

Chapter - 2

Life cycle of malaria parasites

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Introduction

Plasmodia have arisen from the Coccidian stem (table 1) and share most of the typical features of the *Apicomplexa*. They probably represent an evolutionary progress in respect of ancestors (of the *Eimeriidae* family) who just develop in the intestinal tract and have a single host. Plasmodia, in fact, present a sexual multiplication phase in the insect vector, a first asexual multiplication phase in the tissue of the vertebrate host, followed by the main asexual multiplication phase in the blood.

There are four species of Plasmodium of medical interest: *P.vivax*; *P.ovale*; *P.malariae*; *P.falciparum*. Some characteristics of the four species of human Plasmodia are summarized in table 2. *P.falciparum* belongs to the subgenus *Laverania*, while the remainder three species of human malaria belong to the subgenus *Plasmodium*. Although subgeneric designation is rarely used, the distinction is important because it seems probable that the two subgenera present profound phylogenetic differences with relevant clinical significance.

The comprehensive life-cycle of the species of Plasmodium occurring in man is shown in diagrammatic form in figure 1.1. It comprises the exoerythrocytic (B1 to B7) and erythrocytic stages in the vertebrate host (C1 to C8) and the sporogonic cycle in the mosquito (A1 to A10). The humans and other vertebrates act as the intermediate host for the parasite,

while the mosquito, in which the sexual reproduction takes place, is considered to be the definitive one.

