General characters of apicomplexa

The apicomplexa are a monophyletic group composed almost entirely of parasitic (ie, no free-living) species. Formerly the apicomplexa were part of a group called sporozoa and this name is still sometimes used. Recently there have been some suggestions to revert back to the name sporozoa. The parasite belonging to this group of protozoa don't possess any type of locomotion organelles, they show slight amoeboid change of form (body flexion).

Electron microscopyrevealed unique ultrastructural features among the various sporozoa which were subsequently used to redefine the groups. A defining characteristic of the apicomplexa is a group of organelles found at one end--called the apical end--of the organism. This 'apical complex' includes secretory organelles known as micronemes and rhoptries, polar ringscomposed of microtubules, and in some species a conoid which lies within the polar rings.

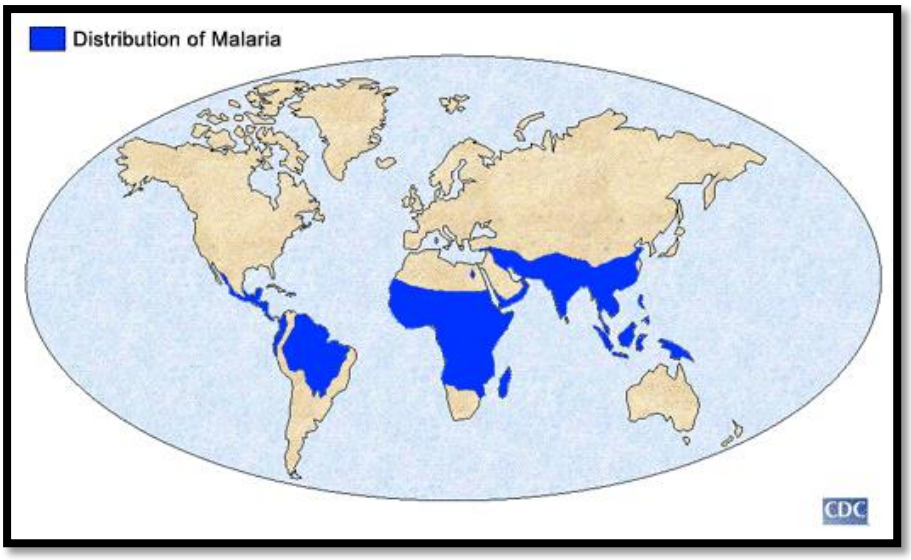
The apicomplexa have complex life cycles that are characterized by three distinct processes , sporogony, merogonyand gametogony (alternation ofgeneration). Also may be classified into two processes schizogony and gametogony.

Sporogony is an asexual reproduction that culminates in the production of sporozoites. Sporozoites will develop into forms that undergo another asexual replication known as merogony. Merogony and the resulting merozoites are known by many different names depending of the species. Similarly, sporogony and gametogony can involve different hosts or cell types. As an alternative to asexual replicationmerozoites can become gametes through a process variously called gametogony, gametogony or gametogenesis. As in other types of sexual reproduction, the gametes fuse to form a zygote which differentiates into a form yielding sporozoites. The apicomplexa are an extremely large and diverse group (>5000 named species).

Malaria

Malaria is the most important tropical disease known to man. It remains a significant problem in many tropical areas, especially in sub-Saharan Africa.

Malaria is spreading as a result of environmental changes, including global warming, civil disturbances, increasing travel and drug resistance There are approximately 100 million cases of malaria worldwide with about 1 million of these proving fatal.



Map illustrating the distribution of malaria throughout the world.

Malaria is caused by protozoa of the Plasmodium species. There are four species which infect both humans and animals; Plasmodium malariae (quartian malaria), Plasmodium vivax (benign tertian malaria), Plasmodium falciparum (malignant tertian malaria, subtertian malaria) and Plasmodium ovale (ovale tertian malaria).

The transmission of the protozoa, Plasmodium requires two hosts, a definitive invertebrate host (vector), and an intermediate vertebrate host (mammals, birds and lizards).

All Plasmodium species undergo the general haemosporina developmental cycle which can be summarized as:

- initial or continual schizogony (reproduction by multiple asexual fission) in the vertebrate host with initiation of gametogony (the formation or production of gametes);

- formation of gametes in the arthropod host and subsequent fertilization and formation of a zygote;

 formation of sporozoites from the zygote by repeated nuclear division followed by cytoplasmic divisions.

The transmission of the parasites between the vertebrate and invertebrate hosts is made by withdrawal or injection during the bloodsucking act, there is little or no exposure to the hazards of the outside world; thus by blood transfusion or inoculation, via the blood stages of the parasite.

Life Cycle

Malaria is transmitted by the female anopheles mosquito. The life cycle of all species of human malaria parasites is essentially the same. It comprises an sexual phase (sporogony) with multiplication in certain Anopheles mosquitoes and an asexual phase (schizogony) with multiplication in the vertebrate host. The latter phase includes the development cycle in the red cells (erythrocytic schizogony) and the phase taking place in the parenchyma cells in the liver (pre-erythrocytic schizogony).

When a female Anopheles mosquito bites an infected person, it ingests blood which may contain the mature sexual cells (male and female gametocytes) which undergo a series of developmental stages in the stomach of the mosquito.Gametocytes do not cause pathology in the human host and will disappear from the circulation if not taken up by a mosquito.

