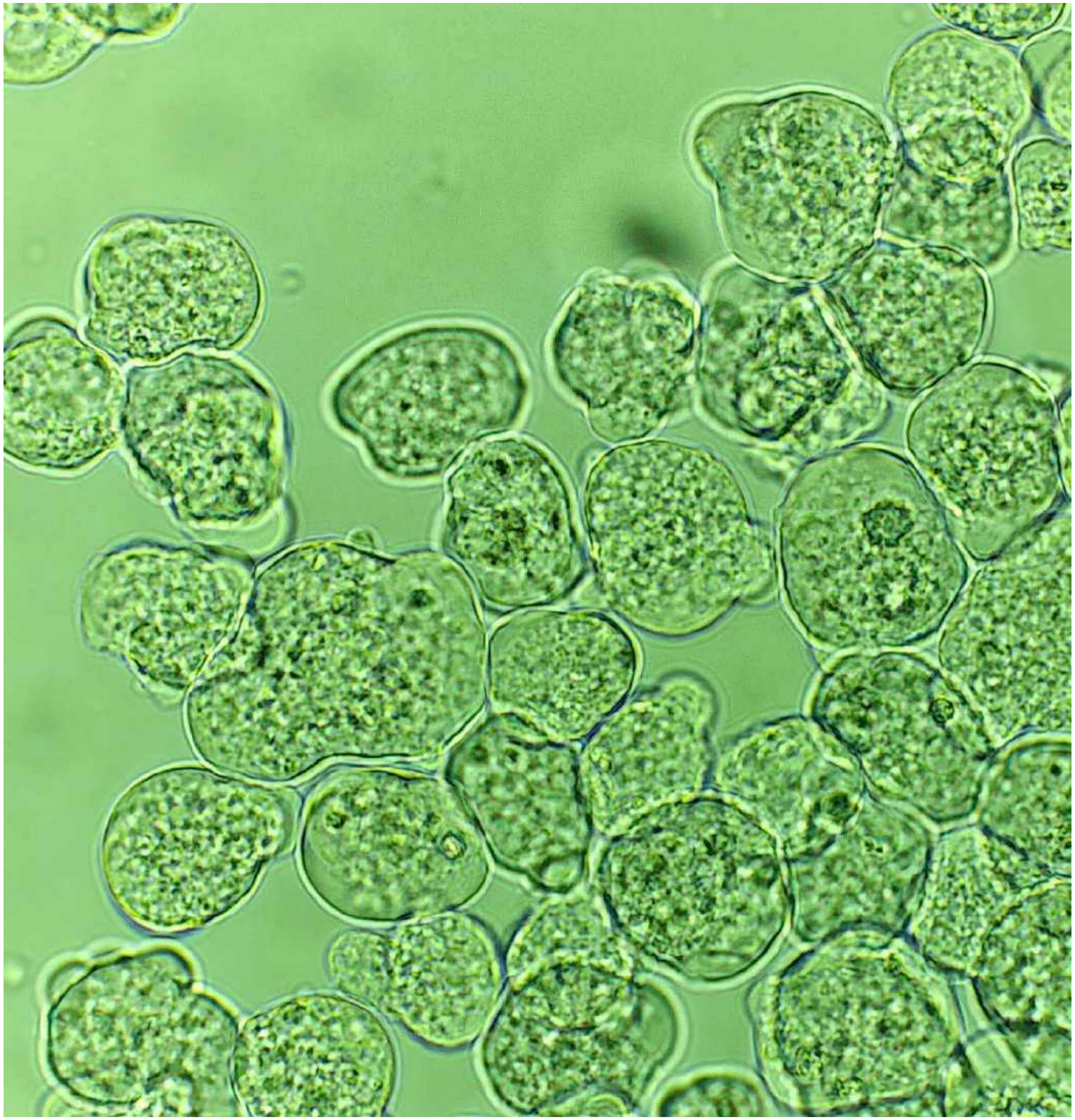
PARASITIC PROTOZOA



Zoology (Gen)

Semester-5

DSE-A

Applied Zoology.ZOOG-DSE-A-5-1-TH

Unit-3

Entamoeba histolytica

Classification of Entamoeba

Kingdom : Protista

Phylum : Sarcomastigophora

Sub phylum : Sarcodina

Super class : Rhizopoda
Class : Lobosa

Genus : Entamoeba

Morphology

Entamoeba histolytica exists in three forms:

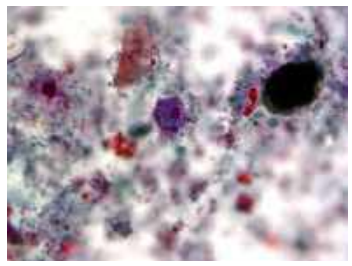
- Trophozoite or feeding stage or free moving stage (Tropho: food/nourishment; zoite: zoon) lives in the mucosa and sub-mucosa of large intestine (colon and cecum). It is the invasive form of the parasite.
- Pre-cyst: It is the transient stage, between the trophozoite and cyst, formed in the lumen of the large intestine
- Cyst: It is the non-feeding infective stage of the parasite found in the lumen of the large intestine

Trophozoite:

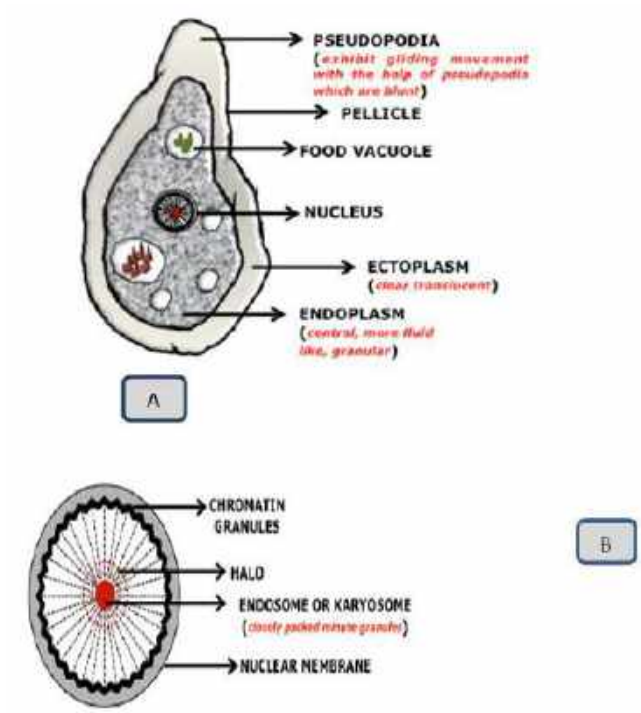
Size: Varies in size from 8µm- 40µm with an average size of about 20µm -25µm. Shape: Not fixed, constantly changing its shape by thrusting out pseudopodia.

Cytoplasm: The cytoplasm is differentiated into a clear, thin translucent ectoplasm and a central fluid like granular endoplasm. Endoplasm contains nucleus, numerous food vacuoles, erythrocytes, granules and tissue debris. The trophozoite is an anaerobic parasite. It lacks mitochondria, golgi bodies and rough endoplasmic reticulum. Contractile vacuole which is a characteristic feature of protistansis also wanting in them. Can you think of a possible reason for this? Well, you may be right!! Entamoeba histolytica is an endoparasite and lives in an isotonic environment and hence does not need to osmoregulate.

Nucleus: As is evident from the figure given below, nucleus is the most distinguishing feature of Entamoeba. Nucleus is spherical in shape, about 3.5µm in size, lined externally by a thin, delicate nuclear membrane. The nuclear membrane is lined internally by a single layer of evenly distributed chromatin granules. The chromatin granules are in the form of small dots. A compact, small karyosome or endosome surrounded by a clear halo (ring) is centrally located. Nuclear striations (spoke like lines) radiate out from the endosome and extend upto the nuclear membrane.



