Parasitology
Second-Semester 2024-2025
Protozoa Lecture: 7
Malaria -1

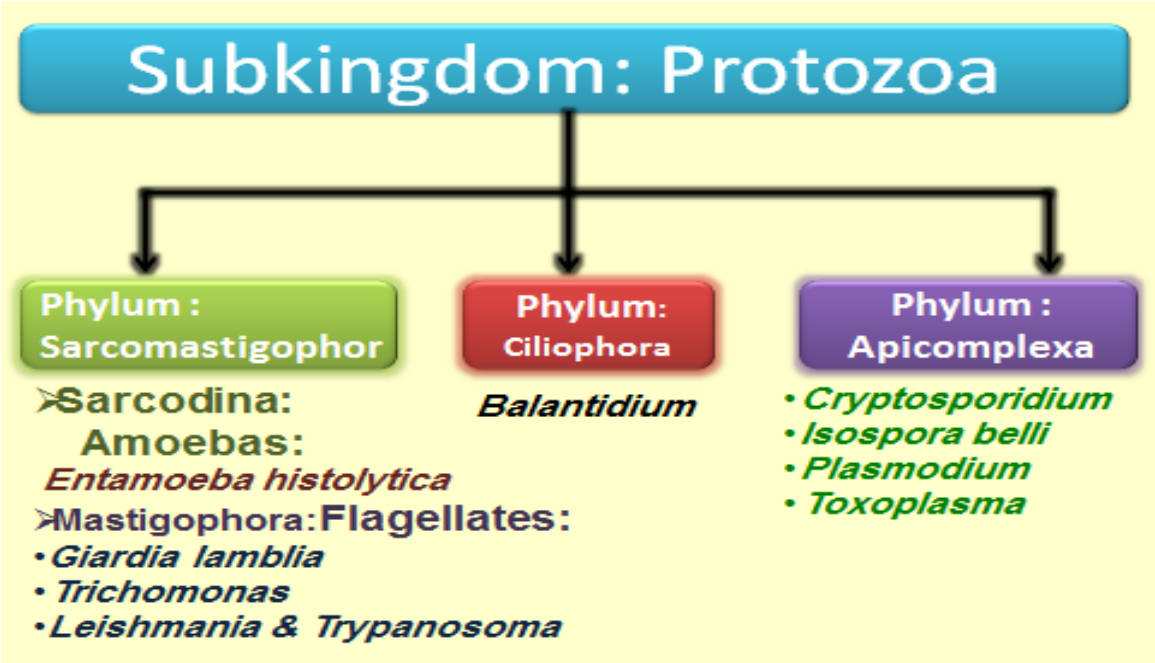
Dr. Azhar **2&3/2/2025**

(Page number 1-15)

Objectives of the lecture 7:

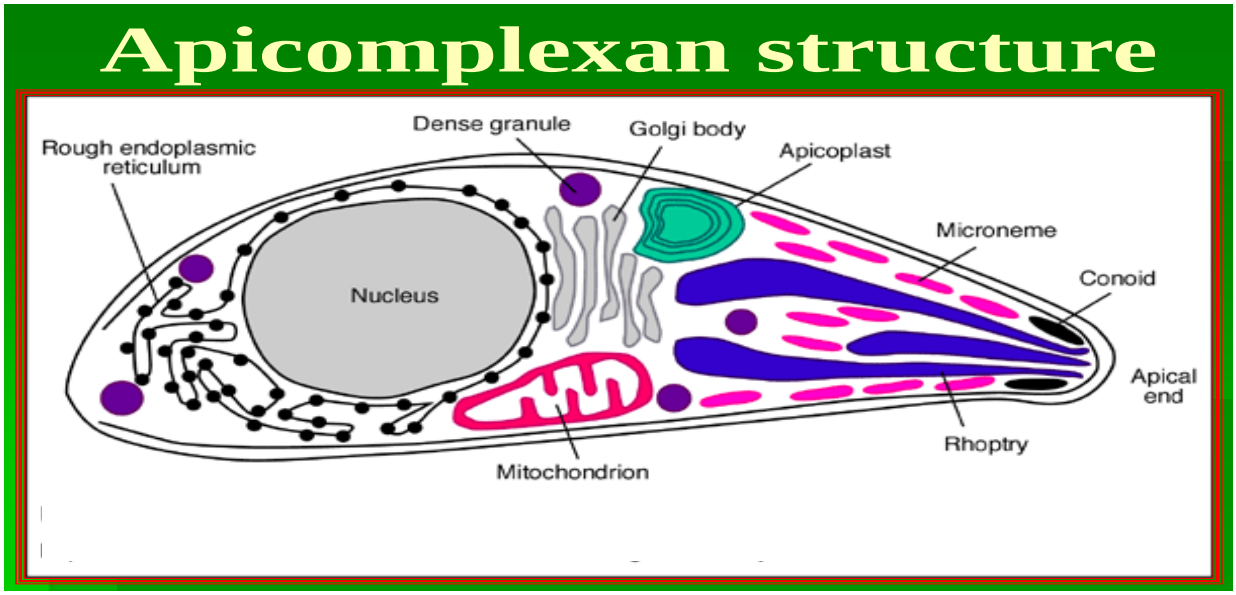
At the end of this lecture the 3rd year student is able to:

1. Define the Phylum **Apicomplexa**.
2. Describe the Apicomplexan structure (for movement).
3. Describe the Phylum: Apicomplexa, Class: Sporozoa genus *Plasmodium*.
4. List and describe the species of the genus ***Plasmodium*.**
5. List and describe the diseases caused by this genus.
6. Describe the Clinical manifestations of malaria.
7. Describe the complications of malaria.



