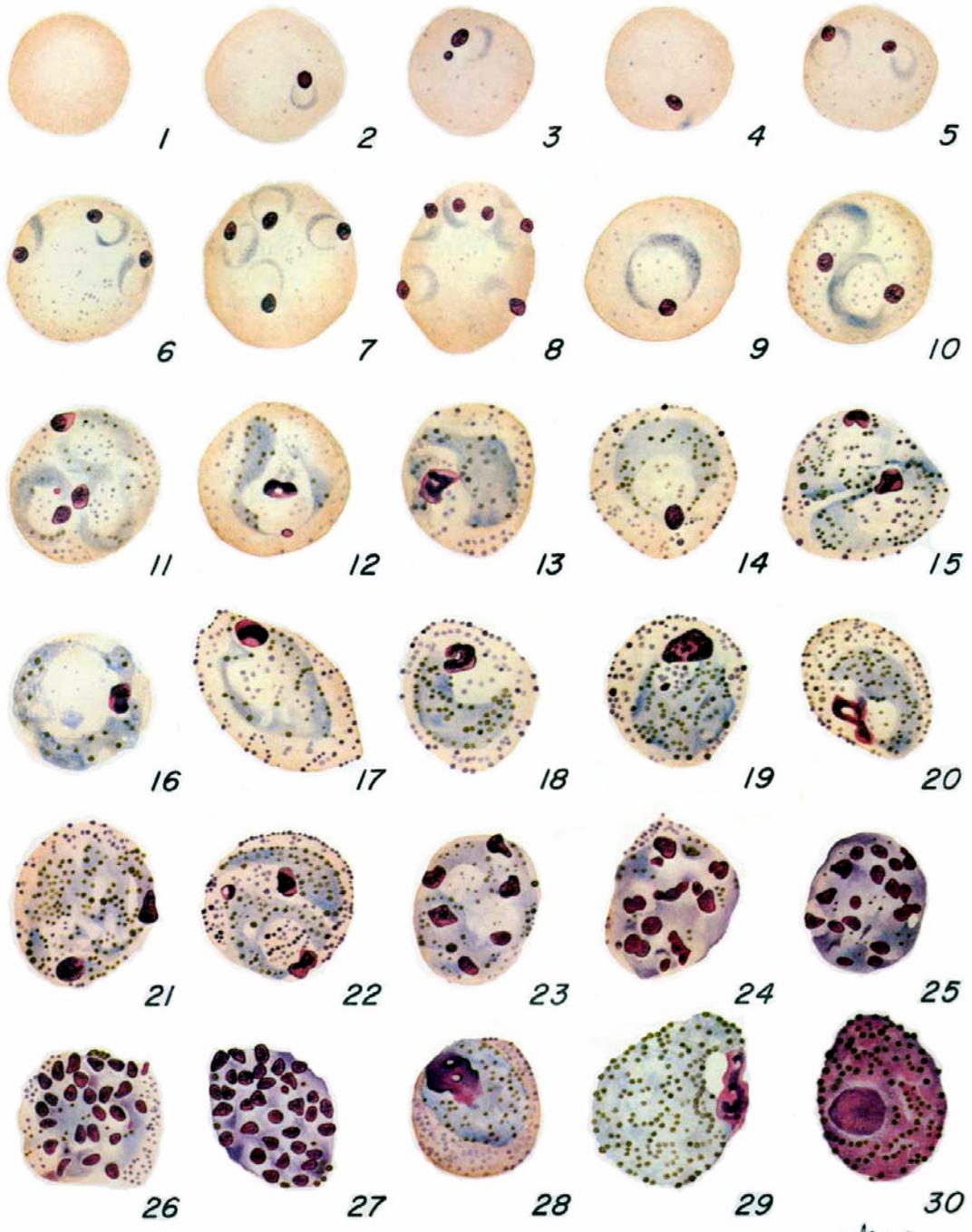


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Plasmodium eylesi Warren, Bennett, Sandosham, and Coatney, 1965

THIS was the second species of malaria described from Malayan gibbons and the third described for the world. A mature male *Hylobates lar* was taken in the Kedah-Perlis area of the country and made available to the authors for study. It was found infected with a malaria parasite (Warren *et al*, 1965, 1965a) which was studied subsequently in five other gibbons.

There was no doubt in the minds of the authors as to its being a new species. They gave it the name *Plasmodium eylesi* in honor of the late Dr. Don E. Eyles who contributed so much to our knowledge of malaria in general and to simian malaria in particular.