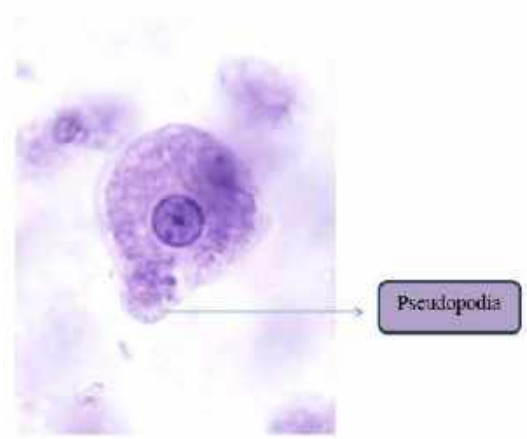
Trichrome stained trophozoite of Entamoeba histolytica



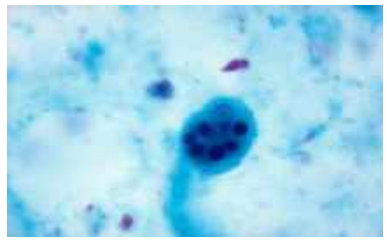
A. Diagrammatic sketch of Trophozoite of Entamoeba histolytica

B. Detailed structure of nucleus of Trophozoite of Entamoebahistolytica

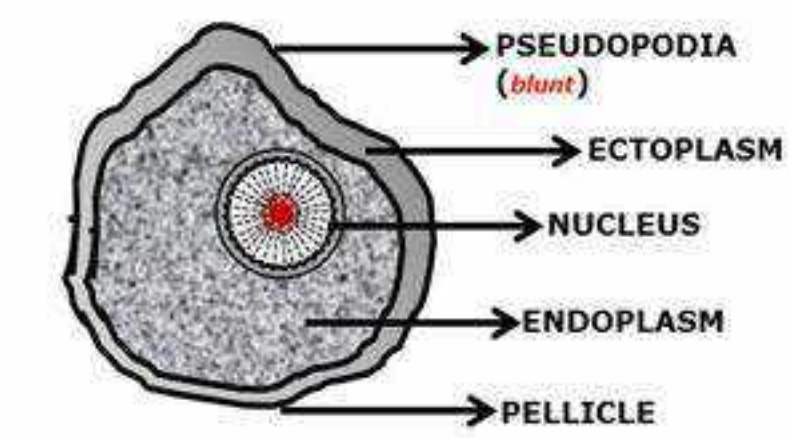
Locomotion: The trophozoite shows slow, gliding movement in one direction. They move about with the help of pseudopodia. Pseudopodia are finger like flowing extensions of the cytoplasm which may be short and wide or long and narrow. Locomotion is brought about by the forward extension of the ectoplasm followed by the granular endoplasm which then flows into the finger like extensions. The direction of movement may change suddenly by giving out pseudopodia at some other site. The movement of the trophozoite is dependent on the consistency of the surrounding medium, age of the parasite and the temperature of the host.



Feeding and digestion: Food consists of bacteria and other cytolysedorganic substances found in the hosts intestine. Food is taken in by Entamoeba either by phagocytosis or by pinocytosis. A food vacuole is formed. The food vacuole contains RBC which may be in various stages of digestion. The digestion is intra-cellular within the food vacuole.



PRE CYST: Smaller in size (10-20µm) than the trophozoite but larger than cyst, round or oval with a blunt pseudopodia. It has a relatively large nucleus that retains all the characteristics of the nucleus of a trophozoite. The trophozoite extrudes food vacuoles before encystment so that the endoplasm is free from red blood cells and other ingested food particles.

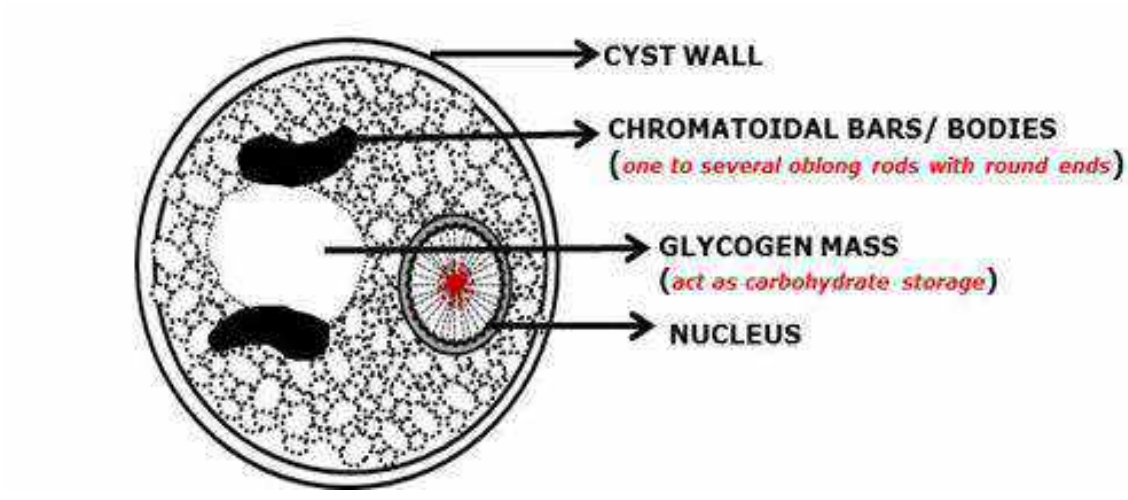


Diagrammatic sketch of pre-cyst of Entamoeba histolytica

CYST: Cyst exists in three forms:

- **Immature uninucleate cyst**
- **Binucleate cyst**
- **Mature quadrinucleate cyst**

The trophozoite becomes completely round and is surrounded by a transparent, highly retractile double walled resistant cyst wall. The cyst wall varies greatly in size from 6-9 μm (small race) to12-15 μm (large race). The cytoplasm is clear and hyaline (transparent/glassy). Before encystment, the parasite eliminates food vacuoles and accumulates considerable amount of food in the form of glycogen mass and black bodies called chromatoidal bodies. These chromatoidal bodies are large, smooth, oblong rods with rounded ends. They may be one to several in number. They are called chromatoidal bars or chromatoidal bodies because they stain as chromatin with haematoxylin stain. It is believed that the chromatoidal bodies contain DNA and phosphates and serve as storage for ribosomes. Cyst when formed is uninucleate but the nucleus within the cyst divides twice to form a quadrinucleate cyst. It is this mature quadrinucleate cyst (cyst containing four nuclei) which is the infective stage. Occasionally, additional division may result in aberrant form with more nuclei. Although the nucleus retains all the characteristics of the trophozoite, it is smaller in size due to successive mitotic division. The mature quadrinucleate cyst lacks chromatoidal bodies and glycogen mass as they are absorbed by the cytoplasm. The chitinous wall around the cyst makes it resistant to the gastric contents of the stomach, adverse environmental conditions and to the chlorine concentration found in the potable water.



Diagrammatic sketch of uninucleate cyst of Entamoeba histolytica

Life cycle

E. histolytica is a monogenetic parasite. Another host is required for the perpetuation of a species.

The infection begins when the host swallows the mature quadrinucleate cyst along with the contaminated food or water. As the cyst wall is resistant to the acidic content of the stomach, the quadrinucleate cyst passes unaltered into the small intestine where excystation takes place. In the intestine, the cyst wall is digested by the action of trypsin in an alkaline medium at a temperature of 37°C. During this process, the cytoplasmic body retracts and loosens from the cyst wall. Pseudopodia are formed at various points and vigorous amoeboid movements occur within the cyst. Frequently, the pseudopodia press against the wall at certain spots as though the imprisoned organism were searching for exit. Eventually, a tetranucleate amoeba known as metacyst (amoeba with four nuclei) emerges out. Immediately on emergence, the four nuclei of the metacyst undergo division to form eight nuclei. Each nuclei gets surrounded by a bit of its own cytoplasm and leads an independent existence. Thus, eight amoebulae are formed. These are known as metacystic or metacyclic trophozoites which are actively motile. The metacystic trophozoites move down to the caecum and ileocloacal region of the intestine. The young amoebulae being actively motile invade the tissues and finally lodge themselves in the mucosa and submucosa of the large intestine-its final abode. They prefer this site as the organic material (food), pH and gases in this part of the large intestine are more stable and ideal for the existence of E. histolytica trophozoites. Here, the trophozoites grow at the expense of living tissues and multiply by simple binary fission. The trophozoites secrete histolysin which causes necrosis and destruction of the host's tissue and helps the parasite to derive nourishment from the dissolved dead tissues.

However, there are some non-invasive trophozoites that remain in the lumen of the large intestine and multiply by binary fission. These trophozoites feed on the hosts nutrients from the surrounding medium. Some of the trophozoites from the cells of the mucosa and submucosa after repeated binary fission move back into the lumen of the intestine.

