Plasmodium Spp.

- **Definition**
- Malaria is a mosquito-borne infectious disease of humans and other animals caused by the genus *Plasmodium*, transmitted by the bite of female anopheles mosquito.
- Malaria is the most important parasitic disease being widespread in tropical and subtropical regions in a broad band around the equator, including much of Sub-Saharan Africa, Asia, and the Americas.

- The disease results from the multiplication of malaria parasites within red blood cells, causing symptoms that typically include fever and headache, in severe cases progressing to coma, and death.
- Name is derived from Italian **Mal' aria or bad air**

Why it is important in Medicine

- Malaria remains the world's most devastating human parasitic infection. Malaria affects over 40% of the world's population. WHO, estimates that there are 350 - 500 million cases of malaria worldwide, of which 270 - 400 million are *Falciparum* malaria, the most severe form of the disease.
- Malaria parasite cause life- threatening protozoan disease.
- It is the most important of all the tropical disease in terms of morbidity and mortality.
- Malaria kills in one year what AIDS kills in 15 years. For every death due to HIV/AIDS there are about 50 deaths due to malaria.
- The incidence of malaria is increasing due to resistance of vectors to insecticides and drug resistant parasite

Malaria parasite infecting humans belong to four species:

- **Causative organism: *Plasmodia***
  - ***Plasmodium vivax*: tertian malaria**
  - ***Plasmodium malariae*: quartan malaria**
  - ***Plasmodium falciparum*: malignant malaria**
  - ***Plasmodium ovale*: tertian malaria**

Life Cycle

A- Human cycle-Shizogony

Included Four stages

- 1 - Pre or exoerthrocytic schizogony
- 2-Erythrocytic schizogony
- 3-Gametogony
- 4-Secondery exoerthrocytic schizogony

B- mosquitoes cycle

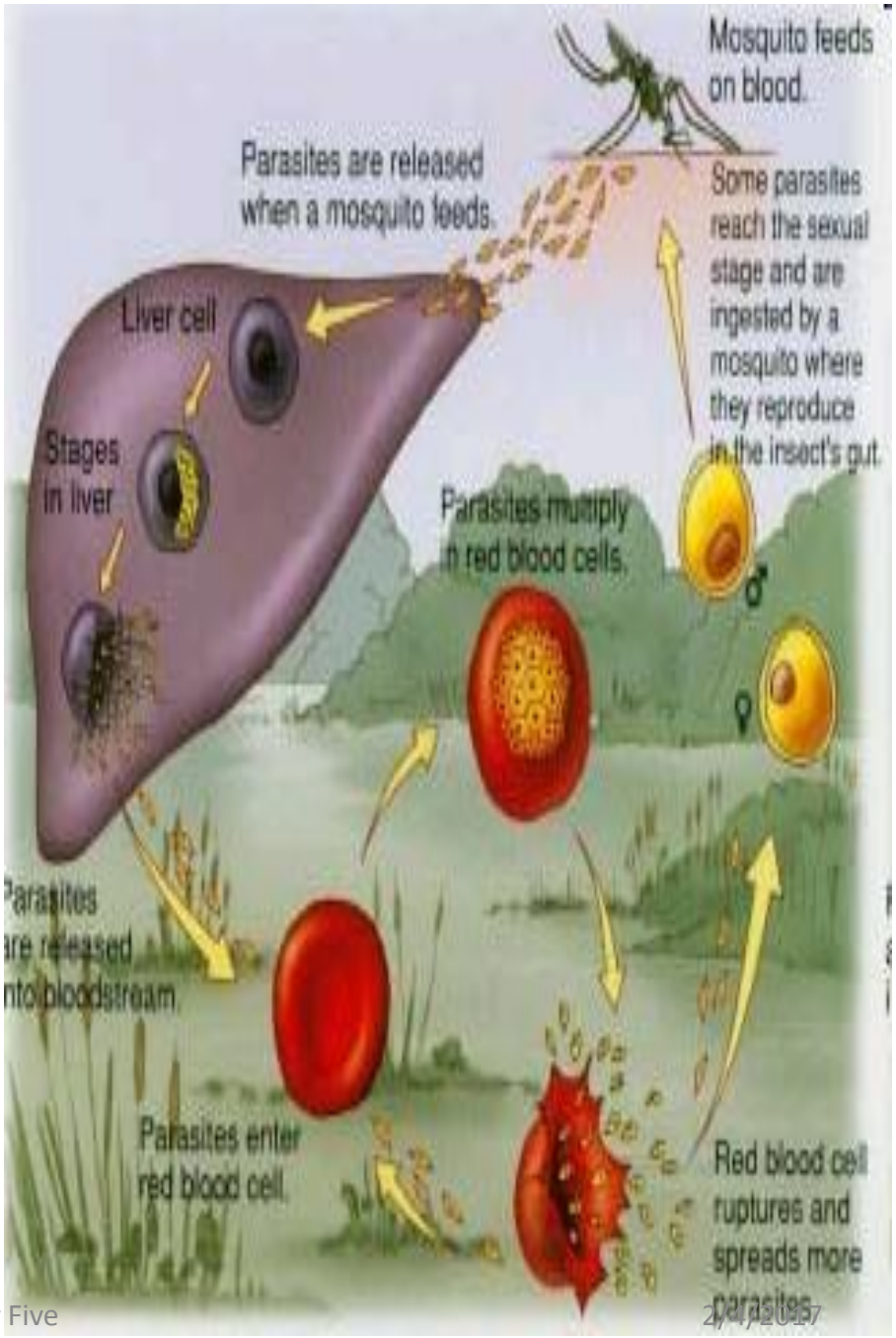
- Sporozoites are infective formsPresent in the salivary gland of female anopheles mosquito
- After bite of infected mosquito sporozoites are introduced into blood circulation.

Man – Intermediate host.

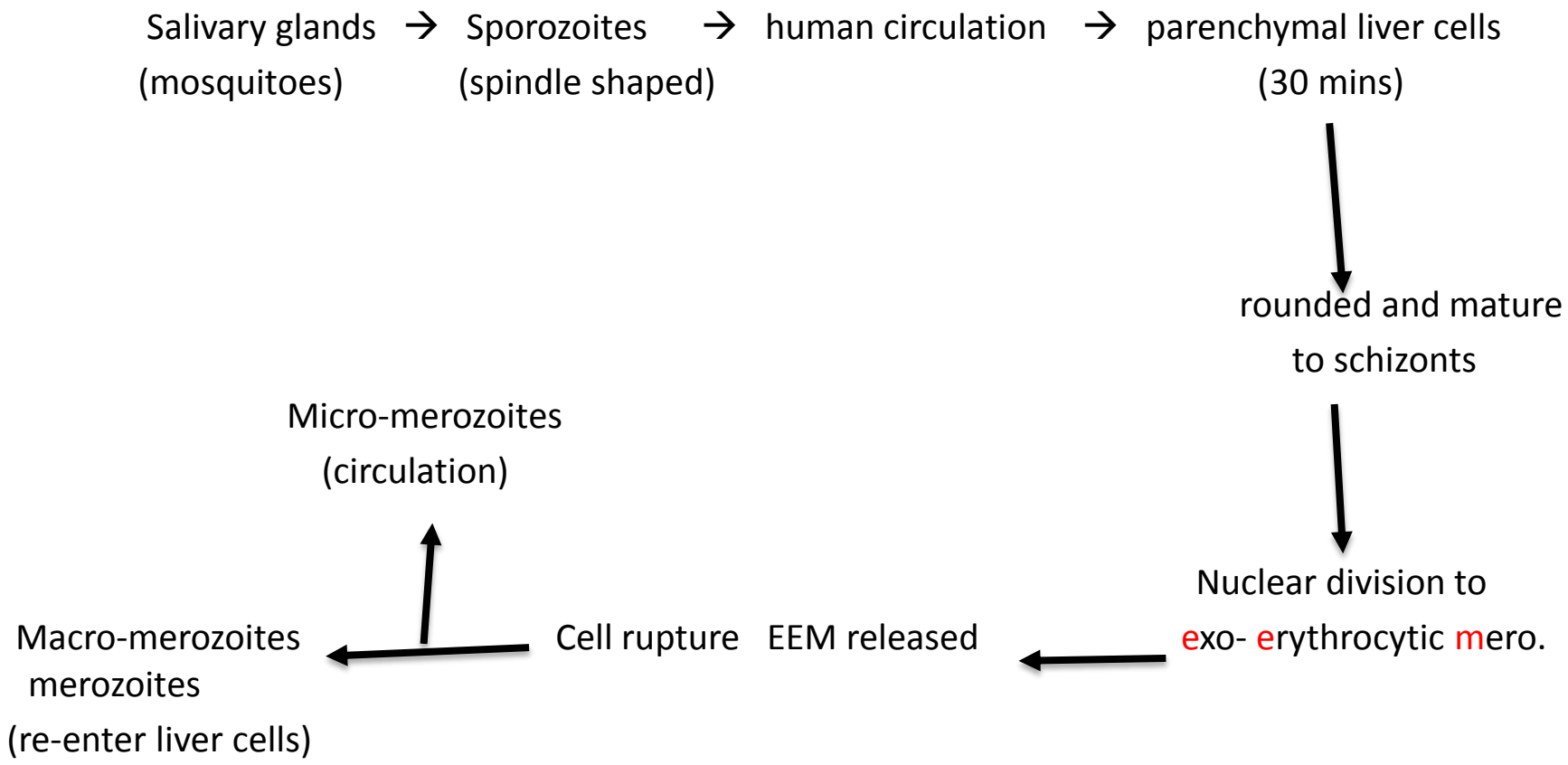
Mosquito – Definitive host

1 - Pre-Erythrocytic Shizogony

- Developmental phase inside the tissue (liver) of man, Site- parenchyma cells (hepatocyte) of liver, Sprozoites are elongated and spindle shaped become rounded inside the liver parenchyma, Multiple nuclear divisions develop to Schizonts, Consists of single generation of pre erythrocytic
- schizont which liberates merozoites
- Duration -- P.V.-8 days, P.O.-9 days
  P.F.-6 days, P.M.-15days
- i)merozoites – enter circulation
  ii)merozoites—re-enter liver cells
- No clinical manifestations or pathological damage