0 10 μ

PLASMODIUM EYLESI

R.H. Nicholson

Cycle in the Blood

PLATE VIII

Immediately after the young parasite enters an erythrocyte, the cell becomes enlarged. Schüffner's stippling becomes evident even with the youngest forms but is not pronounced (Figs. 2-10). Amoeboid forms may occur, but ring forms are by far the most common aspect of the early part of the asexual cycle. Not only are multiple infections present but they are common with as many as six rings in a single cell (Fig. 8). The red-staining nucleus is generally single. The older trophozoites do not fill the cell; they may be slightly amoeboid (Fig. 13) and may surround a vacuole (Fig. 16). Pigment is scarce, granular, and yellowish-brown; Schüffner's stippling continues. The mature trophozoites exhibit a more dense blue-staining cytoplasm (Fig. 19); they are not amoeboid; pigment is granular, and is a yellow-brown to gray. Stippling is scattered and coarse. The host cell is enlarged and sometimes distorted (Fig. 17).

Young schizonts almost fill the enlarged host cell except for small areas where Schüffner's inclusion bodies may be prominent (Fig. 24). The cytoplasm remains dense grayish-blue as schizogony progresses. Pigment remains granular and difficult to distinguish; oval-shaped forms are not uncommon (Fig. 23). Older schizonts are frequently oval (Figs. 25, 27) and the cytoplasm stains a deep bluish-red. The chromatin bodies are randomly distributed (Figs. 26, 27); pigment is yellowish-brown and not clumped. The number of chromatin bodies ranges from 20 to 34 with an average of 25. Fully differentiated mature schizonts have not been observed.

The gametocytes are distinctive, especially the microgametocytes. The fully mature macrogametocytes fill the much enlarged host

cell (Fig. 29). They stain a grayish-blue and exhibit coarse, granular pigment which is scattered rather evenly throughout the parasite. The deep staining, generally oval, nucleus may have a vacuole adjacent to it (Fig. 29). The mature microgametocytes are in an enlarged, circular to oval, host cell which takes a deep brilliant reddish-purple stain with a slightly deeper staining nucleus. The pigment is somewhat prominent and scattered throughout the cytoplasm. Once this parasite is seen, the trained eye will "not forget it" (Fig. 30).

One of the most striking features of this parasite is its tendency for multiple invasion of the host red blood cells, a phenomenon probably associated with the parasites' predilection for reticulocytes. This multiple development in a single host cell is responsible for a second characteristic feature of *P. eylesi*, namely the consistent appearance of parasitized cells with as many as 33 merozoites (Fig. 27).

The parasite has a highly synchronous tertian periodicity.

Sporogonic Cycle

The natural vector of this parasite is unknown. However, the sporogonic cycle has been studied in three laboratory reared species of mosquitoes indigenous to peninsular Malaysia (*Anopheles kochi*, *A. maculatus*, and *A. sundaicus*). Observations began 36 hours after the mosquitoes fed and continued through 14.5 days. Extrinsic incubation took place in an insectary maintained at approximately 27° C. The oocyst measurements are presented in Table 7.

In *A. kochi*, at day 1.5, the mean oocyst diameter was 7.5 μ with a range of 7 to 9 μ .

PLATE VIII.—*Plasmodium eylesi*.

Fig. 1 Normal red cell.

Fig. 2-8. Young trophozoites.

Figs. 9-15. Growing trophozoites.

Figs. 16-20. Mature trophozoites.

Figs. 21-25. Developing schizonts.

Figs. 26-27. Mature or nearly mature schizonts.

Fig. 28. Young macrogametocyte.

Fig. 29. Mature macrogametocyte.

Fig. 30. Mature microgametocyte.

The oocysts continued to grow so that on day 9.5, they had an average size of 53 μ with a range of 27 to 69 μ . Sporozoites were first seen in the salivary glands on day 9.5.

The examination of oocysts in *A. maculatus* and *A. sundaicus* mosquitoes indicated that the growth rate of the parasite was similar in all three species. Sporozoites were present in the salivary glands of each species by day 9.5.

A comparison of the growth rate of *P. eylesi* with that of *P. cynomolgi* in *A. maculatus* mosquitoes (Fig. 24) shows the mean oocyst diameters were quite similar and that sporozoites of each of the parasites were present in the salivary glands at 9.5 days.

The sporozoites in *A. kochi* were infective as shown by their ability to initiate an infection in a gibbon. The prepatent period was 12 days.

Cycle in the Tissue

This part of the cycle is unknown.