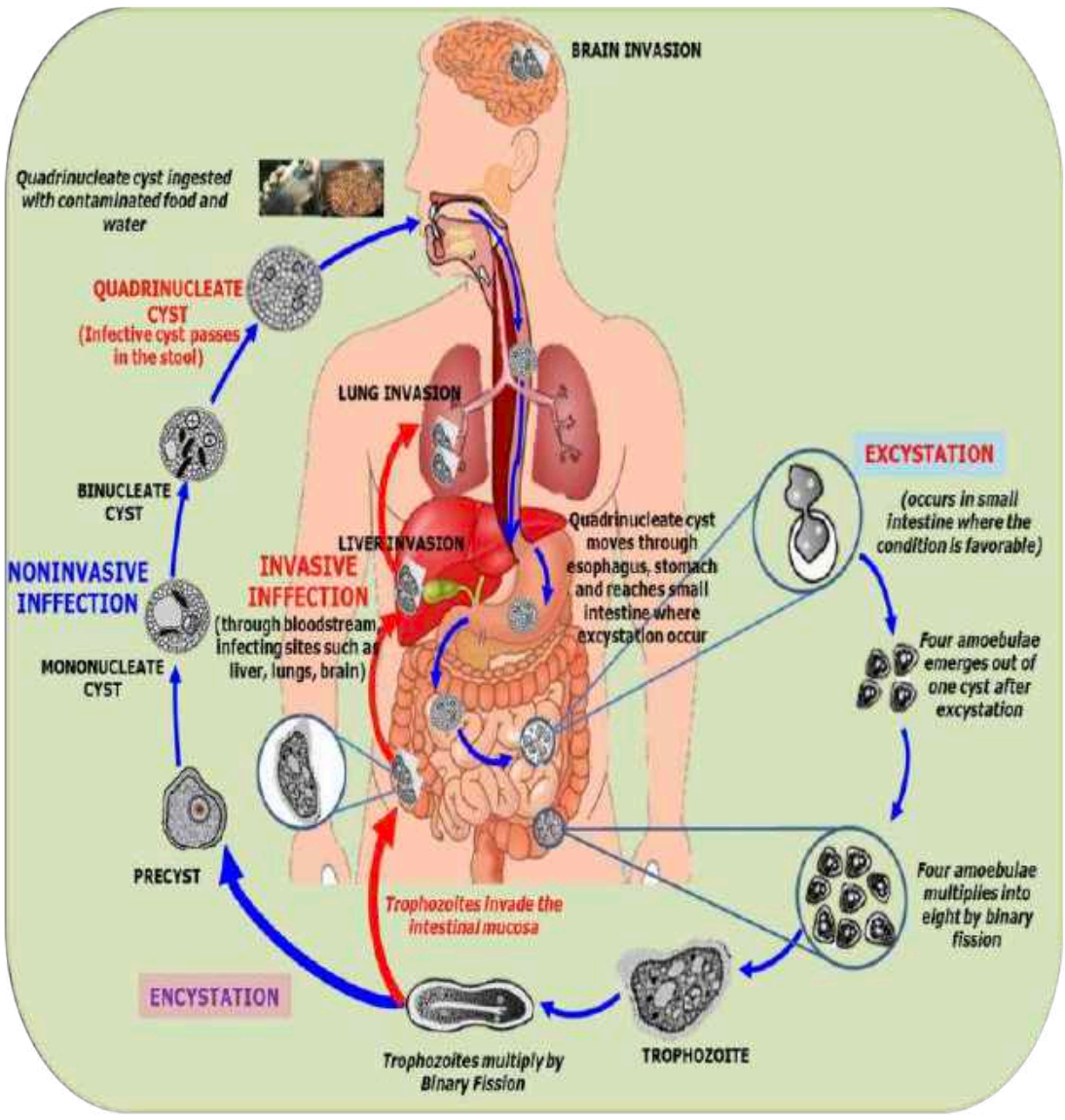
When the conditions become unfavourable for the trophozoites in the lumen of the large intestine, they start to develop a cyst wall. A pre-cyst is first formed which soon becomes a uninucleate immature cyst. The nucleus within the cyst divides to first form a binucleate cyst. The nucleus divides again for the second time to form a quadrinucleate/tetranucleate mature cyst (infective stage). The transformation of trophozoite into a mature quadrinucleate cyst is called encystations and is a means of protection of a species from extinction. Encystation does not take place in the tissues of man: neither in the intestinal mucosa nor in the liver, lungs etc. Thus, actually the metastatic invasion of the trophozoite for all biological purposes is a dead end for the parasite.

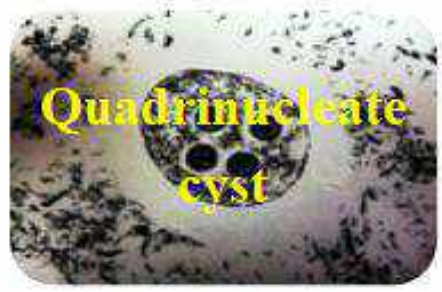
Encystation takes only a few hours and the mature quadrinucleate cyst can remain viable in the lumen of the large intestine for only two days. Mature quadrinucleate cysts are passed out along with the faeces of the host. About 45 million cysts may be voided out from one infective person in a day. The cysts are resistant to the environmental conditions and can live for a few weeks to a few months depending on the temperature (thermal death occurs at 50°C). Moisture is essential for the long existence of the cyst. They can live upto 10 days in a moist stool. The cysts are however, susceptible to dessication.

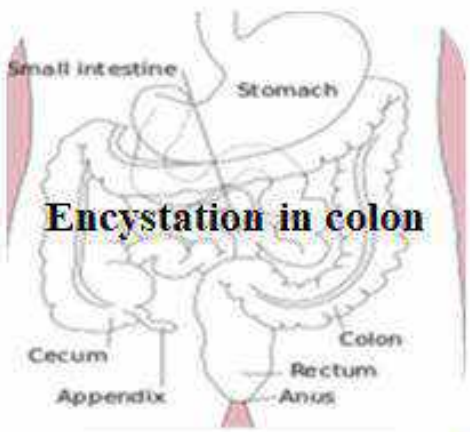
Trophozoites are also voided out along with the cysts in the faeces but they cannot survive outside the body of the host for more than an hour and even if they are ingested by another human being during this period they are killed in the body of the host by the acidic juices of the stomach.

A very important point to note is that both excystation and encystation are not reproductive processes.

Encystation and excystation can take place in the same host; another host is required only for the perpetuation of the species.







NON-INVASIVE COLONIZATION

INVASIVE-EXTRAINTESTINAL COLONIZATION

Mode of Infection: faecal-oral route

Infective Stage: Mature Quadrinucleate cyst

Source of Infection:
Carriers of Entamoeba histolytica are of two types:

1. Contact carriers: People who have never suffered from amoebic dysentery and their health remains unaffected. They are healthy carriers of E. histolytica. They can shed cysts for many years

2. Convalescent carriers: Persons who have recovered from acute amoebic dysentery

Transmission

- Fruits, raw vegetables and food contaminated by faeces containing quadrinucleate cyst