- # Exo-erythrocytic Schizogony

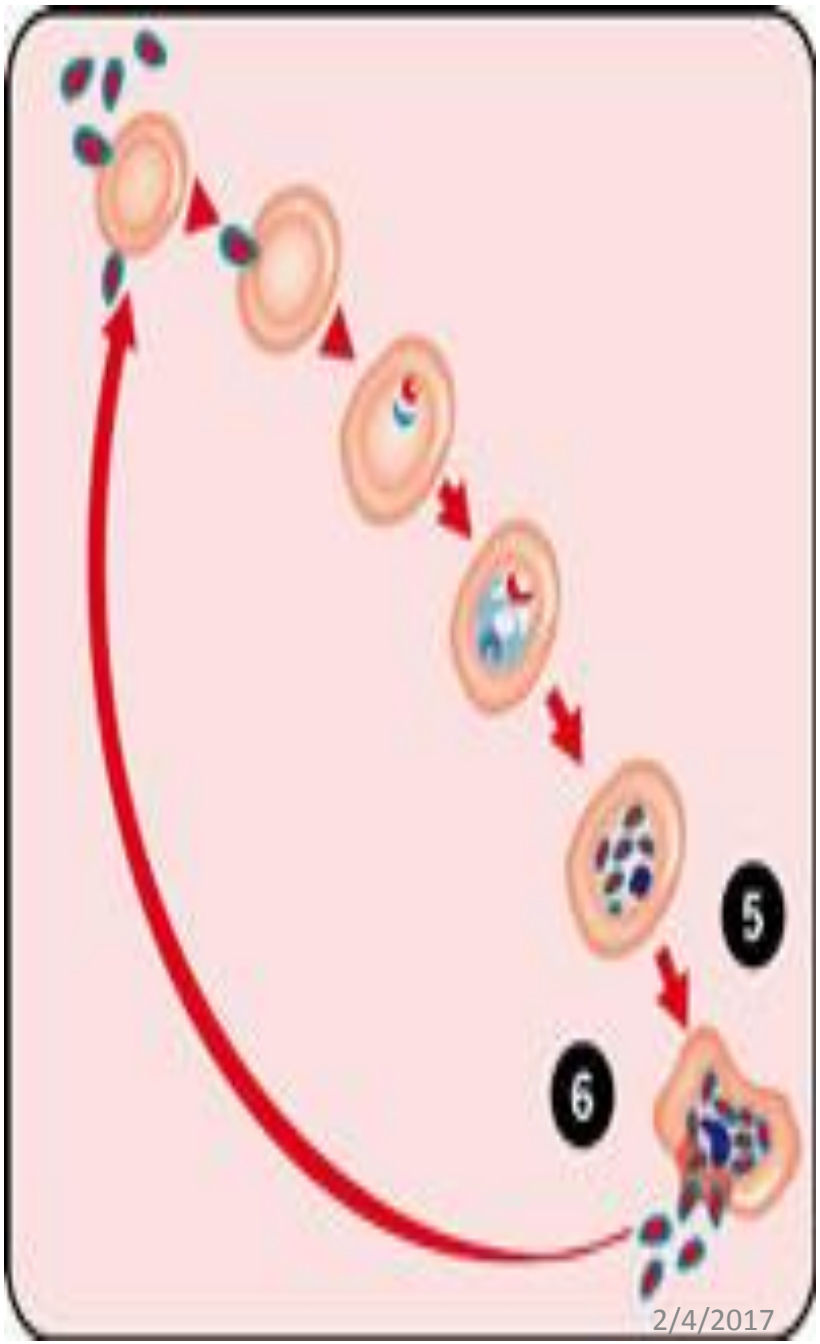


2 - Erythrocytic Schizogony

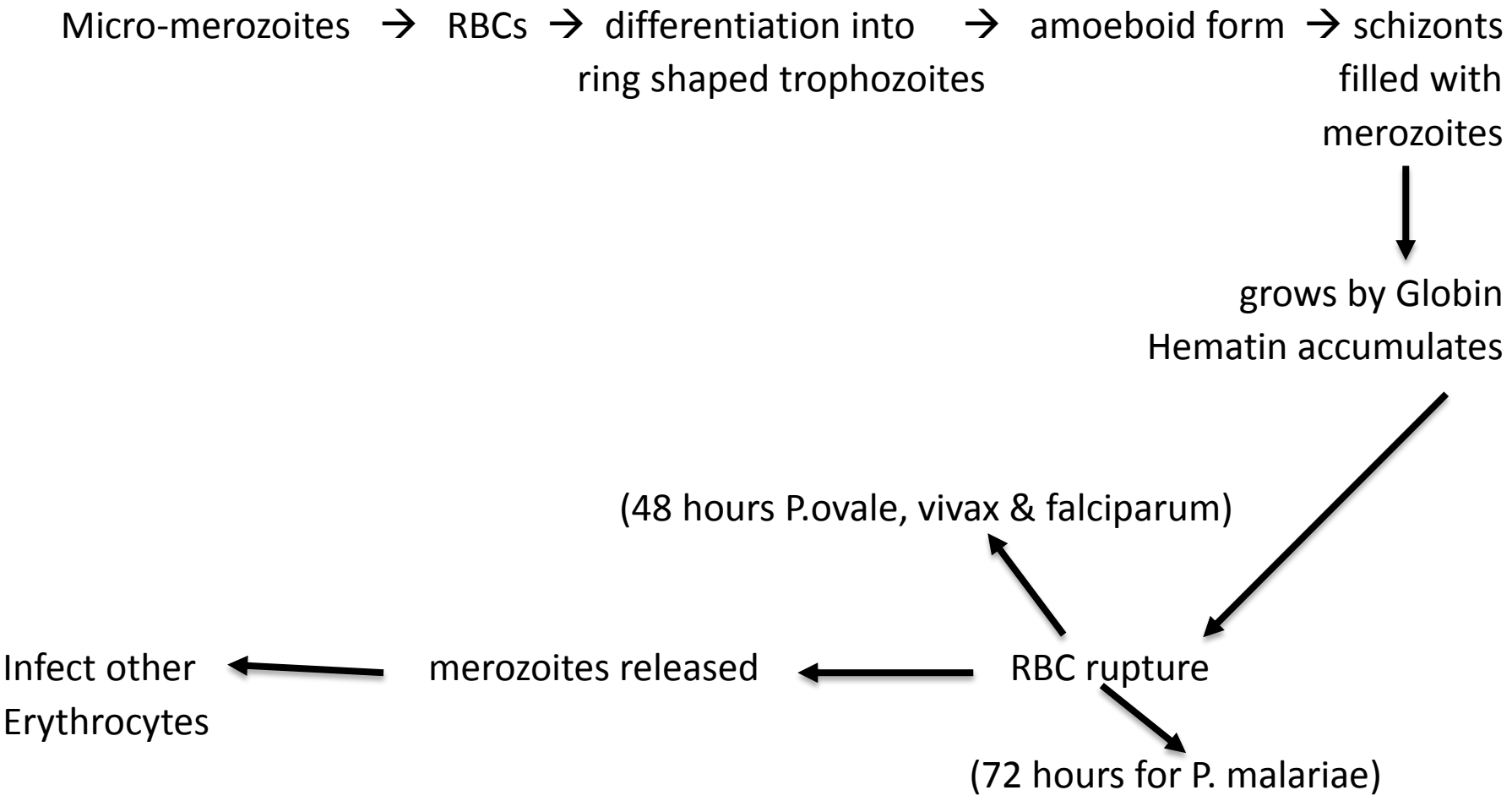
- Merozoites released invade red cells, *P.vivax* & *P. ovale* infects young erythrocytes
- *P.malariae* Infects old erythrocytes, *P.falciparum infects RBC of all ages*
- The Merozoites are pear shape, The receptors for Merozoites are on red cells in the glycoprotein
- Liberated Merozoites penetrate RBC,Three stages occur

1. **Trophozoites 2 .Schizont 3. Merozoite**

- Ruptured red cells release Merozoites which attack new red cells ,Continue with Schizogony, Repeated cycles will continue
- In *P.falciparum* - infected erythrocytes with Schizonts aggregate in the capillaries of brain and other internal organs
- Only ring forms are seen in the blood smears



- **Erythrocytic Schizogony**



- **Erythrocytic merozoites do not reinvade the liver cells.**
- **So malaria transmitted by blood transfusion reproduces only erythrocytic cycle**

- **Affinity of Parasite to Erythrocytes:**
- *P. vivax* Infects young
- *P. malariae* Infects old
- *P.ovale* Infects young
- *P. falciparum* Infects all age groups

- The metabolism of the malaria parasite is largely dependent on the digestion of red cell hemoglobin, which is transformed into malaria pigment. Pigment is absent in the ring stage and becomes detectable only in late trophozoite and the schizont stage.

- The malaria pigment may be yellowish –brown or dark brown in colour.

3 - Gametogeny

- After many cycles of erythrocytic shizogony
- Some merozoites give rise to Gametocytes, capable of sexual reproduction after leaving human host
- Develop in RBCs of the capillaries of internal organs
- Mature gametocytes are seen in peripheral blood
- Microgametocyte of all species are similar in size
- Macro gametocytes are larger in size.
- Duration of maturation- 4 days
- No febrile reactions

4 - Secondary exoerthrocytic schizogony

- In 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 undergoing multiplication without delay. The resting stage of the parasite is known as hypnozoite .
- After a period of weeks, months or years (usually up to 2 years). Hypnozoites are reactivated to become secondary exoerthrocytic schizonts and release merozoites which infect red blood cells producing relapse of malaria.

