Malaria and Piroplasms of Non-Human Primates (29 June 2000)

F.B. Cogswell

Tulane Primate Center, Tulane University, 18703 Three Rivers Road, Covington, LA 70433, USA.

Introduction

Malaria

Malaria in primates is caused by a protozoan parasite of the genus *Plasmodium*. The life cycle includes an obligate sojourn in an *Anopheles* mosquito vector where sexual reproduction takes place (Fig. 1). While taking a blood meal, the female Anopheline injects sporozoites, which go directly to the liver. Hepatic stages include schizonts that develop to release merozoites, which then invade red blood cells. Asexual stages seen in blood films are young trophozoites (often called ring forms), mature trophozoites, and the dividing schizonts that yield the merozoites for a new generation. Sexual stages in the blood are the microgametocytes (male) and macrogametocytes (female).

Some species of *Plasmodium* infecting primates (*P. cynomolgi, P. simiovale, P. fieldi*) also produce latent hepatic stages (hypnozoites) which are responsible for malaria relapse (Fig. 2). Relapse is very specifically defined in malaria as a recurrence of parasites in the peripheral blood (Fig. 3), after adequate blood schizonticidal therapy, and results from the re-invasion of the peripheral blood by merozoites, produced by the latent liver stages [1].

Most primate malaria parasites exhibit tertian periodicity, completing one asexual cycle every 48 hours. Exceptions are *P. knowlesi* in Old World monkeys which has a quotidian or 24 hour cycle, as well as *P. inui* and *P. brasilianum* which have a quartan or 72 hour cycle. The primate malarias are generally thought to have arisen from endocellular, coccidian parasites in the
intestinal tract of reptiles, birds, and amphibians. The earliest primates probably carried the ancestors of present-day malaria parasites when they began to evolve in the Lower Tertiary period. The putative ancestor of present-day primate malaria parasites is Hepatocystis, a ubiquitous parasite of African monkeys and apes. This is a well-adapted, relatively benign parasite, which produces only gametocytes in the circulation. The liver stages are macroscopic and were undoubtedly the first liver stage of primate malaria seen by man, as hunters and cooks prepared monkeys for the table (Fig. 25).

The malaria parasites of New World monkeys, however, are thought to have come from Europe and Africa during the slave trade. There is little evidence of malaria in the pre-Columbian New World, and the two species of monkey malaria in the New World, *P. brasilianum* and *P. simium*, are closely related molecularly, and possibly identical, to the human malaria parasites, *P. malariae* and *P. vivax* [2].

**Babesia**

Other Apicomplexan parasites that may appear in blood smears and confused with malaria parasites, are the piroplasms (family *Babesidae*). *Entopolyploides macaci* is often reported in rhesus macaques (*Macaca mulatta*), African monkeys (*Cercopithecus*), and baboons (*Papio*) but will infect a variety of non-human primates [3]. The organisms are smaller than *Plasmodium*, markedly pleomorphic, and produce no pigment. The infections are usually benign even in splenectomized animals, and do not respond to standard antimalarial therapy. Unlike *Plasmodium*, they are tick-borne.

**Diagnosis**

Diagnosis of malaria, Hepatocystis, or the piroplasms is best accomplished with a thick and thin blood smear, which can be made on the same slide. The thick smear is more sensitive (20 parasites/µl) while the thin smear is used to confirm morphology. The thin smear is fixed in absolute methanol for 2 minutes; the thick smear is left unfixed. Slides are stained in Giemsa for 45 minutes at a pH of 7.25. If time is critical, e.g. if a count is necessary before an animal recovers from anesthesia, a satisfactory stain can be made by doubling the Giemsa concentration and staining for 15 minutes. Other blood stains (Field's, Quickstain) are less than satisfactory, although Wright's stain may be used.

Blood samples can be taken by earstick or tailstick using disposable lancets. It is helpful to shave the tip of the tail or ear before taking a drop of blood in a capillary tube. The thin smear should be fixed as soon as it is dry; the thick smear must be allowed to dry thoroughly before staining. Thick smears should be examined for at least 5 minutes, corresponding to 100 oil immersion fields; thin films must be examined for 15 minutes before a monkey can be declared negative for malaria parasites. In doubtful cases, repeated blood smears can be made daily, taking into consideration that anesthetizing a monkey to make a blood smear may be more stressful than the infection.

The easiest way to quantify infections is by counting the number of parasitized red cells in relation to 10,000 total red cells. This yields a percent parasitemia which is a rough gauge of the severity of infection.

Parasitemia is often expressed as # parasites/mm³ of blood using the following formula:

\[
\text{# infected RBC's} \times \frac{\text{Total RBC's}}{1,000} = \text{parasites/mm³}
\]

One may also count the number of parasitized red cells per leukocyte which, if a CBC is also performed, will also yield an acceptable measure of the level of infection. The accuracy of this method may be compromised if other infections are present which raise the white cell count. Dipstick tests for circulating antigen (hrp2, ldh) may give positive results for *P. cynomolgi*, *P. coatneyi*, and *P. knowlesi* [4]. Other non-human primate malarias have not been tested. PCR is not generally useful in a clinical setting unless one is interested in specific molecular sequences. It is not more sensitive than a well-made thick smear and available primer sequences are limited to *Plasmodium* species used in malaria model work, e.g. *P. cynomolgi*, *P. knowlesi*, and *P. coatneyi*. Serology may be useful in differentiating *Entopolyploides* from *Plasmodium* and this can be done by the Centers for Disease Control and Prevention.
Pathology
Most primate malarias and infections with piroplasms are subclinical, unless the animal is immunosuppressed or splenectomized. Experimental infections of rhesus macaques with *P. knowlesi*, *P. coatneyi*, and less often *P. cynomolgi*, may be characterized by jaundice (Fig. 4), anorexia, listlessness, fever, anemia and splenomegaly in spleen-intact animals. Clinical signs of chills and fever are in response to toxins (*Plasmodium* GPI) exposed during the release of merozoites from the red cell. Pregnant animals may experience more severe anemia, which will have a measurable impact on the health of the fetus [5].