Course of Infection

Infections, induced by the inoculation of parasitized blood, have been studied in four gibbons (Fig. 25). Beginning on the first day of

patent infection the parasitemia increased rapidly, so that by the end of the first week the count was 20,000 per mm^3 where it remained, more or less, until day 20. From then until day 40, the count averaged about 10,000 parasites per mm^3 of blood, and thereafter declined slowly. The details of the actual infection in each animal were different; i.e., gibbon G 19, whose parasitemia reached 90,000 per mm^3 , was treated and then survived a second episode of above 100,000 per mm^3 . It was finally cleared of the infection by a curative dose of chloroquine on the 128th day of the patent infection, whereas the other animals exhibited more moderate parasitemias and were able to handle their infections without treatment.

The original investigators were able to study a single infection induced by the inoculation of sporozoites from *A. kochi* fed on G 19. This was accomplished in gibbon G 8 on 30 May 1964; young parasites appeared in the blood 12 days later. The parasite count increased from a second day count of 401 per mm^3 of blood to a count of 40,900 on day 8 which was the highest count encountered during an observation period of 32 days.

Because of the possible zoonotic nature of these malarias in the higher apes one is always tempted to inquire of Nature whether she will

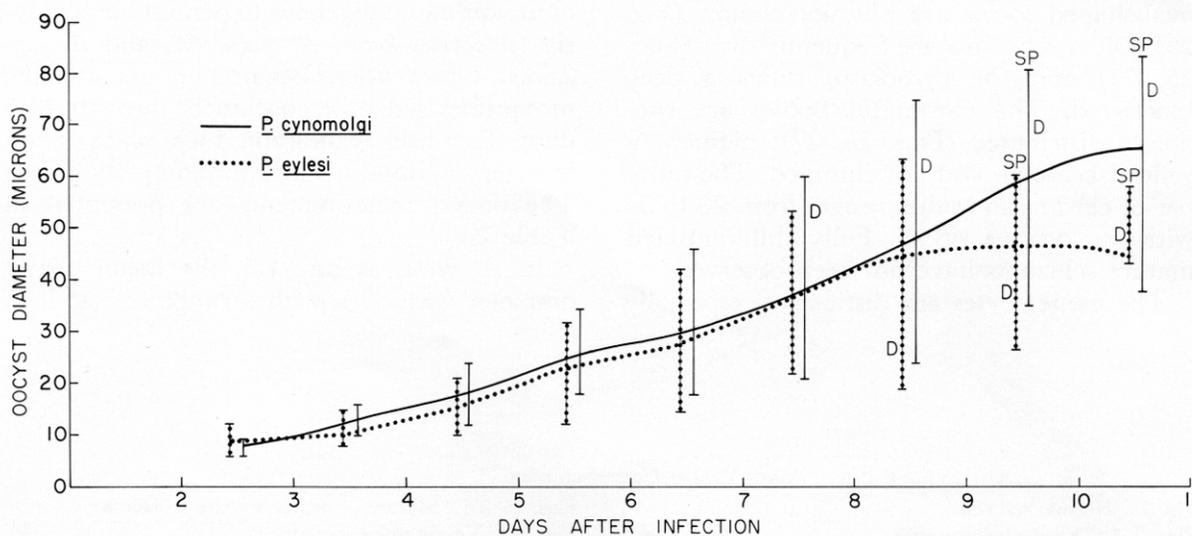


FIGURE 24.—Mean oocyst growth curve and ranges in oocyst diameters of *Plasmodium eylesi* and *P. cynomolgi* in *Anopheles maculatus* mosquitoes. Incubation temperature 27° C. (D = oocyst differentiation; SP = sporozoites present in the salivary glands). (Data courtesy [sic] of Dr. Gordon Bennett).

accept such a parasite in man. With *A. kochi* mosquitoes harboring sporozoites of *P. eylesi* in their salivary glands and lacking certified human volunteers the only hope of a trial in man was for one of the investigators to act as a volunteer. This Dr. Gordon F. Bennett (1968) did. He was bitten by *A. kochi* mosquitoes, known to be infected. On the 15th post exposure day, he exhibited pronounced clinical symptoms comparable to those he had previously experienced when infected with *P. cynomolgi*. The symptoms persisted for about two weeks. During this time, parasites were evident at a very low level for about one week. There is reasonable doubt that Bennett's symptoms, although real, were due to infection with *P.*

eylesi because blood passed from him to a parasite-free gibbon failed to produce an infection in the animal and, also, because the *P. eylesi* infection, if that is what it was, was too low to allow positive identification from parasites encountered in the blood smears.