❖ The relapse: is the situation in which the erythrocytic infection is eliminated and a relapse occurs later because of a new invasion of the RBCs from liver merozoites.

Recrudescence

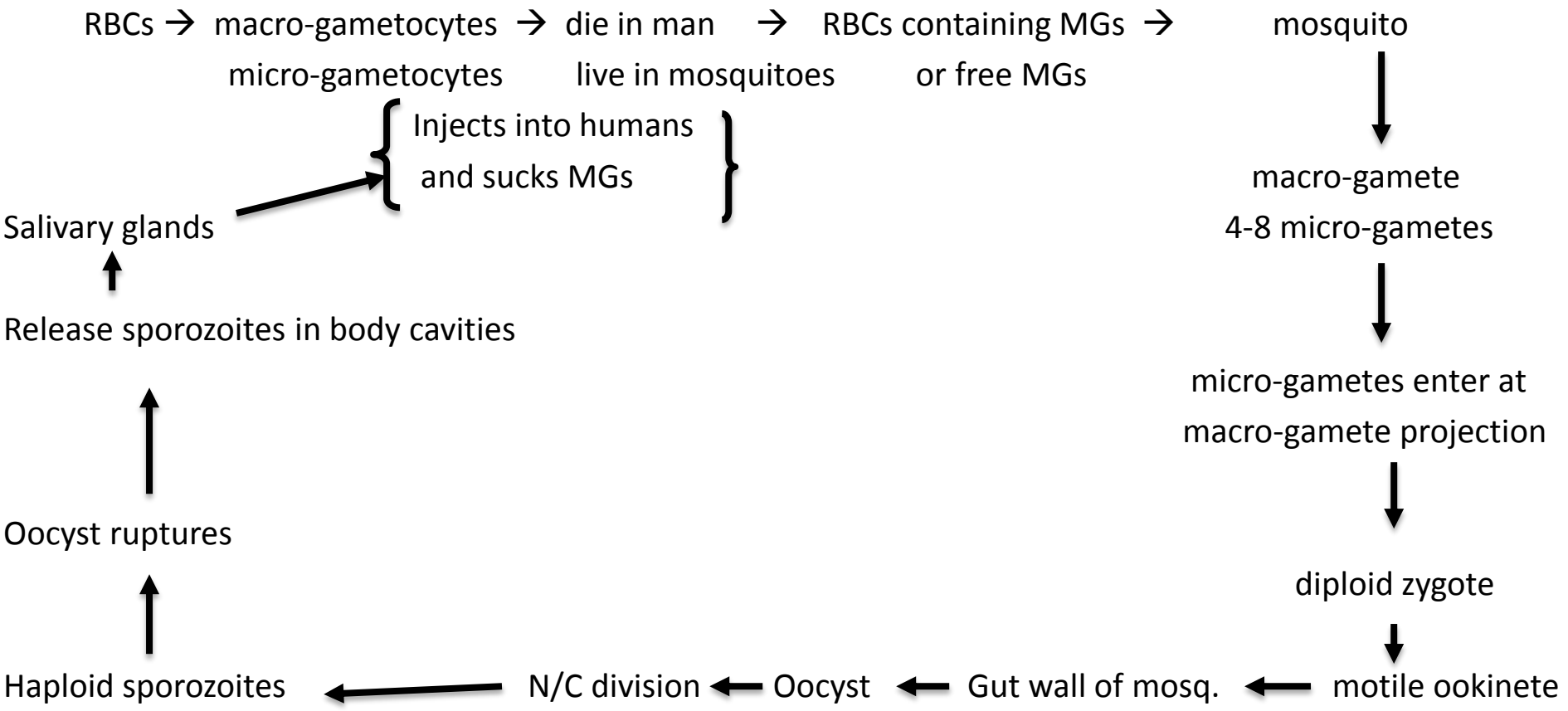
- The situation in which the RBC infection is not eliminated by the immune system or by the therapy and the numbers in the RBCs begin to increase again with subsequent clinical symptoms

- Recrudescence in *P. malaria* is due to survive in the peripheral blood at every low level of parasitaemia for a considerable time (10 years or more).

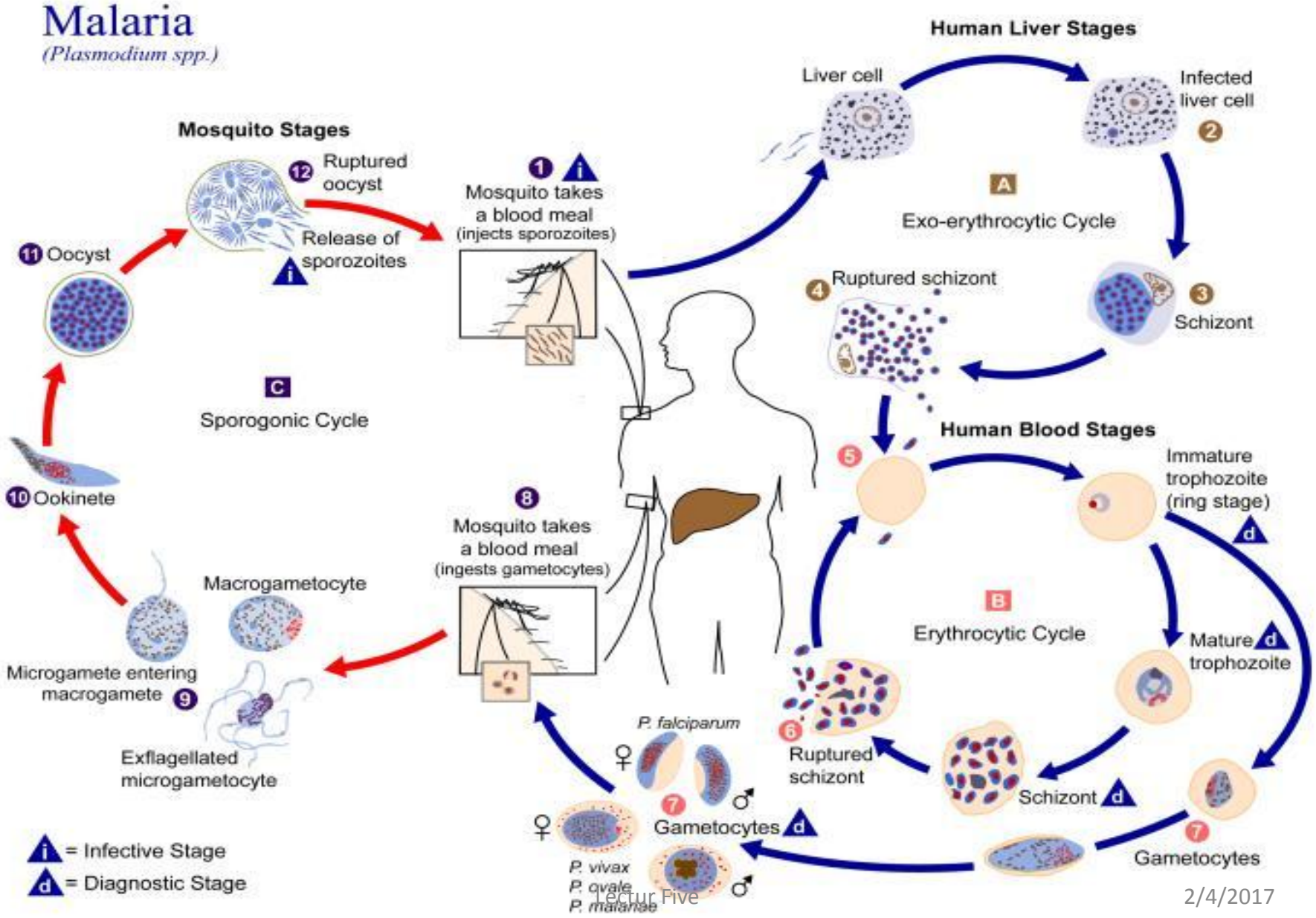
B - Mosquito cycle – Sporogony

- Sexual cycle will be initiated in the humans by the formation of Gametocytes, develop further in the female Anopheles Mosquito, Only mature sexual forms are capable of further development in Mosquito
- In midgut one Microgametocyte develops into 4-8 thread like filamentous structures named Micro gametes (exflagellation). From one macrogametocyte only one macrogamete is formed
- Fertilization occurs when a Microgametocyte penetrate into Macrogametocyte
- Fertilized macrogametocyte is known as Zygote, Zygote matures into Ookinete, Ookinete to Oocyst
- Oocyst matures with large number of Sporozoites (A few hundred to thousands.). Oocyst ruptures and release Sporozoites in the body cavity of Mosquito, There is a specific predilection for salivary glands
- Now capable to transmit the infection to new Host

• Sexual Cycle: Sporogony



Life Cycle


Malaria
(Plasmodium spp.)
Human Liver Stages
Liver cell
Infected liver cell
2
A
Exo-erythrocytic Cycle
Ruptured schizont
4
3
Schizont
Human Blood Stages
5
Immature trophozoite (ring stage)
d
B
Erythrocytic Cycle
Mature trophozoite
d
6
Ruptured schizont
Schizont
d
7
Gametocytes
Gametocytes
d
P. falciparum
P. vivax
P. ovale
P. malariae
7
Gametocytes
d
8
Mosquito takes a blood meal (ingests gametocytes)
Macrogametocyte
Microgamete entering macrogamete
9
Exflagellated microgametocyte
10 Ookinete
11 Oocyst
C
Sporogonic Cycle
Mosquito Stages
12 Ruptured oocyst
Release of sporozoites
i
1
i
Mosquito takes a blood meal (injects sporozoites)
i = Infective Stage
d = Diagnostic Stage

Cycles differs in Different species

Duration of erythrocytic schizogony:

Cycle repeats every 48 hours in

P. falciparum, P. ovale and *P. vivax*

(Repeats every 72 hours In *P. malaria*)

Incubation period Which includes Exo eythrocytic cycle time and one or two erythocytic cycles:

P. vivax 14 days and *P. falciparum* 12 days *p. ovale* 17 days and *P. malariae* infection can start after 28 days.

(Clinical Manifestations are related to cycle of events in relation to RBC).

