Entamoeba histolytica

Morphological features

(a) Trophozoites

Viable trophozoites vary in size from about 10-60μm in diameter. Motility is rapid, progressive, and unidirectional, through pseudopods. The nucleus is characterized by evenly arranged chromatin on the nuclear membrane and the presence of a small, compact, centrally located karyosome. The cytoplasm is usually described as finely granular with few ingested bacteria or debris in vacuoles. In the case of dysentery, however, RBCs may be visible in the cytoplasm, and this feature is diagnostic for E. histolytica.

(b) Cyst

Cysts range in size from 10-20μm. The immature cyst has inclusions namely; glycogen mass and chromatoidal bars. As the cyst matures, the glycogen completely disappears; the chromotiodials may also be absent in the mature cyst.

Life cycle

Intestinal infections occur through the ingestion of a mature quadrinucleate infective cyst, contaminated food or drink and also by hand to mouth contact. It is then passed unaltered through the stomach, as the cyst wall is resistant to gastric juice. In terminal ileum (with alkaline pH), excystation takes place. Trophozoites being actively motile invade the tissues and ultimately lodge in the submucous layer of the large bowel. Here they grow and multiply by binary fission. Trophozoites are responsible for producing lesions in amoebiasis. Invasion of blood vessels leads to secondary extra intestinal lesions. Gradually the effect of the parasite on the host is toned down together with concomitant increase in host tolerance, making it difficult for the parasite to continue its life cycle in the trophozoite phase. A certain number of trophozoites come from tissues into lumen of bowel and are first transformed into pre-cyst forms. Pre-cysts secret a cyst wall and become a uninucleate cyst. Eventually, mature quadrinucleate cysts form. These are the infective forms. Both mature and immature cysts may be passed in faeces. Immature cysts can mature in external environments and become infective.

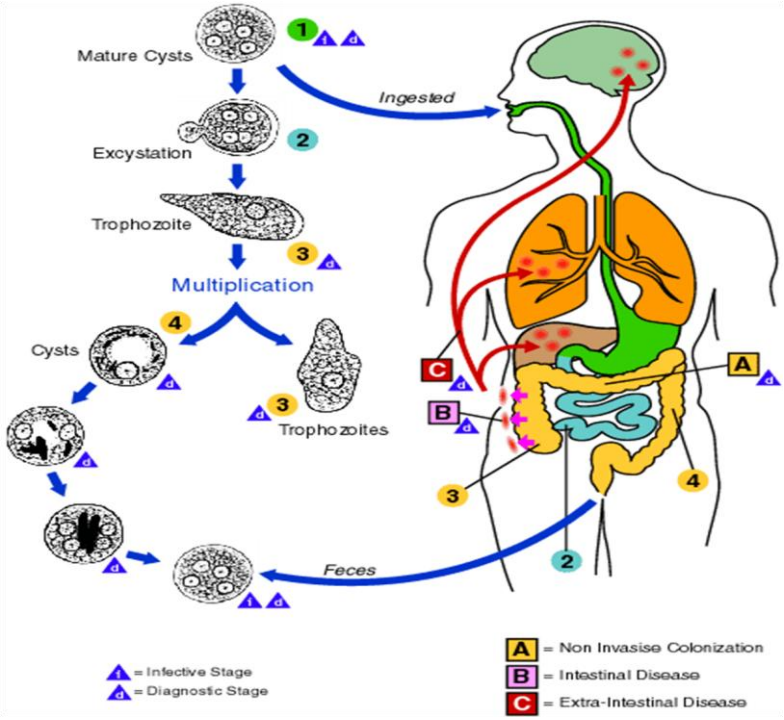

Figure: life cycle of Entamoeba histolytica

Pathogenesis

Trophozoites divide and produce extensive local necrosis in the large intestine. Invasion into the deeper mucosa with extension into the peritoneal cavity may occur. This can lead to secondary involvement of other organs, primarily the liver but also the lungs, brain, and heart. Extraintestinal amebiasis is associated with trophozoites. Amoebas multiply rapidly in an anaerobic environment, because the trophozites are killed by ambient oxygen concentration.