Apicomplexa

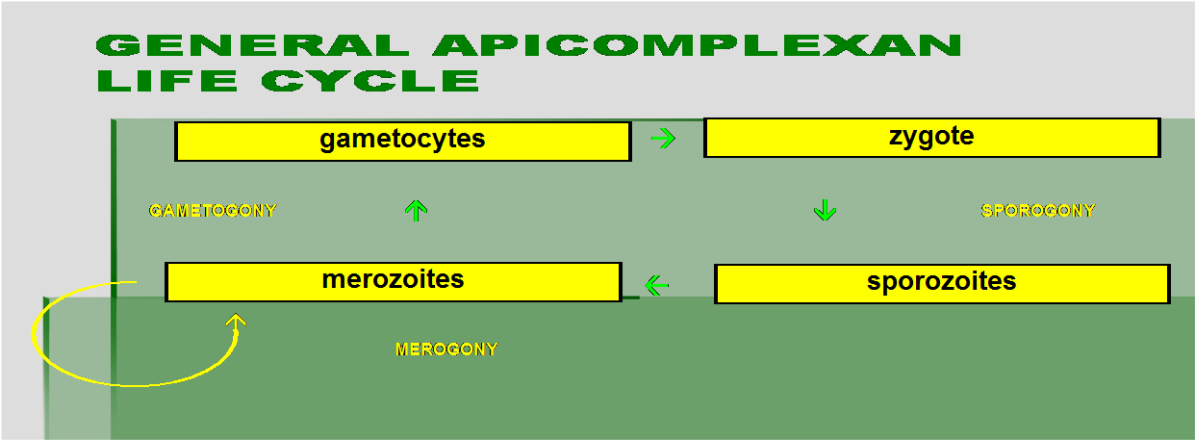
apical complex= apicoplast
unicellular, spore-forming
exclusively parasites (i.e., no free-living)
Motile structures are absent except in certain gamete stages



Complex life-cycle

sexual
(sporogony &gametogony)

asexual
(schizogony or merogony)



Malaria البرداء

- ☑ The cause of much human morbidity and mortality.
- ☑ Forty percent of the world's population lives in endemic areas.
- ☑ Malaria poses a serious barrier to economic progress in many developing countries.
- ☑ 40% of the world’s population lives in endemic areas
- ☑ 3-500 million clinical cases per year
- ☑ 1.5-2.7 million deaths (90% Africa) with the majority of deaths being young children. Malaria is caused by members of the genus ***Plasmodium***.
- ☑ ***Plasmodium*** species are apicomplexa and exhibit a heteroxenous life cycle involving a vertebrate host and an arthropod vector.

- ☑ Vertebrate hosts include: reptiles, birds, rodents, monkeys and humans.
- ☑ Four distinct species infected humans: ***P. falciparum, P. vivax, P. ovale and P. malariae* .** These species are host specific and there are no **zoonosis**.
- ☑ The Fifth Human Malaria Parasite is ***P. knowlesi*** that causes malaria in humans and other primates (**zoonosis**). It has been reported in nearly all the countries in Southeast Asia and in travelers returning from these countries.
- ☑ ***Plasmodium vivax*** more prevalent because it is more tolerant of lower temperatures.
- ☑ The species differ in regards to their morphology, details of their life cycles, and their clinical manifestations.
- ☑ 40% of the world's population lives in endemic areas.
- ☑ 3-500 million clinical cases per year.
- ☑ 1.5-2.7 million deaths (90% Africa) with the majority of deaths being young children.
- ☑ Mammalian ***Plasmodium*** species are transmitted by **female anopheline mosquitoes (pregnant ♀)**.
- ☑ **increasing problem (re-emerging disease):**
    - **resurgence in some areas**
    - **drug resistance (↑ mortality)**

Disease:

***P. falciparum*: (two-day cycle) malignant tertian malaria or falciparum malaria.**

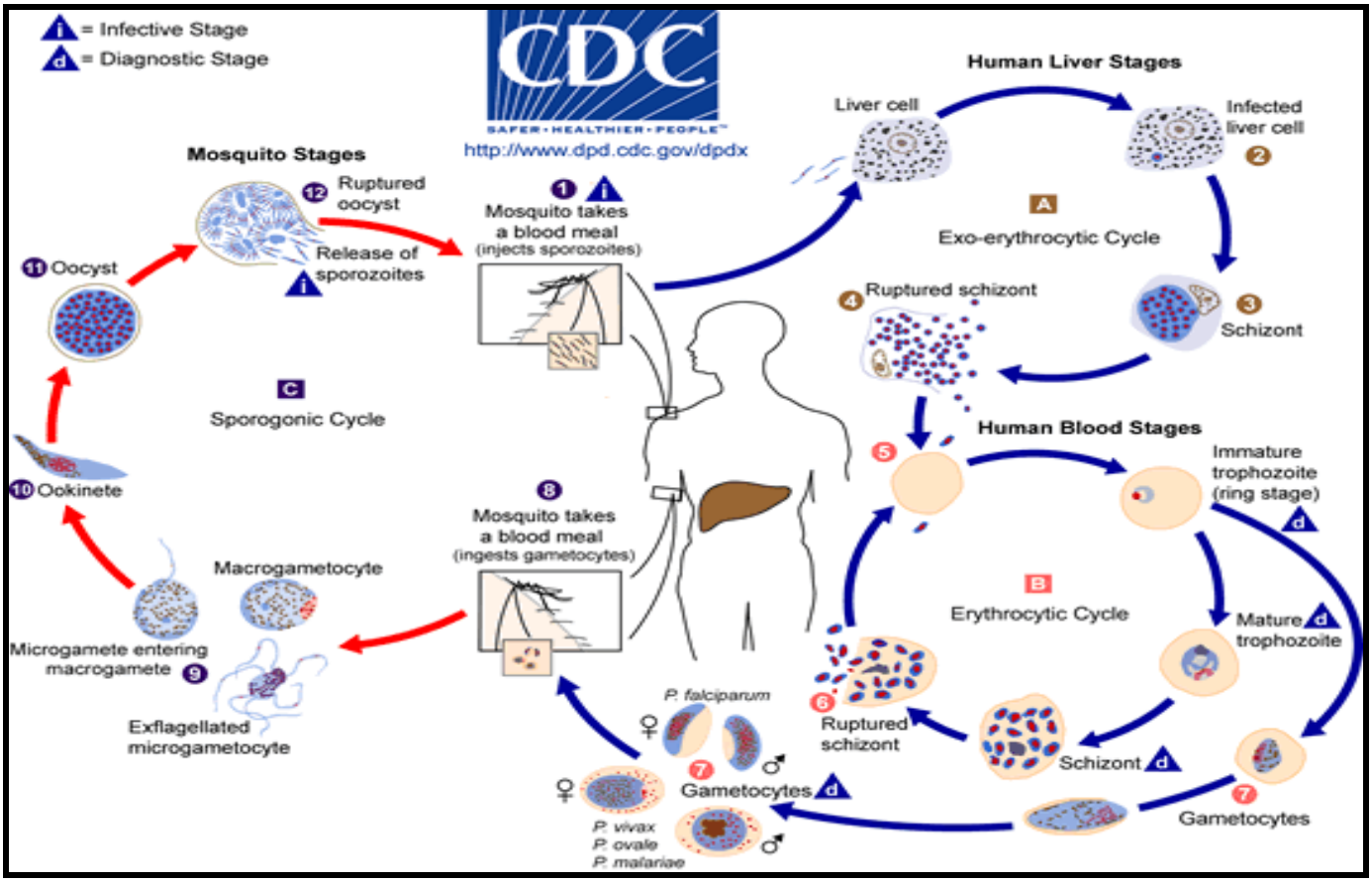
***P. vivax*: (two-day cycle) benign tertian** or **vivax malaria.**

***P. ovale*: (two-day cycle) ovale tertian malaria or ovale malaria.**

***P. malariae*: (three-day cycle) quatrain malaria.**

***P. knowlesi*: (one-day cycle) simian malaria or "quotidian" malaria.**

Life cycle:

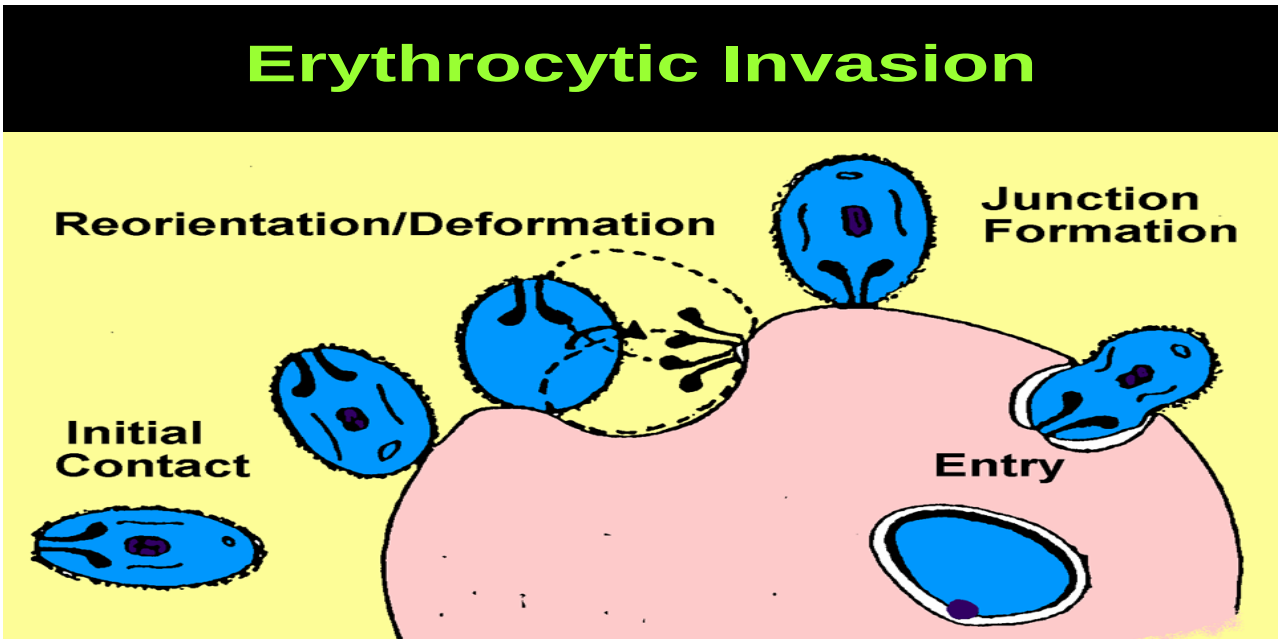


The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female ***Anopheles*** mosquito inoculates sporozoites into the human host❶. Sporozoites within 30-60 minutes will invade liver cells ❷and mature into schizonts❸, which rupture and release merozoites ❹ in cycle of asexual reproduction (schizogony). (if note, in ***P. vivax*** and ***P. ovale*** a dormant stage [**hypnozoites**] can persist in the liver and cause relapses by invading the bloodstream weeks, or even years later.) After this initial replication in the liver (**exo-erythrocytic= pre-erythrocytic schizogony**Ⓐ), the parasites undergo anther asexual multiplication in the erythrocytes (**erythrocytic schizogony**Ⓑ). Merozoites❹ infect red blood cells❺ and undergo a trophic period in which the parasite enlarges. The early trophozoite is often referred to as '**ring form**' because of its morphology. **Trophozoite** enlargement is accompanied by an active metabolism including the ingestion of host cytoplasm and the proteolysis of hemoglobin incompletely into globin and iron porphyrin **hematin (hemazon)**; the later is malaria pigment, a compound of hematin and protein. The ring stage trophozoites mature into **amoeboid stage** (mature trophozoite). The end of the trophic period is manifested by multiple rounds of nuclear division without cytokinesis resulting is a **schizont**. **Merozoites** bud from the mature schizont, also called a **segmenter**, and the rupture of the infected erythrocyte releasing **merozoites**❻. Invasion of erythrocytes reinitiates another round of the blood-stage replicative cycle. Some parasites differentiate into sexual erythrocytic stages (gametocytes)❼. Blood stage parasites are responsible for the clinical manifestations of the disease.

The gametocytes, male (microgametocyte) and female (microgametocyte), are ingested by an ***Anopheles*** mosquito during a blood meal❽. The parasites' multiplication in the mosquito is known as the sporogonic cycleⓒ. While in the mosquito's stomach, the

microgametes penetrates the macrogametes generating zygotes❾. The zygotes in turn become motile and elongated (ookinetes) ❿which invade the midgut wall of the mosquito where they develop into oocysts ⓫. The oocysts grow, rupture, and release sporozoites⓬, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle❶.

In the life cycle there are **2** transfers stages: sporozoite (from mosquito to man) & gametocytes (from man to mosquito) and **3**invasive stages: Merozoite (invade erythrocytes of human), Sporozoite (invade salivary gland of mosquito & hepatocytes of human) &ookinete (invade epithelial cell of mosquito's stomach).



Prepatent period: is defined as the time between sporozoite inoculation and the appearance of parasites in the blood and represents the duration of the liver stage.
Incubation periods are defined as the time between sporozoite inoculation and the first appearance of clinical signs.

Exoerythrocytic cycle

	P. falciparium	***P. vivax***	***P. ovale***	***P. malariae***
Prepatent period	6 days	8 days	9 days	13 days
Incubation period	12 days	13-17 days	13-17 days	28-30 days

Malaria Transmission

- ✓ **Natural (sporozoites/*Anopheles*)**
- ✓ **Blood transfusions**
  - shorter incubation period
  - fatality risk (*P. falciparum*)
  - no relapses possible (*P. vivax/P. ovale*)
- ✓ **Syringe sharing**
- ✓ **Congenital**
  - relatively rare although placenta is heavily infected.

Difference Between Mosquito-borne Malaria and Blood transfusion Malaria

	Mosquito-borne malaria	Blood Transfusion malaria
Mode of transmission	Mosquito bite	Blood or Blood products transfusion
Infective stage	Sporozoite	Trophozoite
Incubation period	Long	Short
Pre-erythrocytic schizogony	Present	Absent
Hypnozoites	May be present	Absent
Severerity	Comparitively less	More complications seen
Relapse	May occur	Does not occur
Radical treatment	Required	Not required

Malaria pigment or Haemozoin pigment

The parasite feeds on the hemoglobin of the erythrocyte. It does not metabolize hemoglobin completely and therefore, leaves behind a hematin-globin pigment called the malaria pigment or haemozoin pigment, as residue. The malaria pigment released when the parasitized cells rupture is taken up by reticuloendothelial cells.

Such pigment-laden cells in the internal organs provide histological evidence of previous malaria infection.

Differences between the species include:

☒ Minor life cycle variations

1. ***P. vivax*** and ***P. ovale*** exhibit the hypnozoite stage and can cause true relapses.
2. Trophozoite- and schizont-infected erythrocytes of ***P. falciparum*** sequester in the microvasculature and are not found in the peripheral circulation.