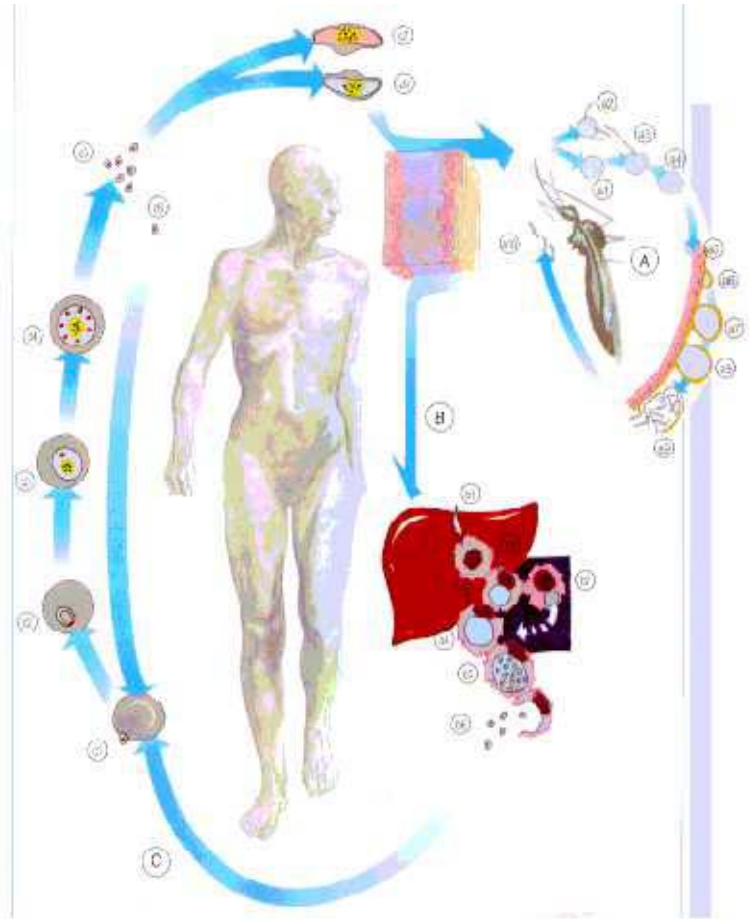


Fig. 1

The exoerythrocytic stages of malaria parasites in humans

Malaria infection in the human host starts when the **sporozoites** are injected into the blood stream during a blood meal by an infectious mosquito. Although it is assumed that one single sporozoite is capable of initiating the infection in man, the number of sporozoites injected by a mosquito bite is supposed to vary from dozens to thousands. It is likely that this number strongly affects the clinical picture: the greater the sporozoite load, the shorter the incubation period and the more serious the symptoms. The sporozoites remain in the circulation for a short period, calculated as 60 minutes at maximum, before they actively enter the liver of the host (Lopez-Antunano, 1980). The Kupffer cells in the liver may be invaded (or the parasite may be phagocytosed) but the sporozoites are not able to develop in those cells and die shortly after invasion. Most parasites however invade the hepatocytes and start the asexual **exo-erythrocytic schizogonic cycle**. The cycle has been studied in detail in liver sections from a rodent model for malaria infection, but observations from liver biopsy in human volunteers are also available for *P.falciparum* and *P.vivax*. The liver sporozoite initially appears as a mononucleated round body in the cytoplasm of the host cell; subsequently it begins to develop and multiply asexually, a mature **schizont** (the multinucleated stage of the parasite) is formed, and finally a large number of **merozoites** are released. The mature schizont is 30-70 μm large, has no pigment (there is no hemoglobin in the hepatocyte), and occupies the entire cell cytoplasm. The length of the schizogonic liver cycle is constant for each Plasmodium species to the extent that it can be considered a taxonomic character: this is the above mentioned prepatent period (5.5 days, 8, 9, and 15 days for *P.falciparum*, *P.vivax*, *P.ovale*, and *P.malariae* respectively). The number of merozoites produced at the end of the cycle is also species dependent: it is estimated as 2,000 for *P.malariae*, 10,000 for *P.vivax/P.ovale*, and up to 30,000 for *P.falciparum* (Garnham, 1966). The liver cycle ends when the mature schizont ruptures and releases the merozoites into the sinusoids of the liver. Released merozoites can only invade a red blood cell: the theory of the continuation of the liver cycle by invasion of a new hepatocyte by the merozoite, is not any more accepted at present.

Biological basis for relapses

Two species of human malaria determine a relapsing infection: *P.vivax* and *P.ovale*. In these two species some of the liver trophozoites immediately start the exo-erythrocytic schizogonic cycle which has been described above, while others remain into the liver in a latent (dormant) stage for varying periods of time and are termed **hypnozoites** (Krotoski, 1985). The length of the period of dormancy varies with the subpopulations of *P.vivax* and *P.ovale*: the relapse, in other words, is not triggered by host factors (waning of immunity) but rather seems to be a genetically determined intrinsic property of the parasite. A single inoculation of sporozoites of a relapsing species contains a mixture of genetically distinct parasites that give rise to discrete subpopulations of exoerythrocytic trophozoites. The number of relapses, as their periodicity, seems to be a characteristic of the parasite strain. At one side of the spectrum of possibilities is the *P.vivax hibernans* strain who has a homogeneous population of sporozoites characterized by a latency of 250 days or more. At the other side is *P.vivax chesson* strain with an heterogeneous population of sporozoites, some programmed for immediate development, others to determine relapses at intervals of 2 to 3 months for a period of up to 2 years (Coatney, 1950). Actually, the two group of strains are considered to differ to the extent to justify their separation into two subspecies (Garnham, 1966).

The erythrocytic stages of malaria parasites in humans

The erythrocytic stages of malaria parasites has several important implications in clinical practice: first, this is the only stages causing the complex and varying spectrum of symptoms characterizing the disease in humans; secondarily, the recognition of parasites in the blood of a patient allows the diagnosis of the infection and the differentiation of the various species of the causing agent.

The time required to complete the erythrocytic cycle is a fixed characteristic of the parasite species (table 2): *P.falciparum*, and *P.vivax*, have a 48-hour development period, in *P.ovale* it lasts 50 hours, while *P.malariae* has a longer cycle of 72 hours. Theoretically the periodicity

of the erythrocytic cycle would determine the classical cyclical presentation of symptoms every other day in tertian malaria infections and every third day in quartan malaria. In practice, however, the typical periodicity of malaria paroxysm cannot be recognized in the initial periods, since most parasite populations are heterogeneous and continuous fever therefore results from the completion of asynchronized schizogonic cycles.