Laboratory diagnosis

In intestinal amoebiasis:

• Examination of a fresh dysenteric faecal specimen or rectal scraping for trophozoite stage. (Motile amoebae containing red cells are diagnostic of amoebic dysentery).

• Examination of formed or semiformed faeces for cyst stage. (Cysts indicate infection with either a pathogenic E.histolytica or non-pathogenic E.dispar.)

Extraintestinal amoebiasis

• Diagnosed by the use of scanning procedures for liver and other organs.

• Specific serologic tests, together with microscopic examination of the abscess material, can confirm the diagnosis.

Balantidiasis

The intestinal protozoan Balantidium coli is the only member of the ciliate group that is pathogenic for humans. Disease produced by B. coli is similar to amebiasis, because the organisms elaborate proteolytic and cytotoxic substances that mediate tissue invasion and intestinal ulceration.

Life cycle

The life cycle of B. coli is simple, involving ingestion of infectious cysts, excystation, and invasion of trophozoites into the mucosal lining of the large intestine, caecum, and terminal ileum. The trophozoite is covered with rows of hair like cilia that aid in motility. Morphologically more complex than amebae, B. coli has a funnel-like primitive mouth called a cytostome, a large (macro) nucleus and a small (micro) nucleus involved in reproduction.

Clinical features

As with other protozoan parasites, asymptomatic carriage of B. coli can exist. Symptomatic disease is characterized by abdominal pain, tenderness, tenesmus, nausea, anorexia, and watery stools with blood and pus. Ulceration of the intestinal mucosa, as with amebiasis, can be seen; a secondary complication caused by bacterial invasion into the eroded intestinal mucosa can occur. Extra intestinal invasion of organs is extremely rare in balantidiasis.

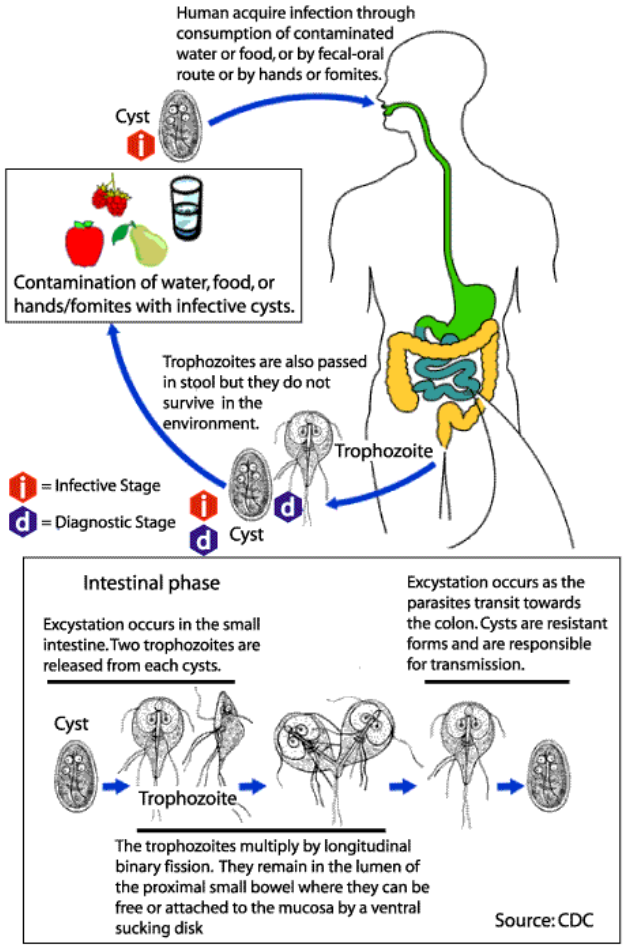

Figure: life cycle of Balantidium coli

Laboratory Diagnosis

Microscopic examination of faeces for trophozoite and cysts is performed. The trophozoite is very large, varying in length from 50 to 200µm and in width from 40 to 70µm. The surface is covered with cilia.

