Univ. of Mustansiriyah
College of Pharmacy
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# Medical Parasitology

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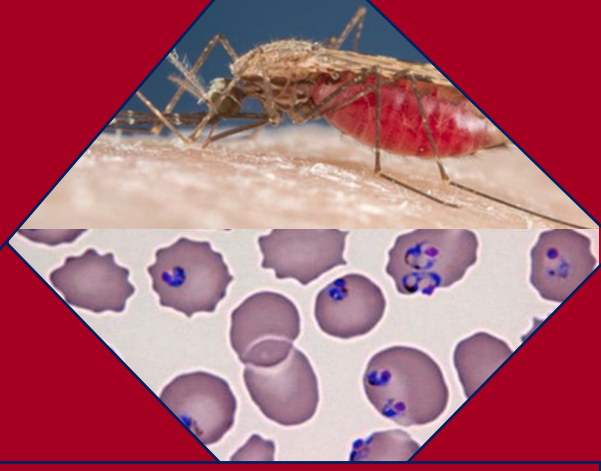


## Lec. 4 Sporozoa - Malaria parasite

## Sporozoa

Sporozoa belong to the **phylum Apicomplexa**. This phylum contains two classes namely **Haematozoea** and **Coccidia**. The parasites of **class Haematozoea** occur in the blood of their vertebrate hosts; it contains two orders: **Haemosporida**, containing the genus ***Plasmodium*** which causes **malaria**, and Piroplasmida, containing the genus *Babesia* which is rare and accidental parasite of man, they complete their life cycle in two hosts. Parasites of **class Coccidia** either undergo the whole of their life cycle in a single host, typically in the epithelial cells of the gut, or divide a similar cycle between two hosts. The class **Coccidia** contains one order, **Eimeriida**, which contains five genera: ***Toxoplasma***, *Cryptosporidium*, *Cyclospora*, *Isospora* and *Sarcocystis.*

## Malaria Parasite

Malaria parasites belong to the genus ***Plasmodium***. There are approximately 156 named species of *Plasmodium* which infect various species of vertebrates. Four are known to infect humans: ***P. vivax, P. falciparum, P. malariae*** **and** ***P. ovale***. They cause a disease called malaria, the most important of all tropical diseases in terms of morbidity and mortality. Worldwide, around two billion individuals are at risk; 100 million develop overt (distinct) clinical disease and 1.5-2.7 million die every year. Nearly 85% of the cases and 90% of carriers (many asymptomatic) are found in tropical Africa. The incidence of malaria is increasing due to the resistance of vectors to insecticides and drug resistant parasites.

Of the four species that infect humans, *P. vivax* and *P. falciparum* are responsible for 95% of infections. *P. vivax* has widest distribution, extending throughout the tropics, subtropics and temperate zones. *P. falciparum* is generally confined to (limited to) the tropics, *P. malariae* is sporadically distributed and *P. ovale* is confined mainly to central West Africa and some South Pacific Islands.

## Life cycle

Malaria parasites exhibit a complex life cycle involving alternating cycles of asexual division (**schizogony**) occurring in man (intermediate host) and sexual development (**sporogony**) occurring in female ***Anopheles*** mosquito (definitive host). Therefore, malaria parasites exhibit an alternation of generations and an alternation of hosts.

## Human Cycle

The sporozoites are the infective form of the parasite. They are present in the salivary glands of the female *Anopheles* mosquito. Man gets the infection by the bite of an infected mosquito. It usually bites at night or during the twilight hours, either right after sunset or before sunrise. During the act of biting, the proboscis of the mosquito pierces the skin and saliva containing sporozoites is injected directly into the blood stream. The cycle in man comprises of following stages:

1. Primary exo-erythrocytic or pre-erythrocytic schizogony.
2. Erythrocytic schizogony.
3. Gametogony.
4. Secondary exo-erythrocytic or dormant schizogony.

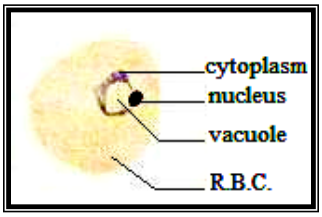
### Primary exo-erythrocytic or pre-erythrocytic schizogony

Within one hour all the **sporozoites** leave the blood stream and enter into liver parenchyma cells. The sporozoites which are elongated, spindle-shaped bodies become rounded inside the liver cells. They undergo a process of multiple nuclear division, followed by cytoplasmic division and develop into primary exoerythrocytic schizont or pre-erythrocytic schizont. In different species, it varies in size from 24-60µm in diameter and contains 2,000-30,000 merozoites. Primary exoerythrocytic schizogony consists of only one generation. The duration of this cycle of *P. falciparum, P. vivax, P. ovale* and *P. malariae* is 6, 8, 9 and 13-16 days, respectively. When primary exoerythrocytic schizogony is complete, the liver cell ruptures and releases several thousand individual parasites (**merozoites**) into the blood stream.

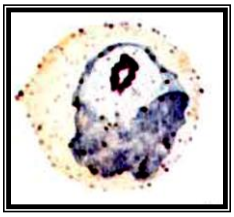
### Erythrocytic schizogony

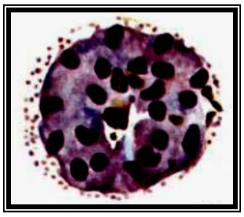
The merozoites liberated from primary exoerythrocytic schizogony enter the blood stream and invade red blood cells where they multiply at the expense of the host cells. Here they pass through the stages of **trophozoites**, **schizonts** and **merozoites**. Depending on the

species, 6-32 nuclei are produced followed by cytoplasmic division, and the red cell ruptures to release the individual merozoites, which then infect fresh red blood cells.



Ring stage.