The blood phase of the life-cycle is initiated when the merozoites from liver schizonts are discharged into the circulation (Garnham, 1988). The **merozoite** is 1 μm in diameter, consisting of a single nucleus and adjacent cytoplasm. It invades almost immediately an erythrocyte to enter its **trophozoite** stage. A vacuole is produced by the parasite which assumes the characteristic **ring form** (the young trophozoite). Within 12-24 hours, as the parasite grows, the cytoplasm expands, the vacuole slowly disappears and a characteristic parasitic pigment becomes visible within the cytoplasm. At the end of this phase the trophozoite has a single nucleus, a large cytoplasm, no vacuole, and a variable amount of pigment. The nucleus starts to divide approximately 30 hours after invasion in the case of *P.falciparum*, *P.vivax*, and *P.ovale*, while in *P.malariae* this requires approximately 40 hours. As nuclear division produces two or more nuclei the parasite enters the stage of a **schizont**. Nuclear division continues until an appropriate number of merozoites is produced: approximately 36 for *P.falciparum*, 24 for *P.vivax* and *P.ovale*, 12 for *P.malariae*. At the end of this phase the schizogonic cycle is completed, the erythrocyte ruptures releasing the merozoites into the blood stream and determining the typical malaria paroxysm. The merozoites discharged into the circulation invade new erythrocytes to repeat the schizogonic cycle until the process is inhibited by the specific immune response or by chemotherapy. In the course of a schizogonic cycle (within a red blood cell) some of the merozoites become differentiated into sexual forms (the gametocytes); the mechanisms at the basis of this differential development are unknown. Gametocytes appear early (approximately from the third generation) in infections caused by *P.vivax*, *P.ovale*, and *P.malariae*, while at least 10 generations are thought to be required before *P.falciparum* gametocytes appear in the blood, which probably reflects the slow maturation and the sequestration of the immature stages in this species (Carter, 1980).

The morphologic characteristics of the stages of malarial parasites in human blood are described in details in another section of the book. The first stage of the maturation process is

the ring form. In *P.falciparum* (figure 1.2) the ring form has variable size, the smallest are only 1.2 μm in diameter, tend to adhere to the internal surface of the erythrocyte (accolè form, marginal forms) and the nucleus is often divided to show two chromatin dots. As the cytoplasm becomes fleshy and the vacuole reduces in size, the parasites disappear from the peripheral circulation. The ring form is the only asexual stage usually identifiable in the peripheral blood of patients with *P.falciparum* infection (gametocytes may be present after the second week of patent asexual parasitaemia) because more mature stages of this species adhere to the endothelium of post-capillary venules in the tissues. *P.vivax* and *P.ovale* ring forms (figure 1.3 and figure 1.4) have similar features, but the cytoplasm may early present amoeboid tendency and traces of stippling start to appear on the parasitized erythrocyte. In these species trophozoites are seen in the peripheral circulation: while the parasite mature the cytoplasm enlarges and presents amoeboid pseudopodes (the erythrocyte enlarges as well to contain the parasite), the vacuole disappears, the pigment (product of haemoglobin catabolism) becomes prominent, and the stippling are profuse. *P.malariae* trophozoites (figure 1.5) are regular in shape (except those assuming a characteristic band form across the erythrocyte), with a small vacuole, early and abundant pigment, no stippling.

Mature schizonts in *P.vivax/ovale* present a large number of merozoites irregularly arranged and occupying the whole of the red blood cell, with more and more prominent stippling. In *P.malariae* a smaller number of merozoites are often regularly arranged around the central mass of pigment (daisy shape) and no stippling is present.

Gametocytes of *P.vivax/ovale* and *P.malariae* are morphologically similar to the late trophozoite: however, they present a completely matured parasite (no vacuole is visible) with abundant and unagglomerated pigment. *P.falciparum* gametocytes present a typical banana-like shape. Two types of gametocytes are found in the peripheral blood: the female macrogametocytes and the male micro-gametocytes. They can be differentiated by the fact that in the male parasite nuclear material is dispersed (preparing to exflagellation) while in the female parasite it is condensed.

The sporogonic cycle in the mosquito

Female mosquitoes seek a blood meal as a protein source for egg production. The cycle of blood meal and oviposition continues throughout the life of the female mosquito and requires repeated contacts with the vertebrate host, allowing for ingestion of malaria parasites, their multiplication and maturation, and transmission to other individual hosts during subsequent feedings.