- Contaminated water

- Unhygienic habits

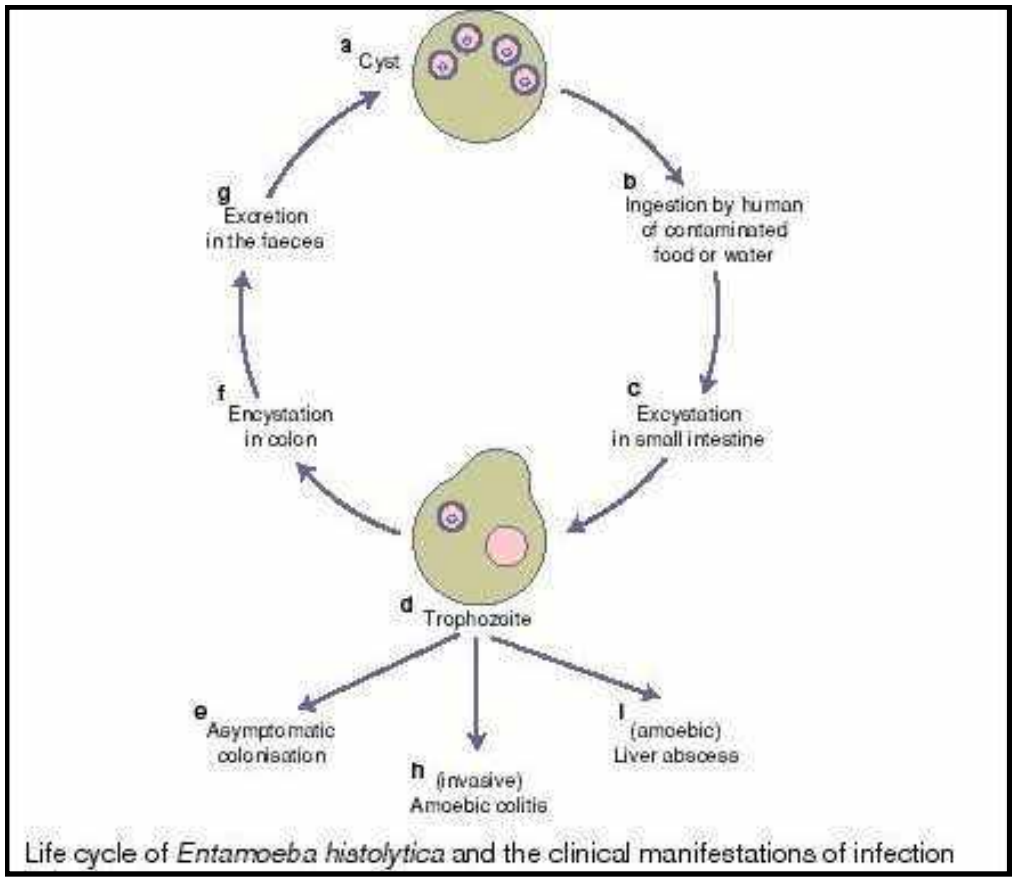
- Mechanical vectors like houseflies and cockroaches. Houseflies may act as carrier of the cysts from faeces to unprotected food and water. The droppings of cockroaches too have been found to contain mature cysts and thus they also serve as a source of infection.

- Sexual transmission of Entamoeba histolytica has been reported in about 20-30% homosexuals.

Life history at a glance

- **Host :Man is the only host (Definitive)**
- **Infective stage : Mature quadrinucleate cyst (Metacyst)**
- **Route of infection : Fecal – oral**
- **Site of lesion : Large intestine**
- **Diagnostic stage : Cyst & trophozoite**
- **Pathogenic stage : Trophozoite**

- E. histolytica passes its life cycle in single host, the man
- and it has 2 stages, 1. trophozoites 2. cysts.
- Mature Cysts containing four nuclei (metacyst) is the infective form, which spread via the ingestion of faecally contaminated food or water.
- When mature cysts are ingested via food and drinks their excystation occurs within the lumen of the small intestine. During excystation, nuclear division is followed by cytoplasmic division, giving rise to eight young trophozoites known as amebula.
- These young trophozoites, reside in the lumen of the cecum and large intestine, where they adhere to the colonic mucus and epithelial layers.
- Approximately 90% of individuals infected with E. histolytica are asymptomatically colonized. Re-encystation of the trophozoites occurs under certain unfavorable conditions like dehydration within the lumen of the colon, resulting in the excretion of cysts in the faeces and continuation of the life cycle.
- Alternatively, the trophozoites can invade the colonic epithelium, causing amebic colitis (in ~10% of infected people).
- E. histolytica can spread in the bloodstream (hematogenously) after it has penetrated the colonic epithelium and can establish persistent extraintestinal infections, most commonly amebic liver absce



Life cycle of *Entamoeba histolytica* and the clinical manifestations of infection

PATHOGENESIS

Intestinal amoebiasis :

– The trophozoites are pathogenic form which colonize the intestine by adhering to colonic mucin glycoproteins, via a galactose and N-acetyl- D-galactosamine (Gal/GalNAc)–specific lectin.

– Host cells are killed via the induction of an apoptotic cascade. Apoptotic killing occurs by a novel pathway that is not blocked by bcl-2 and does not require fas, or the TNF-α receptor.

– Recently the amebas have been demonstrated to activate “effector” caspases immediately before destruction of the host cell. Inhibition of these human caspases blocks killing by the amebas.

– Trophozoites also contain a pore-forming protein that is probably involved in the destruction of endocytosed bacteria.

– The purified protein is also capable of inducing necrotic death of eukaryotic cells.

– Motility and resistant to destruction by lectin mediated complment activation also play a role.

– Enzymes like proteases destroy extracellular matrix.

Features of amoebic ulcer :

– Trophozoites establish themselves in cecum and proximal colon, and then invade tissues. This is frequently mild and self-limiting, with minimal symptoms. However, can cause extensive destruction of the colonic mucosa, and invade other organs.

– Initially produces small, discrete erosions or minute crypt lesions. These then extend through the mucosa, into the submucosa, and expand laterally to produce flask-shaped lesions. These may merge and cause denudation of overlying mucosa, producing a minute hole of communication. These flasks shaped ulcer usually have ragged margins, and bases due to digesting effect of proteases.

– The floor remains full of altered blood from oozing of the sidewalls. Base of the ulcer usually remains limited to muscularis mucosa, rarely reach to submucosa, and up to muscularis externa.

Extra intestinal Amoebiasis

E. histolytica can metastasize in any organ which hepatic amoebiasis is the commonest.

Hepatic amoebiasis – Ameba can reach the liver via the portal vein from which they extend progressively in all directions, producing liver cell necrosis and liquefaction, to form an amebic liver abscess, which will extend eventually into adjacent structures. They stay small or continue to grow – center of abscess is full of necrotic fluid, outer wall full of trophozoites. If abscess ruptures, organisms are available to eat other organs. The necrotic fluid is well comparable to ancovy sauce as per its colour and consistency. Microscopically, this fluid contains nil to very few pus cells, trophozoites can be found but never cysts.

Pulmonary amoebiasis -develop generally when liver abscess ruptures through diaphragm.

Other ectopic sites include brain, skin, penis (possibly acquired sexually). Rare sites include kidneys, spleen, male and female genitalia and pericardium.

Plasmodium vivax

Classification of Plasmodium

Kingdom : Protista

Sub Kingdom : Protozoa

Phylum : Apicomplexa
Class : Sporozoa
Sub Class : Coccidia

Order : Haemosporida
Sub Order : Aconoidina

Family : Haemosporidae

Genus : Plasmodium

Morphology of Plasmodium

The blood-stages of human Plasmodium species exhibit different morphology and modification in the

host erythrocyte. These differences can be used to distinguish the four species (Table 1).

P. falciparum blood stages are characterized by the presence of slightly smaller and numerous ring stages than the other species. Erythrocytes having multiple infections are seen more often in P. falciparum than in the other species. Distinct crescent-shaped gametocytes of P. falciparum appear late in the infection.

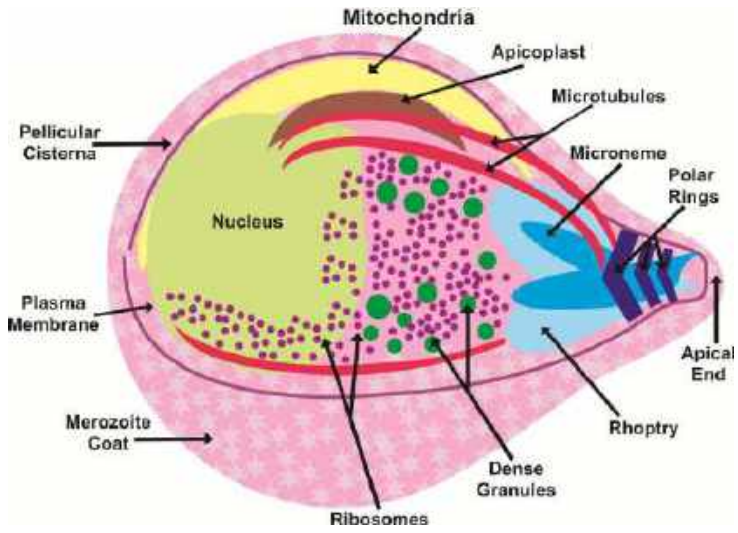
P. vivax with enlarged infected erythrocytes and granules 'Schüffner's dots', over the erythrocyte cytoplasm, manifests at caveola-vesicle complexes that form on the erythrocyte membrane. The trophozoite of P. vivax has an ameboid appearance. The schizonts can have more than 20 merozoites.

P. ovale also exhibits Schüffner's dots with an enlarged erythrocyte. It is difficult to distinguish the infection from P. vivax. In general, P. ovale is a more compact parasite than P. vivax. This insistence is most evident in the growing trophozite stage. Merozoites are fewer per schizont. Elongated host erythrocytes are found in case of P. ovale.