Trophozoite.


Schizont.

The parasitic multiplication during the erythrocytic phase is responsible for bringing on the clinical attack of malaria. Erythrocytic schizogony may be continued for a considerable period, but with time, the infection tends to die out. *P. falciparum* differs from the other malaria parasites in that its developing erythrocytic schizonts aggregate in the capillaries of the brain and other internal organs, so that only young ring forms are found in peripheral blood.

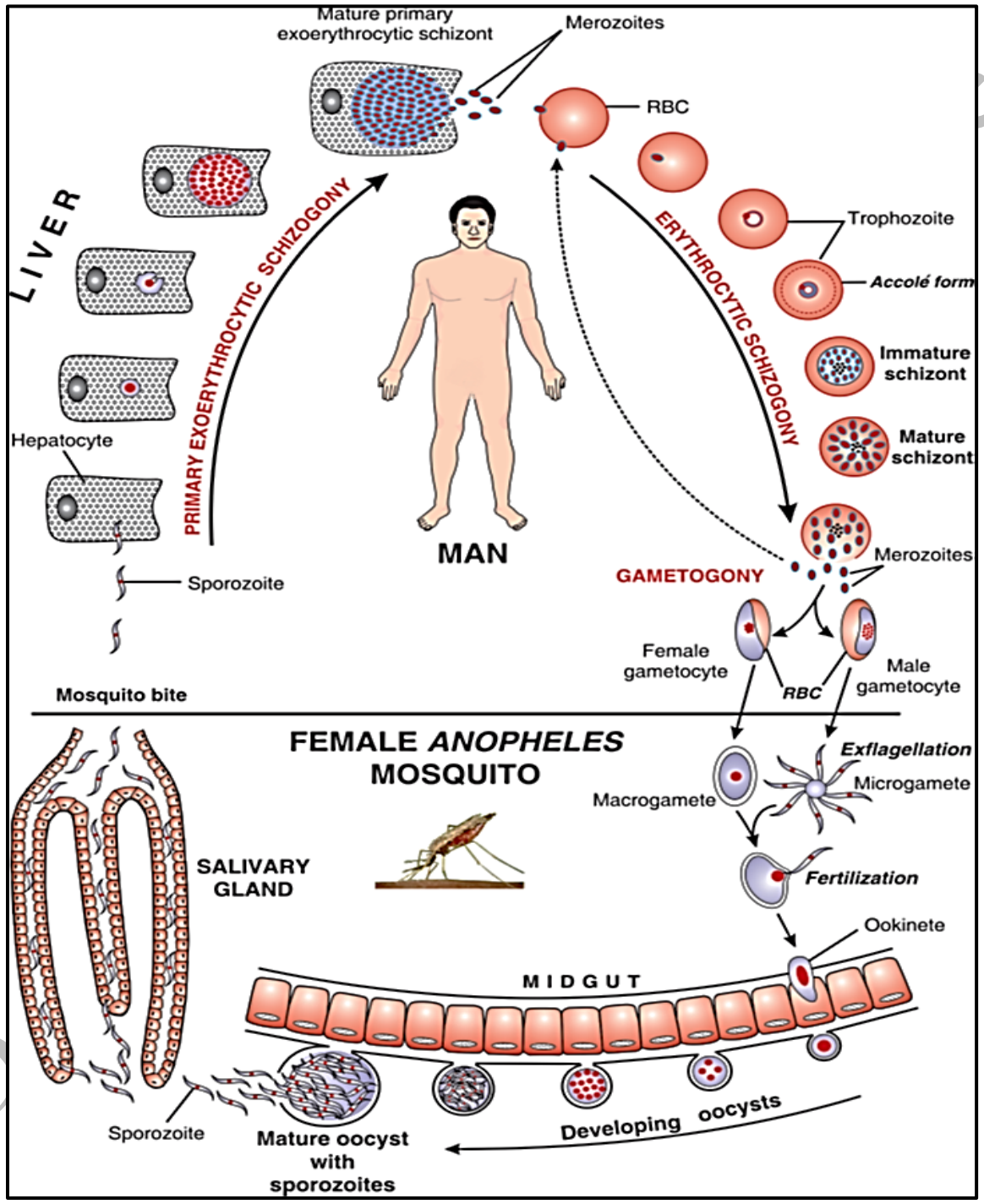
The malaria parasite's metabolism largely depends on the digestion of red cell haemoglobin, which is transformed into malaria pigment. The pigment is absent in the **ring stage** and becomes detectable only in the late trophozoite and the schizont stage. The malaria pigment may be yellowish-brown or dark brown in colour.