Giardia lamblia

Important features

The life cycle consists of two stages, the trophozoite and cyst. The trophozoite is 9-12 µm long and 5-15µm wide anteriorly. It is bilaterally symmetrical, pear-shaped with two nuclei (large central karyosome), four pairs of flagella, two axonemes, and a suction disc with which it attaches to the intestinal wall. The oval cyst is 8-12µm long and7-10µm wide, thick-walled with

four nucleus and several internal fibera? Each cyst gives rise to two trophozoites during excystation in the intestinal tract.

Transmission is by ingestion of the infective cyst.

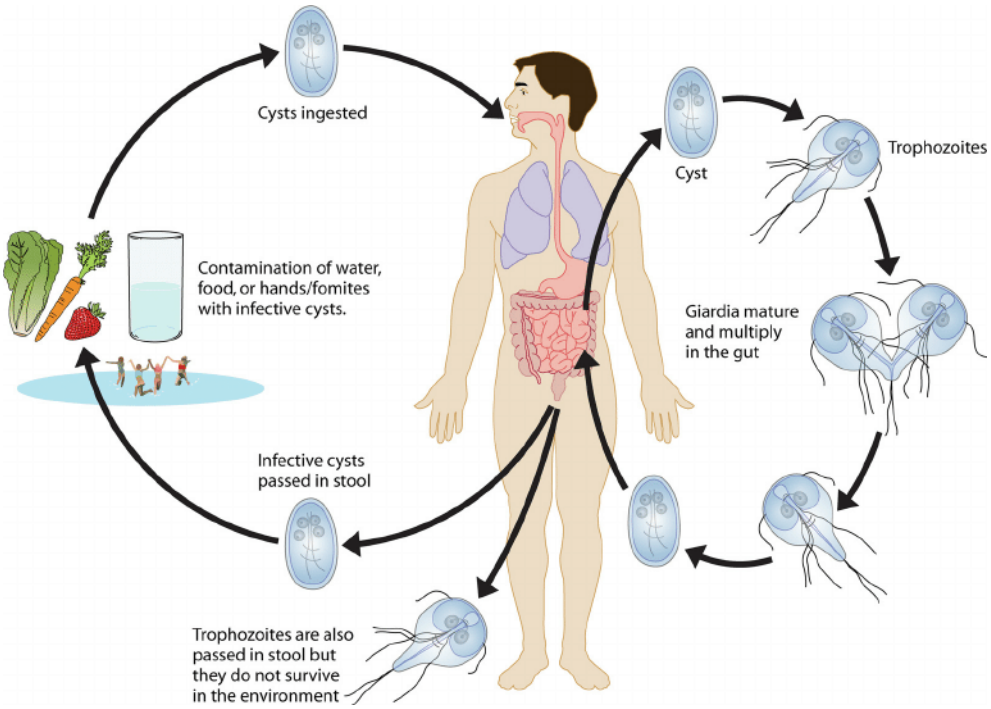

Figure: Life cycle of Giardia lamblia.

Pathogenesis

Infection with G.lamblia is initiated by ingestion of cysts. Gastric acid stimulates excystation, with the release of trophozoites in duodenum and jejunum. The trophozoites can attach to the intestinal villi by the ventral sucking discs without penetration of the mucosa lining, but they only feed on the mucous secretions. In symptomatic patients, however, mucosa-lining irritation may cause increased mucous secretion and dehydration. Metastatic spread of disease beyond the GIT is very rare.

Clinical features

Clinical disease: Giardiasis Symptomatic giardiasis ranges from mild diarrhea to severe malabsorption syndrome. Usually, the onset of the disease is sudden and consists of foul

smelling, watery diarrhea, abdominal cramps, flatulence, and streatorrhoea. Blood & pus are rarely present in stool specimens, a feature consistent with the absence of tissue destruction.

Immunity

The humoral immune response and the cellular immune mechanism are involved in giardiasis. Giardia – specific IgA is particularly important in both defense against and clearance of parasite.

Laboratory diagnosis

Examination of diarrhoeal stool- trophozoite or cyst, or both may be recovered in wet preparation. In examinations of formed stool (e.g. in asymptomatic carriers) only cysts are seen. Giardia species may occur in "showers", i.e. many organisms may be present in the stool on a given day and few or none may be detected the next day. Therefore one stool specimen per day for 3 days is important.