Host erythrocyte preference

1. ***P. vivax*** and ***P. ovale*** prefer reticulocytes (immature erythrocytes)
2. ***P. malariae*** prefers senescent erythrocytes
3. ***P. falciparum*** exhibits no preference

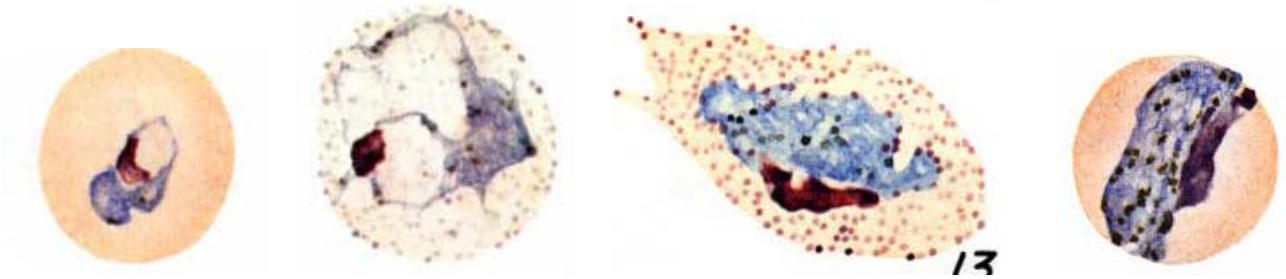
Morphological Differences

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. **<u>Ring stage</u>**: of all four species are very similar and difficult to distinguish. ***P. falciparum*** rings tend to be a little smaller and more numerous than the other species. The presence of a large number of rings in the absence of more mature stages, as well as multiply-infected erythrocytes, is highly suggestive of ***P. falciparum*****.**

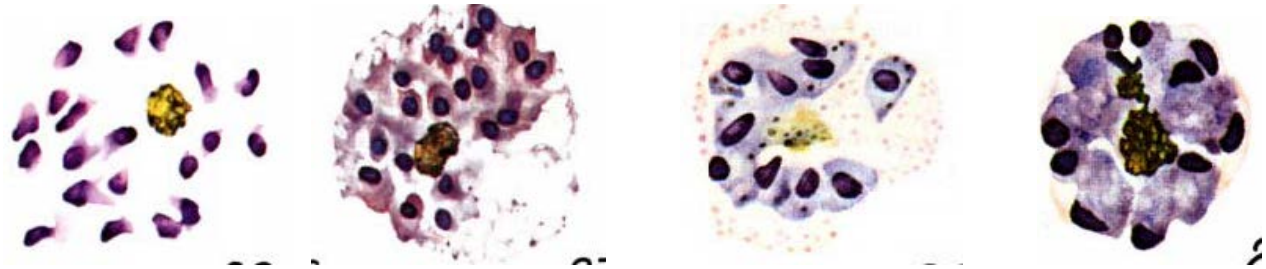
P. falciparum ***P. vivax*** ***P. ovale*** ***P. malariae*** **Normal RBC**

2. **<u>Mature trophozoit</u>**: erythrocytes infected with ***P. vivax*** and ***P. ovale*** are enlarged and exhibit Schüffner's dots as the rings mature into mature trophozoites. The trophozoites of ***P. vivax*** are often **amoeboid**, whereas ***P. ovale*** tends to be more compact. The ***P. malariae*** trophozoite is very compact and extending across the diameter of the host and known as **band form**, the host erythrocyte is not enlarged.



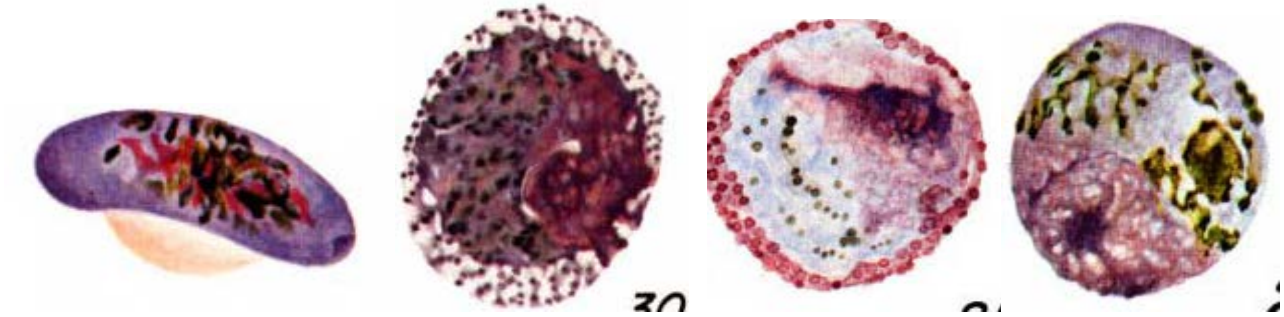
P. falciparum ***P. vivax*** ***P. ovale*** ***P. malariae***

3. **<u>Mature schizont</u>**: asexual forms of ***Plasmodium*** **spp.** Schizonts of ***P. falciparum*** are rarely found in the peripheral circulation (sequestered in blood of viscera). The typical number of merozoites produced per schizont is: ***P. vivax*** 14-20 (up to 24), ***P. ovale*** 6-12 (up to 18), ***P. malariae*** 8-10 (up to 12), and ***P. falciparum*** 16-24 (up to 36).

