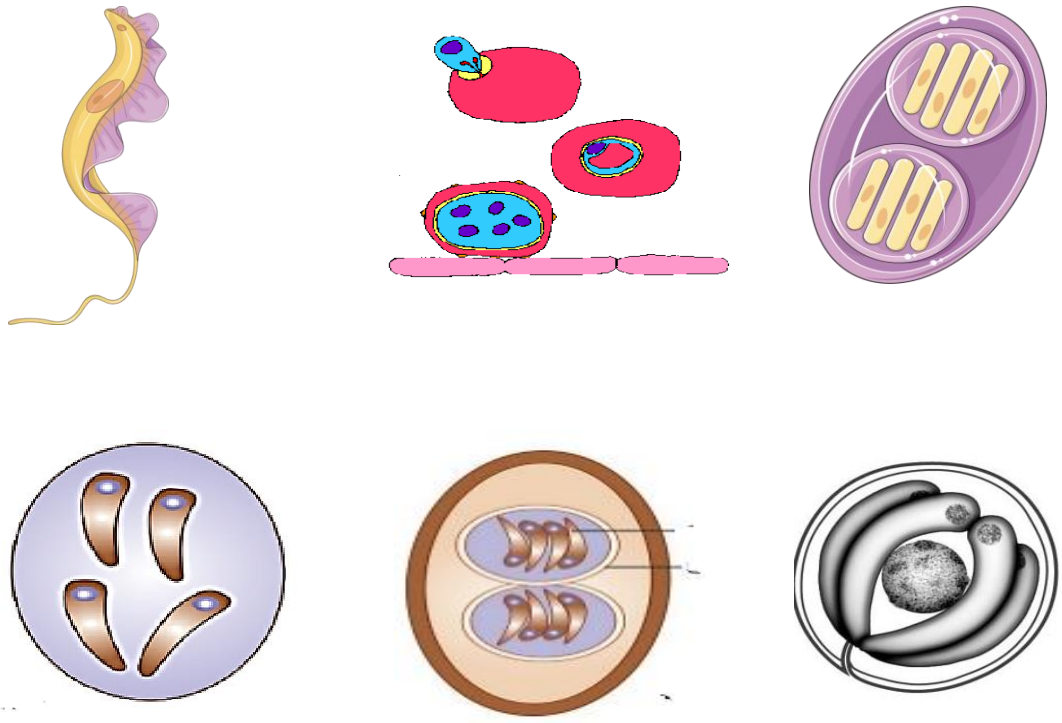
University of Baghdad
College of Sciences
Biology Department

Protozoan Parasitology
Part 2
المرحلة الثانية (صباحي+ مسائي)



➢ Trypanosomiasis

Etiologic agents

- *Trypanosoma brucei* complex – African trypanosomiasis (sleeping sickness)
- *Trypanosoma cruzi* – American trypanosomiasis (Chagas' disease)

Important features

These species may have four stages in their life cycle:
Amastigote, Promastigote, Epimastigote, Trypomastigote

- In human trypanosomes of the African form, however, the amastigote and promastigote stages of development are absent.

- The typical structure of trypanosome is an elongated spindle-shaped body with tapers at both ends, a centrally situated nucleus, a kinetoplast posterior to nucleus, an undulating membrane arising from the kinetoplast and proceeding forward along the margin of the cell membrane and a single free flagellum at the anterior end.

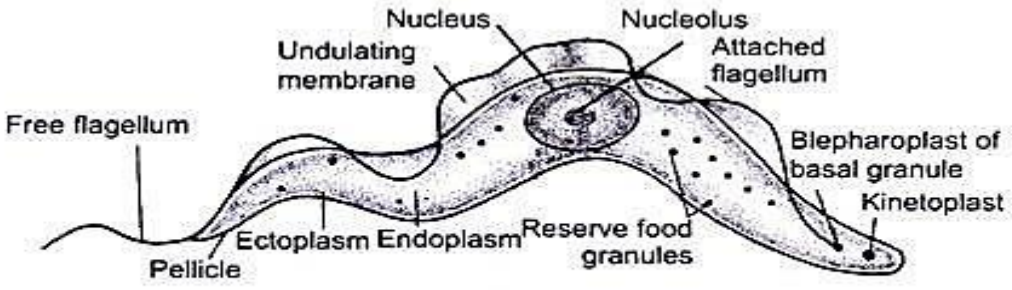
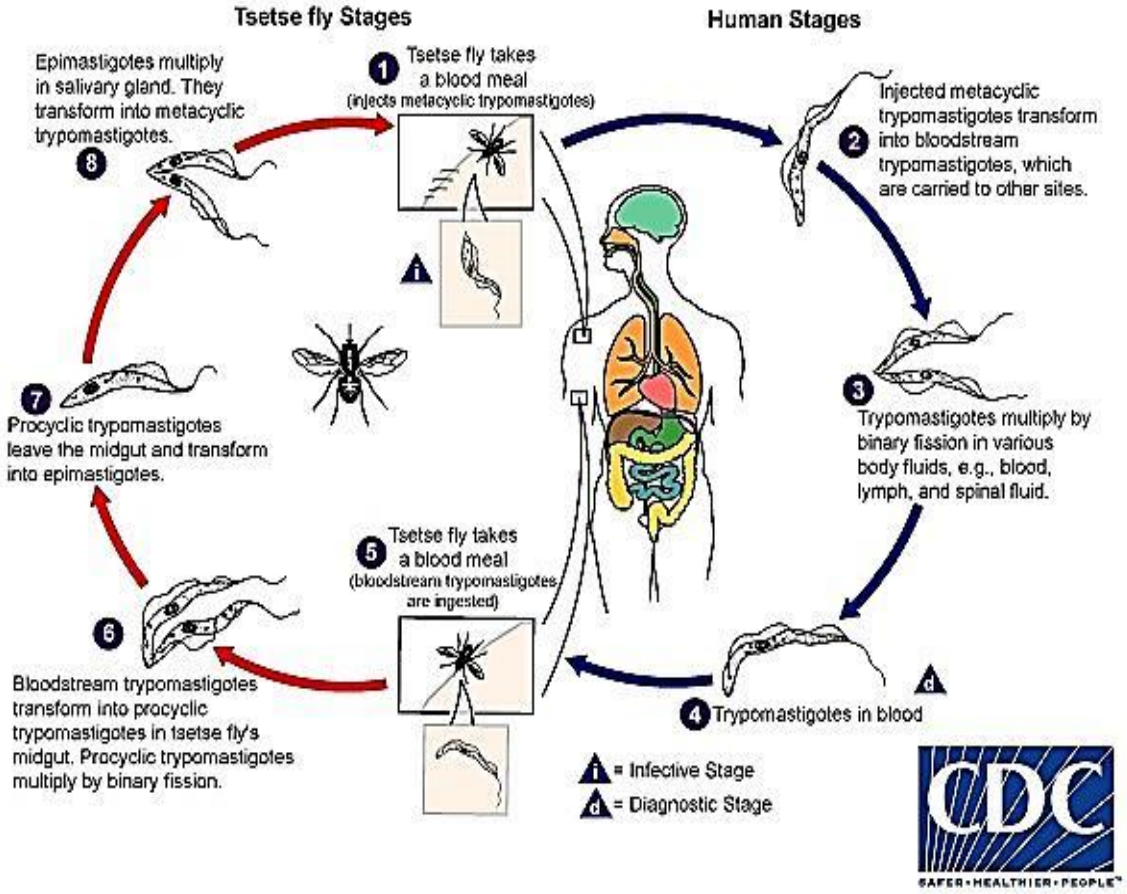


Fig. 9.22 *A Trypanosoma*

➢ African trypanosomiasis (*Trypanosoma brucei* complex)

Trypanosoma brucei gambiense & *Trypanosoma brucei rhodesiene* are causative the agents of the African typanosomiasis, transmitted by insect bites. The vector for both is the **tsetse fly**.



Life cycle of *Trypanosoma brucei*

During a blood meal on the mammalian host, an infected tsetse fly (genus *Glossina*) injects **metacyclic trypomastigotes** (infective stage) into skin tissue. The parasites enter the lymphatic system and pass into the bloodstream (**anterior station development**). Inside the host, they transform into bloodstream trypomastigotes ❷, are carried to other sites throughout the body, reach other body fluids (e.g., lymph, spinal fluid), and continue the replication by binary fission ❸. The entire life cycle of African trypanosomes is represented by extracellular stages. The tsetse fly becomes infected with bloodstream trypomastigotes when taking a blood meal on an infected mammalian host ❹, ❺). In the fly's midgut, the parasites transform into procyclic trypomastigotes, multiply by binary fission ❻, leave the midgut, and transform into epimastigotes ❼ . The epimastigotes reach the fly's salivary glands and continue multiplication by binary fission ❽. The cycle in the fly takes approximately 3 weeks. Humans are the main reservoir for *Trypanosoma brucei gambiense*, but this species can also be found in animals. Wild game animals are the main reservoir of *T. b. rhodesiense*.