P. malariae exhibit compact stages and does not modify the host erythrocyte except few elongated trophozoites which stretch across the erythrocyte to form a band like structure. Schizonts will typically have 8-10 merozoites, often arranged in a rosette pattern, with a clump of pigment in the center.

Structure of Plasmodium Merozoite

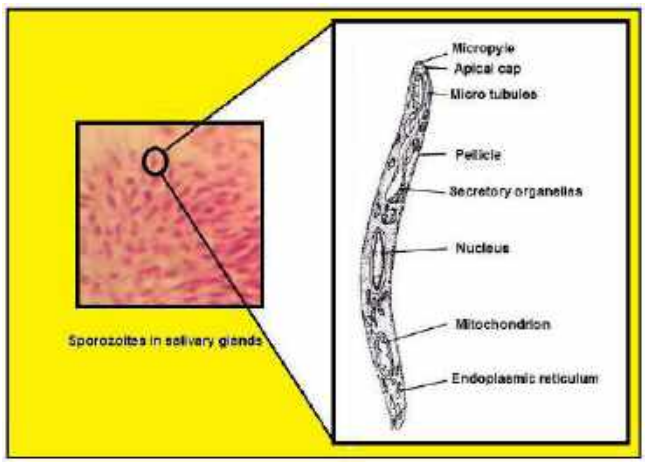
Merozoite is an ovoid cell and measures approximately 1.5 micron in length and 1 micron in width. The apical end is like truncated cone-shaped projection demarcated by the polar rings. Three types of membrane-bound organelles, Rhoptries (two prominent pear-shaped), micronemes (ovoid bodies) and dense granules (spheroid vesicles) are present at the anterior end of the Merozoite (Figure 2). The function of these organelles is related to the binding and entry of the Merozoite into the host cells. Merozoites are basically short-lived and need to invade a new host erythrocyte just after the release. A trilaminar pellicle surrounds the merozoite, which is composed of a plasma membrane and two closely aligned inner membranes. Beneath this inner membrane complex is a row of subpellicular Microtubules, radiating posteriorly from the polar ring of the apical end. The inner membrane complex and subpellicular Microtubules function as a cytoskeleton giving rigidity to the Merozoite. Mitochondrion are generally acristae or with very few cristae. The „apicoplast" (plastid) is believed to be the evolutionary homologue of the plant chloroplast. A single vesicular nucleus with a centrally located nucleolus is also present in Merozoite.



Merozoite of Plasmodium

The Infective Stage: Sporozoite

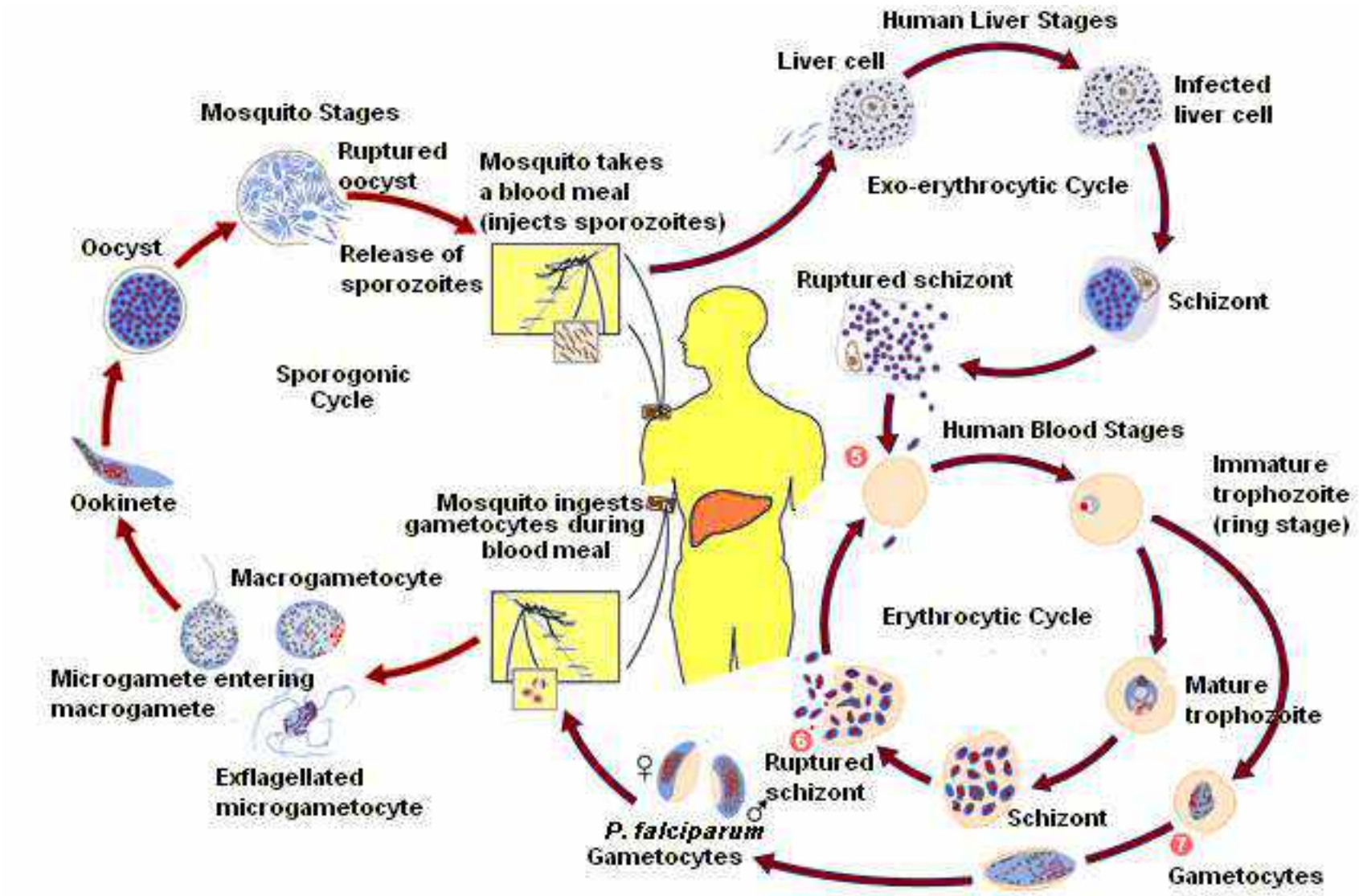
The most versatile of the invasive stages of the Plasmodium life cycle are the Sporozoites. During their passage from the mosquito vector to the vertebrate host, sporozoites exhibit diverse behaviors, including gliding locomotion, invasion, migration and egress from target cells. These functions are performed by rhoptries and micronemes, which are the only secretory organelles around the apical cap (Figure 3). Finally the sporozoites invade hepatocytes and transform into exoerythrocytic stages, continuing the cycle to the erythrocytic part in RBC.



Typical structure of sporozoite of Plasmodium

Life Cycle of the Malaria Parasite

The life cycle of malaria parasite is very complex. It‟s a digenetic parasite, i.e. it requires two hosts to complete its cycle . The life cycle of all species of human malaria parasites is essentially the same. It comprises an exogenous sexual phase (sporogony) with multiplication in certain Anopheles mosquitoes and an endogenous asexual phase (schizogony) with multiplication in the vertebrate host. The latter phase includes the development cycle in the red cells (erythrocytic schizogony) and the phase taking place in the parenchyma cells in the liver (pre-erythrocytic schizogony). The vertebrate act as the intermediate (secondary) host for the parasite, while the mosquito is considered to be the definitive (primary) one as the sexual reproduction takes place in mosquito.



Life cycle of malaria parasite

Exo-Erythrocytic Stages of Human Malaria Parasites

Sporozoites are the infective stage. Malaria infection in the human starts with their injection into the blood stream during a blood meal by an infectious mosquito. The circulatory period of sporozoites is short for about 60 minutes, after which they actively enter the liver of the host. The sporozoites die shortly after invasion in the Kuppfer cells of liver, as these are resistant to sporozoites. Most of the sporozoites enter the hepatocytes to begin the asexual exo-erythrocytic schizogonic cycle. The liver trophozoite, a mononucleated round body, divides asexually to form a mature multinucleated schizont which finally releases a large number of merozoites. The number of merozoites produced at the end of the cycle is also dependent on the species of the parasite. The merozoites are released into the sinusoids of the liver by the rupture of liver schizonts. Released merozoites invade red blood cells. Two species of human malaria parasite show relapses are P. vivax and P. ovale where some of the liver trophozoites immediately start the exo-erythrocytic schizogony while others remain in a dormant stage and are termed as hypnozoites.