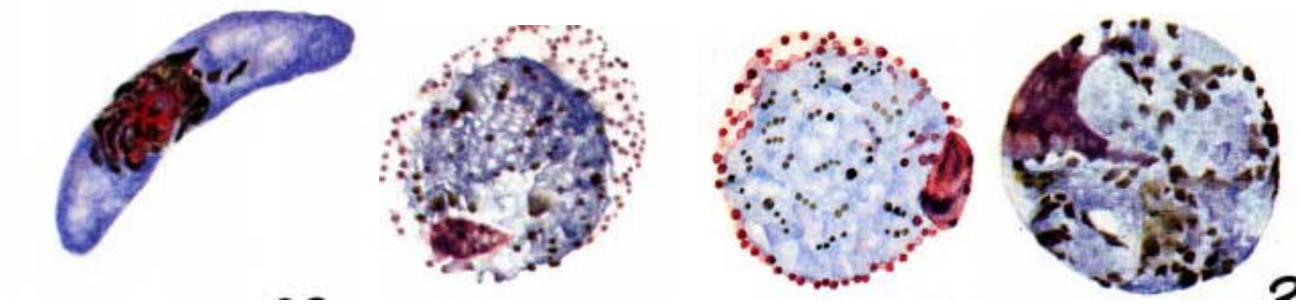


P. falciparum	*P. vivax*	*P. ovale*	*P. malariae*
(Sequestered)	(14-20, up to 24)	(6-12, up to 18)	(8-10, up to 12)

4. **<u>Gametocyte</u>*: P. falciparum*** exhibits crescent-shaped gametocytes whereas the other species are all round to oval. ***P. vivax*** and ***P. ovale*** gametocytes are in enlarged erythrocytes with Schüffner's dots and are difficult to distinguish from each other. ***P. malariae*** gametocytes do not modify the host erythrocyte. Gametocytes can be distinguished from trophozoites by their large size (nearly filling the erythrocyte) and a single nucleus. Mature microgametocytes (male gametes) tend to stain lighter than macrogametocytes (female gametes) and have a more diffuse nucleus.



Microgametocytes



Macrogametocytes

Comparison of the Characteristics of Plasmodia Causing Human Malaria

	P. vivax	*P. falciparum*	*P. malariae*	*P. ovale*
Hypnozoites	Yes	No	No	Yes
Erythrocyte preference	Reticulocytes	Young erythrocytes, but can infect all stages	Old erythrocytes	Reticulocytes
Stages found in peripheral blood	Rings, trophozoites, schizonts, gametocytes	Only rings and gametocytes	As in vivax	As in vivax
Ring stage	Large, 2.5 µm, usually single, prominent chromatin	Delicate, small, 1.5 µm, double chromatin, and multiple rings common, Accole forms found.	Similar to vivax, but thicker	Similar to vivax, more compact
Late trophozoite	Large irregular, actively amoeboid, prominent vacuole	Compact, seldom seen in blood smear	Band form characteristic	Compact, coarse pigment
Schizont	Large filling red cell	Small, compact, seldom seen in blood smear	Medium size	Medium size
Number of merozoites	12–24 in irregular grape-like cluster	8–24 grape-like cluster	6–12 in daisy-head or rosette pattern	6–12 irregularly arranged
Microgametocyte	Spherical, compact, pale blue cytoplasm, diffuse nucleus	Sausage or banana-shaped pale blue or pink cytoplasm, large diffuse nucleus	As in vivax	As in vivax
Macrogametocyte	Large, spherical, deep blue cytoplasm, compact nucleus	Crescentic, deep blue cytoplasm, compact nucleus	As in vivax	As in vivax
Infected erythrocyte	Enlarged, pale, with Schuffner's dots	Normal size, Maurer's clefts, sometimes basophilic stippling	Normal, occasionally Ziemann's stippling	Enlarged, oval fimbriated, prominent Schuffner's dots
Duration of schizogony (days)	2	2	3	2
Prepatent period (days)	8	5	13	9
Average incubation period (days)	14	12	30	14
Appearance of gametocyte after parasite patency (days)	4–5	10–12	11–14	5–6
Duration of sporogony in mosquito (25°C) (days)	9–10	10–12	25–28	14–16
Average duration of untreated infection (years)	4	2	40	4

Recrudescence and Relapse Malaria

Both terms mean renewal of malaria manifestation without a new infection occur.

Recrudescence malaria عودة الملاريا:

In *P. falciparum* and *P. malariae* infections after the primary attack, sometimes there is a period of latency, during which there is no clinical illness, but some parasites persist in some erythrocytes, although the level of parasitemia is below the fever threshold or sometimes below the microscopic threshold. Erythrocytic schizogony continues in the body at low levels and gradually the number of parasites builds up to cross the fever threshold. Fresh malarial attacks then develop. These new malarial attacks that appear after a period of latency usually within 8 weeks after the primary attack and resulting from persistence of the erythrocytic cycle of the parasites are called recrudescence. Recrudescence may be due to waning immunity of the host or possibly due to antigenic variation.

In *P. falciparum* infections, recrudescence is seen for 1–2 years, while in *P. malariae* infection, they may last for long periods, even up to 50 years.

Relapse malaria انتكاس الملاريا:

It is seen in *P. vivax* and *P. ovale* infections. In both these species, 2 kinds of sporozoites are seen, some of which multiply inside hepatocytes promptly to form schizonts and others which remain dormant. These latter forms are called hypnozoites (from hypnos: sleep). Hypnozoites remain insdie the hepatocytes as uninucleated forms, 4–5 µm in diameter, for long periods. Reactivation of hypnozoites leads to intitiation of fresh erythrocytic cycles and new attacks of malarial fever. Such new attacks of malaria, caused by dormant exoerythrocytic forms, reactivated usually from 24 weeks to 5 years after the primary attack are called relapses.