Figure 4. Malaria icterus in a rhesus macaque following an experimental infection with *Plasmodium cynomolgi*. (Photo courtesy G. Baskin). - To view this image in full size go to the IVIS website at www.ivis.org . -

Grossly the lungs, liver, and spleen are gray and the blood thin. Histologically, tissue macrophages are filled with malaria pigment and there are hemosiderosis and parasitized RBC’s. Intravascular clotting with thrombi and parasitized erythrocytes is common. Often there is pulmonary and cerebral edema [4].

Treatment
Most primate malarias can be treated with chloroquine at a dosage of 7 mg/kg base for 5 days (total = 35 mg/kg). This can be given as an IM injection or per os via nasogastric tube. The bitter taste of 4-aminoquinolines precludes putting it in food. Chloroquine is effective against the circulating trophozoite (feeding) stage of the parasite but will not affect hepatic stages nor circulating gametocytes. Mefloquine can be used, especially if an isolate is suspected to be chloroquine-resistant, at a dose of 20 mg/kg, one dose, per os. Hepatic stages of *Plasmodium* require treatment with an 8-aminoquinoline (primaquine) at a dose of 3 mg base/kg body weight per os for 7 days [6]. This may be necessary in sporozoite-induced (i.e. natural) infections with *P. cynomolgi*, *P. simiovale* and *P. fieldi* where malarial relapse is a consideration.

Information on the treatment of non-human primate piroplasms has not been reported. Human infections with Babesia microti have been successfully treated with a combination of clindamycin and oral quinine. Adult dosage for adults is clindamycin, 1.2 g IV b.i.d. or 600 mg orally t.i.d. plus quinine 650 mg orally t.i.d., both for 7 days [7]. Dosages for children (more similar in body weight and surface area to non-human primates) have not been reported.

*Plasmodium*
The morphology of the circulating blood stages, described from Romanowsky stained blood films (Giemsa, Wright's) are diagnostic for each *Plasmodium* species. The size of the parasitized red cell (enlarged or not), the presence or absence of Schüffner's stippling (erythrocyte membrane caveolae presenting as “dots” on a stained blood film), Maurer's clefts (narrow clefts which appear as coarse stippling), and the number of merozoites in a mature schizont, are characteristics that aid in species identification. The developing trophozoites are often described as appliqué, or accoléé forms, where the parasite appears as a fine blue line apparently applied to the margin of the red cell. Macrogametocytes (female) and microgametocytes (male) are the circulating sexual forms whose staining characteristics are an aid to species diagnosis. The following descriptions and accompanying plates are largely from The *Primate Malarias* [8], (used with permission), and are based on Giemsa-stained thin blood smears. Table 1 and Table 2 are adapted from Collins, 1988 [9] and serve as a guide to determining which species of *Plasmodium* may be expected to be found in a given animal.
<table>
<thead>
<tr>
<th>Natural Host</th>
<th>Plasmodium sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pan troglodytes</em></td>
<td><em>P. schwetzi, P. reichenowi</em></td>
</tr>
<tr>
<td><em>Gorilla gorilla</em></td>
<td><em>P. schwetzi, P. reichenowi</em></td>
</tr>
<tr>
<td><em>Hylobates lar</em></td>
<td><em>P. eylesi, P. jefferyi, P. youngi</em></td>
</tr>
<tr>
<td><em>Hylobates moloch</em></td>
<td><em>P. hylobati</em></td>
</tr>
<tr>
<td><em>Pongo pygmaeus</em></td>
<td><em>P. pitheci, P. sylvaticum</em></td>
</tr>
<tr>
<td><em>Macaca arctoides</em></td>
<td><em>P. cynomolgi</em></td>
</tr>
<tr>
<td><em>M. cyclopis</em></td>
<td><em>P. cynomolgi, P. inui</em></td>
</tr>
<tr>
<td><em>M. fascicularis</em></td>
<td><em>P. cynomolgi, P. coatneyi, P. fieldi, P. inui, P. knowlesi</em></td>
</tr>
<tr>
<td><em>M. mulatta</em></td>
<td><em>P. cynomolgi, P. inui</em></td>
</tr>
<tr>
<td><em>M. nemestrina</em></td>
<td><em>P. cynomolgi, P. fieldi, P. inui, P. knowlesi</em></td>
</tr>
<tr>
<td><em>M. radiata</em></td>
<td><em>P. cynomolgi, P. fragile, P. inui</em></td>
</tr>
<tr>
<td><em>M. sinica</em></td>
<td><em>P. cynomolgi, P. fragile, P. simiovale</em></td>
</tr>
<tr>
<td><em>Cynopithecus niger</em></td>
<td><em>P. inui</em></td>
</tr>
<tr>
<td><em>Cercocebus aterrimus</em></td>
<td><em>P. gonderi</em></td>
</tr>
<tr>
<td><em>C. atys</em></td>
<td><em>P. gonderi</em></td>
</tr>
<tr>
<td><em>C. galeritus agilus</em></td>
<td><em>P. gonderi</em></td>
</tr>
<tr>
<td><em>Mandrillus leucophaeus</em></td>
<td><em>P. gonderi</em></td>
</tr>
</tbody>
</table>
### Table 2. *Plasmodium* species of New World Monkeys

