

*Plasmodium simiovale* sp. nov., a new Simian Malaria Parasite  
from Ceylon\*

by

A. S. DISSANAIKE

Department of Parasitology, University of Ceylon

PATHMATHEVY NELSON

and

P. C. C. GARNHAM

Department of Parasitology, London School of Hygiene  
and Tropical Medicine



Two new malaria parasites were recently described from *Macaca sinica* in Ceylon (Dissanaike, Nelson and Garnham, 1965). Since then, another parasite that was isolated earlier in the year, has been found to differ in many respects from hitherto reported species from monkeys. This parasite is described in the present paper, and is named *Plasmodium simiovale* n.sp. This name denotes that the parasite is a simian counterpart of the human *P. ovale*.

In March 1965, two toque monkeys, *Macaca sinica*, were shot near the Mi-Oya river, by the 7 mile post on the Puttalam-Anuradhapura road in N.W. Ceylon. This was the same locality from which the original isolations of *P. cynomolgi ceylonensis* and *P. shortti* (Ceylon strain) were made. Both monkeys (M 73 and M 74) were negative by ordinary blood examination, but when the blood, taken from the hearts of these monkeys, was inoculated into two clean toque monkeys (M 66 and M 68 respectively) parasites appeared after two weeks, M 66 showing *P. shortti* and another parasite which seemed to be "new", and M 68 *P. shortti* alone.

Blood from M 66 was later inoculated into another laboratory toque monkey (M 78) and the same two species of parasites appeared. At this stage, chilled blood from M 78 was sent to the London School of Hygiene and Tropical Medicine and was inoculated into a Rhesus monkey, No. 377. Table I summarises the isolations and subsequent transfer of the parasite. It will be noted that mosquito transmission got rid of the *P. shortti*, and sporozoites of *P. simiovale* alone appeared. A pure line was thus obtained and is preserved at the London School of Hygiene and Tropical Medicine at a temperature of  $-70^{\circ}\text{C}$ , and in passage in rhesus monkeys.

### Sporogonic Stages

Laboratory bred *Anopheles maculipennis atroparvus* and *A. stephensi*, maintained at a temperature of  $26^{\circ}\text{C}$ , were used in the studies. Other than a 5 day old oocyst ( $15\mu$  in diameter, with the pigment in lines on the periphery) seen in the gut of one *A. stephensi*, no stages were found in this mosquito.

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TABLE I

	M 73	Negative	M 74	Negative
CEYLON	M 66	<i>P. shortti</i> and "new" parasite	M 68	<i>P. shortti</i> only
	M 78	<i>P. shortti</i> and "new" parasite		
L.S.H. & T.M.	377	<i>P. shortti</i> and <i>P. simiovale</i>		
	378			
	(-ve)			
	379	<i>P. simiovale</i>		
	380	<i>P. simiovale</i>		

— denotes blood inoculation  
 == denotes mosquito infection

In *A. m. atroparvus*, oocysts measuring 23–35 $\mu$  were seen on the seventh day. The pigment was in the form of dark, discrete grains, arranged in irregular lines. On the 9th day, the oocysts were 35–45 $\mu$ . The pigment granules were obscure in some, and concentrated over a small area in others. On the 11th day, oocysts measured 40–50 $\mu$  and sporozoites were present. The sporozoites measured 12–14 $\mu$  and invaded the salivary glands on the 13th day.

### Blood Stages

The shortest prepatent period after sporozoite infection was found to be 24 days. After the inoculation of a small quantity of infected blood, parasitaemia could be detected in five days or less.

*P. simiovale* exhibits a striking synchronicity of asexual development in the blood. The periodicity is tertian, and schizonts rupture between 11 a.m. and noon on alternate days. Schizogony (i.e. division of the nucleus) begins about twelve hours before this; one hour after rupture of the mature schizonts, rings only are to be found in the blood stream. The uninucleate state is thus seen to occupy three quarters of the 48 hours' cycle.

The parasite invades mature corpuscles. The young ring forms (about 2 hours' old, and measuring 1.5 $\mu$ ) are non-amoeboid, with a round nucleus often projecting or lying inside the vacuole. Quite early, the invaded corpuscle begins to increase in size (7.5 instead of the normal 7 $\mu$ ) and stippling commences in the erythrocyte, when the parasite is still a small ring. A little later, the surface of the cytoplasm may exude small dimples, but neither at this stage nor later is the parasite actively amoeboid; it preserves a compact form. The cytoplasm of the larger rings soon begins to show a few vacuoles, which are such conspicuous features of the older parasite.