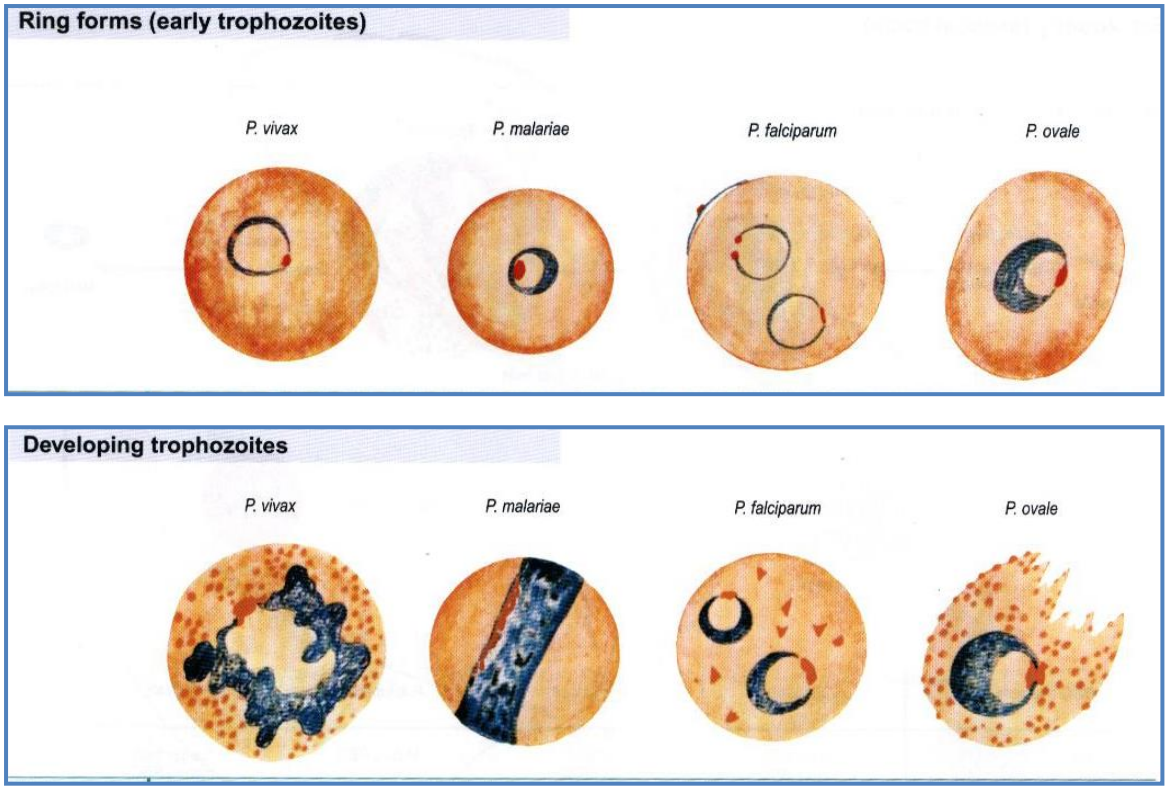
The gametocytes are large parasites which fill up the erythrocyte, but only contain one nucleus. The mature gametocytes are round in shape except in *P. falciparum* in which they are crescent or banana shape. In all species the female gametocytes is large (Macro gametocyte)and has cytoplasm staining dark blue with small compact nucleus staining deep red. In the smaller male gametocytes (microgametocyte) nucleus is diffuse pigmented granules. Gametocytes appear in circulation in the *P. ovale* appear after4-5 days of first appearance of asexual forms and 10-12 days in *P. falciparum* .Persons with gametocytes in circulation is a carrier or reservoir but not cause any clinical symptoms and are essential for transmission of infection.

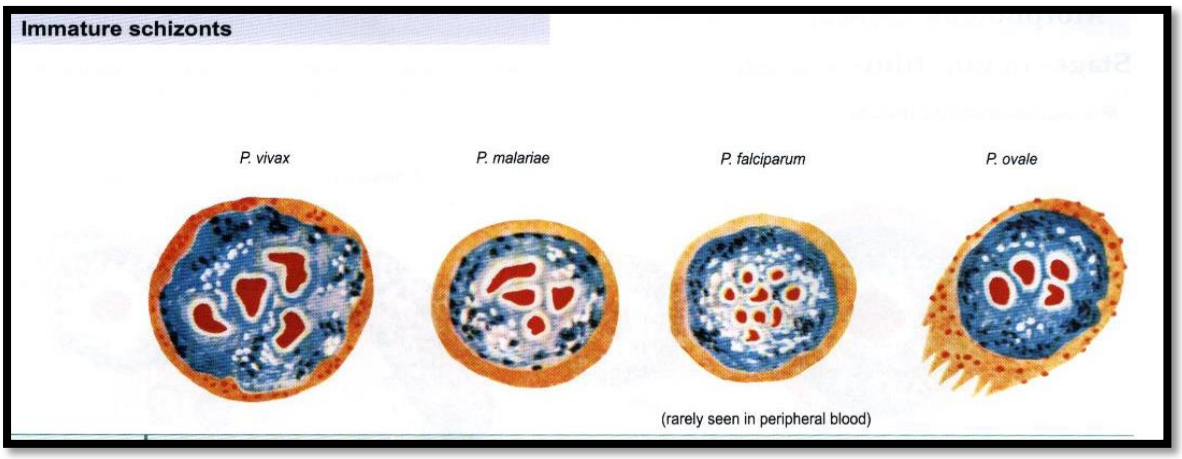
Exflagellation (the extrusion of rapidly waving flagellum-like microgametes from microgametocytes) occurs resulting in the production in a number of male and female gametes. Fertilization occurs producing a zygote which matures to an ookinete. The ookinete is motile invasive stage (invading and exiting)which will