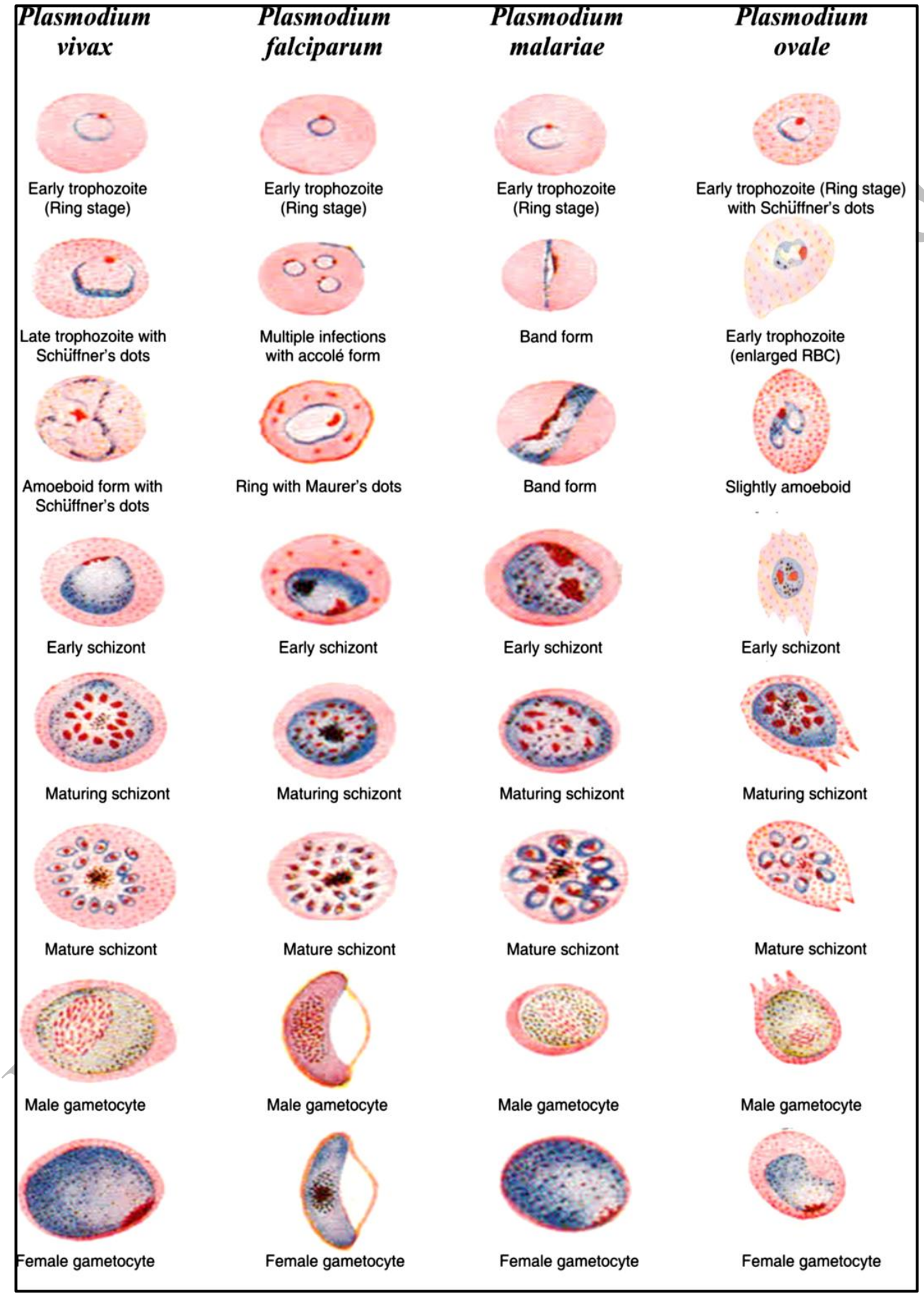
## Gametogony

After malaria parasites have undergone erythrocytic schizogony for a certain period, some merozoites develop within red cells into male and female gametocytes known as **microgametocytes** and **macrogametocytes** respectively. They develop in the red blood cells of the capillaries of internal organs like the spleen and bone marrow. Only mature gametocytes are found in the peripheral blood. They do not cause any febrile condition in the human host. These are produced for the propagation (reproduction) and continuance of the species.

A variety of factors have been shown to stimulate gametocytogenesis, including hyperparasitaemia, anaemia and antimalarial drug treatment and it appears that the parasite is capable of sensing the hostile conditions, and by transforming into gametocytes, it prepares to escape into a new host. The host carrying gametocytes is known as **carrier**. The microgametocytes of all four species of *Plasmodium* are smaller in size. Cytoplasm stains light blue and the nucleus (chromatin) is diffuse and large. On the other hand, the macrogametocytes are larger, the cytoplasm stains deep blue and the nucleus is compact and small.

Although the longevity of mature gametocytes may exceed several weeks, their half-life in the blood stream may be only 2 or 3 days, while waiting for the mosquito to take them up.




Plasmodium vivax
Plasmodium falciparum
Plasmodium malariae
Plasmodium ovale
Early trophozoite (Ring stage)
Early trophozoite (Ring stage)
Early trophozoite (Ring stage)
Early trophozoite (Ring stage) with Schüffner's dots
Late trophozoite with Schüffner's dots
Multiple infections with accolé form
Band form
Early trophozoite (enlarged RBC)
Amoeboid form with Schüffner's dots
Ring with Maurer's dots
Band form
Slightly amoeboid
Early schizont
Early schizont
Early schizont
Early schizont
Maturing schizont
Maturing schizont
Maturing schizont
Maturing schizont
Mature schizont
Mature schizont
Mature schizont
Mature schizont
Male gametocyte
Male gametocyte
Male gametocyte
Male gametocyte
Female gametocyte
Female gametocyte
Female gametocyte
Female gametocyte

## Secondary exo-erythrocytic or dormant schizogony

In the case of *P. vivax* and *P. ovale*, some sporozoites on entering into hepatocytes enter into a resting (dormant) stage before undergoing asexual multiplication while others undergo multiplication without delay. The resting stage of the parasite is rounded, 4-6µm in diameter, uninucleate and is known as **hypnozoite**. After a period of weeks, months or years (usually up to 2 years) hypnozoites are reactivated to become secondary exo-erythrocytic schizonts and release merozoites which infect red blood cells producing a **relapse** of malaria. Therefore, **the relapse is** the situation in which the erythrocytic infection is eliminated and reactivated later because of a new invasion of the RBCs from the liver merozoites produced after the hypnozoite is reactivated.