Extrinsic incubation period: take different periods for their development of sexual cycles in Anopheles (8-35 days).

- **Trophozoites:** Growing form in human blood (ring form and all stages onwards except fully grown gametocytes and Schizonts)
- **Schizont:** Asexually dividing form, Immature schizont , Mature schizont
- **Schizogony:** Asexual reproduction → N/C divides → Merozoites in RBC and liver
- **Sporogony:** Sexual reproduction forming sporozoites (mosquitoes)
- **Sporozoite:** The morphological form which develops in the mosquito salivary gland and is injected when the mosquito feeds, infecting humans.
- **Gametocyte:** From some trophozoites or merozoites in RBCs It is infective to mosquito
- **Gametes:** From micro and macro-gametocytes
  Macro-gamete/female (nuclear reduction 1:1)
  Micro-gamete/male (exflagellation 1:4-8)
- **Zygote:** Fertilized macro-gamete
- **Ookinete:** Active motile zygote
- **Oocyst (Spore):** Rounded, immotile ookinete, membranous cyst wall, containing many sporozoites

Mode of transmission

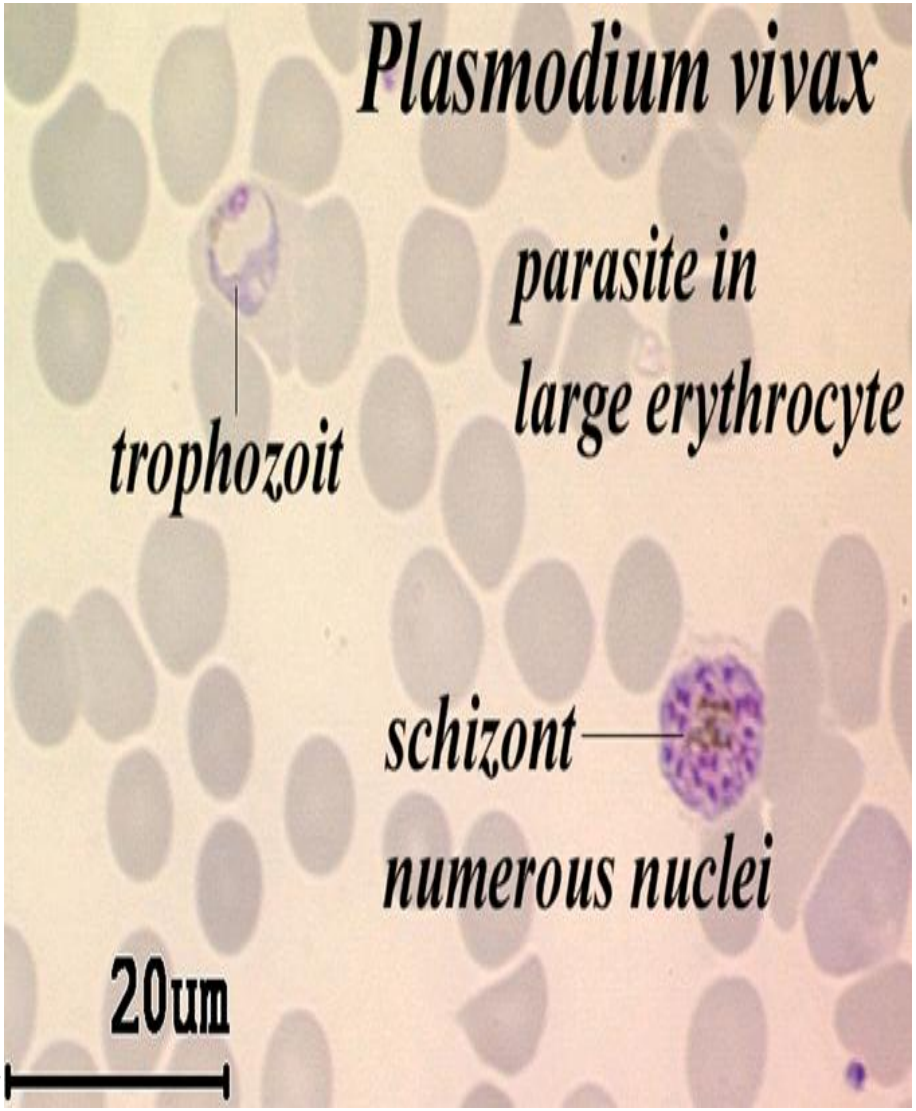
- Malaria is transmitted by the bite of an infective female Anopheles mosquito.
- Other methods: Transfusion of blood from a patient of malaria. that is known as transfusion malaria.
- Transmission of infection to fetus in utero through some placental defect. This is known as congenital malaria.
- By the use of contaminated syringes particularly in drug addicts.
- The other methods are also known as trophozoite-induced malaria. In this condition there is no primary and secondary exoerthrocytic schizogony, incubation period is short and there is no relapse

Morphology

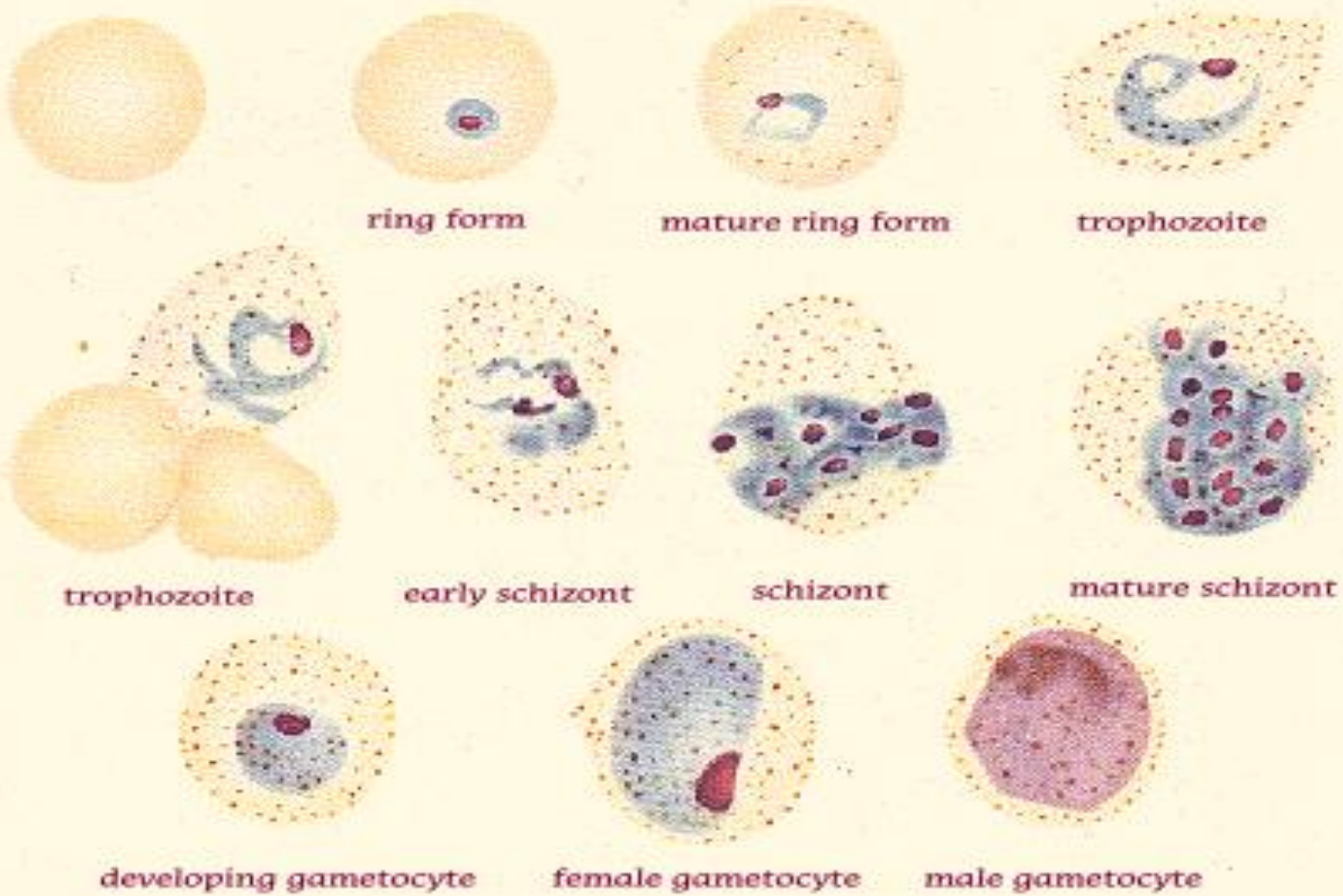
FEATURE	*P.VIVAX*	*P.MALARIAE*	*P.FALCIPARUM*	*P.OVALE*
Infected RBCs	**Enlarged, pale, fine stippling(schuffner's dots) primarly invade reticulocytes, young RBCs**	**Not enlarged,no stippling,primarly invades older RBCs, Ziemann's dots on prolonged staining**	**Not enlarged, coarse stippling-Maurer's clefts, invades all RBCs**	**Enlarged,oval,fimbriated RBCs,pale,conspicous James dots**
Ring stage trophozoites	**Large rings (1/3–1/2 red cell diameter). Usually one chromatin granule; ring delicate.**	**Large rings (1/3 red cell diameter). Usually one chromatin granule; ring thick.**	**Small rings (1/5 red cell diameter). Often two granules; multiple infections common; ring delicate, may adhere to red cells.**	**Large rings (1/3 red cell diameter). Usually one chromatin granule; ring thick.**
Pigment in developing trophozoites	**Fine; light brown; scattered.**	**Coarse; dark brown; scattered clumps; abundant.**	**Coarse; black; few clumps.**	**Coarse; dark yellow-brown; scattered.**
Older trophozoites	**Very pleomorphic.**	**Occasional band forms.**	**Compact and rounded.**	**Compact and rounded.**
Mature schizonts (no. of merozoites)	**More than 12 merozoites (12–24).**	**Large merozoites (6–12). Often in rosette.**	**merozoites (6–32). Very rare in peripheral blood.**	**large merozoites (6–14). Often in rosette (irregular).**
Gametocytes	**Round or oval.**	**Round or oval.**	**Crescentic.**	**Round or oval.**