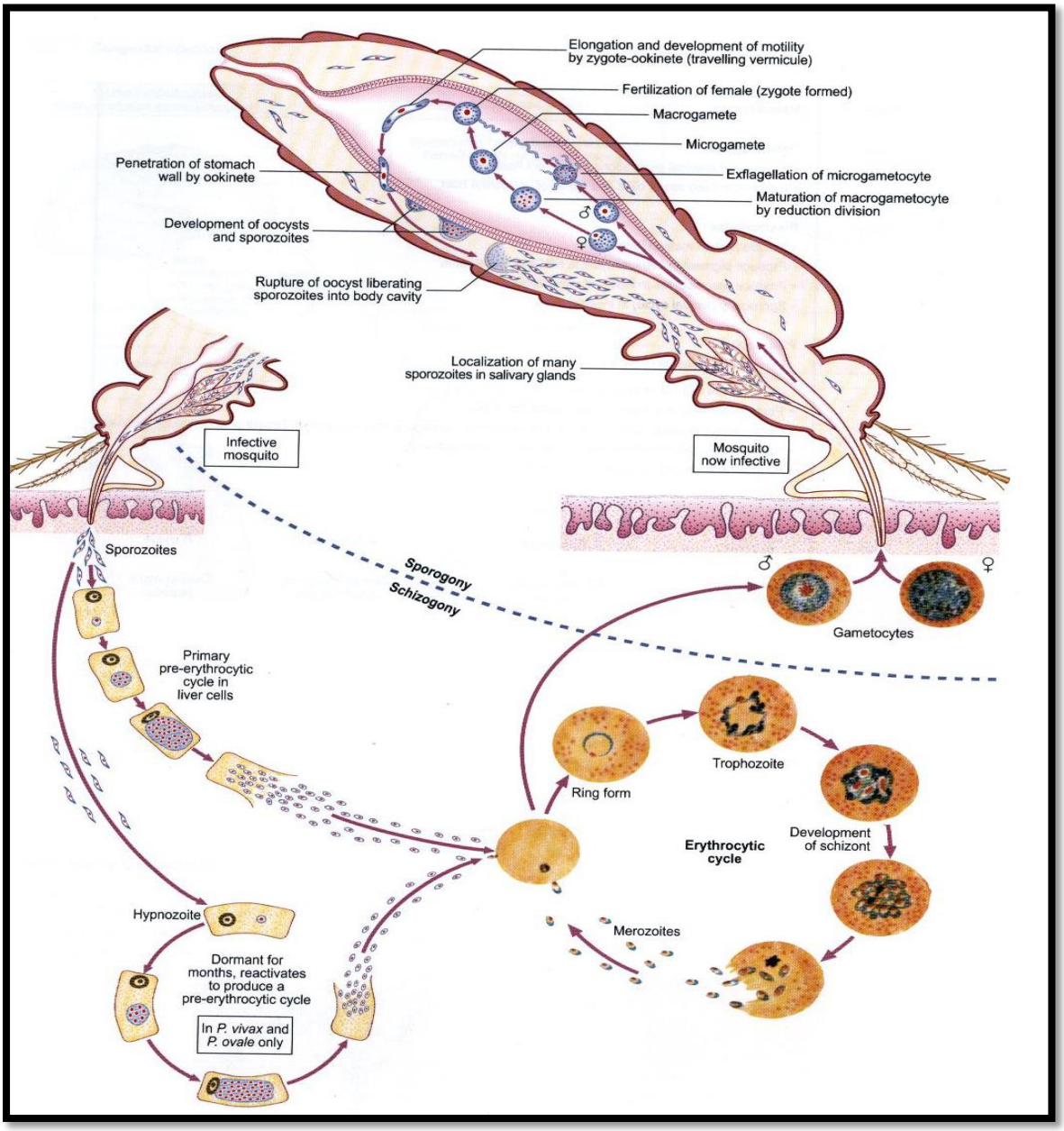
transverse both the peritrophic matrix and the midgut epithelium of the mosquito. After reaching the extracellular space between the epithelial cells and the basal lamina, the ookinete develops into an oocyst.

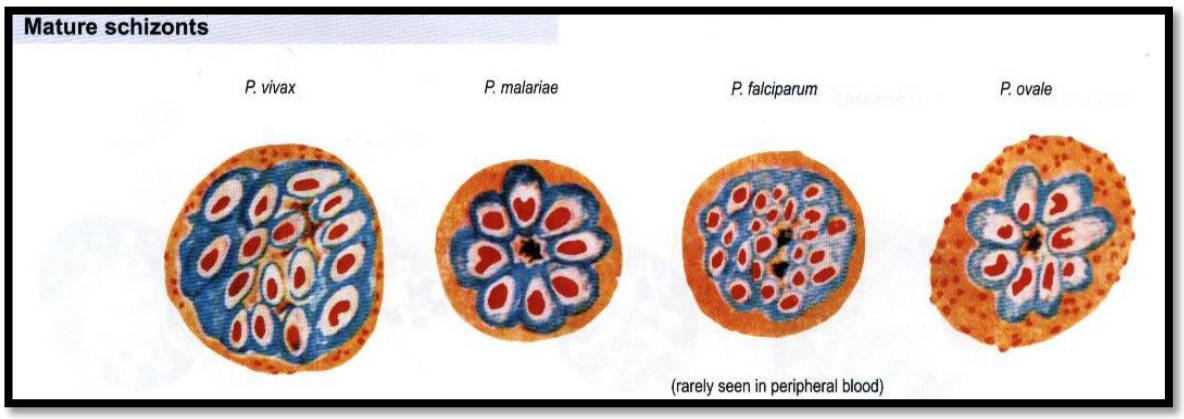
The length of the developmental stage in the mosquito not only depends on the Plasmodium species but also the mosquito host and the ambient temperature. This may range from eight days in Plasmodium vivax to as long as 30 days in Plasmodium malariae.