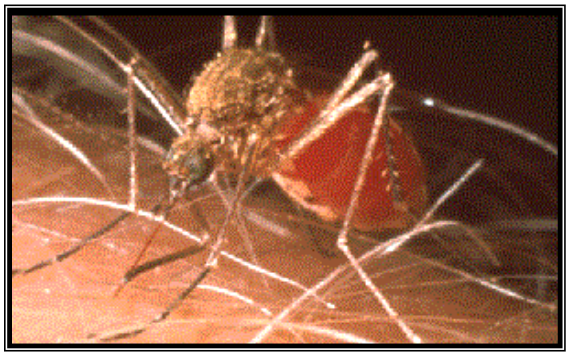
Hypnozoites are not formed in the case of *P. falciparum* and *P. malariae,* therefore, relapse does not occur in the disease caused by these species. On the other hand, the situation in which the RBC infection is not eliminated by the immune system or by therapy and the numbers in the RBCs begin to increase again with subsequent clinical symptoms is called a **recrudescence**.

Drug resistance creates a situation in which the initial peak of parasitemia is only partially controlled and a recrudescence of resistant parasites occurs shortly after. All species of *Plasmodium* may cause a recrudescence. *P. malariae* can survive in the peripheral blood at a very low level of parasitemia for a considerable time (10 years or more), occasionally producing detectable peaks with a recrudescence of clinical symptoms.

## Mosquito cycle

The sexual cycle actually starts in the human host itself by the formation of gametocytes which are present in the peripheral blood. Both asexual and sexual forms of the parasite are ingested by the **female *Anopheles* mosquito** during its blood meal from the patient.

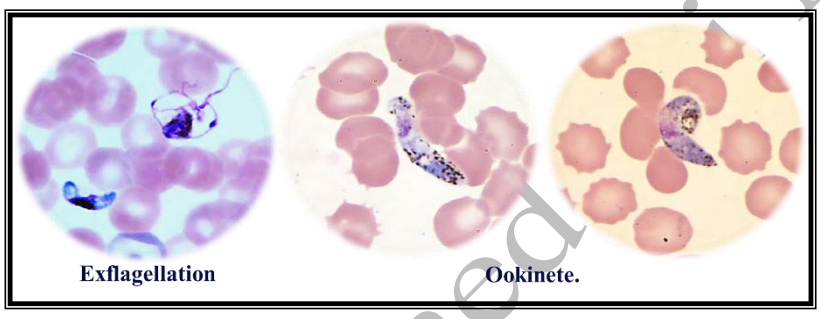
*Anopheles* mosquito (the vector)

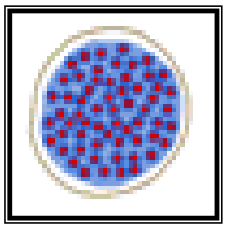


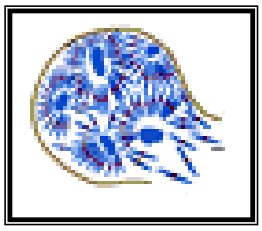
Feeding mosquito

The female mosquito has a stout (strong) proboscis which can pierce the human skin like a needle. On the other hand, the proboscis of the male is not stout, it is flexible; hence it cannot pierce through the skin. In the mosquito, only the mature sexual forms are capable of further development and the rest die. In order to infect a mosquito, the blood of a human carrier must contain at least **12 gametocytes/µl** and the number of female gametocytes must be more than the number of males.

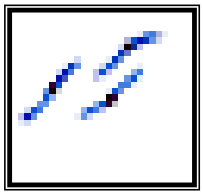
In the stomach of the mosquito, eight thread-like filamentous structures called **microgametes** are formed from one microgametocyte by the process of **exflagellation**. The macrogametocyte does not show any exflagellation; it develops into a **macrogamete**, its nucleus shifts to the surface, where a projection is formed. Fertilization occurs when a microgamete penetrates this projection. The fertilized macrogamete is known as **zygote**. This occurs in 20 minutes to 2 hours. In the next 24 hours, the zygote lengthens and matures into a motile vermiculate stage called **ookinete**. It penetrates the epithelial lining of the stomach of the mosquito and comes to lie between the external border of the epithelial cell and peritrophic membrane where it develops into **oocyst**.





Oocyst.