Host Specificity

The natural host of *P. eylesi* is the white-handed gibbon, *Hylobates lar*. The parasite is not transferable to the rhesus monkey (*Macaca mulatta*) by the inoculation of infected blood.

The natural invertebrate host of *P. eylesi* is unknown. On an experimental basis, 12 species of anopheline mosquitoes indigenous to its area

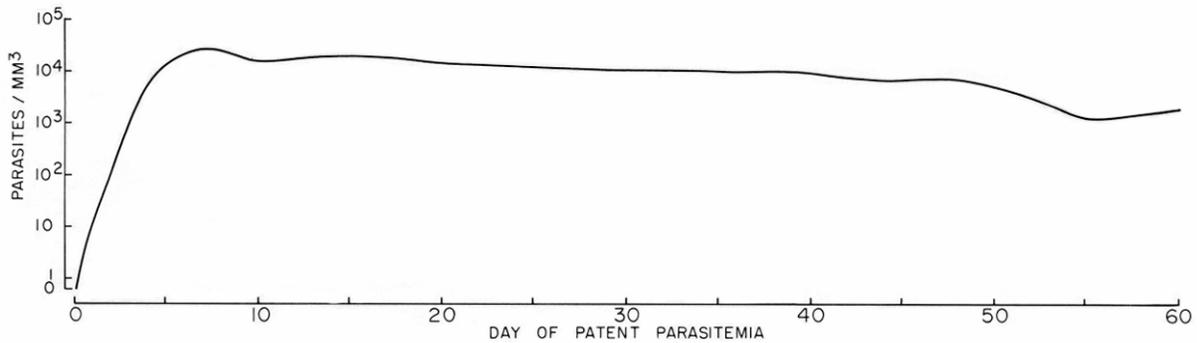


FIGURE 25.—Median parasitemia curve for infections of *Plasmodium eylesi* (4 blood-induced and one sporozoite-induced) in five gibbons, *Hylobates lar*.

TABLE 7.—Oocyst diameters of *Plasmodium eylesi* in *Anopheles kochi*, *A. maculatus*, and *A. sundaicus*.

Days after infection	<i>A. kochi</i>			<i>A. maculatus</i>			<i>A. sundaicus</i>		
	No.	Range	Mean*	No.	Range	Mean	No.	Range	Mean
1.5	6	7-9	7.5						
2.5	48	7-12	9	17	6-12	9	52	6-14	10
3.5	76	8-17	12	62	8-15	10	60	10-15	12
4.5	204	11-24	18	112	10-21	15	96	12-24	18
5.5	188	12-35	24	215	12-32	26	113	15-39	25
6.5	279	15-50	33	65	14-42	26	98	21-57	36
7.5	268	23-63	42†	81	22-53	38	111	24-56	45
8.5	334	21-68	48†	151	19-63	45†	50	41-68	52†
9.5	210	27-69	53†**	100	27-60	46†**	211	29-75	54†**
10.5	128	26-71	54†**	12	43-58	44†**	138	30-71	54†**
11.5							110	38-65	52†**
Totals	1741	7-71		815	6-63		1039	6-75	

* Measurements expressed in microns.
 † Oocyst differentiation.
 ** Sporozoites present in the gland.

of the world became infected when allowed to feed on a gibbon parasitized with *P. eylesi*. These were *A. kochi*, *A. maculatus*, *A. sundaicus*, *A. leucosphyrus*, *A. umbrosus*, *A. roperi*, *A. letifer*, *A. b. introlatus*, *A. riparis macarthuri*, *A. vagus*, *A. sinensis*, and *A. lesteri*. All but the latter three species delivered sporozoites to the salivary glands. The intensity of the infections varied from one species to another (Table 8). *Anopheles vagus* was the most susceptible followed by *A. kochi*, *A. maculatus*, *A. sundaicus*, *A. umbrosus*, and *A. lesteri*. Comparative feedings were not made with the other species.

Infections were obtained in *A. kochi*, *A. maculatus* and *A. sundaicus* mosquitoes when they were allowed to feed on two different gibbons (G 11 and G 19) between the 6th and the 18th days of patent parasitemia (Fig. 26). Maximum infection (100 percent) was obtained, from feedings on both animals, on day 10.

A comparison of the salivary gland infection rates (Table 9) shows that when the gut infection levels are comparable, the percentage

and intensity of infection of the salivary glands is greater in *A. kochi* than in *A. maculatus*.