Erythrocytic Stages of Human Malaria Parasites

The intricate and varying spectrum of symptoms characterizing the disease in humans is due to Erythrocytic stages of malaria parasite. The recognition of parasites in the blood of a patient allows the diagnosis of the infection and the differentiation of the various species. The time required to complete the erythrocytic cycle is a fixed characteristic of the parasite species (Table 3). Merozoites initiate the blood phase of the life-cycle on rupture and discharge of liver schizonts into the circulation. The merozoites possess a single nucleus and adjacent cytoplasm with a diameter of 1 μm. It invades immediately an erythrocyte to develop in trophozoite stage. The young trophozoite feeds on erythrocyte, produces a vacuole which assumes the characteristic ring form. This stage is referred to as signet ring stage. Approximately in 18 hours expansion of cytoplasm and disappearance of vacuole takes place slowly, and there is an appearance of a characteristic parasitic pigment within the cytoplasm. Pigment, known as haemozoin (Yellowish brown malarial pigment, haemozoin), is formed due to the parasite ingestion of haemoglobin and decomposition of the same into protein and haematin. Protein is used as food whereas unused haematin forms toxic.

Mature trophozoite has a single nucleus, a large cytoplasm without vacuole and inconsistent amount of pigment. P. falciparum, P. vivax and P. ovale takes approximately 30 hours to start nuclear division after invasion while P. malariae takes approximately 40 hours. Nuclear division leads to the production of the schizont stage. Nuclear division continues until an appropriate number of merozoites are produced.

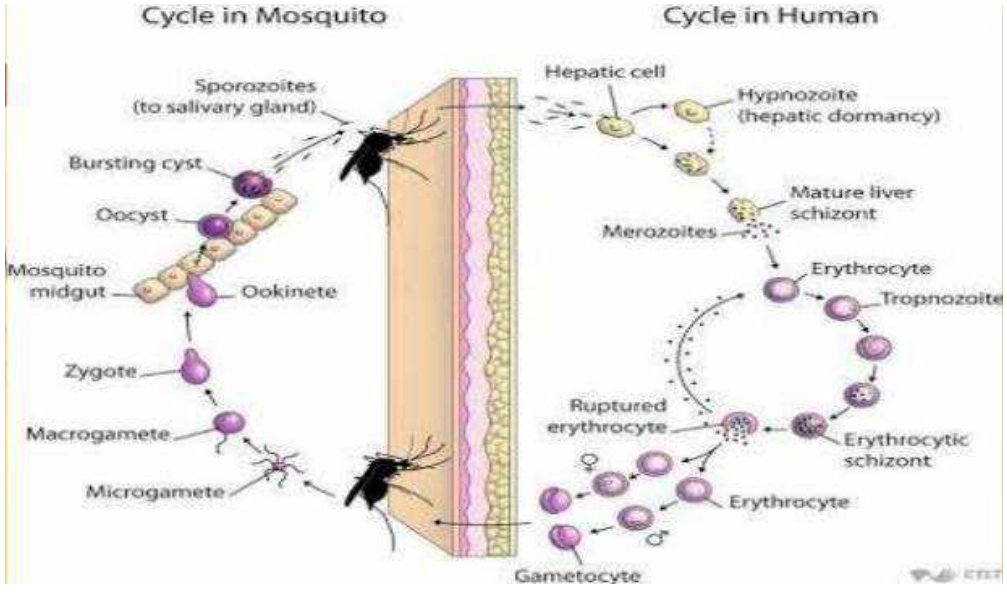
Species	***P. vivax***	***P. ovale***	***P. malariae***	***P. falciparum***
Pre-erythrocytic cycle (days)	8	9	13	5-6
Pre-patent period (days)	11-13	10-14	15-16	9-10
Incubation period (days)	13	17	28	12
Number of merozoites per tissue schizont	10,000	15,000	2,000	40,000
Hypnozoites	present	present	absent	absent
Erythrocytic cycle (hours)	48	50	72	48

Characteristic features of four species of human Plasmodia

A typical malaria paroxysm is determined by erythrocytic rupture to release the merozoites into the blood stream. Merozoites released into the circulation invade new erythrocytes to repeat the schizogony until the process is inhibited by the specific immune response or by chemotherapy. Some of the merozoites differentiate into sexual forms (the gametocytes) in the course of erythrocytic schizogony which remain inside the RBCs (Figure 4.). Gametocytes become visible approximately from the third generation in case of P. vivax, P. ovale, and P. malariae while in case of P. falciparum it requires approximately 10 generations for appearance of gametocytes which probably reflects the slow maturation and the sequestration of the immature stages in this species. Gametocytes of P. vivax, P. ovale and P. malariae are morphologically similar to the late trophozoite while P. falciparum gametocytes present a crescent shape. In the peripheral blood two types of gametocytes, the female macro-gametocytes and the male micro- gametocytes are present. They can be differentiated on the basis of nuclear material which is dispersed in male parasite (preparing to ex-flagellation) while condensed in female parasite.

Sexual Cycle in the Mosquito

Protein, required for egg formation of female Anopheles mosquitoes, comes through the blood meal. Oviposition continues throughout the life of the female mosquito which requires repeated contacts with the vertebrate host for blood meal. These subsequent feedings allow malaria parasite‟s multiplication, maturation and transmission to other individuals. The sexual cycle starts with the ingestion of mature female and male gametocytes by a suitable species of Anopheles during a blood meal. In the midgut of insect, the female gametocytes get rid of the cover of red blood cell to remain free in the extra-cellular space as macrogamete. The male gametocyte nucleus divides into eight sperm like flagellated micro-gametes through ex-flagellation which arrive at midgut and actively move to fertilize a macrogamete. After fertilization a zygote is formed, which develops into slowly motile ookinete in approximately 15 hours. The ookinete ruptures the peritrophic membrane and the epithelium of the midgut to inhabit below the basal lamina of the outer gut wall. In 24-72 hours after the blood meal the ookinete develops into a non motile oocyst. Narrow and curved sporozoites are produced from oocyst on maturation which are actively motile and about 15 µm in length. The sporozoites migrate and reach the salivary glands by making small perforations in the wall of cyst. Sporozoites penetrate the basal membrane of salivary gland and settle into the salivary duct. During the mosquito blood feeding, the salivary fluid content (which has anti-clotting properties) with sporozoites are actively injected into the human host to start another asexual cycle. Mosquito, a poikilothermic host, affect the speed of the cycle since it strongly depends on the temperature and other climatic factors.



PATHOGENESIS

- Clinical signs of malaria can be attributed to two factors
  - Host inflammatory response
    - Produces chills and fever
    - Correlated with maturation of merozoites, rupture of RBCs
    - Toxins released from burst RBCs can stimulate secretion of TNF by macrophages
    - TNF overproduction and toxicity can cause most or all of malaria symptoms
  - Anemia
    - Caused by destruction of RBCs

Incubation

- **Pre-patent Period**
  - Time taken from infection to symptoms
    - *Plasmodium falciparum* 6-12 days
    - *Plasmodium vivax* 10-17 days
    - *Plasmodium ovale* 12-16 days
    - *Plasmodium malariae* 28-30 days

➢ **Benign malaria**

- Milder in nature
- Caused by all 4 sps
- Characterised by febrile paroxysm ,anemia , & splenomegaly

✓ Febrile paroxysm

- Fever comes depending on sps
- It occurs every 4 th day (72 hr cycle for *P .malariae*) & every 3 rd day (48 hr for other sps)
- Paroxysm corresponds to the release of successive broods of merozoites into the blood stream ,at the end of RBC cycle

Paroxysm of fever is comprised of 3 stages

cold stage
- •feeling of intense cold
- •vigorous shivering
- •lasts 15-60 minutes

hot stage
- •intense heat
- •dry burning skin
- •throbbing headache
- •lasts 2-6 hours

sweating stage
- •profuse sweating

✓ **Anemia**

- After a few paroxysm of fever ; patients develops anemia
- Causes
- Parasite induced RBC destruction - lysis of RBC due to release of merozoites
- Bone marrow suppression leading to decrease RBC production
- Increased fragility of RBC
- Autoimmune lysis of coated RBC

✓ **SPLENOMEGALY**

- After few weeks of febrile paroxysm - spleen gets enlarged
- It is due to massive proliferation of macrophages that engulf parasitized & nonparasitized coated RBC