Epidemiology

T. burcei gambiense is limited to tropical west and central Africa, correlating with the range of the tsetse fly vector. The tsetse flies transmitting *T.b. gambiense* proximity to human dwellings. People who work in such areas are at greatest risk of infection.

T. burcei rhodeseinse is found primarily in East Africa, especially the cattle-raising countries. *T.b. rhodeseines* also differs from *T.b. gambiense* in that domestic animal hosts (cattle and sheep) and wild game animals act as reservoir hosts. This transmission and vector cycle makes the organism more difficult to control than *T.b. gambiense*.

Clinical features

Although both species cause sleeping sickness, progress of the disease is different.

T. gambiense induced chronic infection over a few years. The earliest signs are an ulcer at the site of the fly bite. As reproduction of organisms continues, the lymph nodes are invaded, and fever, myalgia, arthralgia, and lymph node enlargement results. Swelling of the posterior cervical lymph nodes is characteristic of Gambian sleeping sickness. Chronic disease progresses to CNS involvement with lethargy, meningoencephalitis, mental retardation, and general deterioration. In the final stages, the patient becomes difficult to arouse or obtain a response from, eventually progressing to a comatose state. Death is the result of CNS damage and other infections, such as pneumonia.

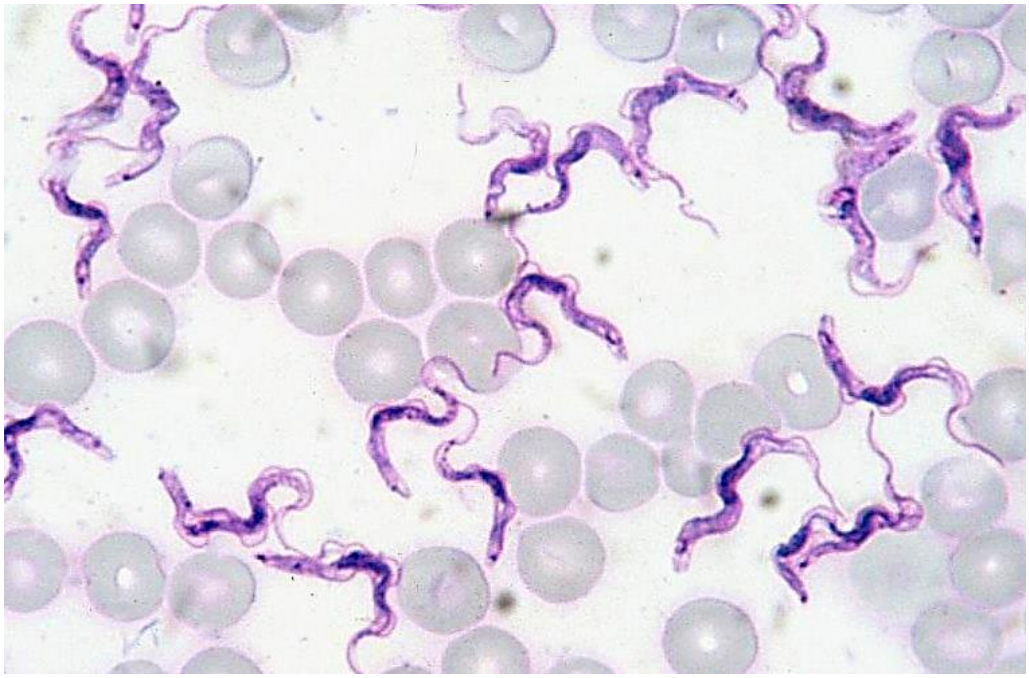
T. rhodesiense induced acute infection, rapidly progressive disease that is usually fatal. This more virulent organism also **develops in greater numbers in the blood**. Early in the infection, CNS invasion occurs, resulting in lethargy, anorexia, and mental disturbance leading to death.

Immunity

Both the humoral and cellular immunity involve in these infections. The immune responses of the host to the presence of these parasites, however, is faced with antigenic variation, in which organisms that have changed their antigenic identity can escape the host immune response and initiate another disease process with increased level of parasitemia.

Laboratory

- Examination of thin and thick films
- In aspiration from lymph nodes and concentrated spinal fluid.



Trypomastigote stage of *Trypanosoma burcei* complex

Treatment

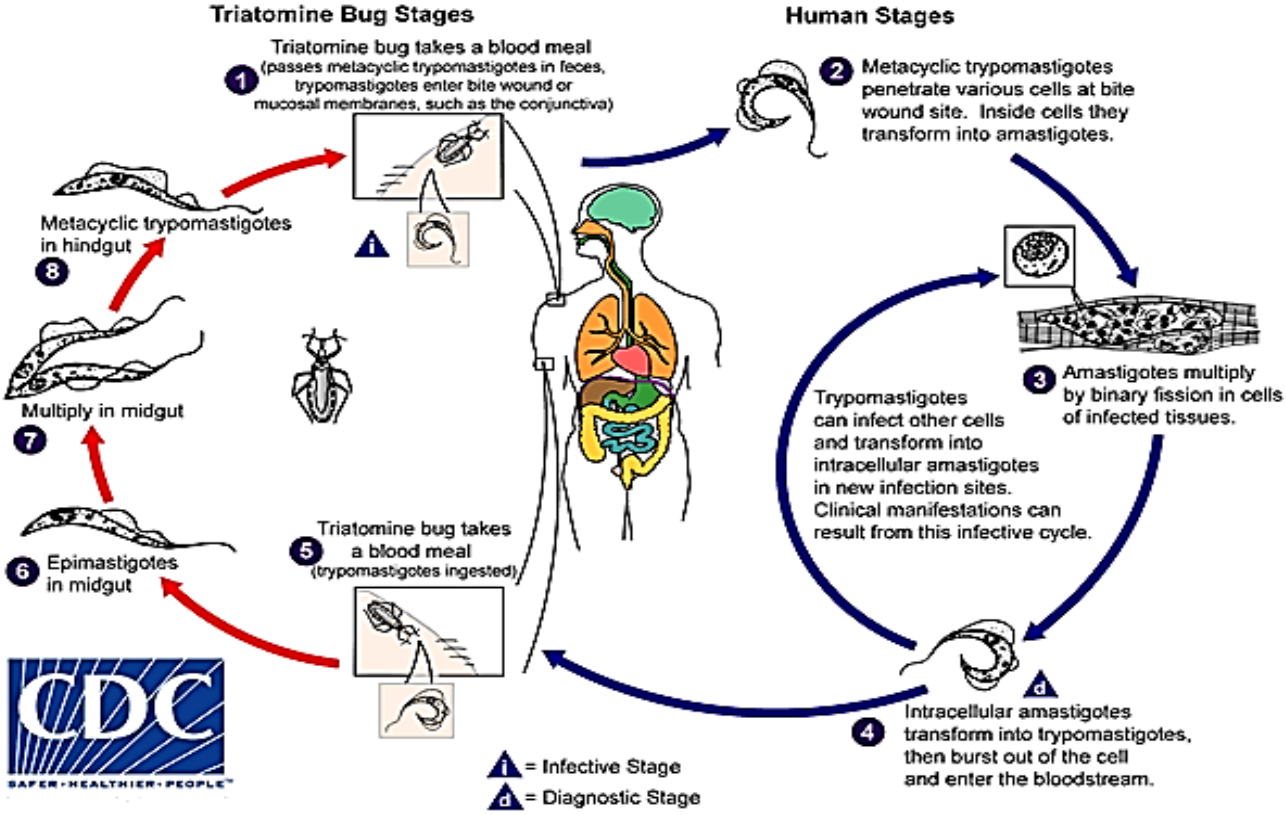
The same treatment protocol is applied for these parasites. For the acute stages of the disease the drug of choice is suramin with pentamidine as an alternative. In chronic disease with CNS involvement, the drug of choice is melarsoprol.

Prevention

• Control of breeding sites of tsetse flies and use of insecticides.
• Treatment of human cases to reduce transmission to flies.
• Avoiding insect bite by wearing protective clothing & use of screen, bed netting and insect repellants.

➢ American trypanosomiasis

Trypanosoma cruzi is a pleomorphic trypanosome that includes an additional form of amastigote in its life cycle. The vector for transmission are **reduviid bugs**.