It is possible that a single human infection was obtained through the agency of mosquito bites (*A. kochi*) but further work is needed before this observation can be inserted into the realm of fact.

Antigenic Relationships and Immunity

Not much is known about antigenic relationships and immunity as applied to this species, although it is well to point out that the animal in which *P. eylesi* was induced by sporozoite inoculation was already carrying a low grade infection with *P. youngi*. This may show that infection with *P. youngi* did not exclude infection with *P. eylesi*, although it must be recognized that the previous and current *P. youngi* infection may have modified the course of the superimposed *P. eylesi* infection.

TABLE 8.—Comparative infectivity of *Plasmodium eylesi* in *Anopheles kochi*, *A. vagus*, *A. maculatus*, *A. sundaicus*, *A. umbrosus*, and *A. lesteri*.

Mosq. species Comparison*	Number tests	Number of mosquitoes		Percent infection		GII** ratios
		Standard	Other	Standard	Other	
Kochi						100
Kochi : Vagus	1	10	10	60.0	30.0	113.3
Kochi : Mac	3	156	179	59.0	55.3	47.0
Kochi : Sund	1	10	21	100	100	36.0
Kochi : Umb	1	35	7	77.1	28.6	3.6
Kochi : Les	2	18	12	100	50.0	1.4

* Kochi = *Anopheles kochi*, Vagus = *A. vagus*, Mac = *A. maculatus*, Sund = *A. sundaicus*, Umb = *A. umbrosus*, Les = *A. lesteri*.

** GII = Gut Infection Index = average number of oocysts per 100 guts; the GII ratio is the relationship of the GII of *A. kochi* to another species where the GII of *A. kochi* = 100.

TABLE 9.—Comparison of salivary gland infection rates of *Plasmodium eylesi* in *Anopheles kochi* and *A. maculatus* (gut infection rates were 27 oocysts per gut for each species).

Days after infection	<i>A. kochi</i>		<i>A. maculatus</i>	
	Positive/Dissected	PGI*	Positive/Dissected	PGI
8.5	0/24		0/10	
9.5	16/34	3.4	7/45	2.1
10.5	24/36	3.3	7/18	2.1
11.5	22/37	3.2	21/32	2.4
12.5	15/20	2.7	18/35	2.9
13.5	7/17	3.7	8/23	3.0
14.5	6/14	2.5	2/10	1.5
Totals	90/182	3.2	63/173	2.5

- PGI = Positive Gland Index = Average gland rating of salivary glands found to be positive.

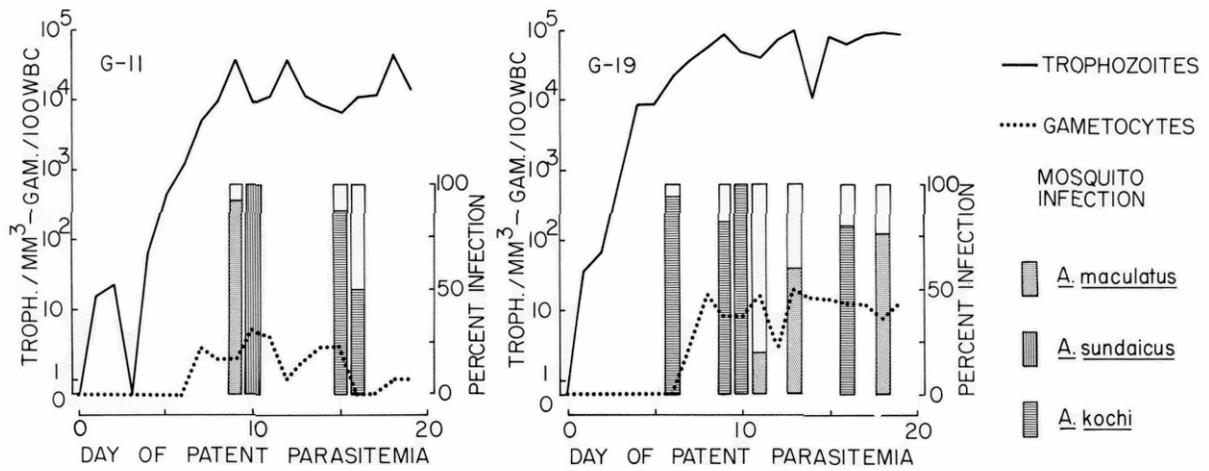


FIGURE 26.—Relationship of parasitemia to mosquito infection in two different gibbons, *Hylobates lar*, infected with *Plasmodium eylesi*.

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