➢ **Falciparum malaria (malignant tertian malaria)**

- Possess a no of virulence factors
- So, disease is more acute & severe in nature
- Sequestration of the parasites :
- Important feature of the pathogenesis is its ability to sequester (holding back) the parasites in the blood vessels of deep visceral organs like brain, kidney etc.
- Leads to blockage of vessels, congestion & hypoxia of internal organs

Trypanosoma gambiense

Classification of Trypanosoma

Kingdom : Protista

Phylum : Kinetoplasta
Class : Kinetoplastida

Order : Trypanosomatida

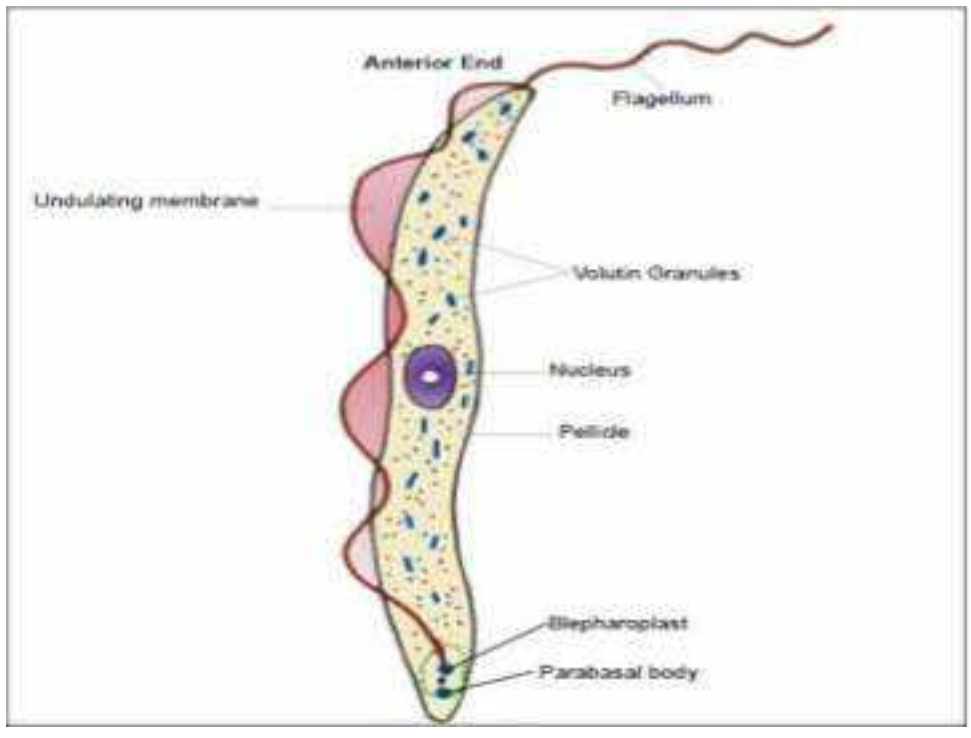
Family : Trypanosomatidae

Genus : Trypanosoma

Morphology

T. gambiense are microscopic, elongate, flattened and have fusiform body pointed at both ends and covered by a membranous pellicle which maintains the form of body. It measures about 10 µm to 40 µm in length and 2.5 µm to 10 µm in width. A single flagellum

arises from a basal body situated near the posterior end and curves in a spiral form round the body forming undulating membrane, thrown into 3 or 4 folds depending upon the length of the parasite. The undulating membrane is believed to be an adaptation for locomotion in the blood. The flagellum is free at the anterior end. The nucleus is large and oval, situated in the centre of the body and the cytoplasm contains numerous greenish refractile granules called volutin granules. These granules store food particles mainly glycogen and phosphate. At the base of the flagellum is located the basal granule or blepharoplast close to which is another granule, the parabasal body.



Morphology of Trypanosoma gambiense.

Life cycle

T. gambiense is a digenetic parasite which requires two hosts for completing the life cycle (figure 6). The primary host is humans and the intermediate host is blood sucking insect Tsetse fly of the genus Glossina. The mammals like pig, antelopes and buffaloes often act as reservoir host harbouring the parasite. When Tsetse fly sucks the blood from infected individual or wild mammal, it carries Trypanosomes to its mid gut where they divide asexually by longitudinal binary fission. Here the parasite changes their morphology and give rise to metacyclic forms which are short and stumpy. At this stage, the fly is said to be infective. When the infected tsetse fly bites a healthy human host, it releases these metacyclic rypanosomes in the blood stream of host and repeats the life cycle. Sexual reproduction is unknown in T. gambiense.
It is essentially a parasite of connective tissue in human where it multiplies readily. It consumes large amount of glucose and invades the regional lymph nodes through the lymphatic systems and also invades the blood system causing parasitaemia. It finally localizes in the brain. It is to be noted that African sleeping sickness is a disease which affects the central nervous system.

Life cycle in human:

Infection:

The infection by parasite is initiated when tsetse fly harboring the infective metacyclic form, bite the healthy individual. When the fly bites, it releases trypanosomes into blood stream which develop into long slender form and multiply asexually by binary longitudinal fission at the site of innoculation. These become ‘stumpy’ via ‘intermediate forms. Consequently the parasites invade the blood stream and causes parasitaemia. The trypomastigote forms, mainly the short stumpy forms are taken up by the tsetse fly along with its blood meal and undergo a series of complex biological development inside the insect host before becoming infective to man.

Multiplication:

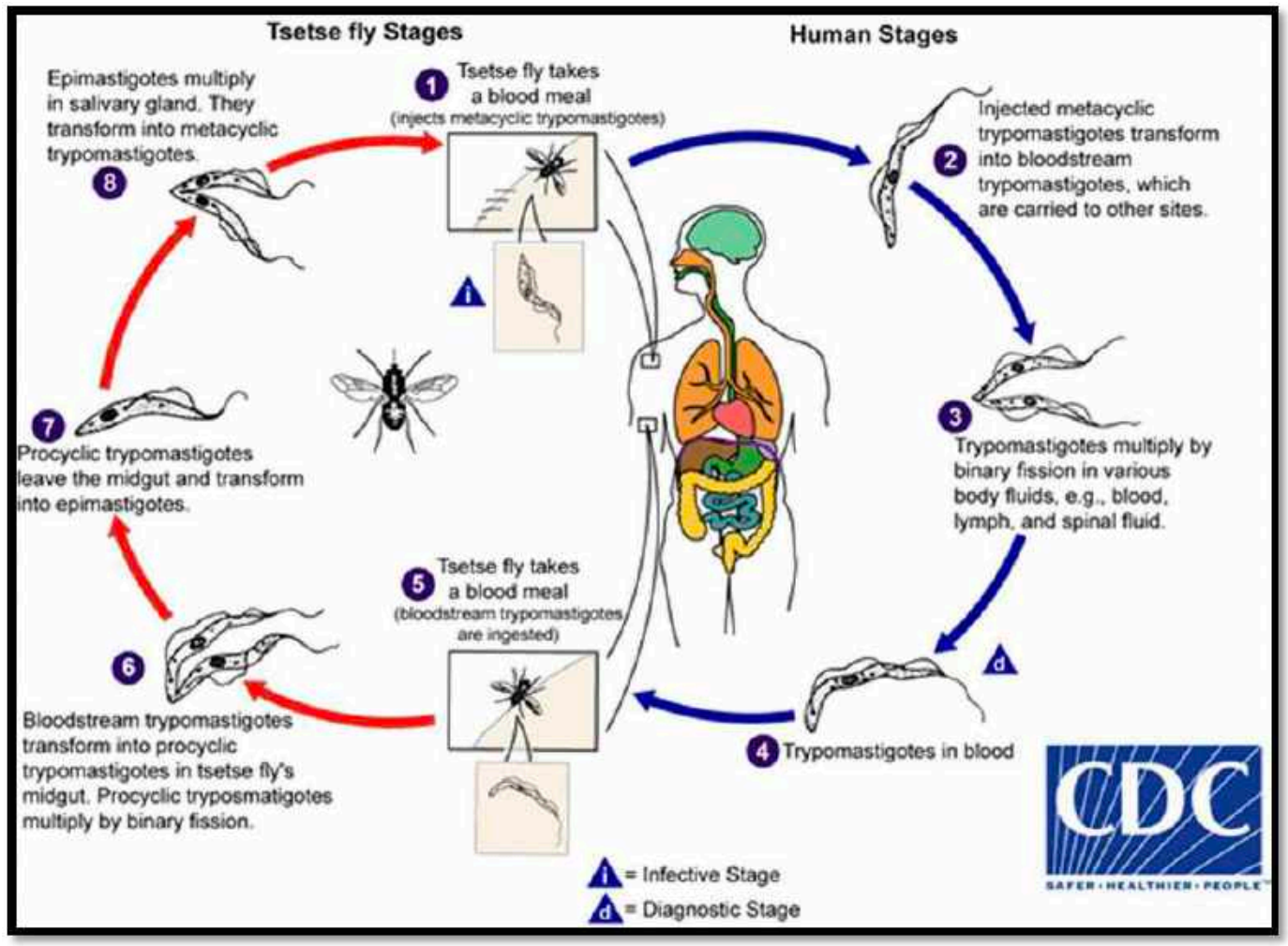
All stages of parasites in humans are extracellular as they are present in the blood cells. In human blood, the metacyclic forms which are devoid of free flagellum become transformed into long slender forms equipped with long flagella. These stages can freely swim by beating of their free flagellum along with the vibratile movements of the undulating membrane. They multiply asexually by longitudinal binary fission and obtain energy by anaerobic process of glycolysis.