Plasmodium vivax

- Ring stage large, usually single, prominent thicker chromatin
- Number of merozoites 12 to 24 irregular grape like clusters
- RBC enlarged
- Pigment fine golden
- Schuffner's dots present (small red dots)
- Schizont large filling the Red blood cell
- Gametocytes – spherical or globular,
- Size much larger than red cell

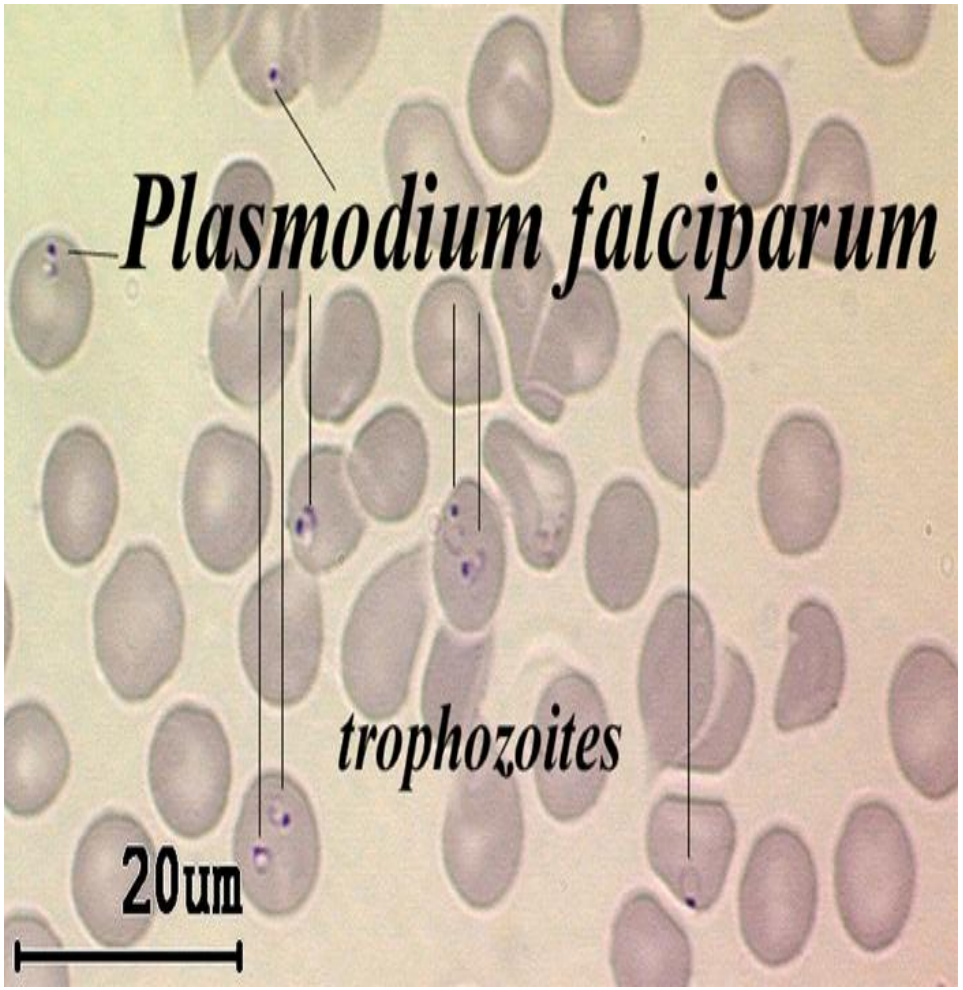


P. vivax

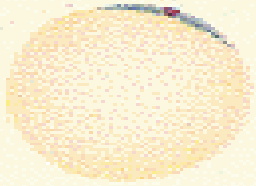

ring form
mature ring form
trophozoite
trophozoite
early schizont
schizont
mature schizont
developing gametocyte
female gametocyte
male gametocyte

Plasmodium falciparum

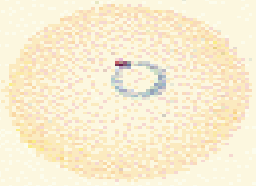
- Ring stage delicate, small. Double chromatin and multiple rings common
- RBC is normal size
- **Maurer's dots** , large red spots sometimes basophilic stippling
- Pigment dark brown or blackish one or two solid blocks
- Gametocytes Crescent, larger than a red cell



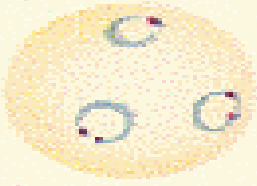
P. falciparum



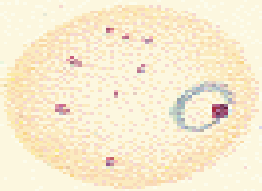
marginal form



ring form

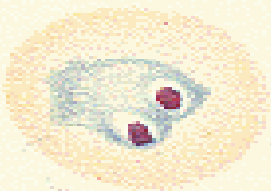


double dotted rings

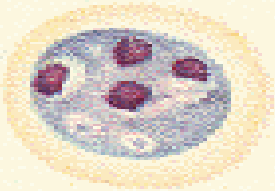


ring form

young trophozoite

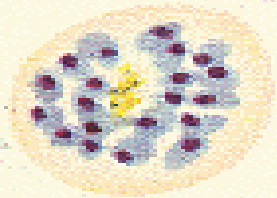


trophozoite



early schizont

schizont



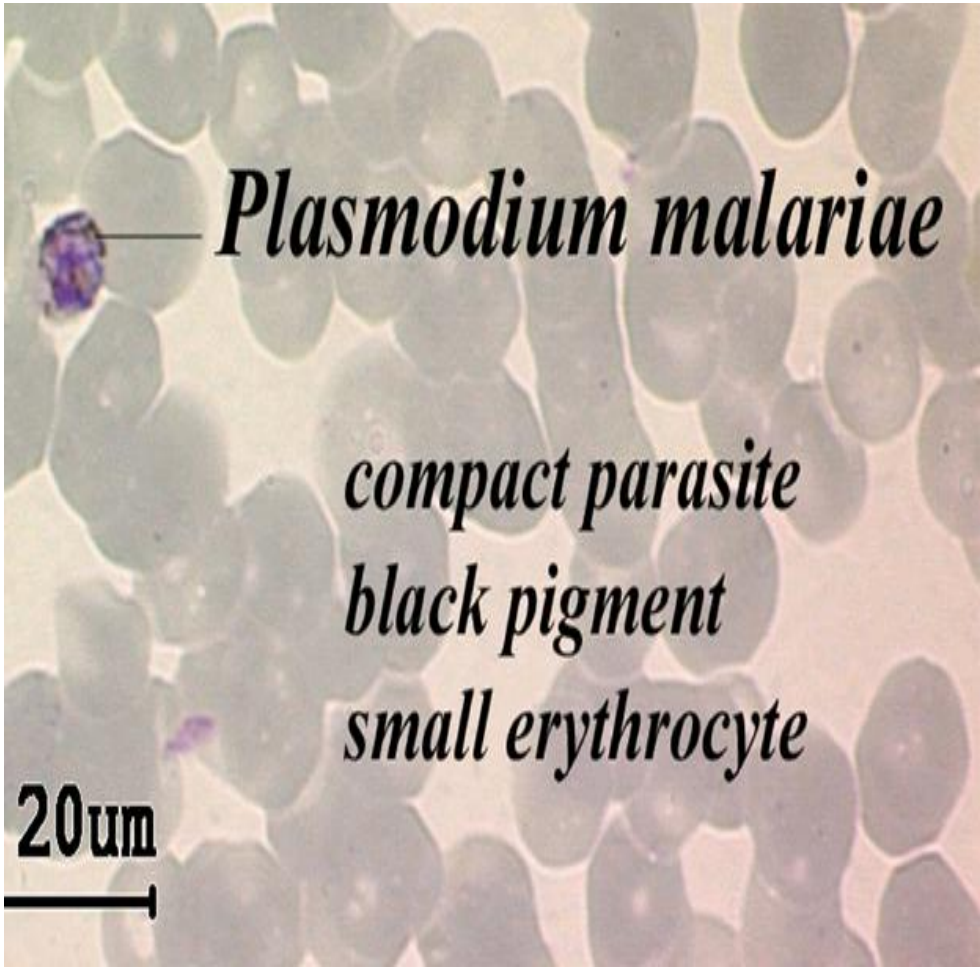
mature schizont

female gametocyte

male gametocyte

Plasmodium malariae

- RBC Normal size
- Contain Ziemann's stippling
- Pigment dark brown coarse granules
- Ring stage similar to *P. vivax* but thicker
- Schizont nearly fils red cell.
- Gametocytes Spherical or globular
- Size much larger than a red cell