Toxoplasma gondii

Toxoplasma gondii causes toxoplasmosis. The definitive host is the domestic cat and other felines. Humans and other mammals are intermediate hosts. T. gondii is usually acquired by ingestion and transplacental transmission from an infected mother to the fetus can occur. Human–to–human transmission, other than transplacental transmission, does not occur. After infection of the intestinal epithelium, the organisms spread to other organs, especially the brain, lungs, liver, and eyes. Most primary infections in immunocompetent adults are asymptomatic. Congenital infection can result in abortion, stillbirth, or neonatal disease with encephalitis, chorioretinitis and hepatosplenomegaly. Fever, jaundice, and intracranial calcifications are also seen. For the diagnosis of acute and congenital infections, an immunofluorescence assay for detection of antibody is used. Microscopic examination of Giemsa–stained preparations shows crescent–shaped trophozoite. Cysts may be seen in the tissue. Treatment is with a combination of sulfadiazine and pyrimethamine.

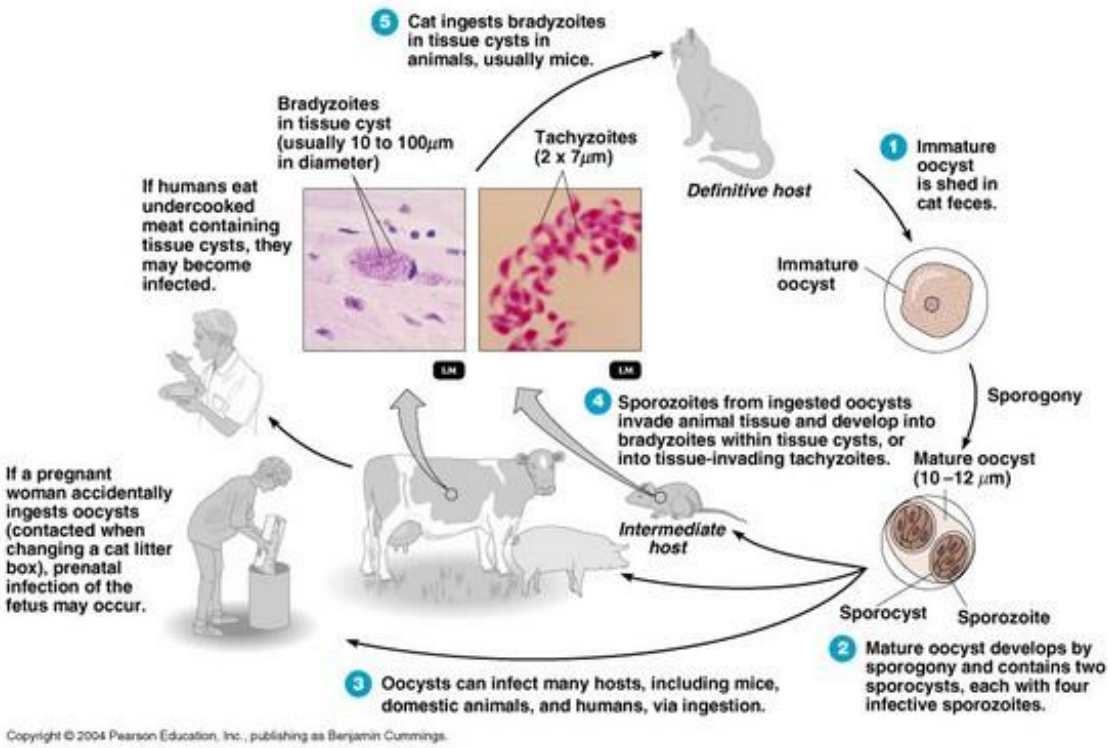

Figure: Life cycle of Toxoplasma gondii

Leishmania Species

Clinical disease - Veseral leishmaniasis

- Cutaneous leishmaniasis

- Mucocutaneous leishmaniasis

The species of leishmania exist in two forms, amastigote (aflagellar) and promastigote (flagellated) in their life cycle. They are transmitted by certain species of sand flies (Phlebotomus & Lutzomyia)