Life cycle of *Trypanosoma cruzi*

An infected triatomine insect vector (or "kissing" bug) takes a blood meal and releases **trypomastigotes** (**infective stage**) in its feces near the site of the bite wound (**posterior station development**). Trypomastigotes enter the host through the wound or through intact mucosal membranes❶. Common triatomine vector species for trypanosomiasis belong to the genera *Triatoma* and *Rhodnius*. Inside the host, the trypomastigotes invade cells near the site of inoculation, where they differentiate into intracellular amastigotes❷. The amastigotes multiply by binary fission❸ and differentiate into trypomastigotes, and then are released into the circulation as bloodstream trypomastigotes❹. Trypomastigotes infect cells from a variety of tissues and transform into intracellular amastigotes in new infection sites. **The bloodstream trypomastigotes do not replicate** (different from the African trypanosomes). The "kissing" bug becomes infected by feeding on human or animal blood that contains circulating parasites❺. The ingested trypomastigotes transform into epimastigotes in the vector's midgut❻. Parasites multiply and differentiate in the midgut ❼ and differentiate into infective metacyclic trypomastigotes in the hindgut ❽

Epidemiology

T. cruzi occurs in both reduviid bugs and reservoir animals in North, Central, and South America. Human disease is found most often among children in South and Central America, where there is correlation between infected wild animal reservoir hosts and presence of infected bugs whose nests found in human dwellings.

Clinical features

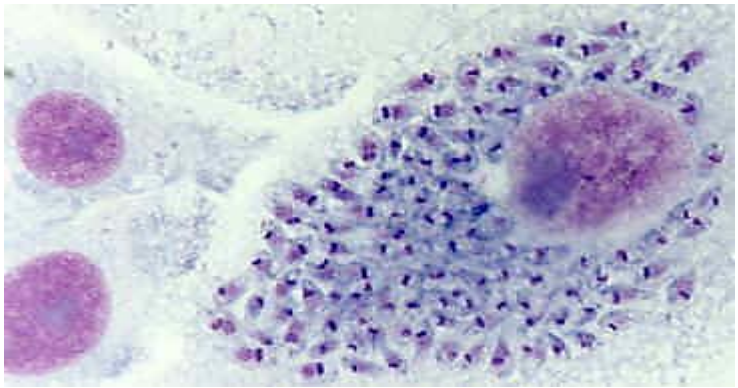
The acute phase, the organism occurs in blood as trypomastigote and in the reticuloendothelial cells as amastigote. The amastigotes can kill cells and cause inflammation. Cardiac muscle is the most frequently and severely affected tissue. Chagas' disease may be asymptomatic acute or chronic disease. One of the earliest signs is development at the site of the bug bite of an erythematous and indurated area called a **chagoma**. This is followed by a rash and edema around the eyes and face. Acute infection is also characterized by fever, chills, malaise, myalgia, and fatigue.

The chronic phase, the organism persists in the amastigote form. The chronic Chagas' disease is characterized by hepatosplenomegaly, myocarditis, and enlargement of the esophagus and colon as a result of the destruction of nerve cells (E.g. Auerbach's plexus) and other tissues that control the growth of these organs. Involvement of the CNS may produce granulomas in the brain with cyst formation and a meningoencephalitis. Death from chronic Chagas' disease results from tissue destruction in the many areas invaded by the organisms, and sudden death results from complete heart block and brain damage.

Laboratory diagnosis

Examine thin or thick stained preparations for trypomastigotes.

Biopsy of lymph nodes, liver, spleen, or bone marrow may demonstrate organisms in amastigote stage.



Amastigote stage of *Trypanosoma cruzi* in skeletal muscle

Xenodiagnosis - which consists of allowing an uninfected, laboratory-raised reduviid bug to feed on the patient and, after several weeks, examining the intestinal contents of the bug for the organism.

Immunity

Unlike African trypanosomiasis, the antigenic variation is less common in *T.cruzi* infection. Therefore, the humoral and cellular immune responses function in the immune system.

Treatment

The drug of choice is nifurtimox. Alternative agents include allopurinol & benzimidazole.

Prevention

- Bug control, eradication of nests
- Treating infected person & exclusion of donors by screening blood.
- Development of vaccine.

➢ COCCIDIA (SPOROZOA)

Coccidia are members of the class sporozoa, Phylum Apicomplexa. Apical complex is present at some stage and consists of elements visible with electron microscope. The life cycle is characterized by an alternation of generations, i.e. sexual (gametogony) and asexual (schizogony) reproduction and most members of the group also share alternative hosts. The locomotion of a mature organism is by body flexion, gliding, or undulation of longitudinal ridges. The genus ***Plasmodium*** is the causes of **malaria**.

➢ Malaria

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

Life cycle

The life cycle of malaria is occured 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 a 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 three stages:
Two in man- Exo- erythrocytic schizogony, - Erythrocytic schizogony
One stage in mosquito – Sporogony

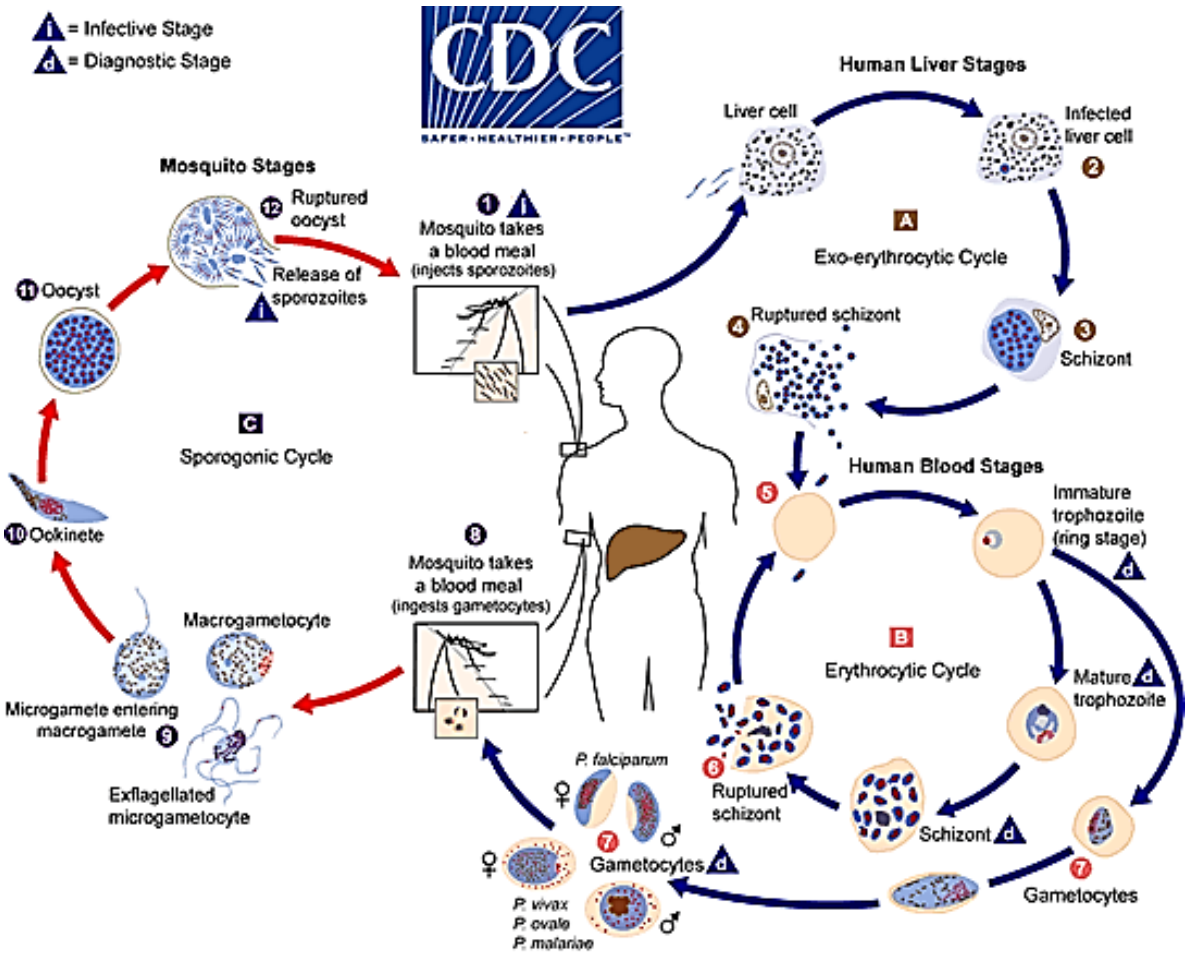
The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates **sporozoites (infective stage)** into the human host ❶ .

Exo- Erythrocytic schizogony 🅐**:** Sporozoites reach the blood stream and within 30 and infect liver cells ❷ and mature into schizonts ❸, which rupture and release merozoites ❹. (Of note, in *P. vivax* and *P. ovale* a dormant stage [hypnozoites] can persist in the liver (if untreated) and cause **relapses** by invading the bloodstream weeks, or even years later.) After this initial replication in the liver (exo-erythrocytic schizogony 🅐), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony 🅑).

Erythrocytic schizogony B: Merozoites infect red blood cells 5. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites 5. Some parasites differentiate into sexual erythrocytic stages (gametocytes) 7. Blood stage parasites are responsible for the clinical manifestations of the disease. The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an *Anopheles* mosquito during a blood meal 8.