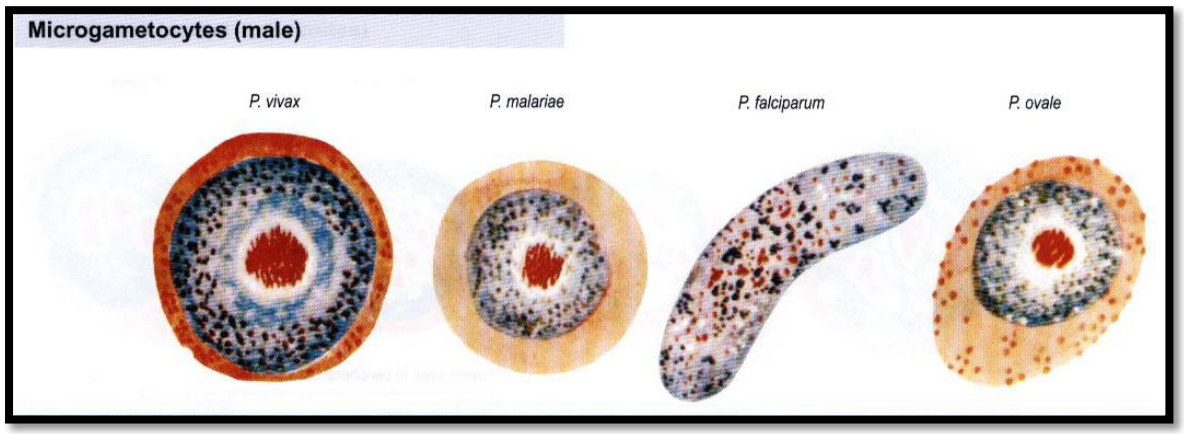
The sporozoites migrate from the body cavity of the mosquito to the salivary glands and the mosquito now becomes infective. Sporozoites enter into the blood stream of a host when the mosquito feeds on blood. Following the inoculation, the sporozoites leave the blood within 40 minutes and enter the parenchymal cells of the liver (hepatocytes). In all four species, asexual development occurs in the liver cells, a process known as pre-erythrocyticschizogony, to produce thousands of tiny merozoites which are released into the circulation after about 16 days. However in P. vivax and P. ovale some sporozoites differentiate into hypnozoites which remain dormant in hepatocytes for considerable periods of time. When they are "reactivated" they undergo asexual division and produce a clinical relapse.

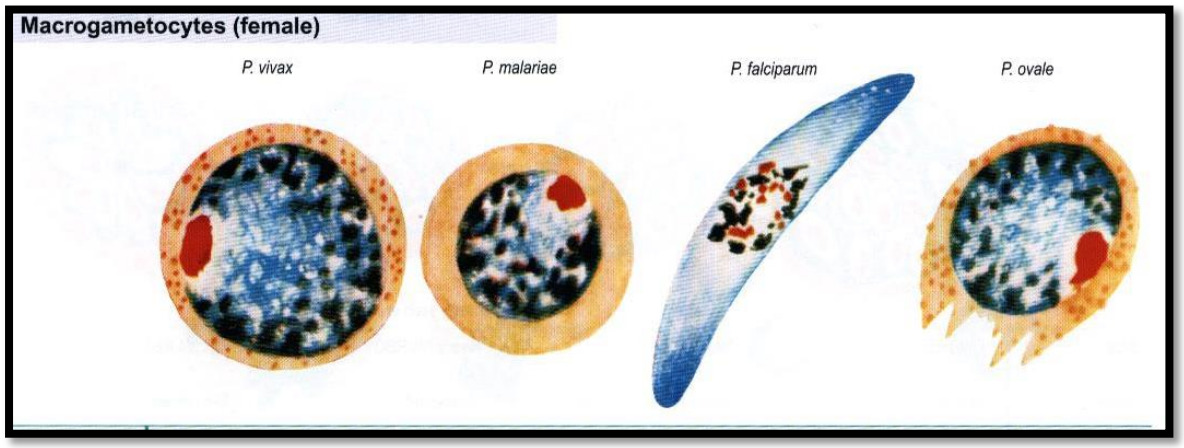



Immature schizonts
P. vivax
P. malariae
P. falciparum
P. ovale
(rarely seen in peripheral blood)