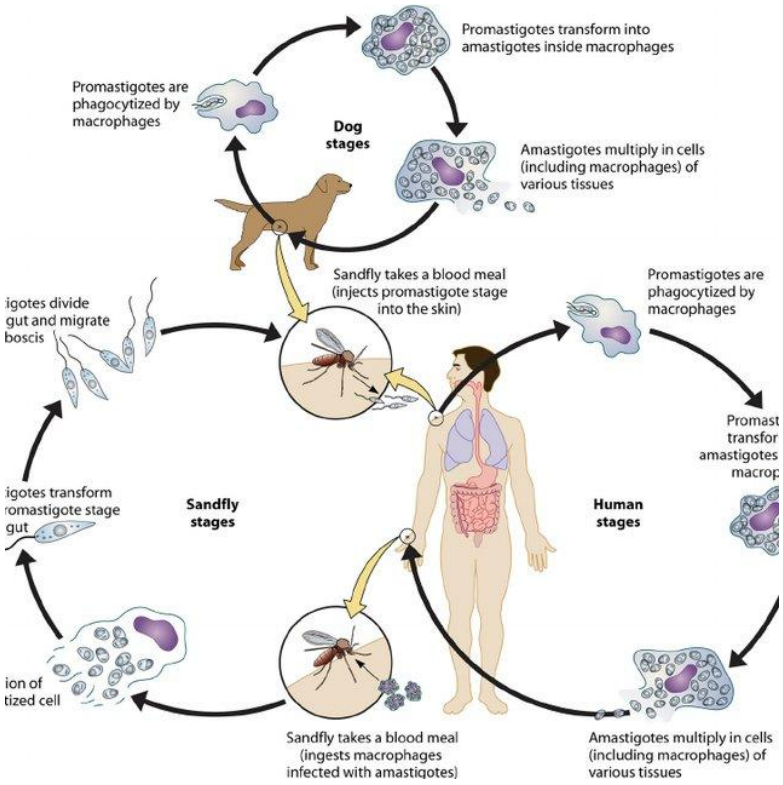

Figure: Life cycle of Leishmania species

Leishmania donovani

Important features

The natural habitat of L.donovani in man is the reticuloendothelial system of the viscera, in which the amastigote multiplies by 48 simple binary fission until the host cells are destroyed, whereupon new macrophages are parasitized. In the digestive tract of appropriate insects, the developmental cycle is also simple by longitudinal fission of promastigote forms. The amastigote stage appears as an ovoidal or rounded body, measuring about 2-3µm in length; and the promastigotes are 15-25µm lengths by 1.5-3.5µm breadths.

Pathogenesis

In visceral leishmaniasis, the organs of the reticuloendothelial system (liver, spleen and bone marrow) are the most severely affected organs. Reduced bone marrow activity, coupled with cellular distraction in the spleen, results in anaemia, leukopenia and thrombocytopenia. This leads to secondary infections and a tendency to bleed. The spleen and liver become markedly

enlarged, and hypersplenism contributes to the development of anaemia and lymphadenopathy also occurs. Increased production of globulin results in hyperglobulinemia, and reversal of the albumin-to-globulin ratio.

Clinical features

Symptoms begin with intermittent fever, weakness, and diarrhea; chills and sweating that may resemble malaria symptoms are also common early in the infection. As organisms proliferate & invade cells of the liver and spleen, marked enlargement of the organs, weight loss, anemia, and emaciation occurs. With persistence of the disease, deeply pigmented, granulomatous lesion of skin, referred to as post-kala-azar dermal leishmaniasis, occurs. Untreated visceral leishmaniasis is nearly always fatal as a result of secondary infection.

Immunity

Host cellular and humoral defence mechanisms are stimulated.

Laboratory diagnosis

- ✓ Examination of tissue biopsy, spleen aspiration, bone marrow aspiration or lymph node aspiration in properly stained smear (e.g. Giemsa stain).
- ✓ The amastigotes appear as intracellular & extra cellular L. donovan (LD) bodies.
- ✓ Culture of blood, bone marrow, and other tissue often demonstrates the promastigote stage of the organisms.
- ✓ Serologic testing is also available.