At 10 hours, the ring has grown to  $3\mu$ , in a corpuscle of  $8\mu$ , stippling is greater, the nucleus is larger and vacuoles are more obvious in the cytoplasm. The erythrocyte itself is now faintly flushed, and 8 hours later (18th hour of the cycle), the reddening of the corpuscle is most striking. This discolouration continues until the parasite is fully grown. The trophozoites of this stage are  $4.5\mu$  in diameter, while the red blood cell is  $8.5\mu$ . Small brownish yellow grains of pigment appear in the cytoplasm, which remains compact, and the central vacuole begins to disappear.

At 26 hours, the trophozoite has usually entirely lost its vacuole, and the large nucleus is displaced to one side. Pigment granules are more numerous and vacuoles more conspicuous, but the characteristic feature of this stage is the marked distortion of the corpuscle, which becomes oval and/or fimbriated in about 60% of the infected cells. The trophozoite now measures about  $5\mu$  and the average dimension of the cell is  $9\mu$  ( $12 \times 7\mu$ ). This effect upon the corpuscle is not absolutely limited to this period of growth, but at other times, the distortion is much less frequently seen; it is not the result of a more rapid drying of the blood film, because films dried immediately in a current of hot air do not show the phenomenon to any extent at other times of the cycle.

At 34 hours, most of the infected corpuscles ( $9.5\mu$  in size) no longer appear oval or fimbriated, and they contain large round parasites ( $7.5\mu$ ) with a big nucleus, many grains of coarse pigment, and several well marked vacuoles in the cytoplasm.

Eight hours later (42nd hour of the cycle), the uninucleate bodies are replaced by schizonts, containing 2 to 6 nuclei, but little altered in size. White circular or cleft like vacuoles are prominent in the blue staining cytoplasm, in which the pigment granules still remain discrete. The nuclei are particularly large and measure  $1.1\mu$ — $2.3\mu$ .

The nuclei undergo further division during the next six hours and 12 to 14 are found in the mature form at noon on the third day. The schizont is about  $9\mu$  in size, while the infected cell has enlarged to a diameter of  $10\mu$ . The cell is usually round, though occasionally it has an oval shape. Vacuoles are still conspicuous and the pigment does not conglomerate until the final separation of the parasite into merozoites. Twelve to 14 ovoid merozoites ( $3\mu$  in length) are produced and these are usually rather haphazardly arranged around the brownish yellow pigment granules, at last clumped together. The erythrocyte at this stage has been greatly changed and it often balloons out into a large sac before the final rupture.

The cytoplasm of the parasite throughout schizogony stains a bright blue colour with a Romanowsky stain, and this is perhaps due to the concentration of all the pigment into granules, so that none remains to dirty the cytoplasm.

Spherical gametocytes ( $9\mu$  in diameter) appear soon after the establishment of asexual parasitaemia, and females appear to be much more numerous than males, though the identification of the former is difficult because the large asexual parasites often nearly fill the corpuscle before the nucleus starts to divide, and the pigment remains unclumped until

TABLE II  
Table showing Differential Characters of Certain Primate Species of *Plasmodium*

	Vertebrate Host	Locality	Periodicity	EFFECT ON ERYTHROCYTE			Amoeboidity	Merozoites	Other characters	Duration of Sporogony at 26°C
				Enlargement	Distortion	Stippling				
<i>P. simiovale</i>	<i>M. sinica</i>	Ceylon	48 hours	+	Temporary fimbriation	Schüffners, flush	Nil	12—14	Large nuclei, vacuoles	13 days
<i>P. shortti</i>	<i>M. sinica</i> and <i>M. radiata</i>	Ceylon and India	72 hours	Slight	Nil	Ziemanns	+	12—14	—	12 days
<i>P. cynomolgi ceylonensis</i>	<i>M. sinica</i>	Ceylon	48 hours	+	Nil	Schüffners, slight flush	+	12—16	—	8 days
<i>P. fieldi</i>	<i>M. nemestrina</i>	Malaya	48 hours	Nil	Occasional fimbriation	Schüffners	Nil	12—13	Eosinophilic masses	—
<i>P. simium</i>	<i>Alouatta fusca</i>	Brazil	48 hours	+	Occasional fimbriation	Heavy Schüffners	+	12—27	Large nuclei	
<i>P. ovale</i>	Man	Cosmopolitan chiefly Africa	49 hours	+	"ovale"	Heavy Schüffners	Nil	8—12	Large nuclei	15 days

schizogony is completed. The nucleus of the macrogametocytes lies on the margin of the parasite, and numerous pigment granules, but apparently no vacuoles are present in the cytoplasm. The microgametocyte stains the characteristic pink colour with Giemsa's stain and the diffuse nucleus occupies about a third of the total area of the parasite. The pigment resembles that of the female.