<table>
<thead>
<tr>
<th>Monkey Host Species</th>
<th>Plasmodium species</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alouatta fusca</em></td>
<td><em>P. brasilianum, P. simium</em></td>
</tr>
<tr>
<td><em>A. palliata</em></td>
<td><em>P. brasilianum</em></td>
</tr>
<tr>
<td><em>A. seniculus straminea</em></td>
<td><em>P. brasilianum</em></td>
</tr>
<tr>
<td><em>A. villosa</em></td>
<td><em>P. brasilianum</em></td>
</tr>
<tr>
<td><em>Aotus sp.</em></td>
<td><em>P. brasilianum</em></td>
</tr>
<tr>
<td><em>Ateles fusciceps</em></td>
<td><em>P. brasilianum</em></td>
</tr>
<tr>
<td><em>A. geoffroyi</em></td>
<td><em>P. brasilianum</em></td>
</tr>
<tr>
<td><em>A. g. griseescens</em></td>
<td><em>P. brasilianum</em></td>
</tr>
<tr>
<td><em>A. paniscus</em></td>
<td><em>P. brasilianum</em></td>
</tr>
<tr>
<td><em>A. p. paniscus</em></td>
<td><em>P. brasilianum</em></td>
</tr>
<tr>
<td><em>A. p. chamek</em></td>
<td><em>P. brasilianum</em></td>
</tr>
<tr>
<td><em>Brachyteles arachnoides</em></td>
<td><em>P. brasilianum, P. simium</em></td>
</tr>
<tr>
<td><em>Callicebus moloch ornatus</em></td>
<td><em>P. brasilianum</em></td>
</tr>
<tr>
<td><em>C. torquatus</em></td>
<td><em>P. brasilianum</em></td>
</tr>
<tr>
<td><em>Cebus albifrons</em></td>
<td><em>P. brasilianum</em></td>
</tr>
<tr>
<td><em>C. apella</em></td>
<td><em>P. brasilianum</em></td>
</tr>
<tr>
<td><em>C. capucinus</em></td>
<td><em>P. brasilianum</em></td>
</tr>
<tr>
<td><em>C. c. capucinus</em></td>
<td><em>P. brasilianum</em></td>
</tr>
<tr>
<td><em>C. c. imitator</em></td>
<td><em>P. brasilianum</em></td>
</tr>
<tr>
<td><em>Chiropotes chiropotes</em></td>
<td><em>P. brasilianum</em></td>
</tr>
<tr>
<td><em>Lagothrix cana</em></td>
<td><em>P. brasilianum</em></td>
</tr>
<tr>
<td><em>L. infumata</em></td>
<td><em>P. brasilianum</em></td>
</tr>
<tr>
<td><em>L. lagotricha</em></td>
<td><em>P. brasilianum</em></td>
</tr>
<tr>
<td><em>Saimiri boliviense</em></td>
<td><em>P. brasilianum</em></td>
</tr>
<tr>
<td><em>S. sciureus</em></td>
<td><em>P. brasilianum</em></td>
</tr>
</tbody>
</table>

**A. Malaria Parasites of Old World Monkeys (Cercopithecidae)**

*P. coatneyi* (Fig. 5)

*Cycle in Blood* - The young ring forms are smaller than in other simian parasites and the typical young trophozoite has a single or double chromatin body. Marginal, appliqué or accolé forms are common. Maurer's
clefts are prominent and characteristic of this species. The early schizonts stain a deep blue, are compact, round, and occupy at least half the cell. The older and mature schizonts fill the host cell and produce about 20 merozoites.

Figure 5. *Plasmodium coatneyi*, cycle in blood. 1: Normal red cell. 2,3,6,7: Young trophozoites. 4,5, 8 - 11: Growing trophozoites. 12,13: Mature trophozoites. 14 - 17: Early schizonts. 18 - 21: Developing schizonts. 22,23: Nearly mature and mature schizonts. 24: mature macrogametocyte. 25: Mature microgametocyte. - To view this image in full size go to the IVIS website at www.ivis.org . -

Course of Infection - *P. coatneyi* causes a tertian infection in all animals studied. In the kra monkey (*Macaca irus*), the parasite produces a mild low-grade infection that may persist for a long time. In rhesus monkeys the infection may be explosive with peak counts on the order of 500,000/mm³, and is often fatal. In *M. fasicularis*, the peak parasitemias range from 15,000 to 57,000/mm³. Sequestration of mature schizonts necessitate blood smears taken at 24 hour intervals. The prepatent period is 10 - 14 days and peak parasitemia (in rhesus) is between the 7th and 9th day of patent parasitemia, with a characteristic 2nd rise three weeks later.

*P. cynomolgi* (Fig. 6)
Cycle in Blood - The asexual blood cycle occupies 48 hours. The young trophozoites (ring forms) exhibit a spherical nucleus, sometimes with only a wisp of cytoplasm. The nucleus is generally single but may be double; double infection of the red cell is not uncommon. The erythrocyte is enlarged and Schüffner's stippling and pigment become prominent as the trophozoite develops. Mature schizonts contain 14 - 20 merozoites; the usual number is 16. The cytoplasm of the macrogametocyte is compact and the pigment is heavy and scattered. The nucleus is compact, eccentrically located and may have a dense, deep staining portion. The nucleus of the microgametocytes is diffuse, taking up most of the parasite. The cytoplasm stains reddish purple in contrast to the light blue of the macrogametocytes.