P. malariae

ring form

early band form

band form

early schizont

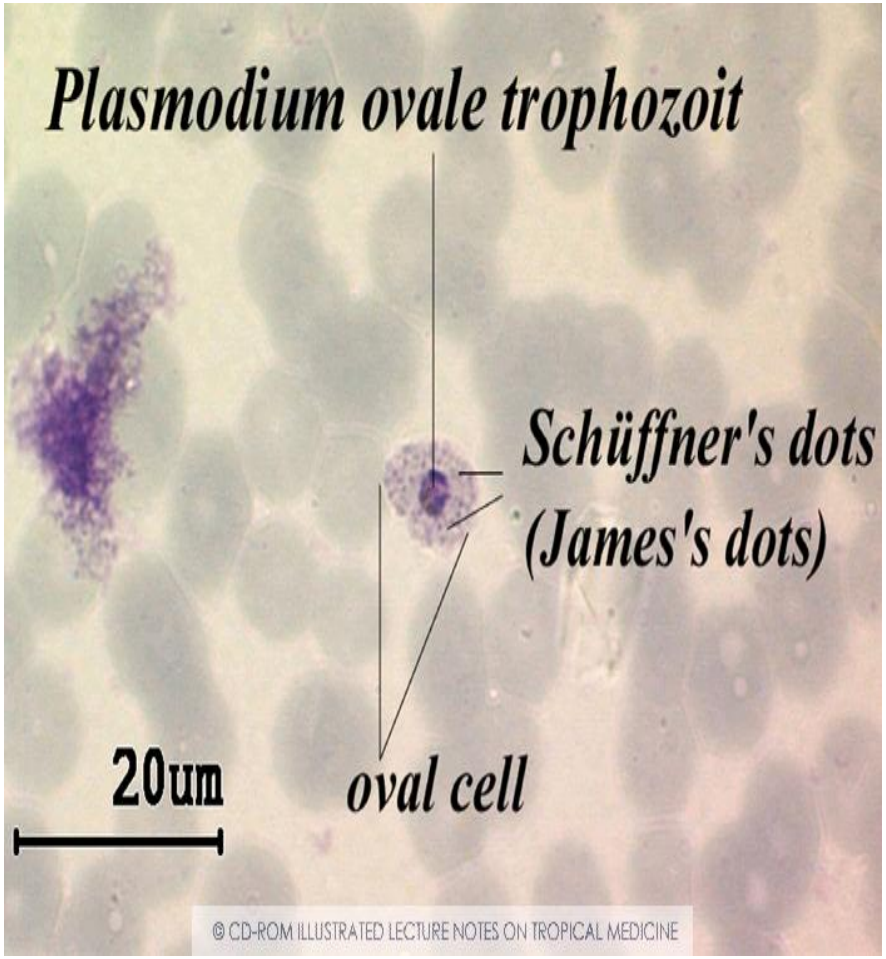
mature schizont

female gametocyte

male gametocyte

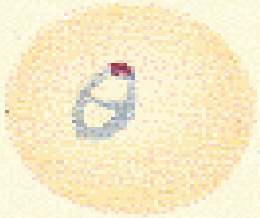
Plasmodium ovale

- Infected RBC slightly larger
- Contain Schuffner's dots
- pigment black coarse granules
- Schizont fills three quarters
- Merozoites 6 -14 fills three quarters
- Gametocytes Spherical or globular

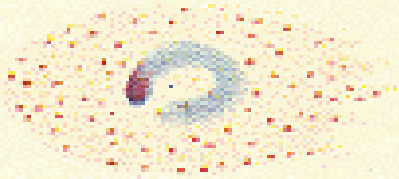


P. ovale

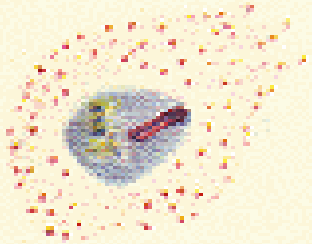
young ring



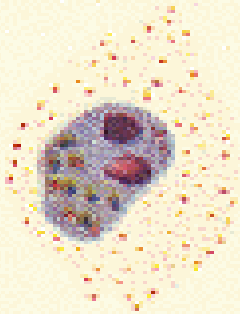
older ring



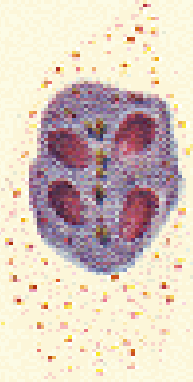
comet form



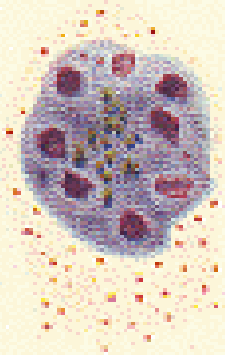
trophozoite



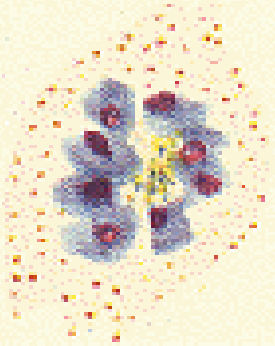
trophozoite



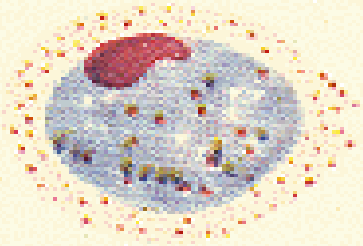
young schizont



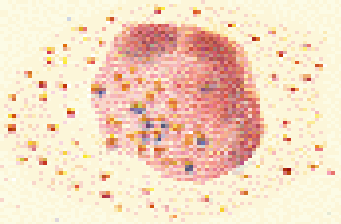
schizont



mature schizont



female gametocyte



male gametocyte

How Malaria present Clinically

The clinical features of malaria vary from mild to sever and complicated according to spp. Of the parasites

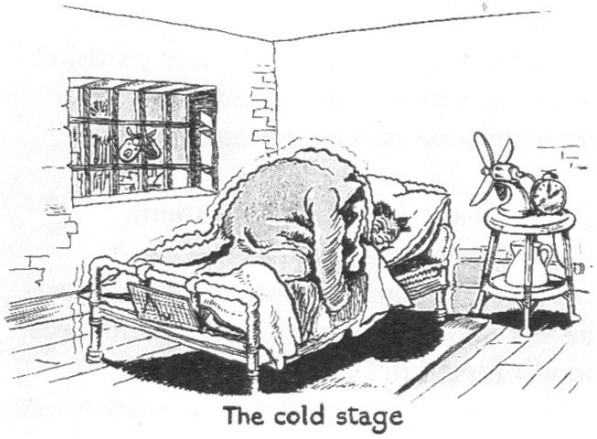
The typical picture of malaria consists of:

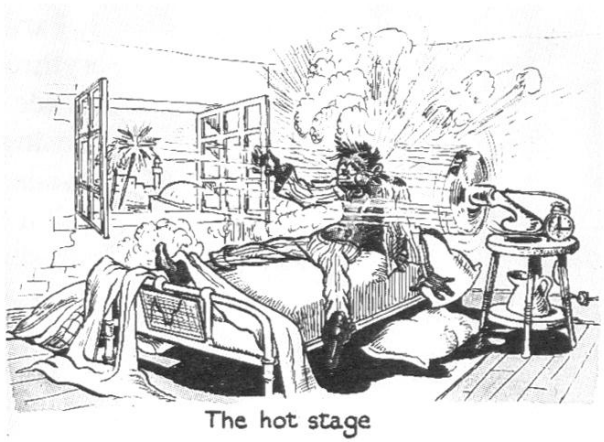
febrile paroxysm

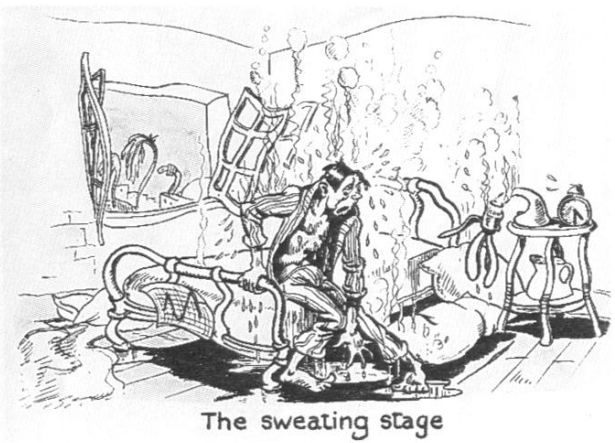
anaemia

splenomegaly.

primary fever: the typical attack has 3 stages:


The cold stage


The hot stage


The sweating stage

Febrile paroxysm

- It generally begins in the early afternoon and comprises of three successive stages –
- the cold stage ,lasting for 1/4-3 hours, the patient experiences intense cold and shivering .
- This is followed by hot stage , lasting for 30minutes-6 hours, when the patient feels intense hot. Patient develops high fever(40.0-40.6°C),sever headache, nausea and vomiting.
- Thereafter, fever ends by crisis accompanied by profuse sweating. (sweating stage).
- The periodicity of the attack varies with the species of the infecting parasite.
- The periodicity is 48 hours in *P. vivax* (benign tertian)and *P. ovale* (ovale tertian), in 72 hours in *P. malariae* (quartan).
- However, with *P. falciparum* , the cycles of different broods of parasite do not become synchronized as they do in other species.

- Febrile paroxysms follow the completion of erythrocytic schizogony when the mature schizont ruptures releasing merozoiter, malarial pigment and other parasitic debris.
- Macrophages and polymorphs phagocytize these and release endogenous leading to pyrexia.
- **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 mechanical destruction of parasitized cells, reduced erythropoiesis in the bone marrow and lysis and phagocytosis of uninfected red cells.
- In addition, in a small number of patients with malignant tertian malaria there is autoimmune destruction of red cells.

Splenomegaly

After a few paroxysms, spleen gets enlarged and becomes palpable. Splenomegaly is due to massive proliferation of macrophages which phagocytize both parasitized and non-parasitized red blood cells.