Elongation and development of motility by zygote-ookinete (travelling vermicule)
Fertilization of female (zygote formed)
Macrogamete
Microgamete
Exflagellation of microgametocyte
Maturation of macrogametocyte by reduction division
Penetration of stomach wall by ookinete
Development of oocysts and sporozoites
Rupture of oocyst liberating sporozoites into body cavity
Localization of many sporozoites in salivary glands
Infective mosquito
Mosquito now infective
Sporozoites
Sporogony
Schizogony
Gametocytes
Primary pre-erythrocytic cycle in liver cells
Ring form
Trophozoite
Development of schizont
Erythrocytic cycle
Merozoites
Hypnozoite
Dormant for months, reactivates to produce a pre-erythrocytic cycle
In P. vivax and P. ovale only


Mature schizonts
P. vivax
P. malariae
P. falciparum
P. ovale
(rarely seen in peripheral blood)


Microgametocytes (male)
P. vivax
P. malariae
P. falciparum
P. ovale

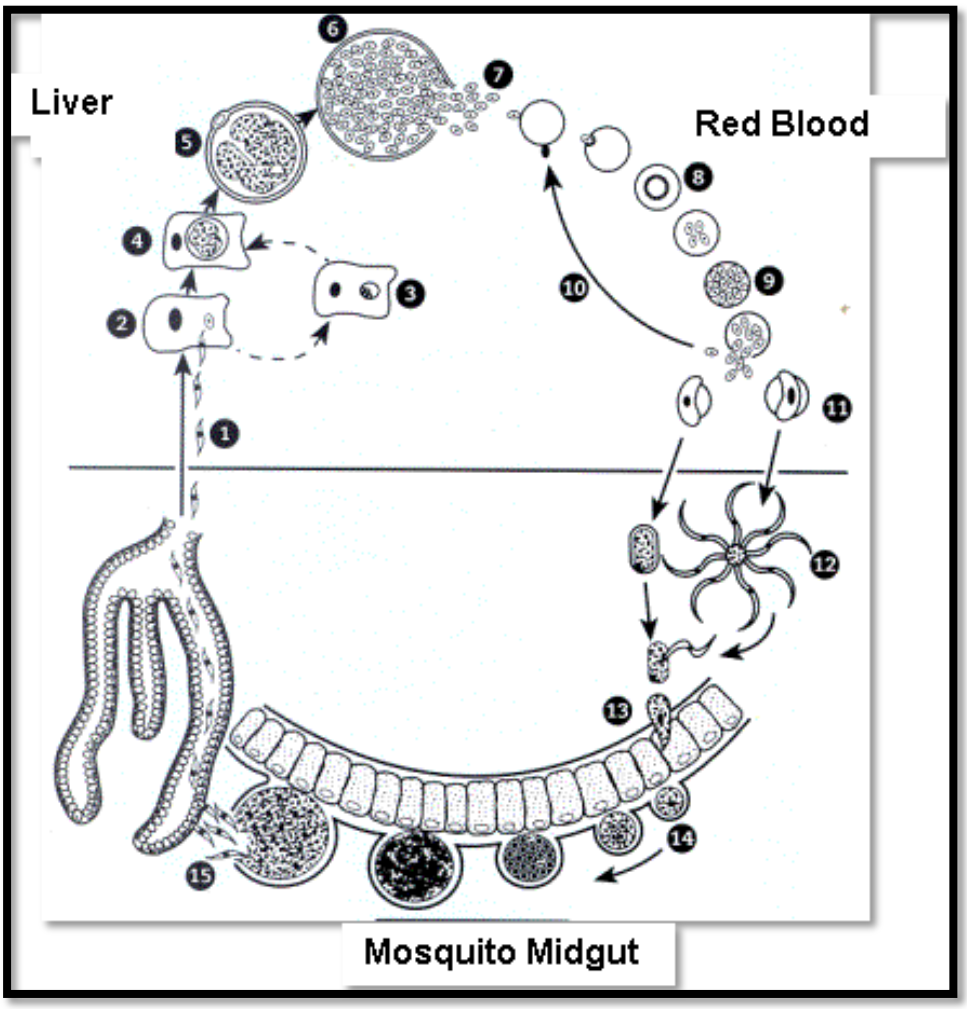

Macrogametocytes (female)
P. vivax
P. malariae
P. falciparum
P. ovale

In P. falciparum and P. malariae hypnozoites are not formed and the parasite develops directly into pre-erythrocytic schizonts.

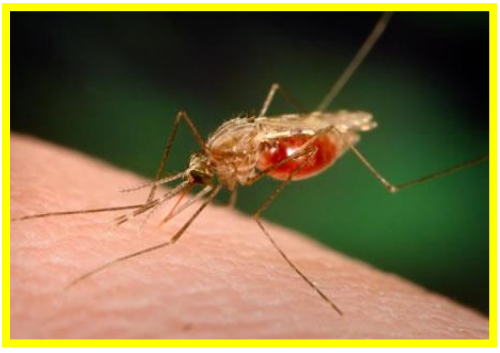
Merozoites released from the infected liver cells invade erythrocytes. The merozoites recognize specific proteins (glycophorin)on the surface of the erythrocyte .the differences in glycoprotein of RBCs of different species may account for species specificity of malarial parasites.

The invasion phenomenon is determined by the type of antigen present on the surface of the red blood cell. For instance, merozoite engulfment requires at least one of two Duffy antigens (Fya + or Fyb +).