Figure 6. *Plasmodium cynomolgi* cycle in blood. 1: Normal red cell. 2 - 7: Young trophozoites. 8 - 13: Growing trophozoites (double infection in 13). 14,15: Nearly mature and mature trophozoites. 16 - 20: Developing schizonts. 21 - 23: Nearly mature and mature schizonts. 24: mature macrogametocyte. 25: Mature microgametocyte. - To view this image in full size go to the IVIS website at www.ivis.org . -

Course of Infection - The prepatent period is from 7 to 16 days with an average of 9.8 days. Peak parasitemias are on the order of 200,000/mm³ by day 8. The parasitemia will generally fall in spleen-intact animals to 18,000/mm³ by day 13 and gradually move downward with fluctuations to approximately 500/mm³ by day 60, depending on the parasite isolate and species of monkey. Animals which succumb to infection will usually do so at the second peak parasitemia around day 16. The B and M strain are the best studied and a monograph by Schmidt [6] is perhaps the definitive source of information on the course of infection in this parasite. *P. cynomolgi* infections will relapse at fixed intervals from latent hepatic tissue stages (hypnozoites). The first relapse may appear as early as day 20 after therapy with a blood schizonticide. The number and frequency of relapses is a function of the number of sporozoites inoculated and may be quite high.

*P. fieldi* (Fig. 7)
Cycle in Blood - The asexual blood cycle is 48 hours. Young trophozoites (ring forms) are about 3 µm in diameter and may have double chromatin bodies; multiple infections of the red cell are not common. Older trophozoites enlarge the red cell slightly and Schüffner's stippling takes a deep red stain. Some host cells are
oval shaped. Immature schizonts exhibit deep blue staining with large red nuclei. The pigment is black and granular. Stippling is heavy and as schizogony proceeds, the eosinophilic masses come together to form a deep red border around the developing schizont. The host cell may be appreciatively enlarged (ballooned out). Mature schizonts produce 4 to 16 large merozoites (mean=12). The brown pigment forms a large mass in the center of the schizont and the host cell may become greatly distorted; this is distinctive for this parasite. The macrogametocytes exhibit a deep red eccentrically placed nucleus seen against a deep blue cytoplasm. The microgametocytes show dark pink cytoplasm with a reddish nucleus containing a deep red bar shaped mass. The pigment granules are heavy and the host cell shows pronounced stippling.

Figure 7. *Plasmodium fieldi*, cycle in blood. 1: Normal red cell. 2 - 4: Young trophozoites. 5 - 10: Growing trophozoites. 11 - 13: Nearly mature and mature trophozoites. 14 - 19: Developing schizonts with typical host cell distortion. 20,21: Mature schizonts with host cell "ballooning". 22,23: Developing and mature macrogametocytes. 24: Mature microgametocyte. - To view this image in full size go to the IVIS website at www.ivis.org . -

Course of infection - Experimental, sporozoite induced infections in spleen-intact rhesus monkeys showed peak parasitemias of 9,000/mm$^3$ to 20,000/mm$^3$ by day 9 and then a rapid decline to a minimal level (<500/mm$^3$) by day 30. *P. fieldi* has been shown to exhibit true relapse and may recur after adequate blood schizonticidal therapy. The number and frequency of relapses are thought to be a function of the number of sporozoites inoculated.

*P. fragile* (Fig. 8)

Cycle in blood - The asexual blood cycle occupies 48 hours. The young ring forms are delicate and may occur as multiple infections in the host red cell. Older ring forms may display an accessory chromatin dot or the chromatin bodies may number up to 3 or 4. Developing trophozoites exhibit numerous black pigment granules with a yellow sheen. Schizonts do not fill the host cell and generally lie to one side. Mature schizonts produce up to 18 or 19 merozoites with an average of 16. Toward the end of schizogony there is a marked cell distortion and the pigment accumulates in a bulky mass. Gametocytes are abundant and tend to be oval or spherical. The microgametocyte may assume irregular shapes but always displays a prominent, diffuse red staining nucleus. Macrogametocytes show an eccentrically located red nucleus and pigment that appears "shattered" or "disintegrating".

Figure 8. *Plasmodium fragile*, cycle in blood. 1: Normal red cell. 2 - 7: Young trophozoites. 8-10: Growing trophozoites. 11,12: Mature trophozoites. 13 - 22: Developing schizonts. 23 - 26: Nearly mature and mature schizonts. 27,28: Immature and mature microgametocytes. 29,30: Mature macrogametocytes. - To view this image in full size go to the IVIS website at www.ivis.org . -

Cycle in blood - The infection may be rapidly fatal in rhesus monkeys (mortality = 33%) with parasitemias reaching $10^9$ /mm$^3$ by day 10 in sporozoite-induced infections. In *M. fasicularis* inoculated with parasitized blood, the animals exhibited low, though persistent parasitemias. A pattern of deep circulation shizogony, marked by alternate days of high and low parasitemia, may necessitate daily blood smears.

*P. gonderi* (Fig. 9)

Cycle in blood - The asexual blood cycle occupies 48 hours. Merozoites prefer to invade reticulocytes and the developing ring forms are often 2 - 4 per red cell. Schüffner's stippling appears in the cytoplasm with further growth of the parasite and the host cell becomes enlarged and distorted. Mature trophozoites show a deep blue staining cytoplasm, a large irregular red staining nucleus, and pigment in small aggregates. Pigment in the
developing schizont is more condensed and the stippling is prominent. Older schizonts fill the red cell and show purple cytoplasm with reddish nuclei on the periphery of the parasite. The mature schizont may not fill the host cell and may contain 12 - 20 merozoites with an average of 16. The cytoplasm of the host cell is hypochromic almost to the point of being inapparent so that the schizont may appear free. Microgametocytes stain a light purplish pink, with dark pigment granules scattered in the cytoplasm. The macrogametocyte stains deep blue with scattered pigment.