Sporogony (extrinsic cycle in mosquito): The parasites' multiplication in the mosquito is known as the sporogonic cycle C. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes 9. The zygotes in turn become motile and elongated (ookinetes) 10 which invade the midgut wall of the mosquito where they develop into oocysts 11. The oocysts grow, rupture, and release sporozoites12, which make their way to the mosquito's salivary glands and migrate to the salivary glands. Now the mosquito is infective. Inoculation of the sporozoites 1 into a new human host and repeated the malaria life cycle.

Life cycle of *Plasmodium* species



➢ *Plasmodium falciparum*

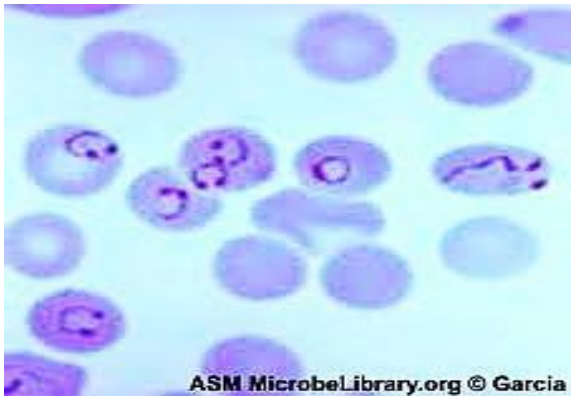
Plasmodium falciparum invades young and old RBCs cells. The infected red blood cells also do not enlarge and become distorted.

- 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.
- 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 sequestered in deep capillaries, liver and spleen.
- Peripheral blood smears characteristically contain only young ring forms and occasionally crescent shaped gametocytes.

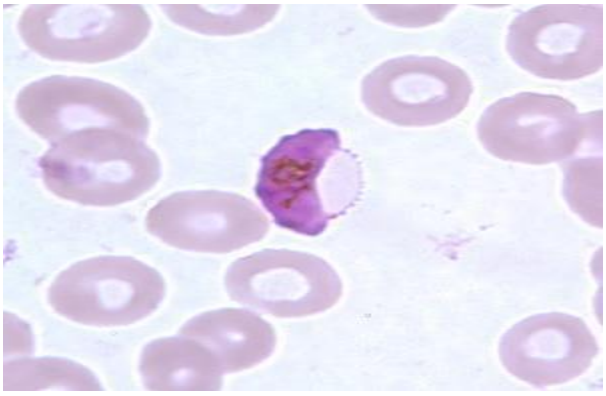
Clinical features

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 chills and fever as well as severe nausea, vomiting and diarrhea. The periodicity of the attacks then becomes tertian (36 to 48 hours). 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.

P. falciparum



Ring form of with multiple infection of erythrocyte



Mature gametocyte

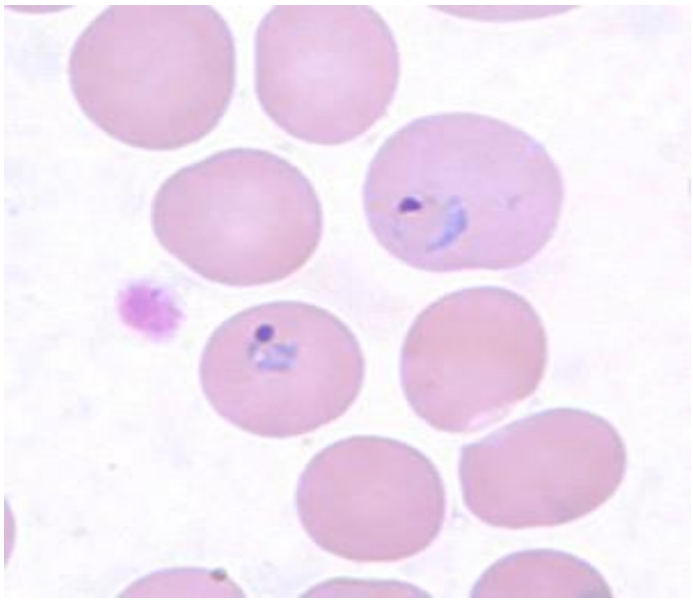
- ***Plasmodium vivax***

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 suffered from flu-like symptoms, such as headache, muscle pains, 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 and exhaustion. Since *P.vivax* infects only the reticulocytes, the parasitemia is usually limited to around 2 to 5% of the available RBCs.



***Plasmodium vivax* ring form**

- ***Plasmodium malariae***
- *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.

- ***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:

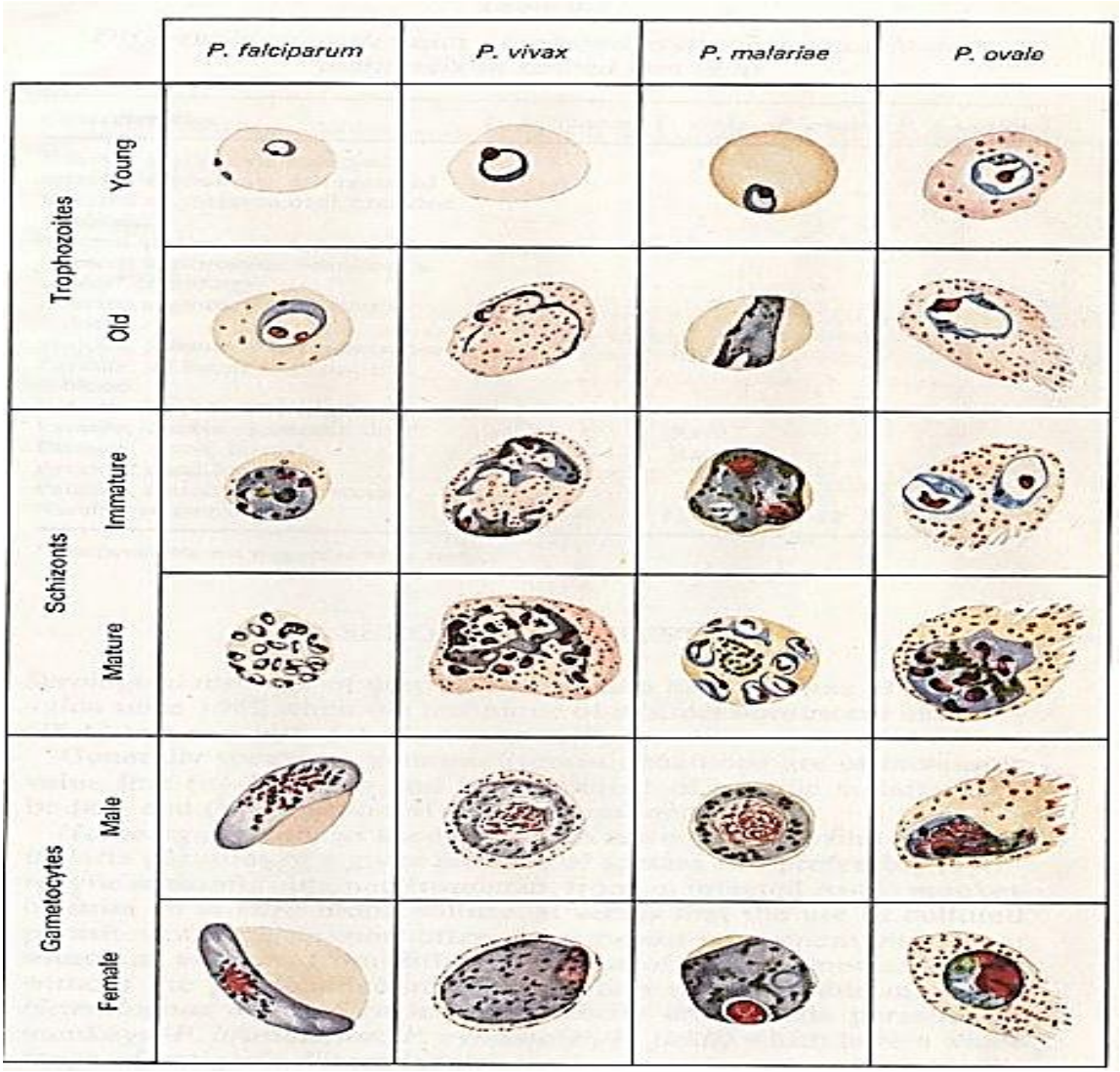
- The host cell becomes enlarged and distorted, usually in an oval form.
- Schiffner's dots appear as pale pink granules.
- The infected cell border is commonly fimbriated or ragged
- Mature schizonts contain about 10 merozoites.

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.

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



Treatment

- Quinine was used historically; however the development of more effective alternatives such as quinacrine, chloroquine, and primaquine in the 20th century reduced its use.
- Modern drugs used include mefloquine (*Lariam*), doxycycline, and the combination of atovaquone and proguanil hydrochloride (*Malarone*).

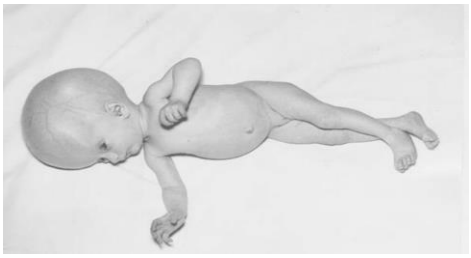
Prevention

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

➢ *Toxoplasma gondii*

History and Distribution

Toxoplasma is an important apicomplexan parasite which causes toxoplasmosis, commonly known as a cat litter disease. This genus has only one species *T. gondii* which can infect any nucleated mammalian cells.