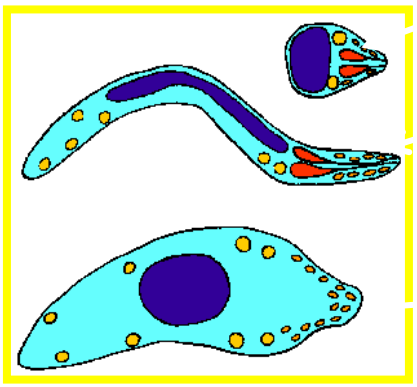
Once in the circulation, the merozoites invade the red cells and develop into trophozoites. In the course of their development they absorb the hemoglobin of the red cells and leave as the product of digestion a pigment called hemozoin, a combination of hematin and protein. This iron-containing pigment is seen in the body of the parasite in the form of dark granules, which are more obvious in the later stages of development.



Merozoites are pear-shaped bodies ,about 1.5um in length possessing apical complex (rhoptry) by which attach to the RBC and secret substance producing pit and the merozoitesphagocytosed and the cell membrane seals itself to form vacuole (parasitophorous v.).Merozoites in RBCs appears a round shape when stained Giemsa or Romanowsky stains , cytoplasm blue and karyosome red with unstained vacuole give parasite ring appearance so called (ring stage) .



Female of Anopheles mosquito



merozoite

- **non-motile**
- **invades erythrocytes**

sporozoite

- **motile**
- **invades mosquito salivary glands**
- **invades liver cells**

ookinete

- **motile**
- **invades mosquito gut epithelial cells**

Ring stage enlarges and develops and become irregular shape showed amoeboid motility so called (amoeboid stage) in *P.vivax* or may called (band forms) in *P.malariae*. When this stage reach to certain stage of development. Nuclear division marks the end of the trophozoite stage and the beginning of the schizont stage.

After a period of growth the trophozoite undergoes an asexual division, erythrocyticschizogony. When the mature trophozoite starts to divide in the red blood cell, separate merozoites are formed resulting in a schizont. When fully developed, the schizont ruptures the red blood cell containing it, liberating the merozoites into the circulation. These merozoites will then infect new red cells and

the process of asexual reproduction in the blood tends to proceed. Some of the merozoites entering red blood cells do not form trophozoites then schizonts but develop into gametocytes and this process takes place in deep tissue capillaries. This erythrocytic cycle of schizogony is repeated over and over again in the course of infection, leading to a progressive increase of parasitemia.

Erythrocytic schizogongy consists of number of rounds merozoites (depending on species) of nuclear replication followed by a budding process.When nucleus divided and cytoplasm undivided this called early stage schizont.When each daughter nucleus surrounded by cytoplasm, Late stageschizonts in which the individual merozoites become discernable are called segmenters. The host erythrocyte ruptures and releases the merozoites. These merozoites invade new erythrocytes and initiate another round of schizogony.

The intermittent fevers often associated with malaria are due to the synchronous rupture of infected erythrocytes and rupture of schizont releases of merozoites and large quantities of pyrogens. This responsible for febrile paroxysm characterizing malaria.

The interval between entry of the sporozoite into the host and earliest manifestation of clinical illness is called incubation peroid. This is different from prepatent period which taken from the time of entry of sporozoites to the first appaerance of parasites in peripheral blood.

Infections with all four strains of malaria have many clinical features in common. These are related to the liberation of fever-producing substances, especially during schizogony.

The common features are:

Fever: Often irregular. The regular pattern of fever does not occur until the illness has continued for a week or more; where it depends on synchronized schizogony.

Anemia: The anemia is hemolytic in type. It is more severe in infections with P. falciparum because in this infection cells of all ages can be invaded. Also, the parasitemia in this infection can be much higher than in other malarias.

Splenomegaly: The spleen enlarges early in the acute attack of malaria. When a patient has been subjected to many attacks, the spleen may be of an enormous size and lead to secondary hypersplenism.

Jaundice: A mild jaundice due to hemolysis may occur in malaria. Severe jaundice only occurs in P. falciparum infection, and is due to liver involvement.

Plasmodium falciparum exists in the tropics and sub-tropics, and is responsible for approximately 50% of all malaria cases. The incubation period of P. falciparum malaria is the shortest, between eight and 11 days and has a periodicity of 36–48 hours. It can be differentiated from the other species by the morphology of the different stages found in the peripheral blood. In infections with Plasmodium falciparum usually only young trophozoites and gametocytes are seen in peripheral blood smears, the schizonts are usually found in capillaries sinuses of internal organs and in the bone marrow. The disease it produces runs an acute course and often terminating fatally. It is a significant cause of abortions and stillborns and even death of non-immune pregnant women.

Morphological Differences:-

The blood-stage parasites of human Plasmodium species exhibit differences in their morphology and modify the host erythrocyte differently .These differences can be used to distinguish the four species.

1-P. falciparum :-

- No change in size and color of RBCs.
- Stained RBCs may contain Maurer's spots.
- Blood smears are characterized by the presence of young trophozoites (rings) in the absence of trophozoites and schizonts.
- The ring stages of P. falciparum tend to be slightly smaller than the other species and are generally more numerous. Accole forms, which are characteristic of *P. falciparum,* because of attempted re - invasion of the red blood cell by merozoites.
- The crescent-shaped gametocytes of P. falciparum are very distinctive.

2-P. vivax :-

- The most distinctive features of P. vivax are the enlarged infected erythrocytes.
- Appearance of red granules, called 'Schüffner's dots', over the erythrocyte cytoplasm.
- The growing trophozoite of P. vivax often has an ameboid appearance
  .
- The schizonts can have more than 20 merozoites.