![Figure 9. Plasmodium gonderi, cycle in blood. 1: normal red cell. 2 - 4: Young trophozoites. 5 - 11: Growing trophozoites. 12 - 15: Mature trophozoites. 16-20: Developing schizonts. 21 - 23: Mature schizonts. 24: mature macrogametocyte 25: Mature microgametocyte.](www.ivis.org)

**Course of Infection** - In sporozoite-induced infections, peak parasite counts are on the order of 190,000/mm$^3$ at day 10 of patent parasitemia. The counts then decline to a low level (about 350/mm$^3$). Animals do not generally require chemotherapy for survival.

**P. inui** (Fig. 10)

**Cycle in blood** - The asexual blood cycle occupies 72 hours. The earliest ring forms are 2 to 3 µ in diameter usually with a single fairly large nucleus. The developing ring forms are amoeboid with stippling and pigment evident. Band forms are not uncommon and the host cell may be somewhat enlarged. Young schizonts appear after 48 hours. Mature schizonts produce up to 18 merozoites with an average of 12. Young gametocytes are compact and have little amoebodity and the pigment is more abundant that in the asexual forms. The cytoplasm of the macrogametocyte stains a delicate steel blue with scattered pigment. The microgametocyte is distinctive with reddish purple cytoplasm overlaid with scattered brown pigment granules.

![Figure 10. Plasmodium inui, cycle in blood. 1: Normal red cell. 2 - 4: Young trophozoites. 5 - 10: Growing trophozoites. 11 - 15: Older and mature trophozoites. 16 - 19: Developing schizonts. 20 - 23: Almost mature and mature schizonts. 24: Mature macrogametocyte. 25: Mature microgametocyte.](www.ivis.org)

**Course of Infection** - Although *P. inui* infects many species of monkeys, most studies are confined to rhesus macaques. Infections in this species tend to be moderate and long lasting, many animals maintaining the infections for many years. Sporozoite-induced infections rose to peak parasitemias of 15,000/mm$^3$ by day 14 and dropped to a level of approximately 2,500/mm$^3$ by day 14. Infections in M. fasicularis and M. radiata monkeys are usually less severe than those in the rhesus macaque [10]. There is no evidence that *P. inui* possess a relapse mechanism but infections may appear in response to stress from shipping.

**P. knowlesi** (Fig. 11)

**Cycle in blood** - The asexual blood cycle is quotidian, occupying 24 hours, which is unique among the primate malarias. The young ring forms may appear in large numbers in the peripheral blood. Appliqué forms are common along with regular rings harboring one or more accessory chromatin dots. Band forms are often seen and stippling (though not Schüffner's) appears with the maturation of the trophozoites. Mature schizonts show an average of 10 but as many as 16 merozoites. The gametocytes display a striking color difference from the asexual forms. The macrogametocyte stains a distinctive blue and the eccentric nucleus takes a deep pink stain. The microgametocyte shows medium pink cytoplasm with the dark pink nucleus making up half the
body of the parasite. There is abundant jet black pigment scattered throughout the cytoplasm.

Course of Infection - In the rhesus macaque, *P. knowlesi* produces a fulminating, often fatal infection. The prepatent period is approximately 6 days with the parasitemia exhibiting a first peak around day 11. Peak parasitemias are on the order of 3.5% to as much as 12% in spleen intact animals. Mean time of death in experimentally infected rhesus macaques was 13.6 days [8]. Splenectomized animals may exhibit parasitemias of up to 45%, and this is invariably fatal without immediate treatment.

*P. simiovale* (Fig. 12)

Cycle in blood - The asexual blood cycle is 48 hours. The ring form trophozoites are delicate and may display an accessory chromatin dot. The erythrocyte becomes enlarged and distorted and stippling becomes granular and prominent. Multiple invasion of the host cell is not uncommon. As schizogony proceeds, small, cleft-like vacuoles appear in the cytoplasm; the pigment remains granular. Some host cells are greatly enlarged and display an eosinophilic ring. Mature schizonts produce 12 - 16 merozoites in a host cell that is generally distorted. Spherical gametocytes appear early in the initial parasitemia. The macrogametocytes, which outnumber the microgametocytes, stain a deep blue and exhibit scattered granular pigment. The microgametocyte stains red to pink and completely fills the host cell.

Course of Infection - The infection is generally mild in *M. sinica* and *M. mulatta*, with the parasitemia never reaching a high level. Maximum counts are on the order of 21,000 - 32,000/mm³ [8]. After the initial peak, parasitemias quickly drop to very low levels. *P. simiovale* is a relapsing malaria and hypnozoites have been demonstrated [11].