Malaria

There are four species normally infecting humans, namely, Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, and Plasmodium malariae.

Life cycle

The life cycle of malaria is passed in two hosts (alternation of hosts) and has sexual and asexual stage (alternation of generations).

Vertebrate host –

- Man (intermediate host), where the asexual cycle takes place. The parasite multiplies by schizogony and there is formation of male and female gametocytes (gametogony).

Invertebrate host

- Mosquito (definitive host) where the sexual cycle takes place. Union of male and female gametes ends in the formation of sporozoites (sporogony).

The life cycle passes in four stages: Three in man:-

- Pre - erythrocytic schizogony
- Erythrocytic schizogony
- Exo- erythrocytic schizogony

One in mosquito - Sporogony

Introduction into humans - when an infective female Anopheles mosquito bites man, it inoculates saliva containing sporozoites (infective stage).

Pre- Erythrocytic schizogony

Sporozoites reach the blood stream and within 30 minutes enter the parenchymal cells of the liver, initiating a cycle of schizogony. Multiplication occurs in tissue schizonts, to form thousands of tiny merozoites. Merozoites are then liberated on rupture of schizonts about 7th – 9th day of the bites and enter into the blood stream. These merozoites either invade the RBC's or other parenchymal liver cells. In case of P. falciparum and possibly P. malariae, all merozoites invade RBC's without re-invading liver cells. However, for P. vivax and P. ovale, some merozoites invade RBC's and some re-invade liver cells initiating further Exo-erythrocytic schizogony, which is responsible for relapses. Some of the merozoites remain dormant (hypnozoites) becoming active later on.

Erythrocytic schizogony (blood phase) is completed in 48 hrs in P. vivax, P. ovale, and P. falciparum, and 72 hrs in P. malariae. The merozoites reinvade fresh RBC's repeating the schizogonic cycles.

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

Gametogony

Some merozoites that invade RBC's develop into sexual stages (male and female gametocytes). These undergo no further development until taken by the mosquito.

Sporogony (extrinsic cycle in mosquito)

When a female Anopheles mosquito vector bites an infected person, it sucks blood containing the different stages of malaria parasite. All stages other than gametocytes are digested in the stomach.

The microgametocyte undergoes ex-flagellation. The nucleus divides by reduction division into 6-8 pieces, which migrate to the periphery. At the same, time 6-8 thin filaments of cytoplasm are thrust out, in each passes a piece of chromatin. These filaments, the microgametes, are actively motile and separate from the gametocyte.

The macrogametocyte by reduction division becomes a macrogamete. Fertilization occurs by entry of a micro gamete into the macro gamete forming a zygote.

The zygote changes into a worm like form, the ookinete, which penetrates the wall of the stomach to develop into a spherical oocyst between the epithelium and basement membrane. The oocystes increase in size. Thousands of sporozoites develop inside the oocysts. Oocysts rupture and sporozoites are liberated in the body cavity and migrate everywhere particularly to the salivary glands. Now the mosquito is infective.