3-P. ovale :-

- Also exhibits Schüffner's dots.
- Enlarged erythrocyte, with irregular edges .
- In general P. vale is more compact parasite than P.vivax.
- Fewer merozoites (8-14)are found per schizont.
- P. ovale also has more of a tendency to form elongated host erythrocytes.

4-P. malariae :-

- Is characterized by a compact parasite (all stages).
- Not alter the host erythrocyte or cause enlargement.
- Elongated trophozoites stretching across the erythrocyte, called band forms, are sometimes observed.
- Schizonts will typically have 8-10 merozoites that are often arranged in a rosette pattern with a clump of pigment in the center.
- See small granules called ziemann,s dots.

Duration of erythrocytic phase :-

The duration of this phase varies according to the species. All mature schizonts in body burst at the same releasing merozoites and pyrogens in circulation cause febrile paroxysm it suggested that schizogonic periodicity is related to human circadian rhythm. Tertian and quartan refer to the differences in the periodicity of paroxysms.

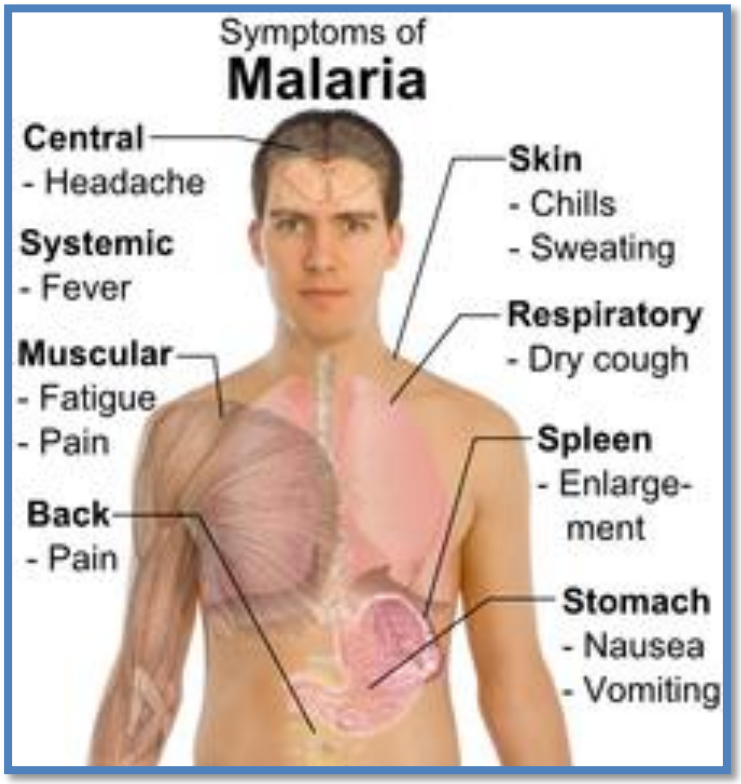
P. vivax------cause benign tertian malaria fever recurs after interval of 48 hours or every third day.

P. falciparum ----cause malignant tertian malaria also called sub-tertian because the cycles are often poorly synchronized and febrile paroxysm recur after intervals of less than the expected 48 hours also called pernicious malaria.

P. malariae -------cause quartan malaria refers to a 72 hour periodicity (occurring every forth day) .

P. ovale ----------cause ovale tertian because of its tertian periodicity and the irregular oval shape of infected RBCs.

Pathogenecity of malaria:-



Clinical manifestation in a typical case are series of febrile paroxysm followed by anemia and splenomegaly.

Febrile paroxysm:-

start generally in early afternoon but actually it may start at any time, each paroxysm shows succession of three stages :-

1-cold stage (lasting 20-60 min).

2- hot stage (lasting 1-4 hours).

3-sweating stage (lasting 2-3 hours).

The total duration of febrile cycle is from (6-10 hours) according to species , the febrile paroxysm synchronizes with erythrocyticschizogony of parasite.

A- with 48 hrs cycle the fever recur every third day tertian fever.

B-with 72 hrs cycle the fever recur every forth day quartan fever.

C-sometimes , especially in early infection with P. vivax there may be two independent broads of parasites with overlapping cycles so the fever recurring at intervals of 24 hrs , this called (quotidian periodicity) .

D- in P.vivax and P.ovale this due to the maturation of two generations of terian parasite on two successive days (tertian duplex).

E-in P.falciparum may not show three successive cold, hot ,sweating stages fever in this case continuous instead of intermittent or is (remittent). This explain that several generation of parasites multiplying at different intervals.

Anemia:- After febrile cycle or fever paroxysm ,anemia of mycrocytic or normocytic , hypochromic develops as resulting of breaking down of red blood cells during the phase of segmentation of the parasite.

This progressive decrease in the number and quantity of circulating erythrocytes, with corresponding reduction in oxygen transport, causing oxygen starvation of the tissues, is followed by multiple thromboses in the smaller blood vessels and progressive decrease in circulating blood volume.

- **Splenomegaly:-**

Enlargement of spleen is the important physical signs of malaria. Haemolysis of infected RBC causes anaemia ,enlarged spleen which contributes to thrombocytopaenia, leucopaenia, and jaundice.

- **liver cell necrosis** producing hypoglycaemia via impaired gluconoegenesis
- **renal ischaemia** and cortical haemorrhage causing renal impairment and failure.

- dysfunction of cells of the small intestine contributing hypoglycaemia to and causing diarrhoea.
- **placental dysfunction** and degeneration causing low fetal birth weight and stillbirth in the pregnant women.