B. Malaria Parasites of Apes (*Hylobatidae* and *Pongidae*)

*P. eylesi* (Fig. 13)

Cycle in blood - The asexual cycle in the blood is a highly synchronous 48 hours. Immediately after the parasite enters the host cell the cell becomes enlarged and Schüffner's stippling is evident but not pronounced. Multiple infections are by far the most common aspect in the early part of the cycle with as many as 6 rings in a single cell. The mature trophozoite enlarges the host cell and produces a granular pigment. Older schizonts are frequently oval with deep bluish red cytoplasm. The number of merozoite ranges from 20 to 34 with an average of 25. Mature macrogametocytes stain grayish blue and exhibit coarse granular pigment. The microgametocytes are in an enlarged oval host cell which takes a very dramatic, deep, brilliant reddish-purple stain.
**Course of Infection** - The course of infection has only been studied in a few gibbons. In blood induced infections, the parasitemia increased rapidly and at the end of the first week was 20,000/mm$^3$ where it remained until day 20. From there until day 40 the count averaged 10,000/mm$^3$ and thereafter declined slowly. In a sporozoite-inoculated animal ring forms appeared in the blood on day 12. The parasite count increased from a 2nd day count of 401/mm$^3$ to a peak on day 8 of 40,900/mm$^3$ [12].

*P. hylobati* (Fig. 14)

*Cycle in blood* - The asexual blood cycle occupies 48 hours. A prominent nucleus and a small fragment of cytoplasm constitute the earliest stage. The cytoplasm increases and multiple chromatin masses are not uncommon. The nucleus begins to change as the trophozoite develops, stretching around the vacuole or branching as it grows. Pigment is present though not prominent; Schüffner's stippling has not been reported. The mature schizont has from 12 - 20 merozoites, with 14 - 16 the most common number. The mature macrogametocyte stains light blue and has a dense, circular to oval nucleus which is usually located at the periphery of the cell. The microgametocyte exhibits a large, diffuse, pink nucleus in a pink-staining cytoplasm.

**Course of Infection** - Very little is known about the course of infection in the natural host (the gibbon), except that it does become latent and may be provoked to exacerbation following splenectomy. In experimental infections in spleen-intact rhesus macaques the infection is transient and eliminated in a few weeks. Splenectomized rhesus inoculated with parasitized blood showed high parasitemias (28%) which persisted for up to four months.

*P. jefferyi* (Fig. 15)

*Cycle in blood* - The asexual cycle in the blood occupies 48 hours. The earliest ring forms display a deep red chromatin dot, occasionally an accessory dot, and a delicate circle of blue-staining cytoplasm. The host cell is not enlarged. Older trophozoites are frequently paisley-shaped with the nucleus sometimes double; the cytoplasm stains a pale blue. The cytoplasm of young schizonts is also pale blue and stippling is absent. The older schizonts are compact and do not fill the host cell. Mature schizonts contain 10 - 18 nuclei; the gold black pigment is clumped. Macrogametocytes stain light blue with a bright red nucleus. Microgametocytes stain reddish-pink with a dark rosé nucleus located at the small end of the parasite.
Course of Infection - This parasite has been observed in only a few gibbons. Infections appear to be of a low level. In splenectomized animals, however, the early and/or acute stages appear to be fulminating in character and may be fatal. In one splenectomized gibbon the parasitemia reach 280/mm³ on day 8. The animal became weak and lethargic; treatment with antimalarials was necessary.

*P. pitheci* (Fig. 16)

Cycle in blood - The earliest forms consist of a deep purple nucleus and a wisp of blue gray cytoplasm, which may appear as a ring or as an elongate smudge. As growth proceeds pigment appears in the form of greenish-brown granules; there is no appreciable host cell enlargement. The developing schizont usually fills the host cell and stippling remains sparse. The mature schizont has 12 - 14 merozoites. The mature macrogametocyte stains grayish-blue with a large, dark staining, eccentrically-located, nucleus. The microgametocyte exhibits a vacuolated, bluish-gray staining cytoplasm and a large, wine-red nucleus.

Course of Infection - The course of infection, as far as is known, runs a chronic course with little if any pathology. The temperature in infected animals was within normal range. Death of one orangutan was ascribed to *P. pitheci* [13] but a thorough post mortem was not done on the animal.

*P. reichenowi* (Fig. 17)

Cycle in blood - The asexual blood cycle occupies 48 hours. The parasite closely resembles *P. falciparum* of man, with crescent-shaped gametocytes and usually only ring stages and gametocytes appearing in the peripheral circulation. The youngest parasites are small rings with a prominent nucleus. A consistent feature is the presence of marginal forms with single or double nuclei; accolé forms are common. The mature schizonts contain 10 - 12 merozoites, but may not be seen in the peripheral circulation, unless the animal is splenectomized. The mature macrogametocyte is crescent-shaped and somewhat slender with pale blue cytoplasm and a red-staining nucleus. The microgametocyte is more robust with bluish-red cytoplasm and a diffuse red-staining nucleus.
Course of Infection - Little is known about the early course of naturally-occurring infections. There is only meager information about naturally-infected and blood-inoculated captive animals.

**P. schwetzi** (Fig. 18)

**Cycle in blood** - The parasite has a 48 hour asexual cycle in the chimpanzee. The earliest ring forms are relatively compact with a dark, round to oval nucleus. Growing parasites enlarge the host cell and stippling is abundant in the older trophozoite stage. The mature schizont has from 12 - 16 distinct nuclear masses and the red cell may be distinctly oval shaped. The macrogametocyte stains uniformly blue with a peripheral wine-red nucleus. The microgametocyte is brightly colored with a reddish-purple cytoplasm and a large, diffuse wine-red nucleus. The pigment is coarse, black to greenish black and the cytoplasmic edge of the parasite is crenated or lace-like.


Course of Infection - *P. schwetzi* appears to invoke no clinical signs, even in young chimps, nor in splenectomized older animals with high parasitemias. It generally occurs as a mixed infection with *P. reichenowi* and *P. rodhaini*.