T. gondii was first discovered by scientists at the 20th century. The genus name is derived from the Greek word toxon, meaning "bow" and referring to the crescent shape of the organism.

T. gondii is an obligate intracellular parasite that has a huge impact on both human and animal health. In humans, it causes serious damage to the unborn child (congenital toxoplasmosis) and in immunocompromised patients. In congenital toxoplasmosis, acute infection leads either to abortion or intellectual retardation for instance hydrocephalus, intracranial calcification and chorioretinitis in the fetus.

In animals, it cause abortion in livestock, and can cause serious economic losses to sheep and goat breeders.

Morphology

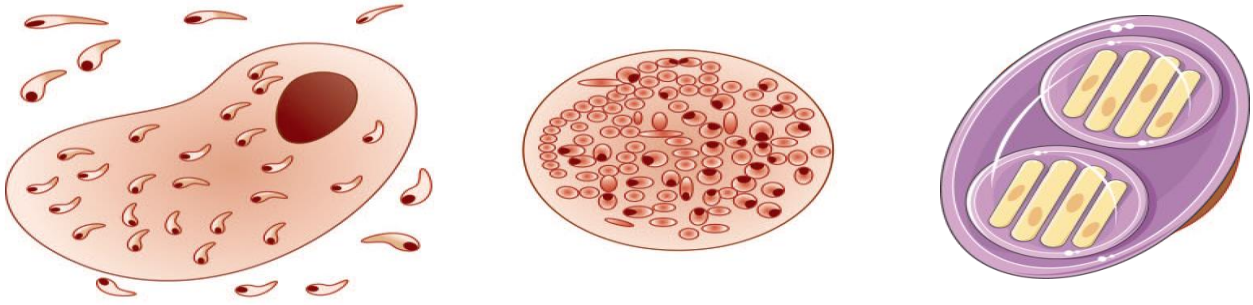
Toxoplasma gondii occurs in 3 forms

A- Trophozoite

B- Tissue cyst

C- Oocyst

All the 3 forms are infectious to man. The trophozoite and tissue cyst represent stages in asexual multiplication (**schizogony**), while the oocyst is formed by sexual reproduction (**gametogony or sporogony**). All 3 forms occur in domestic cats and other felines, which are the definitive hosts and support both schizogony and gametogony. Only the asexual forms, trophozoites and tissue cysts are present in other animals, including humans and birds, which are the intermediate hosts.



A **B** **C**

Figure: *Toxoplasma gondii.* **A.** tachyzoites form within macrophage; **B.** tissue cyst containing bradyzoites; **C.** Oocyst containing 2 sporocysts with sporozoites inside

Trophozoites (Tachyzoites)

The trophozoite is crescent shaped, with one end pointed and the other end rounded. It measures 3–7 μm in length. The nucleus is ovoid and is situated at the blunt end of the parasite. An **apical complex** situated at the pointed end.

The actively multiplying trophozoite is seen intracellularly in various tissues during early acute phase of infection. It can invade any nucleated cell and replicate within cytoplasmic vacuoles by a process called **endogony** (**internal budding**), wherein 2 daughter trophozites are formed, each surrounded by a membrane, while still within the parent cell. When the host cell becomes distended with the parasite, it disintegrates, releasing the trophozoites that infect other cells. During acute infection, the proliferating trophozoites within host cell may appear rounded and enclosed by the host cell membrane. This is, called **pseudocyst** or **colony** and can be differentiated from tissue cysts by staining reactions. The rapidly proliferating trophozoites in acute infection are called **tachyzoites.**

Tissue cyst

Tissue cysts are the resting form of the parasite. They are found during chronic stage of the infection and can be found in the brain (most common site), skeletal muscles, and various other organs. The slowly multiplying parasites within the cyst are called **bradyzoites**. The cyst is round or oval, 10–20 μm in size and contains numerous bradyzoites. Cysts remain viable in tissue for several years. In immunologically normal hosts, the cysts remain silent, but in the immunodeficient subjects, they may get reactivated, leading to clinical disease. It is relatively resistant and when the raw or undercooked meat containing the cysts is eaten, infection occurs.

Oocyst

Oocysts develop only in definitive hosts – in the intestine of cats and other felines but not in humans. It is oval in shape and measures 10–12 μm in diameter.

Each cyst is surrounded by a thick resistant wall. The oocysts are formed by sexual reproduction (gametogony). Cats shed millions of oocysts per day in feces for about 2 weeks during the primary infection. The freshly passed oocyst is not infectious. They undergo sporulation in the soil with formation of 2 sporocysts, each containing 4 sporozoites. The sporulated oocyst is infective. Oocyst is very resistant to environmental conditions and can remain infective in soil for about a year. When the infective oocyst is ingested, it releases sporozoites in the intestine, which initiates infection.

Life Cycle

Toxoplasma gondii completes its life cycle in 2 hosts:

Definitive host: Cats and other felines, in which both sexual and asexual cycle takes place.

Intermediate hosts: Man and other mammals, in which only the asexual cycle takes place. *T. gondii* has 2 types of life cycles:

- Enteric cycle
- Exoentric cycle

Enteric cycle (Enteric cycle occurs in definitive hosts like cat).

-Both sexual reproduction (gametogony) and asexual reproduction (schizogony) are occurs within the mucosal epithelial cells of the small intestine of the cat.

-Cat acquires infection by ingestion of tissue cysts in the meat of rats and other animals or by ingestion of oocysts passed in its feces.

-The bradyzoites are released in the small intestine and they undergo asexual multiplication (schizogony) leading to formation of merozoites.

-Some merozoites enter extraintestinal tissues resulting in the formation of tissue cysts in other organs of the body.

-Other merozoites transform into male and female gametocytes and sexual cycle (gametogony) begins, with the formation of **microgamete** and **macrogamete**.

-A macrogamete is fertilized by the motile microgamete resulting in the formation of an oocyst, which passes through maturation stages (**sporulation**) in the soil after being excreted from host through feces.

-A mature oocyst containing **8 sporozoites**, and it is regard the infective form which may be ingested by rats or other mammals to repeat the cycle.

Exoenteric cycle (Exoenteric cycle occurs in humans, mice, rats, sheep, cattle, pigs and birds) which are the intermediate hosts. Humans acquire infection after:

1- Eating uncooked or undercooked infected meat containing tissue cysts.

2- Ingestion of mature oocysts through food, water contaminated with cat feces.

3- Intrauterine infection from mother to fetus (**congenital toxoplasmosis**)

4- Blood transfusion or transplantation from infected donors.

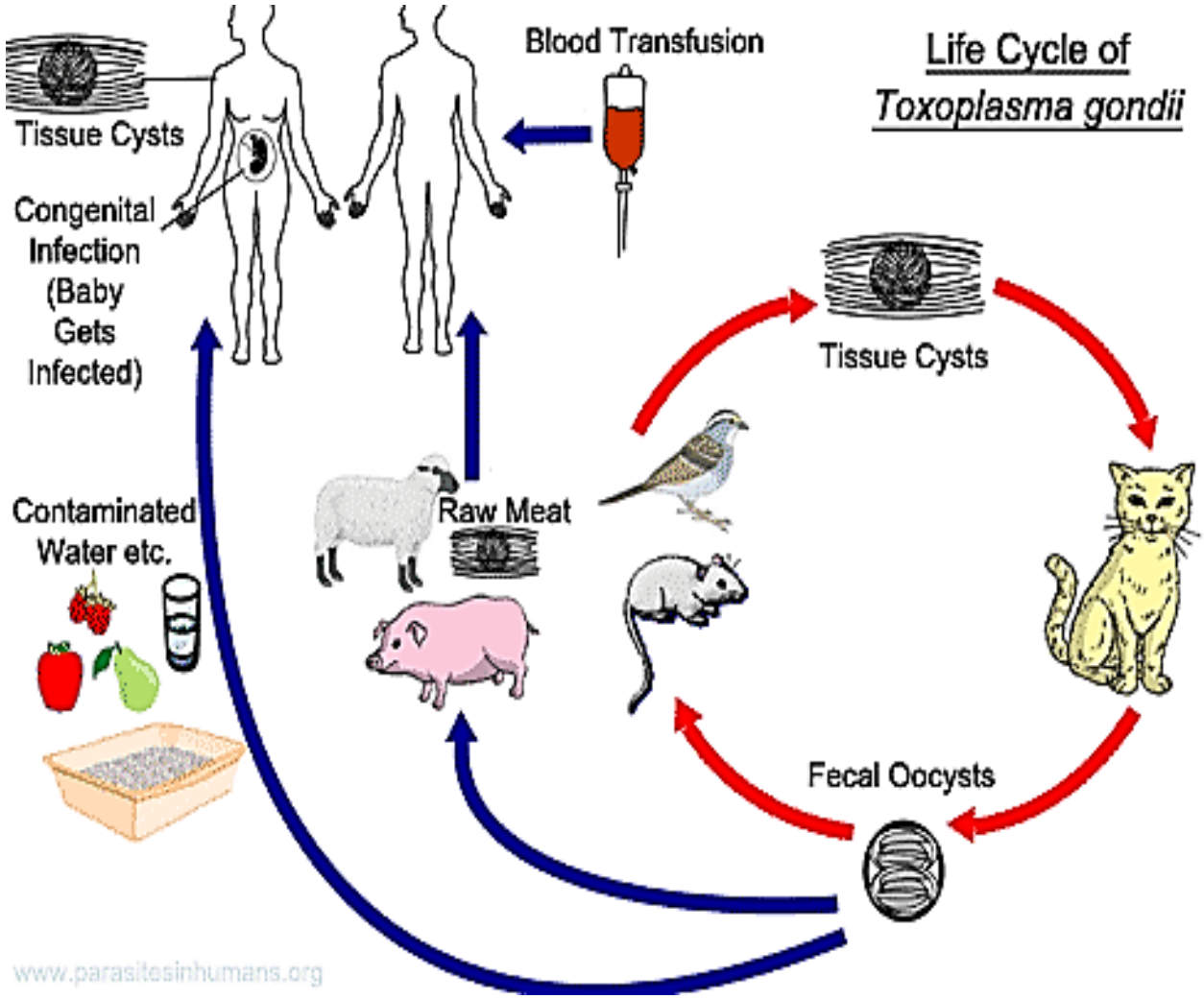
-Sporozoites from oocysts and bradyzoites from tissue cysts enter into intestinal **mucosa** and multiply **asexually** and **tachyzoites** are formed (**endodyogeny**).