The sporogonous cycle in the mosquito takes 8-12 days depending on temperature

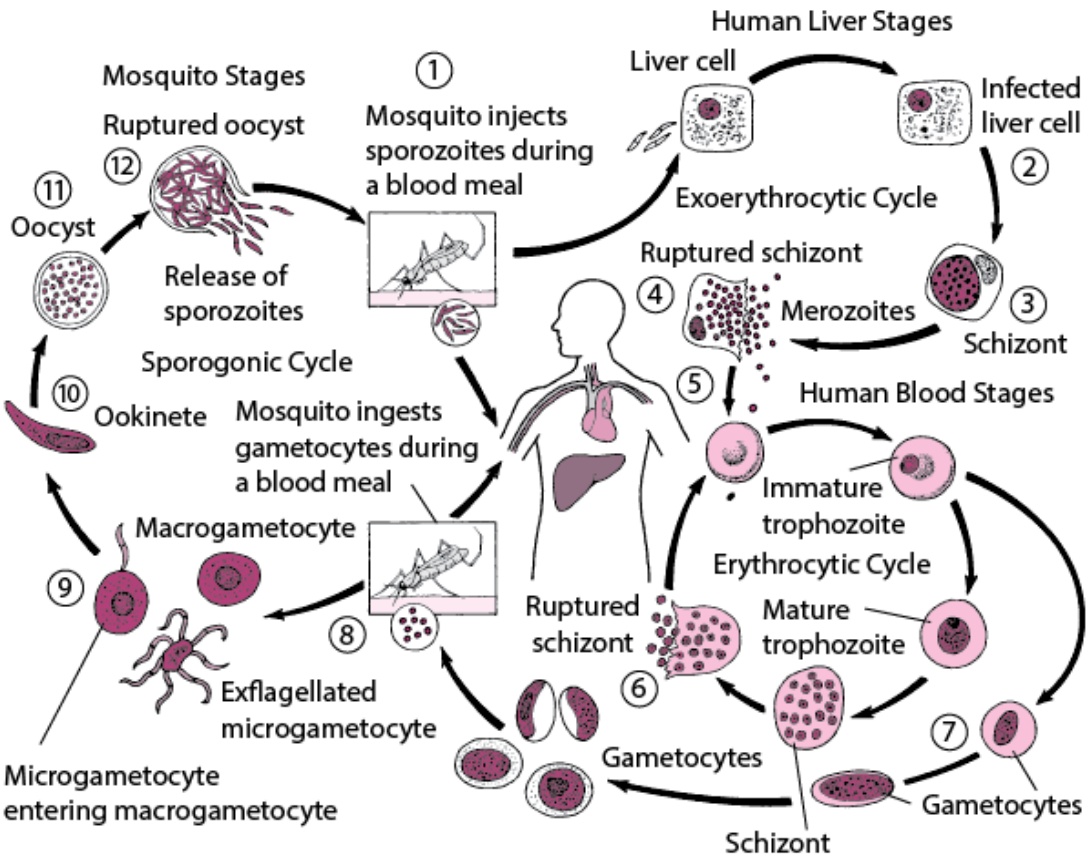

Figure: Life cycle of Plasmodium species

Plasmodium falciparum

Plasmodium falciparum demonstrates no selectivity in host erythrocytes, i.e. it invades young and old RBCs cells. The infected red blood cells also do not enlarge and become distorted.

- ✓ Multiple sporozoites can infect a single erythrocyte, and show multiple infections of cells with small ring forms.
- ✓ The trophozoite is often seen in the host cells at the very edge or periphery of cell membrane at accole position.
- ✓ Occasionally, reddish granules known as Maurer's dots are observed
- ✓ Mature (large) trophozoite stages and schizonts are rarely seen in blood films, because their forms are sequestered in deep capillaries, liver and spleen.
- ✓ Peripheral blood smears characteristically contain only young ring forms and occasionally crescent shaped gametocytes.

Clinical features

Of all the four Plasmodia, P. falciparum has the shortest incubation period, which ranges from 7 to 10 days. After the early flu-like symptoms, P.falciparum rapidly produces daily (quotidian) chills and fever as well as severe nausea, vomiting and diarrhea. The periodicity of the attacks then becomes tertian (36 to 48 hours), and fulminating disease develops. Involvement of the brain (cerebral malaria) is most often seen in P.falciparum infection. Capillary plugging from an adhesion of infected red blood cells with each other and endothelial linings of capillaries causes hypoxic injury to the brain that can result in coma and death. Kidney damage is also associated with P.falciparum malaria, resulting in an illness called "black water" fever. Intravascular hemolysis with rapid destruction of red blood cells produces a marked hemoglobinuria and can result in acute renal failure, tubular necrosis, nephrotic syndrome, and death. Liver involvement is characterized by abdominal pain, vomiting of bile, hepatosplenomegally, severe diarrhea, and rapid dehydration.

