodium* species in blood from humans by 18S rRNA gene subunit-based and species-specific real-time PCR assays. J. Clin. Microbiol. **42:**5636–5643.
98. **Russell, P. F., and B. N. Mohan.** 1939. On experimental malaria infections in certain Anopheles of south-eastern Madras. J. Malaria Inst. India **2:**425–431.
99. **Schuurman, C. J., and A. S. B. Huinink.** 1929. A malaria problem in Java's South-coast. Meded. Dienst Volksgezondh. Mederl.-Indië **18:**120–142.
100. **Schwetz, J.** 1933. Sur les parasites malariens (*Plasmodium*) des singes supérieurs (Anthropoides) africaines. C. R. Soc. Biol. **112:**710–711.
101. **Schwetz, J.** 1933. Sur une infection malarienne triple d'un chimpanzé. Zentbl. Bakteriol. Parasitenkd. Infektkrankh. Abt. I Orig. **130:**105–110.
102. **Schwetz, J.** 1934. Sur le paludisme des pygmées. C. R. Soc. Biol. **115:**1228–1229.
103. **Shute, P. G.** 1951. Mosquito infection in artificially induced malaria. Br. Med. Bull. **8:**56–63.
104. **Shute, P. G., and M. Maryon.** 1951. Studies in the transmission of *Plasmodium malariae* by *Anopheles* mosquitoes. Parasitology **41:**292–300.
105. **Shute, P. G., and M. Maryon.** 1952. A study of human malaria oocysts as an aid to species diagnosis. Trans. R. Soc. Trop. Med. Hyg. **46:**275–292.
106. **Shute, P. G., and M. Maryon.** 1955. Transmission of *Plasmodium malariae* by laboratory-bred *Anopheles maculipennis var. atroparvus* Meigan. Ann. Trop. Med. Parasitol. **49:**451–454.
107. **Siddons, L. B.** 1944. The experimental transmission of quartan malaria by *Anopheles culicifacies* Giles. J. Malaria Inst. India **5:**361–373.
108. **Singh, B., L. Kim Sung, A. Matusop, A. Radhakrishnan, S. S. Shamsul, J. Cox-Singh, A. Thomas, and D. J. Conway.** 2004. A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings. Lancet **363:**1017–1024.
109. **Snounou, G., S. Viriyakosol, W. Jarra, S. Thaithong, and K. N. Brown.** 1993. Identification of the four human malaria parasite species in field samples by the polymerase chain reaction and detection of a high prevalence of mixed infections. Mol. Biochem. Parasitol. **58:**283–292.
110. **Soothill, J. F., and R. G. Hendrickse.** 1967. Some immunological studies of the nephritic syndrome in Nigerian children. Lancet **ii:**629–632.
111. **Stephens, J. W. W., and S. R. Christophers.** 1902. The relation of species of anopheles to malarial endemicity. Rep. Malaria Commun. R. Soc. Ser. **7:**20.
112. **Strickland, C., D. N. Roy, and S. C. Sen Gupta.** 1940. *Anopheles maculatus* and malaria. Trans. R. Soc. Trop. Med. Hyg. **33:**639–652.
113. **Sur, S. N., H. P. Sarkar, and K. M. Banerji.** 1932. Plasmochin as a malarial gametocide. Indian Med. Gaz. **67:**490–493.
114. **Swellengrebel, N. H., W. Schüffner, and J. M. H. Swellengrebel-de Graaf.** 1919. The susceptibility of anophelines to malarial-infections in Netherlands India. Burgerlijk. Geneesk. Dienst. Ned. Indië **3:**1–64.
115. **Tahar, R., P. Ringwald, and L. K. Basco.** 1998. Heterogeneity in the circumsporozoite protein gene of *Plasmodium malariae* isolates from sub-Saharan Africa. Mol. Biochem. Parasitol. **92:**71–78.
116. **Vinetz, J. M., J. Li, T. F. McCutchan, and D. C. Kaslow.** 1998. *Plasmodium malariae* infection in an asymptomatic 74-year-old Greek woman with splenectomy. N. Engl. J. Med. **338:**367–371.
117. **Watson, M.** 1905. Some clinical features of quartan malaria. Indian Med. Gaz. **40:**49–52.
118. **Weijer, C.** 1999. Another Tuskegee? Am. J. Trop. Med. Hyg. **61:**103.
119. **White, R. H. R.** 1973. Quartan malaria nephritic syndrome. Nephron **ii:**147–162.
120. **Young, M. D., and R. W. Burgess.** 1947. The transmission of *Plasmodium malariae* by *Anopheles maculipennis freeborni*. Am. J. Trop. Med. **27:**39–40.
121. **Young, M. D., and R. W. Burgess.** 1961. The infectivity to mosquitoes of *Plasmodium malariae*. Am. J. Hyg. **73:**182–192.
122. **Young, M. D., N. F. Hardman, R. W. Burgess, W. C. Frohne, and C. W. Sabrosky.** 1948. The infectivity of native malarias in South Carolina to *Anopheles quadrimaulatus*. Am. J. Trop. Med. **28:**303–311.
123. **Young, M. D., G. R. Coatney, and S. B. McLendon.** 1941. Studies on induced quartan malaria in Negro patients. III. Measurements of the paroxysmal phases. South. Med. J. **34:**709–712.

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