-Tachyzoites continue to multiply and spread by lymphatic system and blood.

-Some tachyzoites also spread to distant extraintestinal organs like brain, eye, liver, spleen, lung, and skeletal muscles and form **tissue cysts**. The slowly multiplying inside the tissue cysts are known as **bradyzoites**, which remain viable for years.

-Dormant bradyzoites inside the cyst may be reactivated in immune suppression causing renewed infection in the host.

-Human infection is a dead end for the parasite.



Pathogenicity and Clinical Features

Congenital toxoplasmosis

Congenital toxoplasmosis results when *T. gondii* is transmitted transplacentally from mother to fetus. This occurs when the mother gets primary *Toxoplasma* infection, whether clinical or asymptomatic, during the pregnancy.

-The risk of fetal infection rises with progress of gestation; from 25%, when the mother acquires primary infection in first trimester to 65% in the third trimester. The severity of fetal damage is highest when infection is transmitted in early pregnancy.

Acquired Toxoplasmosis

-Infection acquired postnatally is mostly asymptomatic.

-Fever, headache, myalgia, and splenomegaly. The illness may resemble **mild flu**.

-There may be a **typhus-like exanthema** with pneumonitis and myocarditis, which may be fatal.

Toxoplamosis in Immunocompromised Patients

Toxoplasmosis is most serious and often fatal in immunocompromised patients, particularly in AIDS, whether it may be due to reactivation of latent infection.
-Clinical manifestation includes encephalitis, and neuropsychiatric manifestations.
-other organs involved are lungs, pancreas, gastrointestinal tract, eyes, heart, liver.

Laboratory Diagnosis:

Microscopy

Tachyzoites and tissue cysts can be detected in various specimens like blood, sputum, bone marrow aspirate, cerebrospinal fluid (CSF), aminiotic fluid, and biopsy material from lymph node, spleen, and brain. Smear made from above specimens is stained by Giemsa. Tachyzoites appear as crescent-shaped structures with blue cytoplasm and dark nucleus.

Serodiagnosis: Serology is the main stay for diagnosis of toxoplasmosis.
Antibody detection: Diagnosis of acute infection with *T. gondii* can be made by detection of the presence of IgM and IgG antibodies.
--Tests for detecting IgG antibody include:
1. Enzyme-linked immunosorbent assay (ELISA)
2. Indirect fluroscent antibody test (IFAT)
3. Latex agglutination test
4. --Positive IgG titer (>1:10) can be detected as early as 2–3 weeks after infection. Peak level of antibody is observed in blood 4–8 weeks after infection.

--A positive IgM antibody titer indicates an early primary infection. The serum IgM titer can be measured by double sandwich IgM ELISA or IgM immunosorbent assay (IgM-ISAGA). Both assays are equally specific and sensitive. Negative IgM titer and positive IgG titer indicate distant infection.

Molecular Methods

DNA hybridization techniques and polymerase chain reaction (PCR) are increasingly used to detect *Toxoplasma* from different tissues and body fluids.

Treatment: Congenital infection is treated with pyrimethamine and sulfadiazine. For primary prophylaxis Trimethoprim-sulfamethoxazole is the drug of choice

➢ *Isospora belli*

Isospora belli is a coccidian parasite which can cause diarrhea in humans.

Morphology

Oocysts of *Isospora belli* are elongated ovoid and measure 25 µm × 15 µm. Each oocyst is surrounded by a thin smooth 2 layered cysts. Immature oocyst seen in the feces of patients contains two sporoblasts. On maturation, the sporoblast convert into **sporocysts**. Each sporocyst contains 4 crescent-shaped sporozoites. The **sporulated oocyst containing 8 sporozoites** is the **infective stage** of the parasite.

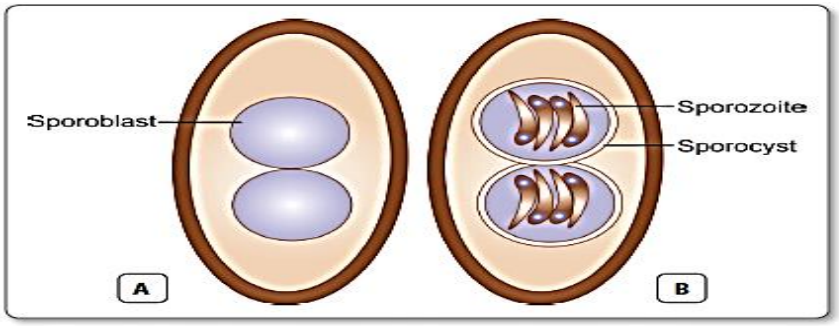
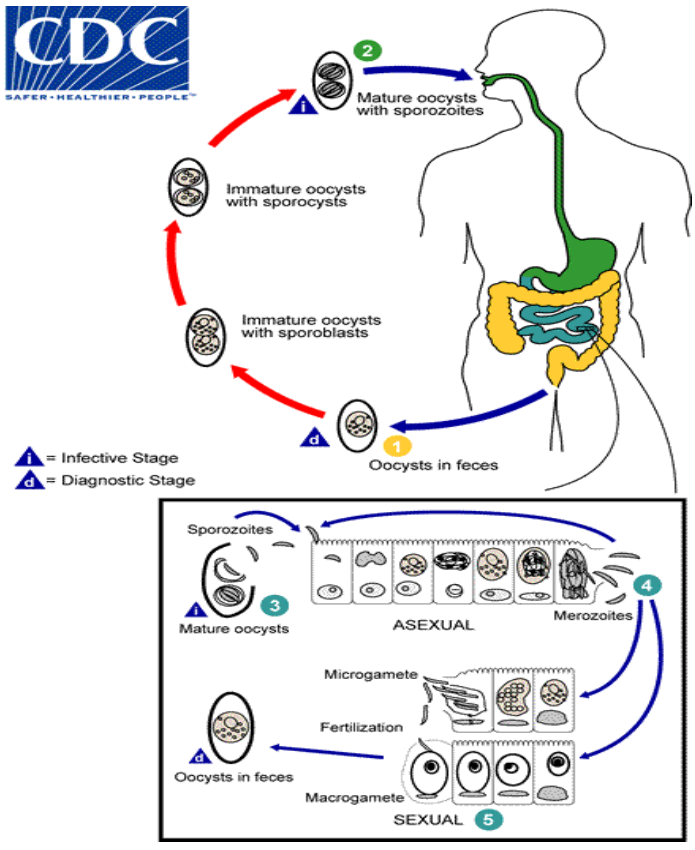


Fig. 7.5: Oocysts of *Isospora belli*. **A.** Immature cyst; **B.** Mature cyst

Life Cycle

Isospora belli completes its life cycle in one host. Man gets infection by ingestion of food and water contaminated with sporulated oocyst. When a sporulated oocyst is swallowed, 8 sporozoites are released from the 2 sporocysts in the small intestine and invade the intestinal epithelial cells. In the epithelium, the sporozoites transform into trophozoites, which multiply asexually (**schizogony**) to produce a number of (**merozoites**). The merozoites invade adjacent epilhelial cells to repeat asexual cycle. Some of the trophozoites undergo sexual cycle (**gametogony**) in the cytoplasm of enterocytes and transform into **macrogametocytes** and **microgametocytes**. After fertilization, a zygote is formed, which secretes a cyst wall and develops into an **immature oocyst**. These immature oocysts are excreted with feces and mature in the soil.