Treatment

Because chloroquine – resistant strains of P. falciparum are present in many parts of the world, infection of P.falciparum may be treated with other agents including mefloquine, quinine, guanidine, pyrimethamine – sulfadoxine, and doxycycline. If the laboratory reports a mixed infection involving P. falciparum and P. vivax, the treatment must eradicate not only P.falciparum from the erythrocytes but also the liver stages of P.vivax to avoid relapses provided that the person no longer lives in a malaria endemic area.

Plasmodium vivax

P.vivax is selective in that it invades only young immature erythrocytes. Infections of P. vivax have the following characteristics:

- ✓ Infected red blood cells are usually enlarged and contain numerous pink granules or schuffner's dots.
- ✓ The trophozoite is ring-shaped but amoeboid in appearance.

- ✓ More mature trophozoites and erythrocytic schizonts containing up to 24 merozoites are present. The gametocytes are round

Clinical features

After an incubation period (usually 10 to 17 days), the patient experiences vague flu-like symptoms, such as headache, muscle pains, photophobia, anorexia, nausea and vomiting. As the infection progresses, increased numbers of rupturing erythrocytes liberate merozoites as well as toxic cellular debris and hemoglobin in to circulation. In combination, these substances produce the typical pattern chills, fever and malarial rigors. These paroxysms usually reappear periodically (generally every 48 hours) as the cycle of infection, replication, and cell lyses progresses. The paroxysms may remain relatively mild or may progress to severe attacks, with hours of sweating, chills, shaking persistently, high temperatures (1030 F to 1060 F) and exhaustion. Since P.vivax infects only the reticulocytes, the parasitemia is usually limited to around 2 to 5% of the available RBCs.

Treatment

Chloroquine is the drug of choice for the suppression and therapeutic treatment of P.vivax, followed by premaquine for radical cure and elimination of gamatocytes

Plasmodium malariae

In contrast with P.vivax and P.ovale, P.malariae can infect only mature erythrocytes with relatively rigid cell membranes. As a result, the parasite's growth must conform to the size and shape of red blood cell.

This requirement produces no red cell enlargement or distortion, but it results in distinctive shapes of the parasite seen in the host cell, "band and bar forms" as well as very compact dark staining forms. The schizont of P.malariae is usually composed of eight merozoites appearing in a rosette.

Clinical features

The incubation period for P. malariae is the longest of the plasmodia, usually 18 to 40 days, but possibly several months to years. The early symptoms are flu-like with fever patterns of 72 hours (quartan or malarial) in periodicity.

Treatment

Treatment is similar to that for P.vivax and P.ovale.

Plasmodium ovale

P. ovale is similar to P. vivax in many respects, including its selectivity for young, pliable erythrocytes. As a consequence the classical characteristics include:

- ✓ The host cell becomes enlarged and distorted, usually in an oval form.
- ✓ Schiffner's dots appear as pale pink granules.
- ✓ The infected cell border is commonly fimbriated or ragged • Mature schizonts contain about 10 merozoites.

Clinical features

The incubation period for P.ovale is 16-18 days but can be longer. Clinically, ovale malaria resembles vivax malaria with attacks recurring every 48-50 hours. There are however, fewer relapses with P.ovale. Less than 2% of RBCs usually become infected.

Treatment

The treatment regimen, including the use of primaquine to prevent relapse from latent liver stages is similar to that used for P.vivax infection.

Laboratory diagnosis

Microscopic examination of thick and thin films of blood is the method of choice for confirming the clinical diagnosis of malaria and identifying the specific species responsible for disease.

Malaria parasites in thick and thin blood films are best stained at pH 7.1 – 7.2 using a Romanowsky stain (contains azure dyes and eosin). The thick film is a concentration method that may be used to detect the presence of organisms.

The thin film is most useful for establishing species identification.

Serologic procedures are available but they are used primarily for epidemiological surveys or for screening blood donors.

Immunity

There is evidence that antibodies can confer hormonal immunity against malaria infection.

Prevention

- ✓ Chemoprophylaxis and prompt diagnosis and treatment.
- ✓ Control of mosquito breeding
- ✓ Protection of insect bite by screening, netting and protective clothing
- ✓ Use of insect repellents.