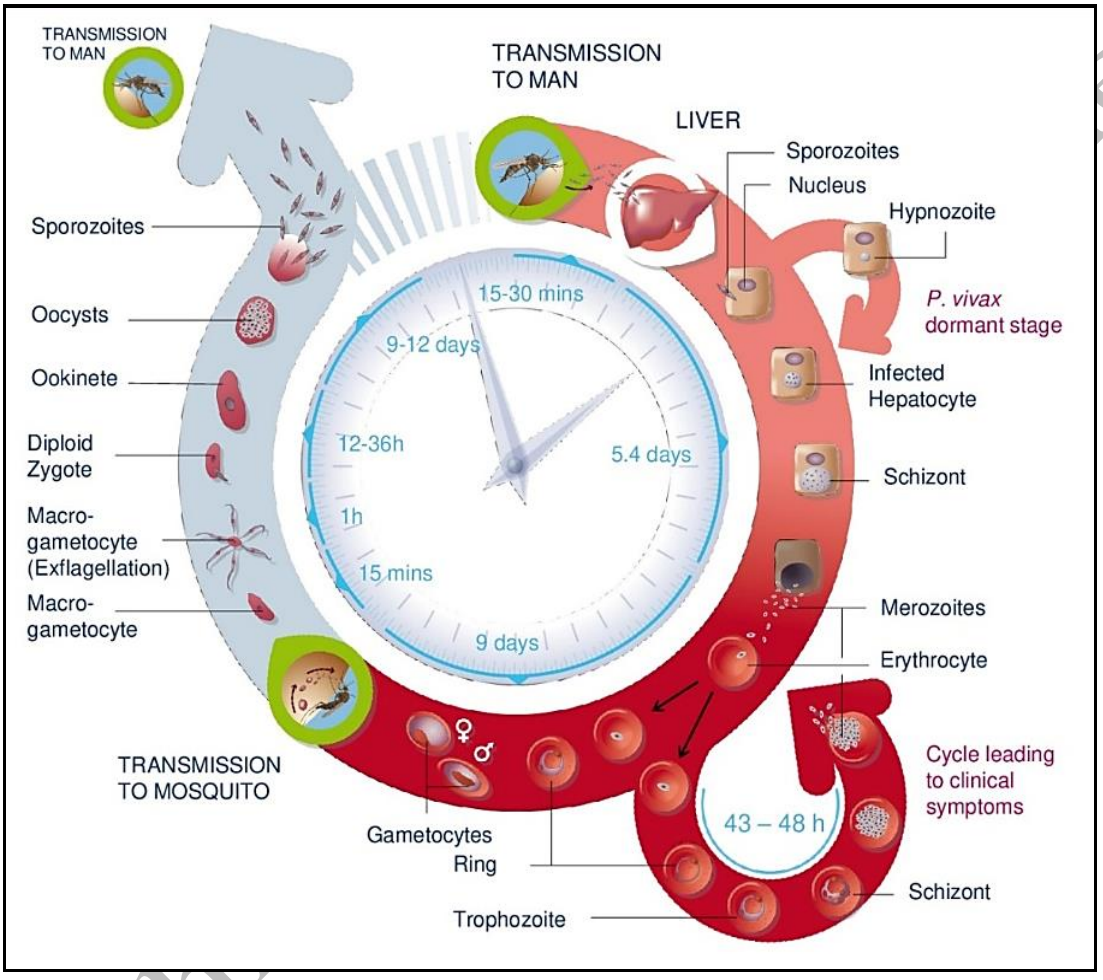

Ruptured mature oocyst.

It is rounded, 6-12µm in diameter with a single vesicular nucleus. It increases in size to reach a diameter of 40-50µm. The **sporozoites** develop inside it; the number of sporozoites in each oocyst varies from few hundreds to few thousands, and the number of oocysts in the stomach wall varies from a few to more than a hundred.


Sporozoites.

On about the 10$^{th}$ day the oocyst is fully mature, ruptures and releases sporozoites in the body cavity of the mosquito. Through the body fluid, the sporozoites are distributed to various organs of the body except the ovaries. They have a special predilection (tendency) for salivary glands and ultimately reach in maximum numbers in the salivary ducts. At this stage, the mosquito is capable of transmitting the infection to man.

Only the sporozoite, merozoite and ookinete are designed for the invasion of the hepatocyte, erythrocyte or midgut epithelial cell of the mosquito respectively, possess a surface coat and the specialized apical end characteristic of Coccidia. Other stages of the life cycle, which are designed for growth and development within the host cell, lack these invasion organelles.




## Pathogenicity

Man develops infection by the bite of an infected female *Anopheles* mosquito. However, theinfection may also be transmitted by:

1. Transfusion of blood from a patient of malaria which is known as transfusion malaria. *Plasmodium* can remain viable in refrigerated blood for up to 10 days.

2. Transmission of infection to the foetus in utero through some placental defect. This is known as congenital malaria.

3. By the use of contaminated syringes, particularly in drug addicts. This is known as mainline malaria.

The above conditions are also known as trophozoite-induced malaria. In this condition there is no primary and secondary exoerythrocytic schizogony, the incubation period is short and there is no relapse. After an incubation period of 12 days for *P. falciparum*, 13–17 days for *P. vivax* and *P. ovale*, and 28-30 days for *P. malariae* patients who develop malaria. The typical picture of malaria consists of **febrile paroxysm**, **anaemia** and **splenomegaly**.

## Febrile paroxysm

It generally begins in the early afternoon and comprises three successive stages cold stage, hot stage and sweating stage. In the cold stage, lasting for 15-60 minutes, the patient experiences intense cold and shivering. This is followed by the hot stage, lasting for 2-6 hours, when the patient feels intensely hot. The patient develops a high fever (40°-40.6°C), severe headache, nausea and vomiting. Thereafter, the fever ends by crisis accompanied by profuse sweating.

The periodicity of the attack varies with the species of the infecting parasite. The periodicity is 48 hours in *P. vivax* (**benign tertian malaria**) and *P. ovale* (**ovale tertian malaria**) and 72 hours in *P. malariae* (**quartan malaria**). However, with *P. falciparum*, the cycles of different broods of the parasite do not become synchronized as they do in other species. Therefore, typical tertian fever is not usual in *P. falciparum* (**malignant tertian malaria**). **Quotidian (daily) periodicity** with the fever occurring at 24 hour intervals may be due to two broods of tertian parasites maturing on successive days or due to mixed infection.

Mixed infection with more than one species of *Plasmodium* is more common than previously suspected. Febrile paroxysms follow the completion of erythrocytic schizogony when the mature schizont ruptures releasing merozoites, malarial pigment and other parasitic debris. Macrophages and polymorphs phagocytose these and release endogenous pyrogens leading to pyrexia. Primary as well as secondary exoerythrocytic schizogony and gametogony do not contribute to clinical illness.

## Anaemia

After a few paroxysms, anaemia of a microcytic or a normocytic hypochromic type develops as a result of:

1. Direct RBC lysis as a result of the life cycle of the parasite.

2. Splenic removal of both infected and uninfected RBCs (coated with immune complexes).
3. Autoimmune lysis of coated infected and uninfected RBCs.
4. Decreased incorporation of iron into heme.
5. Increased fragility of RBCs.
6. Decreased RBC production from bone marrow suppression.