Clinical Features

Infection is usually asymptomatic, mayincludes abdominal discomfort, mild fever, diarrhea, and malabsorption. The diarrhea is usually watery and does not contain blood or pus and is self-limiting, diarrhea lasting for several years seen in immunocompromised persons.

Laboratory Diagnosis

Stool Examination: this techniques may be required when direct wet mount of stools are negative. The staining technique was used of stool smear. In these methods, pink colored acid fast large oocyst (>25 μm) can be demonstrated.

Treatment

Immunodefecient patients with diarrhea and excreting oocysts in the feces should be treated with cotrimoxazole (trimethoprim sulfamethoxazole) in a dose of 2 tablet, 4 times a day for 10 days followed by 2 tablets 2 times a day for 3 weeks.

➢ *Cryptosporidium parvum*

History and Distribution

Cryptosporidium was first observed in the gastric mucosal crypts of laboratory mice by Tyzzer in 1907. Its importance as a pathogen causing diarrhea in animals was recognized in 1971 and the first case of human infection was reported in 1976. C*ryptosporidium* causes of intractable diarrhea, in AIDS patients, and immunocompromised subjects. It is worldwide in distribution. Two species of *Cryptosporidium*, *C. hominis* and *C. parvum* mostly cause human infections.

Habitat

C. parvum inhabits the small intestine. It may also be found in stomach, appendix, colon, rectum and pulmonary tree.

Morphology

The **infective form** of the parasite is **oocyst**. The oocyst is spherical or oval and measures about 5 μm in diameter. The wall of the oocysts is thick, but in 20% cases, wall may be thin. These thin walled oocysts are responsible for autoinfection. Both thin walled and thick walled oocyst contain 4 crescent shaped s**porozoites.** Oocyst can remain viable in the environment for long periods, as it is very hard and resistant to most disinfectants and temperature up to 60°C. It can survive in chlorinated water, but application of ozone and chlorine has been found effective in eliminating the cysts.

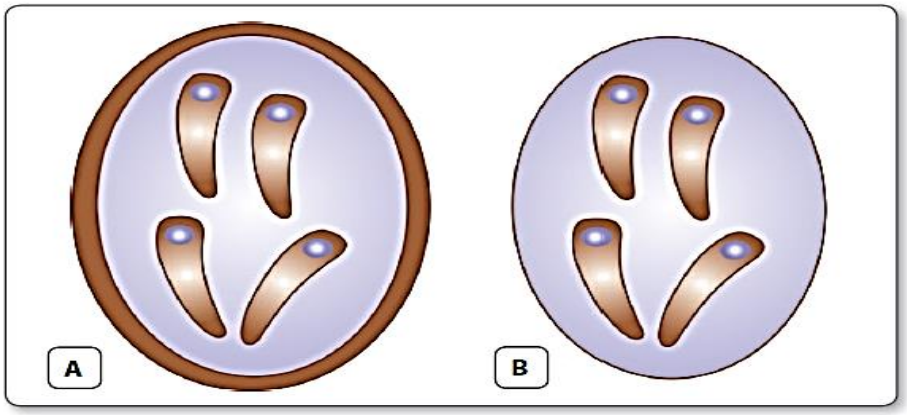
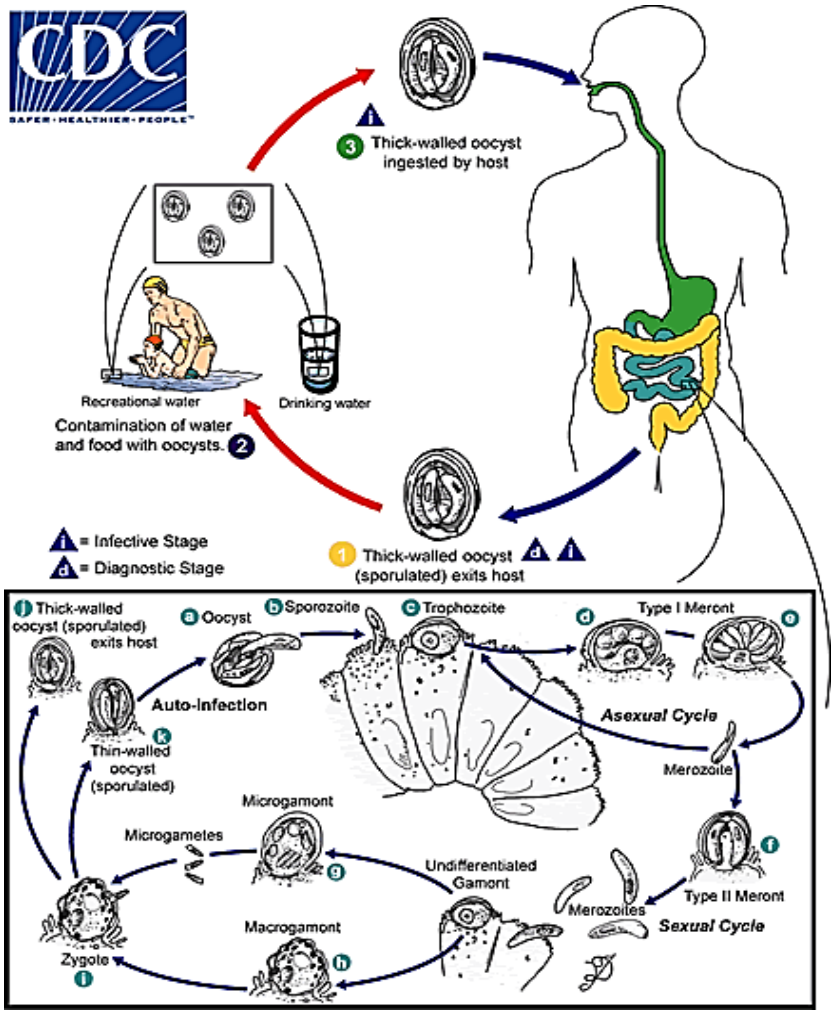


Fig. 7.7: Oocysts of *Cryptosporidium parvum*. **A.** Thick-walled oocyst; **B.** Thin-walled oocyst

Life Cycle: sexual and asexual phases in a single host.



Suitable host: Man.

Reservoirs: Man, cattle, cat, and dog.

Mode of transmission: by ingestion of food and water contaminated with oocysts

Infective form: Sporulated oocysts.

Sporulated oocysts, containing 4 sporozoites, are excreted by the infected host through feces and possibly other routes such as respiratory secretions (1). Transmission occurs mainly through contact with contaminated water. Following ingestion by a suitable host (3), excystation (a) occurs. The sporozoites are released and parasitize epithelial cells ((b), (c)) of the gastrointestinal tract or other tissues such as the respiratory tract. In these cells, the parasites undergo asexual multiplication (schizogony or merogony) ((d), (e), (f)) and then sexual multiplication (gametogony) producing microgamonts (male) (g) and macrogamonts (female) (h). Upon fertilization of the macrogamonts by the microgametes (i), oocysts ((j), (k)) develop that sporulate in the infected host. Two different types of oocysts are produced, the thick-walled, which is commonly excreted from the host (j), and the thin-walled oocyst (k), which is primarily involved in autoinfection. Oocysts are infective upon excretion, thus permitting direct and immediate fecal-oral transmission.

Pathogenicity and Clinical Features

Infection in healthy immunocompetent persons is asymptomatic or causes a self-limiting illness, with watery diarrhea with abdominal pain, nausea, and weight loss. In immunocompromised hosts, especially those with AIDS, diarrhea can be chronic, and profuse, causing significant fluid and electrolyte depletion, weight loss, emaciation, and abdominal pain.

Diagnosis: Demonstration of round oocyst in stool by direct microscopy.