Differences between Recrudescence and Relapse malaria

Recrudescence	Relapse
Seen in *P. falciparum* and *P. malariae*	Seen in *P. vivax* and *P. ovale*
Due to persistence of the parasite at a subclinical level in circulation	Due to reactivation of hypnozoites present in liver cells
Occurs within a few weeks or months of a previous attack	Occurs usually 24 weeks to 5 years after the primary attack
Can be prevented by adequate drug therapy or use of newer antimalarial drugs in case of drug resistance	Can be prevented by giving primaquine to eradicate hypnozoites

Pathogenesis and Clinical Manifestations

The pathology and clinical manifestations associated with malaria are almost exclusively due to the asexual erythrocytic stage parasites. Tissue schizonts (liver schizonts) and gametocytes cause little, if any, pathology. ***Plasmodium*** infection causes an acute febrile illness which is most notable for its periodic fever paroxysms occurring at either 48 or 72 hour intervals.

The severity of the attack depends on:

1. The ***Plasmodium*** species.
2. The state of immunity.
3. The general health and nutritional status of the infected individual.

The disease has a tendency to relapse (due to ***P. vivax, P. ovale*** or recrudesce (due to ***P. falciparum*** & ***P. malariae***) over months or even years.
The **prepatent period** (represents the duration of the liver stage) and i**ncubation periods** (defined as the time between sporozoites inoculation and the onset of symptoms) vary according to species.

Prodromal symptoms

All four species can exhibit non-specific prodromal symptoms a few days before the first febrile attack. These prodromal symptoms are generally described as 'flu-like' and include: headache, slight fever, muscle pain, anorexia, nausea and lassitude. The symptoms tend to correlate with increasing numbers of parasites. These prodromal symptoms will be followed by febrile attacks.

Febrile attacks (the malaria paroxysms)

These paroxysms will exhibit periodicities of 48 hours for ***P. vivax, & P. ovale***, 36-48 hour periodicity for ***P. falciparum***, and a 72-hour periodicity for ***P. malariae***. Patients may also exhibit splenomegaly, hepatomegaly (slight jaundice), and hemolytic anemia during the period in which the malaria paroxysms occur.
The malarial paroxysm (see figure below) will usually last 4-8 hours and begins with a cold stage followed by hot stage which end by the sweating stage.

1- **The cold stage:** begins with sudden onset of chills in which the patient experiences an intense feeling of cold despite having an elevated temperature. This stage is characterized by a vigorous shivering. Lasts within 15-60 min.

Immediately following this cold stage is

2- **The hot stage**: The patient feels an intense heat accompanied by severe headache & dry burning skin. This stage lasts within 2-6 hours.

3- **Sweating stage:** Profuse sweating and the fever will start to decline. The patient is exhausted and weak and will usually fall asleep. Upon awakening the patient usually feels well, other than being tired, and does not exhibit symptoms until the onset of the next paroxysm. This stage lasts within 2-4 hours**.**

Malaria parasites tend to develop synchronously in the human host. In other words, all of the parasites within a host are at approximately the same stage (ie, ring, trophozoite, schizont) as they proceed through schizogony. The malarial paroxysm corresponds to the rupture of the infected erythrocytes and the release of merozoites (Figure above). Studies in ***P. vivax*** have demonstrated a correlation between fever and serum TNF-α (tumor necrosis factor-alpha) level. Presumably antigens or toxins are released when the infected erythrocyte ruptures and lead to the production of TNF-α (pyrogenic) and the febrile attacks will start.

Disease severity and duration				
	P. vivax	***P. ovale***	***P. malariae***	***P.falciparum***
Initial Paraoxysm Severity	Moderate to severe	Mild	Moderate to severe	Severe
Average Parasitemia (mm^3)	20,000	9,000	6,000	50,000-500,000
Maximum Parasitemia (mm^3)	50,000	30,000	20,000	2,500,000
Symptom Duration (untreated)	3-8+ weeks	2-3 weeks	3-24 weeks	2-3 weeks
Maximum Infection Duration (untreated)	5-8 years	12-20 months	20-50+ years	6-17 months
Anemia	++	+	++	++++
Complications			renal	cerebral

P. falciparum **(malignant tertian malaria =2 day cycle)**

In contrast to the other three species, ***P. falciparum*** can produce serious disease with mortal consequences, due in part to:

- The high parasitemias associated with ***P. falciparum*** infections (due to high reproductive capacity of the parasite).
- **Sequestration** of ***P. falciparum***, which is due to the cytoadherence of infected erythrocytes to endothelial cells in the blood vessels of deep tissues.
- The lack of preference for erythrocytes (infects RBCs of all ages).
- The high parasitemia and sequestration result in other complications associated with falciparum malaria, the most notable being **anemia** and **cerebral malaria**.
- Low liver (6 days) and blood (36-48 hrs) periodicities.

The anemia is due in part to the destruction of erythrocytes during blood-stage schizogony. Furthermore, non-infected erythrocytes are destroyed at higher rates during the infection and there is a decreased production of erythrocytes.