Since *P. vivax* and *P. ovale* infect only reticulocytes, therefore, parasitemia is usually limited to around 2-5% of available RBCs. *P. malariae* invades primarily the older RBCs. so that the number of infected cells is somewhat limited. *P. falciparum* in contrast to other *Plasmodium* species tends to invade all ages of RBCs (young and old), and the proportion of infected cells may exceed 50%.

## Splenomegaly

After a few paroxysms, the spleen gets enlarged and becomes palpable. Splenomegaly is due to the massive proliferation of macrophages which phagocytose both parasitized and non-parasitized red blood cells. Non-immune pregnant women are susceptible to all the usual manifestations of malaria. In addition, they have an increased risk of abortion, stillbirth, premature delivery and low birth weight of their infants. Pregnant women are particularly prone to hypoglycemia and pulmonary oedema.

*P. falciparum* is the most pathogenic of the human *Plasmodium* species. It causes a high level of parasitemia with parasite density exceeding 250,000-300,000/ml of blood. Nearly 30-40% of the red blood cells may be parasitized.

Erythrocytic schizogony in *P. falciparum* takes place in the capillaries of the internal organs (spleen, bone marrow, brain, kidney, intestine, heart and placenta). Membrane protuberances (knobs) appear on the surface of the infected red blood cells. These mediate the attachment of the parasitized red blood cells to one another and to the lining of the capillaries and venules as the parasites (except gametocytes) grow older. Thus, only young rings and gametocytes are typically found in the peripheral blood of the patient with *P. falciparum* malaria and in this case they may not be found in a blood film at the time when the clinical picture is most suggestive. The characteristic lesions of *P. falciparum* malaria are due to the blockade of small vessels by sticky parasitized erythrocytes and this leads to tissue hypoxia.

## Pernicious malaria

Pernicious malaria is a complex of life-threatening complications that sometimes take place in acute *P. falciparum* malaria. It is due to heavy parasitization and is of three types:

1. Cerebral malaria: it is a severe complication of *falciparum* malaria and frequently leads to death, even when appropriate therapy has been given. It is characterized by hyperpyrexia, coma and paralysis. Capillaries of the brain are plugged with parasitized red blood cells, each cell containing malaria pigment. In holoendemic areas of malaria, cerebral malaria occurs in children between 6 months and 5 years, most commonly in children aged 3-4 years.

2. Algid malaria: It resembles surgical shock with cold clammy skin, peripheral circulatory failure and profound shock. The patient may also develop vomiting and diarrhea or dysentery.

3. Septicemic malaria: It is characterized by a high degree of prostration, there is high continuous fever with involvement of various organs.

### Blackwater fever

It is a manifestation of repeated infections with *P. falciparum* which were inadequately treated with **quinine**. Sometimes resumption of quinine therapy for the new attack is followed by massive destruction of RBCs, fever, hemoglobinuria and renal failure. The exact mechanism of hemolysis in blackwater fever is not known. An autoimmune mechanism has been suggested. Parasitized and quininized red blood cells, during the previous infection, act as antigens against which antibodies are formed. With subsequent infection and treatment with quinine, there is massive destruction of both infected and uninfected red blood cells. As other antimalarials have replaced quinine, blackwater fever has now become rare.

## Host immunity

### 1. Innate immunity

It refers to inherent, non-immune mechanisms of host defence against malaria. This is due to the age of red blood cells, the nature of hemoglobin, the enzyme content of red blood cells and the presence or absence of certain factors.

-Age of red blood cells: *P. falciparum* infects both young and old red blood cells, while *P. vivax* and *P. ovale* infect only young erythrocytes, and *P. malariae* only old erythrocytes.

-Nature of haemoglobin: The presence of abnormal hemoglobin like thalassemia haemoglobin and foetal haemoglobin confers (allow/ give) resistance against all

*Plasmodium* spp., while sickle cell anaemia trait and haemoglobin E protect against *P. falciparum* and *P. vivax* respectively.

-Enzyme content of red blood cells: A genetic deficiency known as glucose-6-phosphate dehydrogenase (G6PD) trait confers some protection against *P. falciparum* infection. This enzyme is essential for the respiratory process of the parasite.

-Presence or absence of certain factors: The presence of the Duffy factor increases the susceptibility to malaria. It is believed that Duffy factor that presents on the surface of the erythrocytes acts as a receptor for the attachment of malaria parasite. (The Duffy glycoprotein is a receptor for chemicals that are secreted by blood cells during inflammation. It also happens to be a receptor for *Plasmodium vivax*).

## 2. Acquired immunity

Acquired immunity in malaria involves both humoral and cellular immunity. Antibodies against sporozoites and asexual and sexual blood stages develop in malaria patients. The IgM, IgG and IgA antibodies against asexual blood stages may protect by inhibiting red cell invasion and antibodies against sexual stages are believed to reduce malaria transmission.

A variety of cellular mechanisms may play a role in conferring protection against malaria. These include natural killer activity and activated macrophages. The macrophages phagocytose and induce the extracellular killing of target cells. T cells are crucial for malaria immunity; their major function seems to provide help for the production of antibodies and to activate macrophages.

The immunity produced following the infection with malaria parasites is species-specific, stage-specific and strain-specific, and this immunity lasts only till the original infection remains active. This is known as **concomitant immunity** (previously called **premunition** or **infection-immunity**). Malaria parasites like many other microorganisms are capable of periodically changing the expression of their antigens. This provides the parasite with a powerful means for evading host immunity. The ability of *P. falciparum* to remain sequestered (isolated) by cytoadherence to the capillary lining of certain tissues is regarded as a selective advantage as such parasites can avoid frequent passage through the spleen and thus exposure to the immune effector mechanisms. Sequestration does not exist in other human malaria parasites and this is considered the main reason for the difference in disease severity.