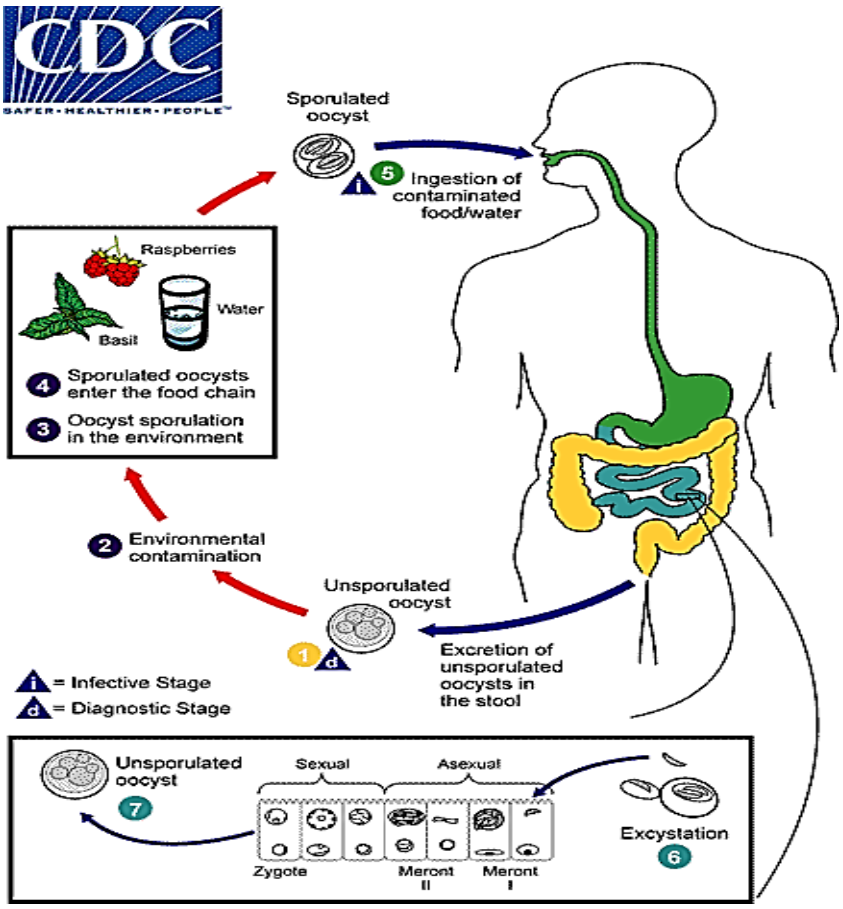
Treatment: Supportive therapy with electrolytes and fluids and early antiretroviral therapy in AIDS patients.

➢ *Cyclospora cayetanensis*

It is a coccidian parasite. It was first reported from Nepal, where it caused seasonal outbreaks of prolonged diarrhea, with peak prevalence in the warm rainy months.

Morphology

The morphological form found in the feces is an oocyst. The oocyst is measuring 8–10 µm in diameter. It contains 2 sporocysts. Each sporocyst contains 2 sporozoites, each sporulated oocyst contains 4 sporozoites.



Life Cycle

Oocyst shed in feces sporulates outside the host. The sporulated oocysts are infectious to humans. Man acquires infection by ingestion of food and water contaminated with feces containing oocysts. Excystation of the sporocyst releases sporozoites. The sporozoites infect enterocytes in the small intestine. The sporozoites develop into unsporulated oocysts, which are excreted in feces.

Pathogenecity and Clinical features

Infection is through feco-oral route by ingestion of contaminated water and vegetables. It causes prolonged diarrhea with abdominal pain, low grade fever, and fatigue. Like other coccidian parasites the infection is more severe in immunocompromised hosts, especially with AIDS.

Diagnosis

Stool Examination: is by direct wet mount demonstration of oocysts in feces.
The oocysts can be stained by Zeihl Neelson stain. Oocysts of *cyclospora* are acid fast and stain red in color.

Treatment

Cyclosporiasis is treated with cotrimoxazole (trimethoprim 160 mg/sulfamethoxazole 800 mg) twice daily for 7 days.

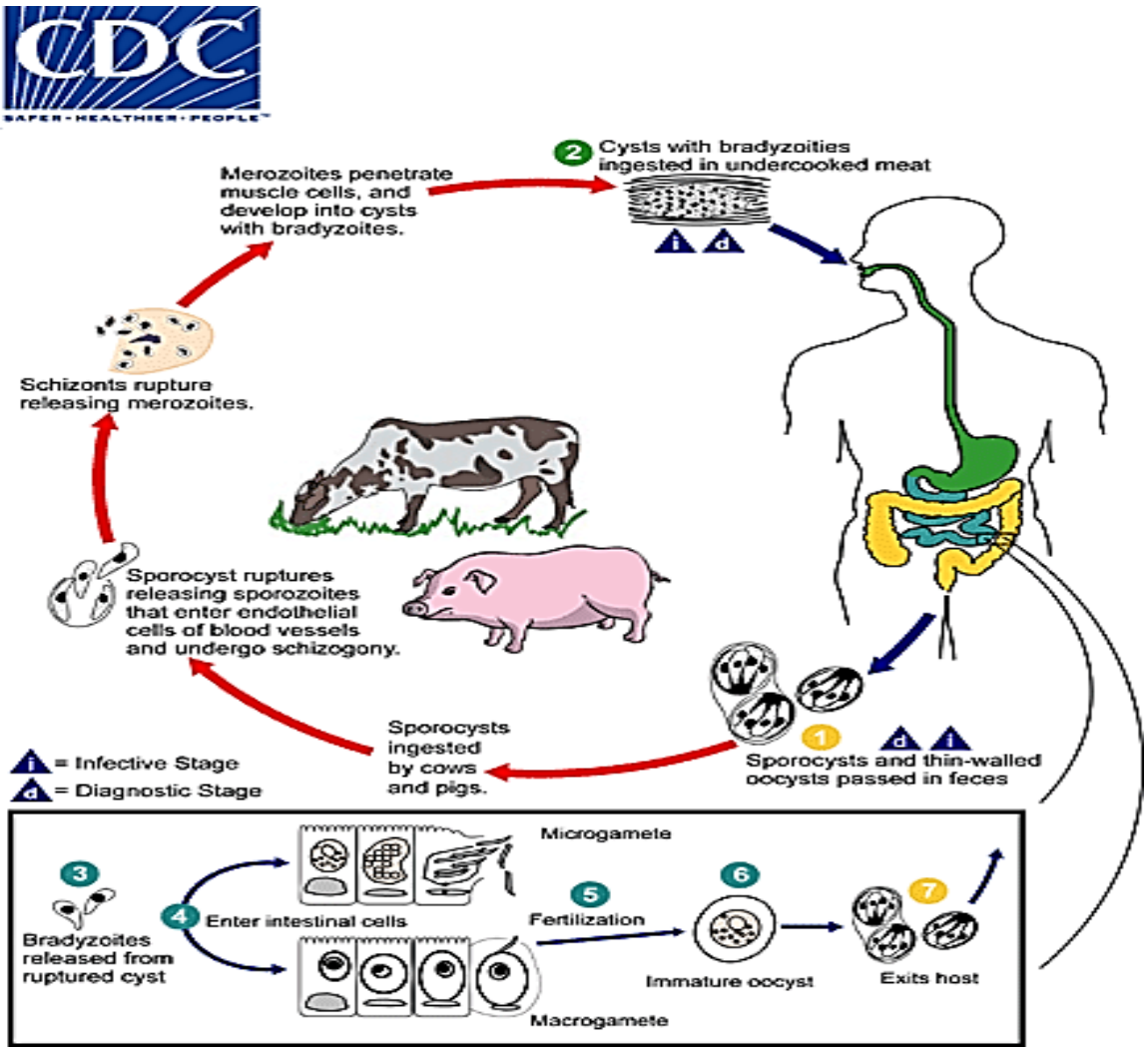
➢ *Sarcocystis*

Three species of genus *Sarcocystis* can infect humans:

- *S. hominis* (transmitted through cattle)
- *S. suihominis* (transmitted through pig)
- *S. lindenmani*.

Humans are the definitive host of *S. hominis* and *S. suihominis* and the intermediate host for *S. lindenmani*.

Sarcocystis species produce cyst in the muscle of the intermediate hosts. These cysts, called *Sarcocysts* contain numerous merozoites (**bradyzoites**). When sarcocyst is eaten by the definitive host, the merozoites are released in the intestine, where they develop into male and female gametes. After fertilization, the zygote develops into an oocyst containing 2 sporocysts, each having 4 sporozoites. These oocysts are shed in feces and are ingested by intermediate host. In the intermediate hosts, the sporozoite invades the bowel wall and reaches the vascular endothelial walls, where they undergo schizogony producing **merozoites** (**tachyzoites**). These spread to muscle fibers and develop into sarcocysts. Cow is the **intermediate host** for *S. hominis.*
Human infection is acquired by eating raw or undercooked beef. Oocysts are shed in human feces, which contaminate grass and fodder eaten by cows. In the case of *S. suihominis,* the pig is the intermediate host and human infection is obtained through eating contaminated pork. Humans are the intermediate host in *S. lindemanni*. Humans apparently get infected by ingestion of oocysts. Sarcocysts develop in the human skeletal muscles and myocardium.



Clinical Features

Intestinal sarcocystosis is usually asymptomatic. Patients may have nausea, abdominal pain, and diarrhea.

Muscular sarcocystosis is also usually asymptomatic but may cause muscle pain, weakness, or myositis, depending on the size of the cyst.

Laboratory Diagnosis

Stool Examination

Sporocysts or oocysts can be demonstrated in feces of human beings.

Muscular Sarcocystosis

Diagnosis can be made by demonstration of sarcocysts in the skeletal muscle and cardiac muscle by biopsy or during autopsy.

Treatment

No specific treatment is available for sarcocystosis.