Metamorphosis:

When absorption of glucose ceases due to antibodies which are produced in blood, is hampered glycolysis. As a result, the trypanosomes stop dividing and shrink to short stumpy forms, which are lacking free flagellum. These stumpy forms do not feed and ultimately die if they are not sucked up by tsetse fly along with the blood meal from infected human.

Relapse of infection:

It has also been reported that some of the long and slender forms of trypanosomes do not undergo any transformation, but change their antigen in blood to which the host has produced the antibodies. These unaltered slender forms continue to survive and multiply in blood leading to future relapses of the infection.



Life cycle in tsetse fly:

Transfer to tsetse fly: When tsetse fly feed on the blood of an infected person, it also sucks short stumpy forms of parasite along with the blood. It is the stumpy forms which continue development in the vector.

Development in mid gut: Further developments of stumpy forms proceeds in the insect mid gut within peritrophic membrane. In the mid gut parasite transforms into long slender form and multiply asexually by longitudinal binary fission. The kinetoplast moves farther from the posterior end of body. The energy yielding process is related to mitochondrial oxidation of pyruvic acid.

Development in salivary gland:

After sometime, the long slender forms migrate into salivary glands via oesophagus and mouthparts of insects. Here, they metamorphose into the crithidial forms with shortened body, reduced free flagellum and the kinetoplast in front of the nucleus. The mitochondria develop an extensive network of cristae and parasite respires more economically as blood glucose gradually declines. The crithidial forms multiply in the lumen of salivary glands and transform into slender metacyclic forms. When the tsetse fly bites a healthy person, it transfers the metacyclic stage into his blood where they initiate another infection.

Sleeping sickness:

T. gambiense causes the disease of West African sleeping sickness. It is different from American sleeping sickness or Encephalitis which is caused by filterable virus.

PATHOGENESIS

Mode of infection:
Inoculative method: by the bite of the infective tsetse fly, Glossina: Both male and female

suck the blood and can transmit the infection. They bite by daylight, usually in the early morning and evening.
The metacyclic stage is introduced by the tsetse fly with the saliva into the subcutaneous pool of blood on which it feeds. Some of the parasites may enter the blood stream directly and majority of them entangled in the tissue space. The initial growth of trypomastigotes occurs in the tissue space which form a favourable nidus or possibly here the organisms can escape the action of antibodies which might be developed. It is to be noted that while the trypomastigotes are multiplying in the subcutaneous tissue, the organisms are either absent or present in small numbers only in the peripheral blood.

It has been suggest that although unlikely yet the connective tissue damage caused by the trypomastigotes may be due to an exaggerated immune response (autoimmune reaction or massive release of kinin) rather than to any direct effect (mechanical damage due to motility) of this relatively non toxic organism. The presence of trypomastigotes in the subcutaneous connective tissue excites host's immune response in two ways.

a) By producing large amount of non specific immunoglobulins which are however not capable of sensitizing the antigen. Antibodies are produced in response to the secretion of an exo-antigen of the trypomastigotes.

b) By heavily infiltrating the site of infection with macrophages, the cells competent to deal with the invaders. The neutrophils take peculiarly little interest in the defense and are therefore not much in evidence.

Thus it will be seen that there is no lack of mobilization of the hosts defensive mechanism but it is the cellular defense which plays the dominant role. The macrophages could be seen to remove the living trypomastigotes in the tissue space. The release of kinins may help to attract macrophages, it also increases the capillary permeability of tissues and may explain the oedematous swollen subcutaneous tissue at the site of infection.

Furthermore, trypanosomes are surrounded by a coat that is composed of variant surface glycoproteins (VSG). These proteins act to protect the parasite from any lytic factors that are present in human plasma. The host's immune system recognizes the glycoproteins present on the coat of the parasite leading to the production of different antibodies (IgM and IgG). These antibodies will then act to destroy the parasites that circulate around the blood. However, from the several parasites present in the plasma, a small number of them will experience changes in their surface coats, resulting in the formation of new VSGs. Thus, the antibodies produced by the immune system will no longer recognize the parasite leading to proliferation until new antibodies are created to combat the novel VSGs. Eventually the immune system will no longer be able to fight off the parasite due to the constant changes in VSGs and infection will arise.

Clinical features:

Bite of tsetse fly causes local irritation which subsides after few days. A trypanosomal chancre may develop at the site of inoculation of trypomastigotes introduced by the bite of the infected tsetse fly. It is a hard painful nodule and fluid withdrawn from it contains actively dividing trypomastigotes. It subsides in a week or two without suppurating. The symptom can appear after several months or a year in Gambian form but symptoms may appear after two weeks in case of Rhodesian form.

It is characterized by the infection of blood stream, involvement and enlargement of lymph nodes and eventually invasion of the central nervous system. The early symptoms are fever, loss of nocturnal sleep, severe headache, and feeling of oppression. A fleeting circulate erythematous rash may appear on the chest and shoulder.

Lymph node enlargement, particularly of the posterior triangle of the neck is a feature of Gambian disease whereas invasion of CNS is very rapid in case of ‘rhodesian’ form. As the CNS is involved, the symptoms of meningo-encephalitis develop resulting in classical sleeping sickness (figure 7). In due course, the patient fall asleep, first at regular interval and then lies prostrate in coma. Finally, the patient becomes thin and exhausted, accompanied by signs of malnutrition. Disruption of the sleep cycle is an important symptom of this stage that gave the disease the name 'sleeping sickness'.

The person infected from disease experience unsystematic and uneven 24-hour rhythm of the sleep-wake cycle. The patient sleeps in daytime and at night time shows periods of wakefulness. Other neurological symptoms of the disease include tremor, confusion, paralysis, general muscle weakness, hemiparesis and paralysis of a limb. Parkinson like movements may also arise due to non-specific movement and speech disorders. The person infected from sleeping sickness may also exhibit psychiatric signs like aggressive behaviour, irritability, psychotic reactions or apathy which can sometimes dominate the clinical diagnosis. If the disease is not treated, it can invariably become fatal, with progressive mental deterioration that leads to coma, systemic organ failure and finally death. In case of T. b. rhodesiense, an untreated infection will leads to death within few months; however, infection with T. b. gambiense will lead to death of the patient after several years when left untreated. Tryptophol is a chemical compound which stimulates sleep in humans. It is the chemical that is produced by the trypanosomal parasite in sleeping sickness. The major mode of transmitting the disease is the bite of an infected tsetse fly but there are several other ways through which people are infected with sleeping sickness.

- ✓ The infection can be spread from pregnant mother to her child because the trypanosomes are able to cross the placenta and cause the disease to the fetus.

- ✓ The mechanical transmission is also possible through other blood sucking insects. However, assessment of epidemiological impact of transmission is very difficult.

- ✓ Accidental infections may also be possible in the laboratories due to pricks from contaminated needles.

Disease management:

Disease management can be done in three major steps.

- ✓ The first step is the screening for potential infection which can be done by serological tests (only available for T. b. gambiense) and confirms major symptoms such as swollen cervical glands.

- ✓ The second stage is to diagnose the presence of the parasite.

- ✓ The last step is staging, which is done to find out the state of disease progression. This involves collection and examination of cerebro-spinal fluid from lumbar region which helps in determining the course of treatment.