# Laboratory diagnosis

Malaria is one of the few parasitic infections considered to be immediately life-threatening, and a patient with the diagnosis of *P. falciparum* malaria should be considered a medical emergency because the disease can be rapidly fatal.

## Microscopy

Diagnosis of malaria can be established by the demonstration of malaria parasites in the blood. Thick and thin smears of blood are prepared on the same or different slides. Blood is taken by pricking a finger or ear lobule before starting treatment with antimalarials. For the preparation of the **thick smear** take a large drop of blood on the slide. Spread it in an area of 12mm square. Dehemoglobinization of the thick smear is done by keeping the slide in distilled water in Koplin's jar in a vertical position for 5-10 minutes till the slide becomes white and then it is dried in air. Both thick and thin smears are stained with **Leishman stain**. The smears are then examined under the oil-immersion lens.

The parasites are most abundant in peripheral blood late in the febrile paroxysm (a few hours after the height of t paroxysm). Therefore, blood for the smear should be collected at this period. All asexual erythrocytic stages, as well as gametocytes can be seen in peripheral blood in infection with *P. vivax*, *P. malariae* and *P. ovale*, but in *P. falciparum* infection, only the ring forms and crescent-shaped gametocytes can be seen. Late trophozoite and schizont stages of *P. falciparum* are usually confined to the internal organs and appear in peripheral blood **only in severe or pernicious malaria**.

The occurrence of multiple rings in an individual red blood cell with **accolé forms** is diagnostic of *P. falciparum* infection. Malaria pigment may be demonstrated inside the monocytes and polymorphonuclear leucocytes. The presence of malaria pigment only, in the absence of malaria parasites, suggests *P. falciparum* infection. Schüffner's, Maurer's, Ziemann's and James's dots can be seen in the red blood cells infected with *P. vivax*, *P. falciparum*, *P. malariae* and *P. ovale* respectively. Red blood cells are enlarged in *P. vivax* infection.

**Thin film** is examined first and if parasites are found, there is no need for examining the thick film. If the parasites are not seen in the thin film in a few minutes, the thick film should be examined. If the parasites are seen in the thick film but the identity is not clear, the thin film is reexamined more thoroughly to determine the identity of the species. The parasites are more along the upper and lower margins of the tail of the film. At least 200-300 oil-immersion fields should be examined before the smears are considered negative.

The staining of films with **acridine orange**, which can be examined either under a fluorescence microscope or a microscope equipped with an interference filter system, allows quicker screening of films, because the parasites are more readily recognized and a lower power lens may be used.

Conventional light microscopy is the established method for the laboratory confirmation of malaria. It offers many advantages:

1. It is sensitive; when used by skilled and careful technicians, microscopy can detect densities as low as 5-10 parasites per µl of blood.

2. It is informative; when parasites are found, they can be characterized in terms of their species (*P. falciparum*, *P. vivax*, *P. malariae* and/or *P. ovale*) and of the circulating stage (e.g. trophozoites, schizonts, gametocytes).

3. It is relatively inexpensive.
4. It can provide a permanent record (the smears) of the diagnostic findings.

However, microscopy suffers from three main disadvantages:

1. It is labor-intensive and time-consuming, normally requiring at least 60 minutes from specimen collection to the result.

2. It is exacting and depends absolutely on good techniques, reagents, microscopes and most importantly well-trained and well-supervised technicians.

3. There are often long delays in providing the microscopy results to the clinician so treatment decisions are often taken without the benefit of the results.

## Rapid diagnostic tests (RDTs)

They are based on the detection of malaria antigens in lysed blood, using immunochromatographic methods. Most frequently they employ a dipstick or test strip bearing monoclonal antibodies directed against the target parasite antigens. The tests can be performed in about 15 minutes. Several commercial test kits are currently available.

## Advantages of RDTs over microscopy

1. RDTs are simple to perform and interpret.
2. They do not require electricity, special equipment or training in microscopy.

3. Health workers with minimal skill can be trained in RDT techniques in periods varying from three hours to one day.
4. Since RDTs detect circulating antigens, they may detect *P. falciparum* infection even when the parasites are sequestered and undetectable by microscopic examination of a peripheral blood smear.

### Disadvantages of RDTs

1. More expensive than microscopy.

2. Kits cannot differentiate between *P. vivax*, *P. ovale* and *P. malariae* nor can they distinguish pure *P. falciparum* infections from mixed infections that include *P. falciparum*.

3. RDTs that detect antigens produced by gametocytes (such as pLDH) can give positive results in infections where only gametocytes are present. Gametocytes do not cause any febrile condition, and those of *P. falciparum* are not affected by schizonticidal drugs. Such positive RDT results can thus lead to erroneous interpretations and unnecessary treatment.

### Other Techniques

Other diagnostic methods include polymerase chain reaction (PCR) and antibody detection by serology ex. indirect haemagglutination (IHA), indirect fluorescent antibody (IFA) and enzyme-linked immunosorbent assay (ELISA). However, serology only measures prior exposure and not specifically current infection.

## Treatment

**Chloroquine** was the standard treatment for acute malaria for many years. However, resistance to this drug in *P. falciparum* is widespread. Less commonly *P. vivax* may also be chloroquine-resistant. **Quinine** is the most reliable alternative to chloroquine for the treatment of malaria caused by chloroquine-resistant strains. **Tetracycline** and **clindamycin** exhibit some antimalarial activity and are used as an adjunct to quinine therapy. **Mefloquine** and **halofantrine** are also active against chloroquine-resistant strains, but resistance to these drugs has also been reported.

Chloroquine and quinine do not eliminate exoerythrocytic parasites in the liver. For this **primaquine** (**8-aminoquinoline drug**) should be used. However, this drug may precipitate hemolysis in individuals who are deficient in the enzyme glucose-6-phosphate dehydrogenase.