More commonly, the patient presents with a combination of the following symptoms

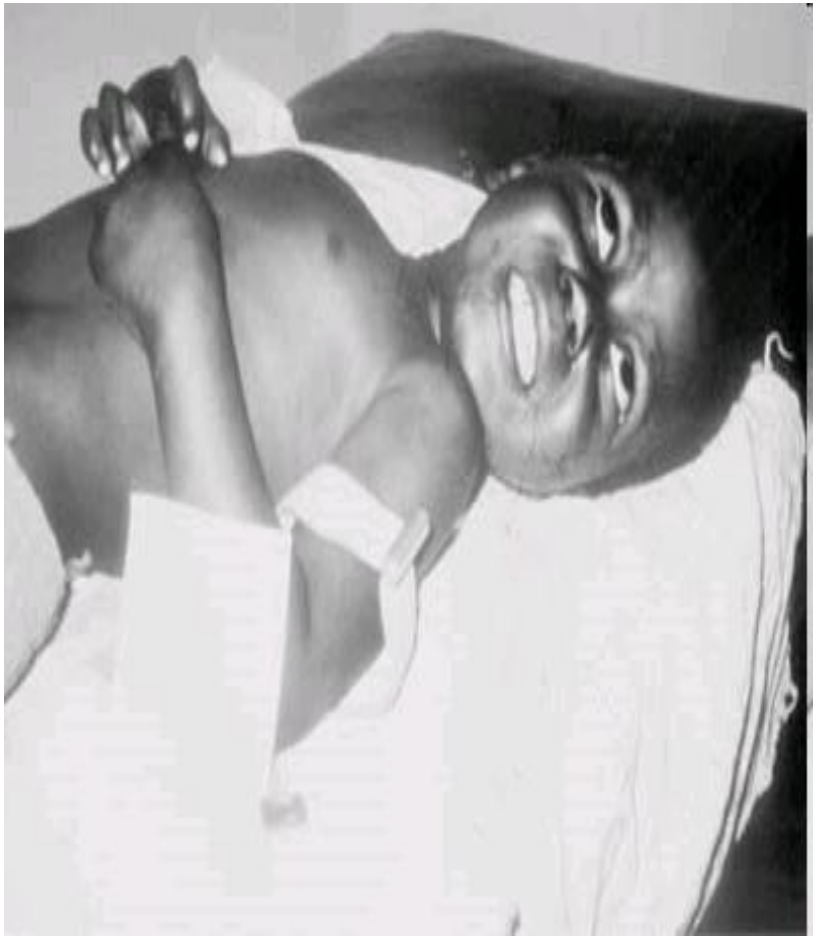
- Fever (Fever and shivering. The attack begins with fever, with the temperature rising as high as 40ºC and falling again over a period of several hours)
- Chills
- Headaches (A poor general condition, feeling unwell and having headaches like influenza)
- Nausea and vomiting
- Body aches (back and joint pain)
- General malaise.
- Gastrointestinal symptoms (Diarrhea, nausea and vomiting often occur as well).
- The common first symptoms – fever, headache, chills and vomiting – usually appear 10 to 15 days after a person is infected. If not treated promptly with effective medicines, malaria can cause severe illness and is often fatal.
- ***P. falciparum*** **is the most pathogenic of the human Plasmodium species. It causes a high level of parasitaemia. Nearly 30-40% of the red blood cells may be parasitized. In contrast to other Plasmodium species, it invades erythrocytes of all ages (young and old).**

Complication of malaria infections (the complications are more common due to *P. falciparum* (malignant malaria) than to other three spp.)

- Can produce fatal complications,
  1.Cerebral malaria
  2.Malarial hyperpyrexia
  3.Gastrointestinal disorders.
  4.Algid malaria
  5 Black water fever can lead to death
  6-acute renal failure
  7- sever anemia
  8-collapse
  9-pulmonary edema

Cerebral Malaria

- Malignant malaria can affect the brain and the rest of the central nervous system.
- Present with Hyperpyrexia
- Can lead to Coma
- Paralysis and other complications.
- Brain appears congested



- Algid malaria

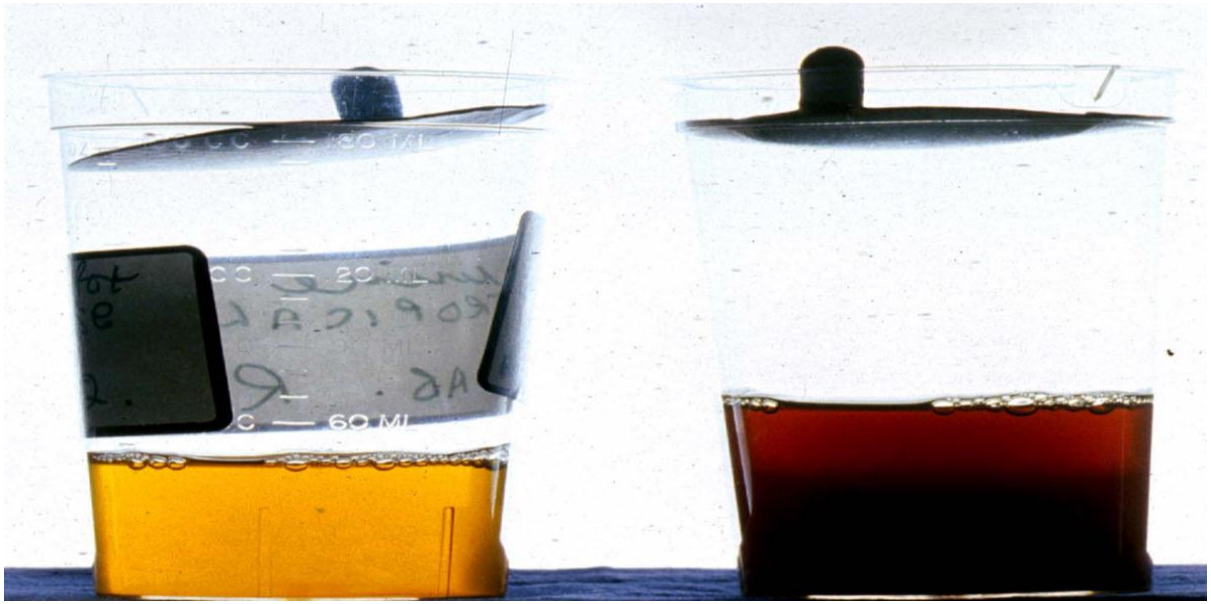
  It resemble surgical shock with cold clammy skin, peripheral circulatory failure and profound shock. Patient may also develop vomiting and diarrhea or dysentery.

- Septicaemic malaria

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

Black water fever

- It is a manifestation of repeated infection with *P. falciparum* which were inadequately treated with quinine.
- Sometimes resumption of Quinine therapy for new attack is followed by massive destruction of RBCs,fever, haemoglobinuria (black color urine)and renal failure.



Other Complications in Malaria

- Pulmonary edema (fluid build up in the lungs) or acute respiratory distress syndrome (ARDS), which may occur even after the parasite counts have decreased in response to treatment
- Abnormalities in blood coagulation and thrombocytopenia (decrease in blood platelets)
- Cardiovascular collapse and shock.
- Acute kidney failure
- Hyperparasitemia, where more than 5% of the red blood cells are infected by malaria parasites
- Metabolic acidosis (excessive acidity in the blood and tissue fluids), often in association with hypoglycemia

Pernicious malaria

It results from anoxia due to obstruction of capillaries in various organs followed by necrosis (death) of tissues. it is always due to *p. falciparum* infection.

According to the organs effected Pernicious malaria may be divided into:

1- Pernicious malaria affecting nervous system

2-Pernicious malaria affecting gastrointestinal system

3- Pernicious malaria affecting other system

- The symptoms often associated with malaria are due to bursting red blood cells and clogged capillaries of major organs. Infection occurs when an infected anopheles mosquito feeds on an individual releasing sporozites into the blood stream. Mosquitos can carry more than one species and thus can infect peoples with more than one species.

- **(the commonest combination seen *P. vivax* and *P. falciparum*).**

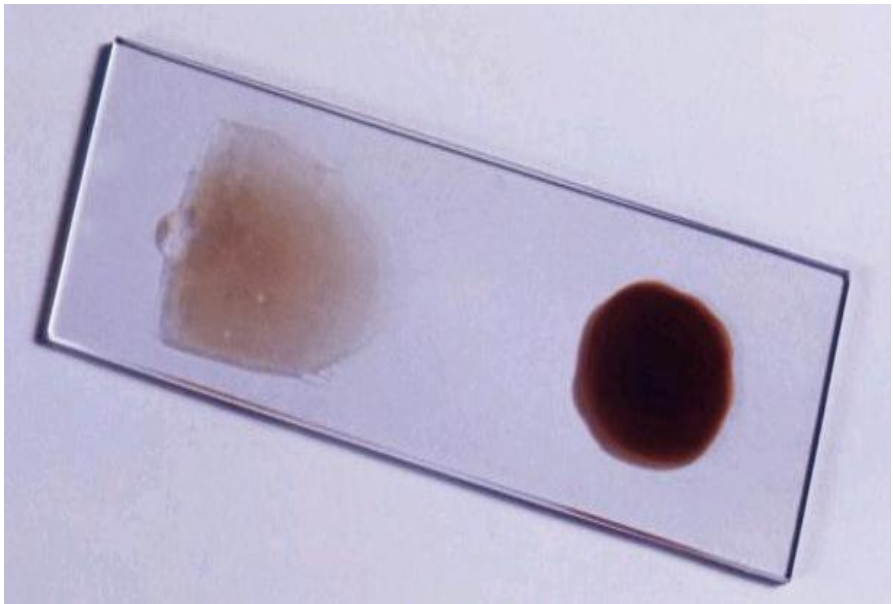
- The clinical picture gets mixed up and fever might occur daily.