The sporogonic cycle initiates when mature female and male gametocytes are ingested by a suitable species of *Anopheles* during a blood meal. As soon as gametocytes reach the midgut of the insect the female gametocyte sheds the red blood cell and remains free in the extracellular space as a **macrogamete**. The male gametocyte nucleus divides into eight sperm-like flagellated **microgametes** each of which also leaves the erythrocyte, reaches the midgut and actively moves to fertilize a macrogamete. Exflagellation of the microgametocyte is triggered by factors present in the mosquito midgut and begins about ten minutes after the blood meal. The result of the fertilization process is the **zigote**, which develops into the elongated, slowly motile **ookinete** within 18 hours from the blood meal. The ookinete actively penetrates the peritrophic membrane and the epithelium of the midgut and settles beneath the basal lamina of the outer gut wall, where it develops into a non motile **oocyst** 24-72 hours after the blood meal. The initial oocyst is 6-8 μm in diameter, has a single nucleus and a thin cyst wall; the oocyst nucleus divides repeatedly leading to the formation of as many as 10,000 new individual nuclei within a mature oocyst of 40-60 μm in diameter. The time required from the establishment of the initial oocyst to its maturation depends on the parasite species, the anopheline species and on the temperature: it varies from 7 to 30 days. The product of the mature oocyst are the **sporozoites**, narrow and curved in shape, actively motile, 10-15 μm in length. The sporozoites leave the cyst passing through small perforations without destroying the wall, at least till most of the parasites have been released, and move into the haemocoelomic space of the insect. The sporozoite migrates and reaches the salivary glands (though most tissues may be invaded) where it penetrates the basal membrane, pass intracellularly through a secretory cell and settles into the salivary duct. Oocyst sporozoites are poorly motile and less infectious and immunogenic than salivary gland sporozoites, showing that significant changes occur during the transfer journey into the hemolymph. When the mosquito feeds, the salivary fluid (which has anti-clotting properties)

and its content of sporozoites are actively injected into the vertebrate host to start another asexual replicative cycle.

Temperature has an important effect on the speed of the cycle in the mosquito, who is a poikilothermic host. Development slows down and ceases for temperature of about 16°C with *P.vivax*; at very high temperature (about 45°C) the cycle is interrupted because the parasite dies. In general, the length of the sporogonic cycle in the mosquito is an irregular variable which strongly depends on the temperature and other climatic factors.

Terminology related to the life cycle of malaria parasites

At the light of the recent advances in the understanding of the life-cycle of malaria parasites, the terminology presented below has been widely accepted and will be used in this chapter (Bruce-Chwatt, 1981).

Prepatent period is defined the time from infection by mosquito bite until the first appearance of the trophozoites in erythrocytes. The prepatent period is fixed and constant for each species of parasites. It has been calculated as 5.5 days in *P.falciparum*, 8 days in *P.vivax*, 9 days in *P.ovale*, and as long as 15 days in *P.malariae*. Measurements were done in the past on volunteers inoculated with sporozoites by mosquito bite and detection of the circulating parasites by means of subinoculation of 500 ml of the volunteer's blood at regular intervals into a second group of volunteers, to test the blood infectiousness (Fairley, 1947). In clinical practice we usually determine detectable parasitaemia by thick smears: this gives an overestimation of the prepatent period, as several intraerythrocytic schizogonic cycles are necessary to build up detectable levels of parasitaemia. The length of the prepatent period in humans have also been confirmed, in the case of *P.falciparum* and *P.vivax*, by studies on experimentally infected human volunteers who had a piece of liver removed by laparotomy at intervals after inoculation (Shortt, 1948; Shortt, 1951)

Incubation period is defined the time from infection by mosquito bite until the first appearance of clinical symptoms. It is variable but usually much longer than the prepatent period as it requires parasitaemia to reach a sufficient density before symptoms occur. The incubation period is thought to be influenced by the initial sporozoite load, and subsequently

by the load of merozoites invading the blood stream: the higher the load the shorter the incubation period. A shorter incubation period is likely to be associated to a more severe disease, as symptoms arise when specific immunity, which starts developing when merozoites are discharged in the blood, is not yet built up.

Recrudescence is the renewed clinical manifestation of the infection due to persistence of erythrocytic forms into the circulation. This is typically observed in *P.falciparum* infection exposed to subcurative drug treatment (latency of few days to few weeks) and in *P.malariae* (latency as long as several years).

Relapse is a renewed clinical manifestation of the infection started by persistent liver merozoites (hypnozoites) which start an exo-erythrocytic cycle months after the invasion of the hepatocyte. This is typically observed in *P.vivax* and *P.ovale* infection treated with drugs which have no action on the parasite liver stages.