**P. silvaticum**

**Cycle in blood** - The youngest ring forms are less than 2 µm in diameter with a prominent, round, dark-staining nucleus, a vacuole and a hair-like circle of cytoplasm. Two or three rings in a single RBC are not uncommon. As the parasite develops, the red cell begins to enlarge; the larger trophozoites are found in red cells measuring 10 µm in diameter. At this stage the stippling is intense; the dots are deep pink in color, closely and evenly distributed, and regular in size becoming coarser as the parasite grows. After 30 - 36 hours of development in the peripheral blood schizonts begin to appear. Up to 20 nuclei have been observed in mature schizonts although merozoites have not been described. The mature microgametocyte is characterized by abundant, dark brown pigment, with a large nucleus (6 µm) seen against a red-hued cytoplasm. The mature macrogametocyte is round to oval with brown bacilliform pigment [14].

Course of Infection - Little is known about the course of infection in sporozoite-induced infections in the orangutan.

**P. youngi** (Fig. 19)

**Cycle in blood** - The asexual blood cycle is 48 hours. The young ring forms measure about 2 µm. Schüffner's stippling appears as the young forms mature; host cells are not enlarged and the cytoplasm stains pale blue. The older schizonts appear to have depleted the host cell of cytoplasm as it stains poorly. There are 12 - 20 merozoites with an average of 14. The macrogametocyte stains pale blue with an eccentric, deep red nucleus. The microgametocyte stains reddish-purple with a large deep reddish-pink nucleus.

Course of Infection - Only blood-induced infections in gibbons have been studied to any appreciable extent. The median parasitemia curve for 12 such animals shows a peak count of 30,000/mm³ occurring on day 13. The parasite level then declined to 100/mm³ by day 50 followed by a secondary rise. Eyles reports that infections in these animals are more severe than is usually seen in malaria infections of lower primates [15].

C. Malaria Parasites of New World Monkeys (Cebidae)

**P. brasilianum (Fig. 20)**

*Cycle in blood* - The asexual cycle require 72 hours. The early trophozoites are small rings with a prominent dark staining nucleus. The parasite nuclei may elongate to form a part of the periphery of the older rings (Fig. 6, Fig. 7) and the ring forms often exhibit accessory dots. Band forms appear during the late trophozoite stage, especially in *Alouatta*. The host cell is enlarged (as opposed to *P. inui*) and each mature schizont produces 8-12 merozoites. There may be only four or up to 16 arranged in a rosette or "daisy head" (Fig. 20). Sexual forms may be scarce. The macrogametocyte which stains a median blue, shows a compact, eccentric, pink-staining nucleus with a dark reddish area. Dark rods are scattered throughout the cytoplasm. The microgametocyte exhibits a large diffuse, dark pink nucleus and exhibits large pigment granules scattered throughout the cytoplasm.

**Figure 20. Plasmodium brasilianum, cycle in blood. 1: Normal red cell. 2 - 5: Young trophozoites. 6 - 13: Growing trophozoites. 14 - 23: Nearly mature and mature trophozoites (note band forms, 16, 19). 24 - 26: Developing schizonts. 27, 28: Mature schizonts. 29: Mature macrogametocyte. 30: Mature microgametocyte. - To view this image in full size go to the IVIS website at www.ivis.org. -**

Course of Infection - Blood -induced infections (and presumably sporozoite-induced as well) as described by Taliaferro and Taliaferrro [16], showed a slow initial rise in the number of parasites to 104 /mm³, depending on the species infected. This peak was followed by a marked diminution in numbers, a low grade blood infection, and then short periods of subpatent parasitemia. The height of parasitemia tended to be more acute in *Cebus capucinus* and *Ateles geoffroyi* than among *Alouatta villosa*. Sharp peaks in temperature occurred, particularly in the spider, howler and night monkey (*Aotus trivirgatus*) and in the tamarin (*Saguinas geoffroyi*).

**P. simium (Fig. 21)**

*Cycle in blood* - The asexual cycle in the blood is 48 hours. The ring forms exhibit a compact dark red nucleus and often show a double chromatin dot. Schüffner's stippling is prominent in all but the youngest rings. The young schizonts are marked by the disappearance of the vacuole; the nucleus elongates and divides and small pigment granules appear in the cytoplasm. In older schizonts the nuclei take a deep reddish-purple stain, are large, oval or crescent shaped. The macrogametocytes have a small dense staining red nucleus, deep blue cytoplasm and scattered granules of pigment. The microgametocytes exhibit a diffuse pink nucleus, pale blue cytoplasm and small diffuse pigment granules.

**Figure 21. Plasmodium simium, cycle in blood. 1: Normal red cell. 2 - 8: Young trophozoites. 9 - 12: Growing trophozoites. 13 - 15: Mature trophozoites. 16 - 18: Young schizonts. 19 - 21: Older schizonts. 22 - 26: Nearly mature schizonts. 27: Mature schizont. 28, 29: Young adult and mature macrogametocyte. 30: Mature microgametocyte. - To view this image in full size go to the IVIS website at www.ivis.org. -**

Course of Infection - Infections in *Alouatta fusca* are characterized by mild signs and moderate to light parasitemias. Splenectomized animals show increased parasitemia, anemia, hair loss, diarrhea and fever as
high as 41.5°C, although these signs may have been caused by failure to accept food by the howler monkeys in captivity [17]. Severe infections can be obtained in splenectomized Saimiri and less severe infections have been reported from A. paniscus and young A. fusca and mild infections in Lagothrix lagotricha. The marmoset (Callicebus jacchus) was found susceptible but the infection was of a low order [18].

D. Malaria Parasites of Lemurs

Little is known about the malaria parasites of lemurs. P. foleyi, often reported, is probably a Hepatocystis since only gametocytes are seen in peripheral blood. P. girardi and P. lemuris are very similar and some authors consider them to be conspecific.