Diagnostic Tools for Human Infections with Malaria

- Blood film examination
- Serology - IFA
- PCR

Laboratory diagnosis

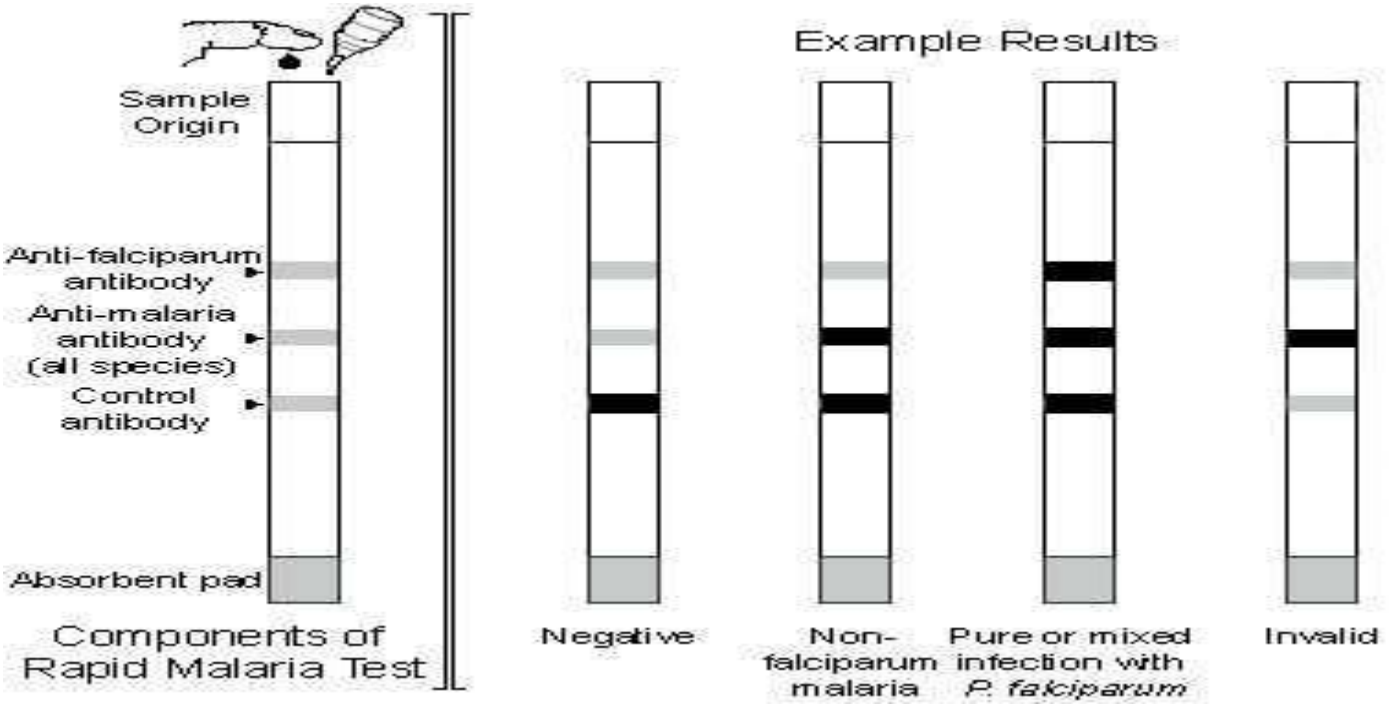
- Diagnosis of malaria can be established by demonstration of malaria parasites in the blood , Thick and thin smears of the blood are prepared on the same or different slides. Thin film is examined first and if parasites are found, there is no need for examining the thick film.

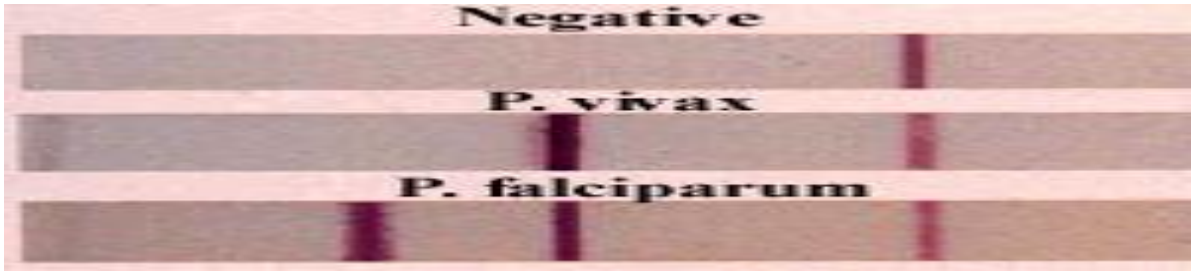


Antigen Detection Methods are Rapid and Precise

- **Antigen Detection**
- Various test kits are available to detect antigens derived from malaria parasites. Such immunologic ("immunochromatographic") tests most often use a dipstick or cassette format, and provide results in 2-15 minutes. These "Rapid Diagnostic Tests" (RDTs) offer a useful alternative to microscopy in situations where reliable microscopic diagnosis is not available. Malaria RDTs are currently used in some clinical settings

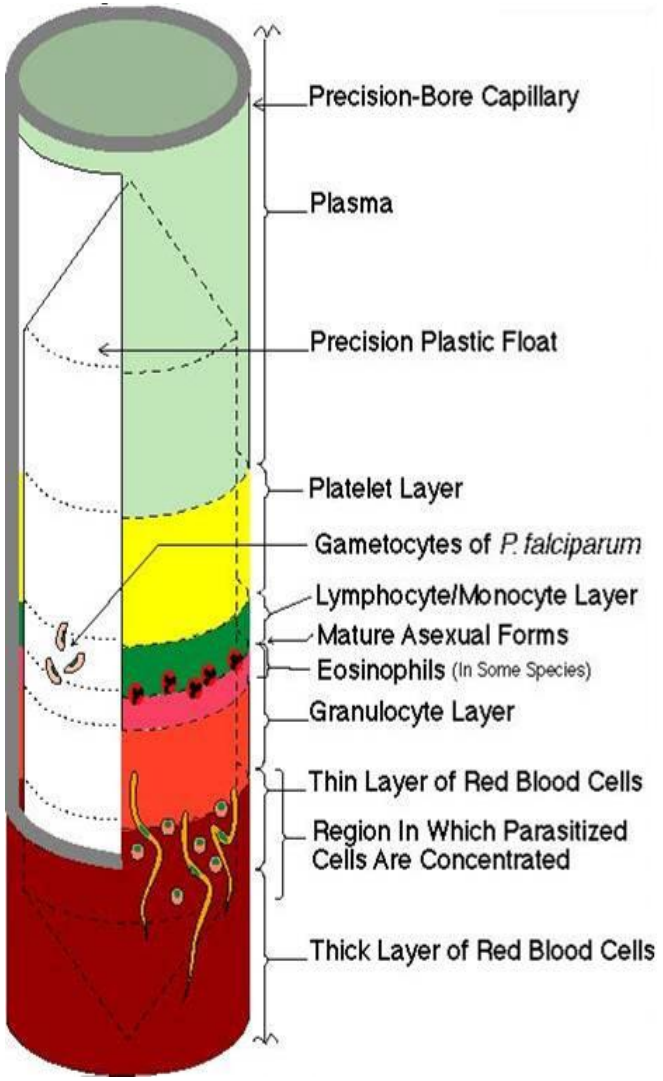
RAPID DIAGNOSTIC TESTS(RDTS)





QUANTITATIVE BUFFY COAT (QBC) TEST

- The QBC test is a new method for identifying the malaria parasite in the peripheral blood. It involves staining of the centrifuged and compressed red blood cell layer with acridine orange and its examination under UV light source.
- It is fast,easy and claimed to be more sensitive than the traditional thick smear examination.



Serology

- Serology detects antibodies against malaria parasites, using either indirect immunofluorescence (IFA) or enzyme-linked immunosorbent assay (ELISA). Serology does not detect current infection but rather measures past experience.

Newer Diagnostic methods

Molecular Diagnosis

- Parasite nucleic acids are detected using polymerase chain reaction (PCR). This technique is more accurate than microscopy. However, it is expensive, and requires a specialized laboratory (even though technical advances will likely result in field-operated PCR machines).

Treatment

Most drugs used in treatment are active against the parasite forms in the blood (the form that causes disease) and include:

Chloroquine was the standard treatment for acute malaria for many years.

Resistance to this druge in *P, falciparum* is widespread. Less commonly *P.vivax* may also be chloroquine-resistant.

Quinine is the most reliable alternative to chloroquine.

Tetracycline and clindamycin exhibit some anti-malarial activity

Prophylaxis

Malaria control depends upon :

- Spraying residual insecticides such as DDT or malathion .
- Spraying the breeding sites with petroleum oils and Paris green (copper acetoarsnite) as larvicides.
- Using larvivorous fish in breeding places.
- Flooding and flushing of breeding places.
- Avoiding exposure to mosquito bites by :

  Wearing long-sleeved clothing and trousers after sunset when the insects are most active.
- Using bed nets impregnated with pyrethroids.
- Application of mosquito repellents containing diethyltolumide to exposed skin.
- Taking chemoprophylaxis.Early diagnosis and prompt treatment of patients .

Development of Vaccines

- Malaria vaccines in development include: pre-erythrocytic or liver-stage vaccines that aim to protect against the early stage of malaria infection; blood-stage vaccines that aim to reduce the severity of disease; and transmission-blocking vaccines that are intended to prevent mosquitoes that fed on an infected person from spreading malaria to new hosts.

Epidemiology

- Recent epidemics have caused a high number of deaths, many in areas previously free of the disease .
- Frequent international air travel has also resulted in increasing numbers of imported cases and deaths in returned travelers and visitors to developed countries previously declared free of the disease.

- A number of factors appear to have contributed to the resurgence of malaria :
- breakdown of control programmes.
- rapid spread of resistance of malaria parasites to chloroquine and other quinolines

and the migration of non-immune populations from areas that are free from malaria to areas where transmission is high.

- armed conflicts have caused displacement of large populations of refugees to areas where living conditions are difficult and the risk of malaria is often high.
- Changing rainfall patterns and land use, leading to new mosquito breeding sites
- changes in vector behaviour have further compounded the problem.

Why vaccines are Difficult

- No licensed vaccine against malaria currently exists
- The parasite has evolved a series of strategies that allow it to confuse, hide, and misdirect the human immune system.
- The parasite changes through several life stages even while in the human host, presenting a different subset of molecules for the immune system to combat at each stage.