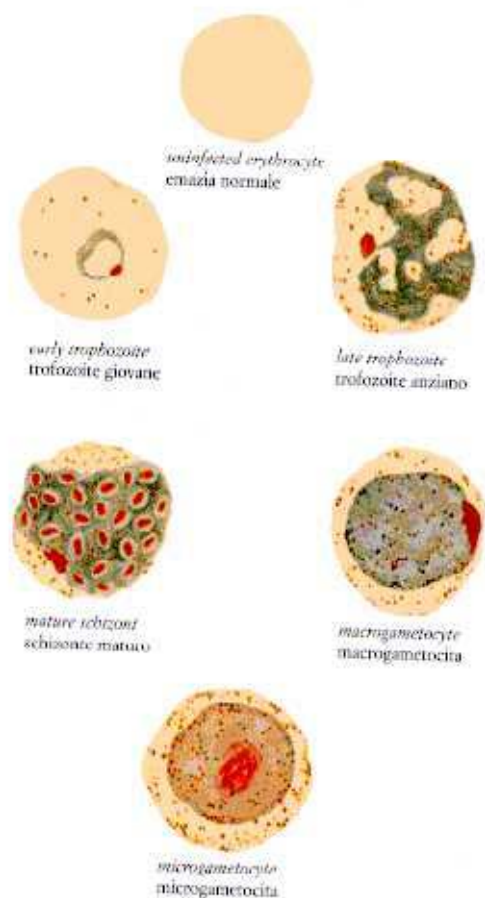


Figure 1.3 Light microscopic morphology of *P.vivax*

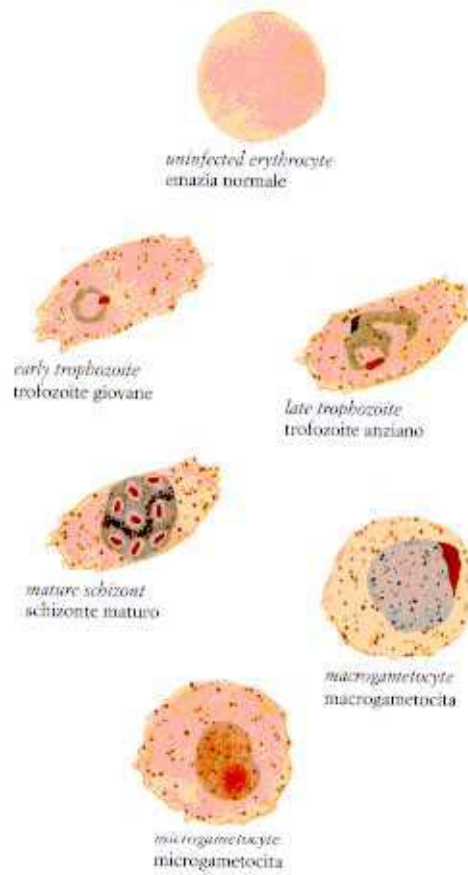


Figure 1.4 Light microscopic morphology of *P.ovale*

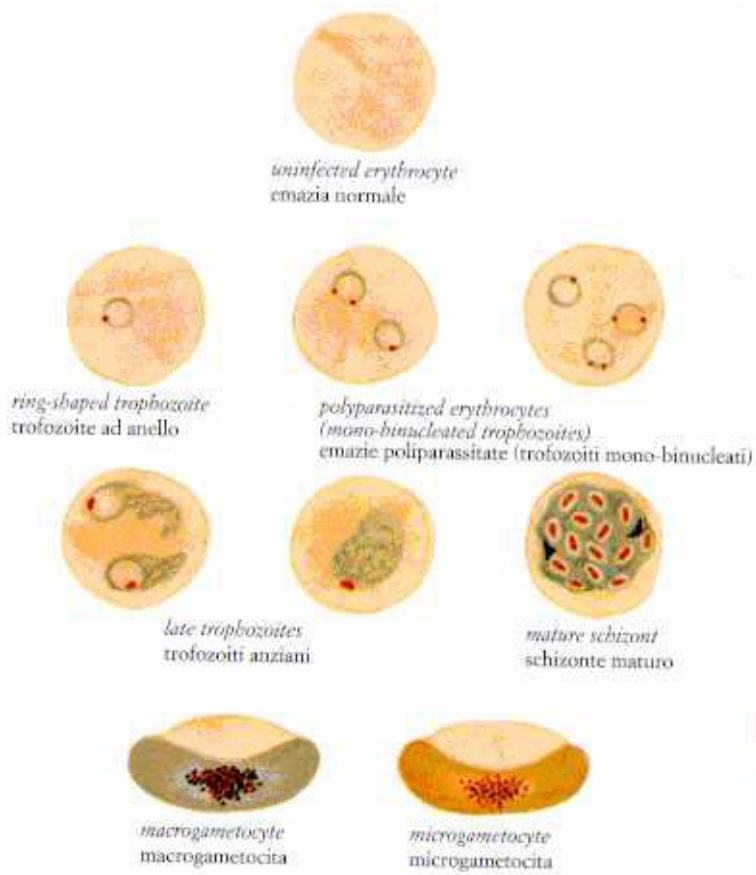


Figure 1.2 Light microscopic morphology of *P.falciparum*

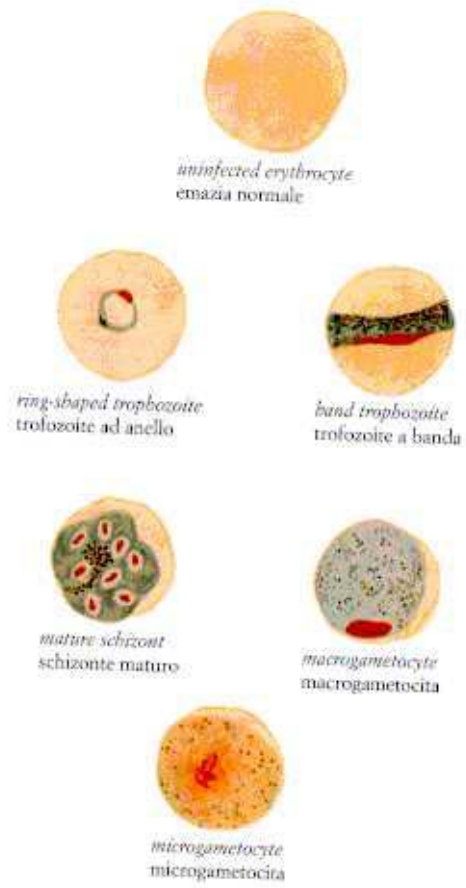


Figure 1.5 Light microscopic morphology of *P. malariae*

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Annex 1 - Color plates and figures

Figure 1.1 Diagrammatic representation of the life cycle of Plasmodia affecting man

A. Sporogonic cycle: Microgametes (a1) meet a macrogamete (a2) in the gut of the mosquito. Fertilization (a3) gives rise to the zygote (a4) which develops into an ookinete (a5). The ookinete penetrates the stomach: on its outer wall the oocyst (a6) matures (a7 - a8) to release motile sporozoites (a9). Sporozoites reaches the salivary glands (a10) from which they can be injected into a vertebrate host.

B. Exoerythrocytic schizogonic cycle: Free circulating sporozoites (b1) penetrate the hepatocytes (b2). In the liver cells the schizogonic cycle takes place (b3 - b4) to produce schizonts (b5) and merozoites (b6) which are released into the circulation. In the case of *P.vivax* and *P.ovale* hypnozoites (b7) dormant parasites that may cause relapses later, are also formed.