P. girardi (Fig. 22)

Cycle in blood - The youngest forms are small solid bodies approximately 1.8 µm in diameter with a large nucleus and a small rim of cytoplasm which stains a deep blue. These stages exhibit a granule of dark pigment which lies on the edge of the cytoplasm. The amount of pigment increases as the parasite matures. The nucleus becomes enlarged and very small vacuoles can be seen in the cytoplasm. In the developing schizont the host cell cytoplasm becomes pallid and distorted. The mature schizont contains 10 - 12 merozoites; stippling is absent. The macrogametocyte is spherical and stains a deep blue with a deep red nucleus. The microgametocyte takes a lilac stain and the peripherally-located nucleus stains a deep red.

Course of Infection - Little is known about the course of infection save that the spleen appears normal in infected animals; splenectomy will allow the infection to come to the fore, after which it subsides.

P. lemuris (Fig. 22)

Cycle in blood - The young trophozoites are small with a rose-red nucleus. The pigment is in granules and there is no evidence of stippling. The schizonts are in enlarged and distorted erythrocytes and display irregularly shaped nuclei. The mature gametocytes are large and irregular in shape. The nuclei are band-like or lobed. The host erythrocyte is greatly enlarged. The macrogametocyte has lavender to purple cytoplasm; the microgametocyte has red-staining nuclei.

Course of Infection - Little is known about the course of infection in the natural host save the morphology of the blood stages.

Piroplasms

The piroplasms of non-human primates are little described. Entopolypoides is the most often reported from rhesus macaques, chimpanzees, Cercopithecus and baboons (Papio cynocephalus). It will infect a wide variety of non-human primates.

Entopolypoides macaci

Cycle in blood - The organisms are smaller that most Babesia species of wild and domestic animals [3]. There are often 2 or more organisms per cell and accolé forms predominate [19]. They are seen as thin dots with a thin dark blue rim of cytoplasm. Older forms are amoeboid with faintly staining cytoplasm. They are markedly pleomorphic and do not form pigment.

Course of Infection - E. macaci appears not to affect the health of baboons, although there may be substantial alterations in hemoglobin and red cell counts [19]. Splenectomy alters the clinical picture by allowing the number of parasites to increase dramatically.
**Hepatocystis**
The state of Hepatocystis taxonomy is still uncertain. It may be that Hepatocystis is a species complex and the slight morphological differences are due to the host species. Garnham [10] has assigned *H. kochi* to *Cercopithecus* sp., *Cercocebus* sp. and *Colobus* sp.; *H. simiae* to *Papio* sp.; *H. bouillezi* to *Cercopithecus mona*; *H. cercopithecii* to *Cercopithecus nictitans* and *C. neglectus*. Here we will only consider *H. kochi* as a type species.

**Hepatocystis kochi** (Fig. 23, Fig. 24, Fig. 25)
The youngest parasite consists of a minute dense spot of chromatin with a tiny loop of cytoplasm. With growth, the chromatin tends to spread in a semicircle or into multiple dots. There is no stippling of the red cell. When mature the parasite is larger than a normal RBC. The mature gametocyte stain poorly in relation to other protozoa. Giemsa-stained preparations reveal a macrogametocyte with a steel blue color; the microgametocyte is a less dense, biscuit-colored object. The nucleus consists of two portions in both male and female gametocytes. In the male there is a large, oval, pale pink area occupying one-third to one-half of the parasite. The nucleus of the female is much smaller and composed of a pale pink area with dense chromatin in the middle. The mature macrogametocyte measures 9.5 µm; the microgametocyte 9.0 µm [10].

![Figure 23. Giemsa stained preparations of Hepatocystis kochi from Cercocebus aterrimus and Cercopithecus aethiops. 1 - 4: Immature gametocytes from Cercocebus. 5,6: Microgametocytes from Cercocebus. 7 - 9: Macrogametocytes from Cercocebus. 10 - 13: Immature gametocytes from Cercopithecus aethiops. 14,15: Immature and mature macrogametocytes from *C. aethiops*. 16: Mature microgametocyte from *C. aethiops*. 22: normal RBC. (After Garnham, 1966).](image)

![Figure 24. Section through a mature schizont of Hepatocystis kochi in Cercopithecus aethiops. (Photo courtesy G. Baskin).](image)

![Figure 25. Schizont of Hepatocystis kochi in the liver of Cercopithecus aethiops (arrow). These may be seen grossly at necropsy in many monkeys of African origin. (Photo courtesy G. Baskin).](image)

**Course of Infection** - Development of the merocyst in the parenchyma of hepatic cells occupies approximately 2 months (Fig. 24, Fig. 25), representing the shortest interval for parasites to appear in the blood after infection. The prepatent period ends with the invasion of the blood by the merozoites of the pre-erythrocytic schizont. This becomes apparent at approximately 66 days post invasion. The gametocytes double in number daily for 3 days and approach maturity. After this time they decline in numbers and disappear in less than a month. Secondary invasions from the liver usually take place and monkeys in captivity have kept their infections for many years. No clinical signs have been ascribed to the parasite; it appears relatively benign in monkeys and does not require treatment.

**References**
4. Baskin GB. Pathology of Nonhuman Primates.
7. Markell EK, John DT, Krotoski WA. In: Medical Parasitology. EDITORS MISSING Philadelphia: W.B. Saunders Co. 1999. - Amazon.com -

All rights reserved. This document is available on-line at www.ivis.org. Document No. A0304.0600 .