The sequestration of ***P. falciparum*** due to **Cytoadherence protein** which is expressed during the **trophozoite** and **schizont** stages and is formed as a result of parasite proteins exported to the erythrocyte membrane causing the blood cells to stick to the walls of small blood vessels of vital organs, especially brain, lung, gut, heart and placenta thereby sequestering the parasite from passage through the general circulation and the spleen. This "**stickiness**" is the main factor giving rise to **hemorrhagic complications** of malaria. High endothelial venules (the smallest branches of the circulatory system) can be blocked by the attachment of masses of these infected red blood cells. The blockage of these vessels causes symptoms such as in **placental** and **cerebral malaria**. In cerebral malaria the sequestrated

red blood cells can breach the blood brain barrier possibly leading to coma. This **Sequestration** of ***P. falciparum***, lead to minimize the removal of infected erythrocytes by the spleen, and increase the parasitemia rapidly.

P. vivax (benign tertian malaria = 2 day cycle): Patients infected with ***P. vivax***, especially for the first time, can be quite ill. However, ***P. vivax*** rarely causes complications or results in death. Relapses can occur for several years.

P. ovale (ovale tertian malaria = 2 day cycle) is the most benign in that the paroxysms tend to be mild and of short duration and relapses seldom occur more than one year after the initial infection.

P. malariae **(quartan malaria= three day cycle)** generally produces a mild disease, but the initial paroxysms can be moderate to severe. It is the most chronic, though, and recrudescences have been documented several decades after the initial infection. This chronicity is sometimes associated with renal complications, which are probably due to the deposition of antigen-antibody complexes in the glomeruli of the kidney.

Other Features of malaria:

- There is an increased activity of the reticuloendothelial system, particularly in the liver and spleen and thus their enlargement, as evidenced by macrophages with ingested infected and normal erythrocytes and hemozoin (malaria pigments).
- Paroxysm may be accompanied by spleno-megaly, hepatomegaly (slight jaundice), hemolytic anemia
- ***P. falciparum*** can be lethal in non-immune (like children)
- Paroxysms become less severe and irregular as infection progresses
- Semi-immune may exhibit little (1-2 days fever) or no symptoms.

Complications of malaria Except for ***P. falciparum***, the pathology associated with malaria tends to be rather benign. Several severe complications can be associated with falciparum malaria with cerebral malaria being the most notable and a frequent cause of death.

Cerebral malaria

- severe complication of falciparum malaria
    - mortality of 30-50%
- a diffuse encephalopathy with loss of consciousness
    - severe headache followed by drowsiness, confusion and coma
    - consciousness ranges from stupor to coma
    - unresponsive to pain, visual, and verbal stimuli
    - convulsions frequently observed

    - onset can be gradual or sudden
- associated with sequestration in micro-vasculature of brain
- Cerebral malaria is associated with retinal whitening, which may be a useful clinical sign in distinguishing malaria from other causes of fever.
- Consequences of severe malaria include coma and death if untreated—young children and pregnant women are especially vulnerable.

Black water fever (hemoglobinuric fever)

Other complication of ***P. falciparum*** which it is characterized by repeated attacks of malaria that have been inadequately treated with quinine. Treatment with quinine for the first time may lead to massive destruction of infected and uninfected erythrocytes which lead eventually to passage of dark red or black urine.

Immunity:

- ✓ The malarial paroxysms will become less severe and irregular in periodicity as the host develops immunity.
- ✓ This immunity is not a sterilizing immunity in that the infection persists longer than the symptoms and individuals can become re-infected.
- ✓ parasitemia does always cause disease (majority of persons living in areas of high endemicity will be parasitemic, but asymptomatic).
- ✓ 'Anti-disease immunity', antibodies against exo-antigens could neutralize their toxic effects.
- ✓ 'Anti-parasite immunity' in that parasitemia tends to be lower in persons previously exposed to malaria.
- ✓ Anti-parasite immunity could include antibodies that prevent merozoite invasion.
- ✓ Humoral and cellular immunity occurs with multiple exposures.
- ✓ Cerebral malaria occurs in non-immune individuals in endemic areas.
- ✓ In P. vivax and P. ovale infections, relapse after a period of dormancy may result from periodic release of merozoites from liver (hypnozoites), owing to the lack of an immune response to the intracellular parasites.
- ✓ Innate, non-acquired immunity to malaria is well demonstrated.

- ✓ Africans or African Americans lacking Duffy blood group antigen F (a-b) are immune to *P. vivax* because this genetic factor appears to be necessary for successful merozoite penetration of the human erythrocyte by this plasmodial species.
- ✓ Sickle cell hemoglobin inhibits growth of *P. falciparum*. This genetic factor is widespread in areas of Africa hyperendemic for falciparum malaria.
- ✓ Other red cell abnormalities such as glucose-6-phosphate dehydrogenase deficiency appear to be protective of the erythrocyte and thereby reduce the severity of plasmodial infection.
- ✓ Currently three vaccination strategies are still being pursued:

    - antisporozoite to prevent liver cell infection.
    - antimerozoite to prevent red blood cell infection.
    - antigametocyte to block transmission.

- ✓ Children exposed to repeated infections in hyperendemic areas develop a high degree of immunity, although this immunity does not imply eradication of infection, but rather a balance between parasite and host.
- ✓ The failure to induce protective antibodies in vaccinated individuals led research investigators to focus on the role of CMI.

End of Protozoa Lecture: 7