C. Erythrocytic schizogonic cycle: Merozoites invade red blood cells (c1) and initiate the maturative process developing into the ring-shaped early trophozoite (c2), the mature trophozoite (c3), and the schizont (c4). Following the rupture of the erythrocytes the schizont releases the merozoites into the circulation (c5). The merozoites re-invade new erythrocytes perpetuating the cycle (c6). Some trophozoites do enter schizogonic division and develop into female (macro-) (c7) or male (micro-) (c8) gametocytes.

Table 1. Classification of human protozoa of the genus Plasmodium

<i>Sub-kingdom</i>	Protozoa
<i>Phylum</i>	Apicomplexa
<i>Class</i>	Sporozoasida
<i>Sub-class</i>	Coccidiasina
<i>Order</i>	Eucoccidiorida
<i>Sub-order</i>	Haemospororina
<i>Family</i>	Plasmodidae

Table 1 - Some characteristics of infection with four species of human Plasmodia

	<i>P.vivax</i>	<i>P.ovale</i>	<i>P.malariae</i>	<i>P.falciparum</i>
Pre-erythrocytic cycle (days)	8	9	13	5-6
Pre-patent period (days)	11-13	10-14	15-16	9-10
Incubation period (days)	13 (12-17)	17 (16-18)	28 (18-40)	12 (9-14)
Number of merozoites/ tissue schizont	over 10,000	15,000	2,000	40,000
Hypnozoites	present	present	absent	absent
Erythrocytic cycle (hours)	48	50	72	48
Average parasitaemia / μ l	20,000	9,000	6,000	20-500,000
Maximum parasitaemia / μ l	50,000	30,000	20,000	2,000,000