Pernicious malaria:-

The term refers to a series of phenomena occurring during the cause of infection with plasmodium falciparum , which is effectively treated threatening of life for patient within 1-3 days.

The pathogenesis of pernicious malaria has been attributed to certain pecular biological features of plasmodium falciparum which cause agglutination of parasitized RBCs leading to blockage of the capillaries vessels of internal organs , these features are:-

1- Erythrocyticschizogony of plasmodium falciparum occurring inside the capillary vessels of the internal organs.

2- Segmenting forms of parasites being unable to alter their shapes during their passages onside the capillaries vessels act as emboli.

3- The growing trophozoites and sexual forms (ovoids) become adhesion to each other as well as the vessels wall, resulting in agglutination and blocking of the vessels.

Parasitaemia , schizonts as well as ring forms are commonly present in large number in peripheral blood.

Black water fever:-

It is manifestation of plasmodium falciparum occurring in previously infected subject and characterized by sudden intravascular hemolysis followed by fever and passage of hemoglobin in the urine.

It is associated with infection by plasmodium falciparum most commonly observed amongest the non-immune population who have resided in malarious countries for 6 months to 1 years and have inadequate doses of quinine for both suppressive prophylaxis and treatment of repeated clinical attacks.

Intravascular hemolysis , the exact mechanism hemolysis in black water fever is not yet clearly known . There appears to be some hemolytic agent involved where by RBCs undergo lysis and liberate a large quantities of oxy-hemoglobin in to the blood stream ,in the plasmodium falciparum the intravascular occurs periodically at the time of schizogony.

Cerebral Malaria

Cerebral malaria is an acute disease of the brain, which is accompanied by fever, convulsions and paralysis or coma. It is due to the sequestration of brain capillaries and venues with parasitised red blood cells and nonparasitised red blood cells.

Laboratory diagnosis:-

Microscopically examination of blood film is considered as most important diagnostic procedure in malaria.

Blood film ,Thick blood film for quick identification of malarial infection, Thin film for identification of species.

Other methods for diagnosis:-

1-culture examination , for differentiation between trophozoite (ring stage) of different species.

2- blood count, little importance.

3-serological diagnosis:-

a-specific complement fixation test and priciptin test.

b-non-specific test, Henry's melano-flocculation.

4-molecular diagnosis by polymerase chain reaction.

Treatment :-

Natural alkaloid (quinine).

Synthetic antimalarial drugs, includes :- 4-aminoquinoline, chloroquinecamoquine, pyrimidines (dorapram, pyrimethmine).

Prophylaxis:-

Protection against mosquito bites.

Systemic use of antimalarial drugs.

Prevention of carriers by 8-aminoquinoline.

Anti-mosquito measures as destruction of adults by DDT and antilarval measures.

Host Immune Response

The immune response of the human host differs somewhat for each of the two stages in the malarial life cycle (i.e., the pre-erythrocytic stage and erythrocytic stage) . It is believed that T-cells, notably CD8 +T cells, play an important role in pre-erythrocytic immunity .

The target for innate immune activity to this stage appears to be parasite-antigen bound molecules expressed on the surface of infected hepatocytes .

On the other hand, CD4 + T cell regulation appears to play a critical role in acquired immunity to the erythrocytic stage of malaria infection.

TABLE 7-1 Diagnostic Differences among the Four Species of Human-Infecting *Plasmodium* (See color plates 1–4 for further reference.)

	Plasmodium vivax	*Plasmodium malariae*	*Plasmodium ovale*	*Plasmodium falciparum*
Duration of schizogony	48 hours	72 hours	49–50 hours	36–48 hours
Motility	Active amoeboid until about half grown	Trophozoite slightly amoeboid	Trophozoite slightly amoeboid	Trophozoite active amoeboid
Pigment (hematin)	Yellowish-brown; fine granules and minute rods	Dark brown to black; coarse granules	Dark brown; coarse granules	Dark brown; coarse granules
Stages found in peripheral blood	Trophozoites, schizonts, gametocytes	Trophozoites, schizonts, gametocytes	Trophozoites, schizonts, gametocytes	Trophozoites, gametocytes
Multiple infection in erythrocyte	Common	Very rare	Rare	Very common
Appearance in infected erythrocyte	Greatly enlarged; pale with red Schuffner's dots	Not enlarged; normal appearance with Ziemann's dots	Slightly enlarged; outline oval to irregular, with Schuffner's dots	Normal size; greenish; basophilic Maurer's clefts and dots
Trophozoites (ring forms)	Amoeboid; small and large rings with vacuole and usually one chromatin dot	Small and large rings with vacuole and usually one chromatin dot; also young band forms	Amoeboid; small and large rings with vacuole	Very small and large rings with vacuole, commonly with two chromatin dots; amoeboid
Segmented schizonts	Fills enlarged RBC; merozoites irregularly arranged around mass of pigment	Almost fills normal-sized RBC; 6-12 merozoites regularly arranged around central pigment mass	Fills approx. $^3/_4$ of RBC; 6-12 merozoites around centric or eccentric pigment mass	Not usually seen in peripheral blood
Gametocytes	Round; fills RBC; chromatin undistributed in cytoplasm	Round; fills RBC; chromatin undistributed in cytoplasm	Round; fills $^3/_4$ of RBC; chromatin undistributed in cytoplasm	Crescentic- or kidney-shaped; chromatin undistributed in cytoplasm