## Drug resistance in malaria parasites

Effective, affordable, and safe treatment of malaria, particularly falciparum malaria, is becoming increasingly difficult as resistance to chloroquine and other antimalarial drugs continues to spread throughout the tropics. Most resistant strains of *P. falciparum* have developed due to:

1. Inadequate drug doses mainly as a result of unregulated drug distribution and prescribing.
2. Lack of adequate drugs.
3. Poor quality of drugs.
4. Incorrect taking of drugs by patients.
5. When insufficient drug is taken to kill the malaria parasites, the mutants survive and multiply. It is also thought that selective pressure favoring naturally occurring resistance mutations which increases in areas of intense malaria transmission.

## WHO definition and classification of drug resistance

Drug resistance has been defined by the World Health Organization as the ability of a parasite to multiply or to survive in the presence of concentrations of a drug that normally destroys parasites of the same species or prevents their multiplication. Three levels of resistance (R) are defined by the World Health Organization:

RI: Following treatment, parasitemia clears but a recrudescence occurs.

RII: Following treatment, there is a reduction but not a clearance of parasitemia.

RIII: Following treatment, there is no reduction of parasitemia.

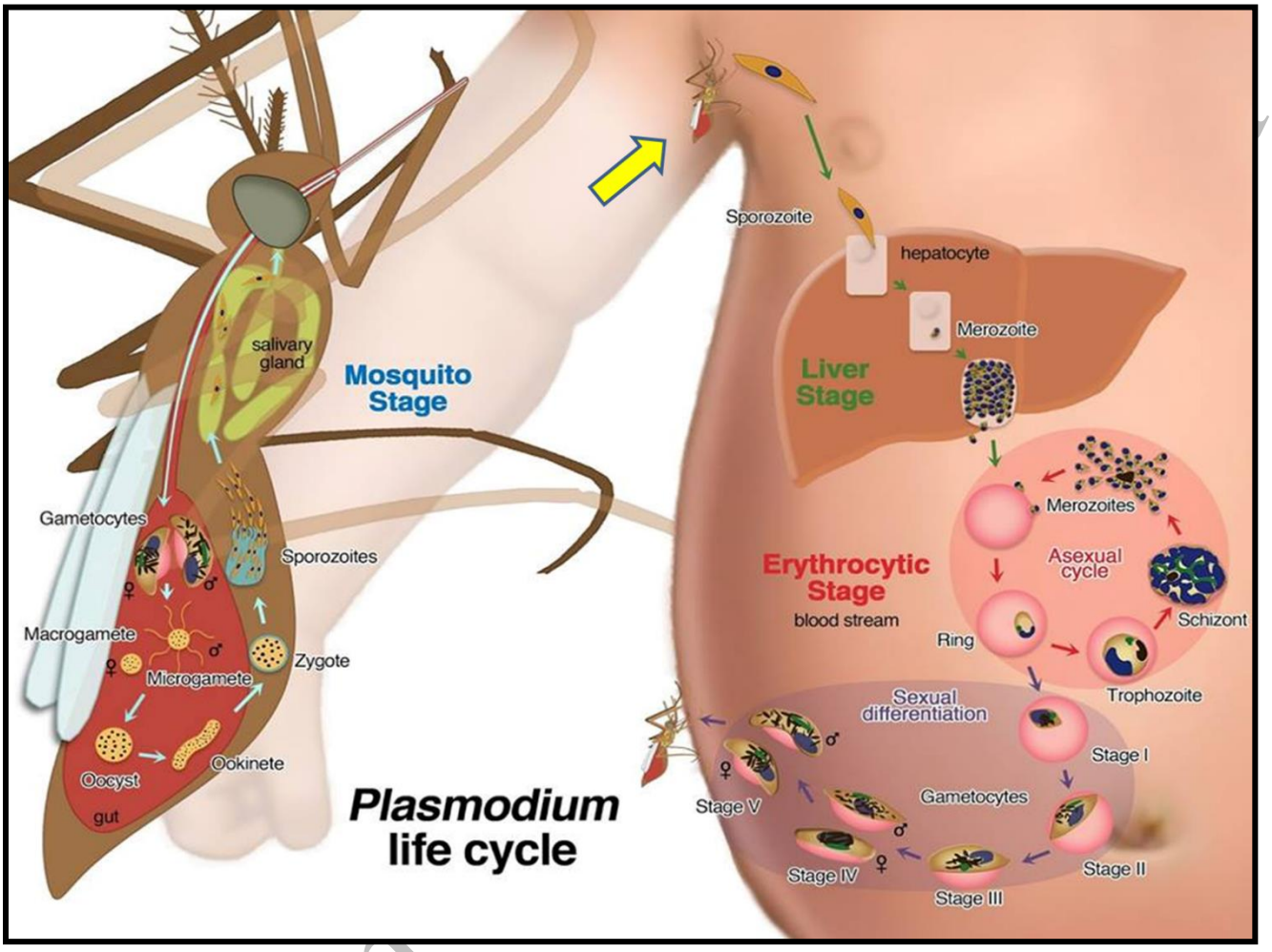
The above method of classifying resistance, based on counting trophozoites in blood films for up to 7 days after treatment and monitoring the patient for any subsequent recrudescence is referred to as *in vivo* testing.

## Vaccine

Despite very intensive research since the mid 1970s, no effective malaria vaccine is yet available.

### *Plasmodium knowlesi*

A fifth species, *P. knowlesi*, which normally infects long-tailed macaques monkeys and pigtailed macaques, is a significant cause of human malaria in Southeast Asia.




"Scheme for the life cycle of *Plasmodium* sp.".