### Course of Infection

Parasitaemia mounts slowly and apparently never reaches a high level; in fact, in nature, the infection is occult and can only be detected by subinoculation of blood into clean animals. In the rhesus, the density of parasitaemia, though low, is higher than that attained by *P. fieldi* in this species of monkey.

The duration of infection is as yet unknown, but at the end of the second week, the blood becomes much disturbed; there is a considerable drop in the polymorphonuclear leucocytes and an increase in the lymphocytes and mononuclears, but the unusual character is the appearance of many normoblasts, without, at this time, any marked anisocytosis or other signs of anaemia. In one rhesus monkey, on the 13th day of parasitaemia, the blood count was as follows:— Total red blood count: 2,480,000 per cu.mm., with 11% of normoblasts in the nucleated cells. Polymorphonuclear leucocytes, 23%. Lymphocytes, 36%. Large mononuclears, 30%.

The blood changes later become profound, and in splenectomised animals the monkey dies of severe anaemia; in one instance, a moribund monkey was revived by injections of iron.

### DISCUSSION

In the first place, *P. simiovale* has to be differentiated from the other species of malaria parasites found in monkeys in Ceylon. *P. fragile* is a much smaller organism, which rapidly erodes the erythrocyte and spends much of its asexual cycle outside the peripheral circulation. *P. shortti* is at once identifiable by its quartan periodicity and lesser degree of stippling. *P. cynomolgi ceylonensis* presents more of a difficulty, because, like *P. simiovale*, it produces Schüffner's dots and has a tertian cycle; the new parasite, however, is not amoeboid, and is characterised by the curious and unique vacuoles in the cytoplasm and the fimbriating effect on the erythrocyte between the 25th and 28th hours of the cycle. No other mammalian species of *Plasmodium* is known to give rise to this type of vacuolation, though a somewhat similar appearance is characteristic of *P. matutinum* both in its erythrocytic and exoerythrocytic stages. Another point of distinction between *P. cynomolgi* and *P. simiovale* is the greater length of sporogony in the latter (13 days instead of 10 days at a temperature of 26°C). There are other minor differences which help in differentiation, such as the large size of the nuclei (*P. simiovale*: 2.3 $\mu$ , *P. cynomolgi*: 2 $\mu$  at the 2 nucleated stage), their late division, the high degree of synchronicity of schizogony, and retention of discrete granules of pigment until the very end of schizogony. The production of 12 to 14 merozoites is less than in most strains of *P. cynomolgi*.

*P. simiovale* bears some resemblance to the human *P. ovale*, from which it differs however in the temporary distorting effect on the corpuscle, the lighter colour of the pigment the greater number of merozoites, the shorter duration of sporogony and the non-infectability of man.

*P. simiovale* and *P. fieldi* appear to possess affinities, although a point of difference, easy to recognise, is the much greater enlargement of the corpuscle produced by *P. simiovale*; moreover, the characteristic eosinophilic masses in the infected erythrocyte are absent in infections of the Ceylon parasite. The lack of amoeboidity is characteristic of both species.

*P. simium* of howler monkeys in Southern Brazil resembles *P. simiovale* to some extent, particularly in the prominent Schüffner's dots and the presence of large nuclei, but many more merozoites are produced by *P. simium*, while the pigment is finer and the cytoplasm more amoeboid.

Table II gives the principal diagnostic characters of these species.

### SUMMARY

1. *Plasmodium simiovale* sp. nov. is described in its blood and mosquito stages from *Macaca sinica* of Ceylon.
2. The parasite has a tertian periodicity of a very high degree of synchronicity, and apparently has affinities with *P. ovale*, *P. fieldi* and *P. simium*.

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### REFERENCES

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### EXPLANATION OF PLATE

- FIGURES 1—5—Early rings  
6—12—Later trophozoites  
13—20—Schizonts  
21—22—Rupturing schizonts  
23—Male gametocyte  
24—Immature female gametocyte  
25—Mature female gametocyte

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