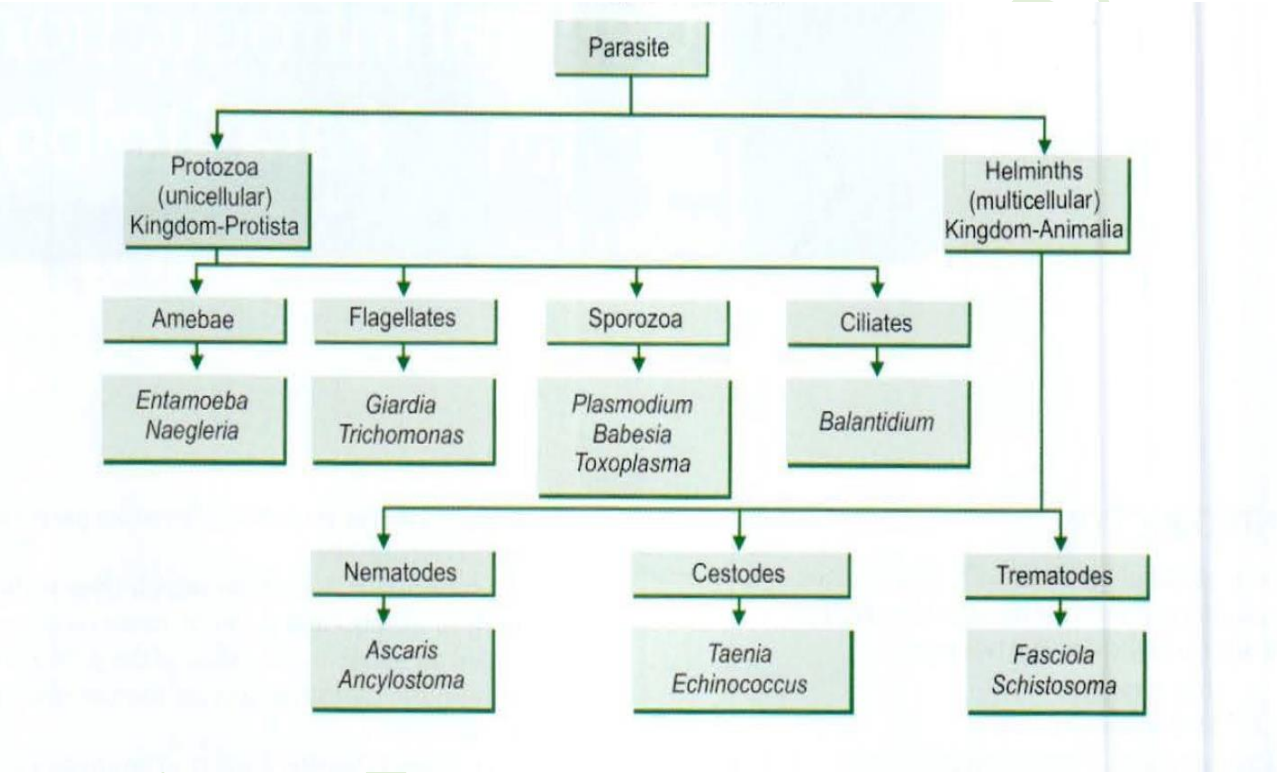
Lec. : 1

Medical Parasitology

Medical parasitology deals with the parasites, which cause human infections and the diseases they produce , It is broadly divided into two parts :

1. Protozoology

2. Helminthology



Types of parasites

PARASITES :

Parasites are living organisms, which depend on a living host for their nourishment and survival. They undergo development in the host. The term "parasite" is usually applied to Protozoa (unicellular organisms) and Helminths (multicellular organisms)

Parasites can also be classified as:

- Ectoparasite : Ectoparasites inhabit only the body surface of the host without penetrating the tissue. Lice, ticks and mites are examples of ectoparasites

- Endoparasite : A parasite, which lives within the body of the host . Most of the protozoan and helminthic parasites causing human disease are endoparasites.

- Free-living parasite: It refers to nonparasitic stages of active existence, which live independent of the host, e.g. cystic stage of *Naegleria fowleri*.

Endoparasites can further be classified as:

- Obligate parasite : The parasite, which cannot exist without a host , e.g. *Toxoplasma* gondii and *Plasmodium*.

- Facultative parasite: Organism which may either live as parasitic form or as free-living form, e.g. *Naegleria fowleri*.

- Accidental parasites: Parasites, which infect an unusual host are known as accidental parasites. *Echinococcus granulosus* infects man accidentally, giving rise to hydatid cysts.

- Aberrant parasites: Parasites, which infect a host where they cannot develop further are known as aberrant or wandering parasites, e.g. *Toxocara canis* (dog roundworm) infecting humans.

HOST :

Host is defined as an organism, which harbors the parasite and provides nourishment and shelter to parasite and is relatively larger than the parasite.

The host may be of the following types :

- Definitive host: The host, in which the adult parasite lives and undergoes sexual reproduction is called the definitive host, e.g. mosquito acts as definitive host in malaria .The definitive host may be a human or any other living being. However, in majority of human parasitic infections , man is the definitive host (e.g. filaria, roundworm, hookworm).

- Intermediate host : The host, in which the larval stage of the parasite lives or asexual multiplication takes place is called the intermediate host. In some

parasites, two different intermediate hosts may be required to complete different larval stages. These are known as first and second intermediate hosts .

- Paratenic host : A host, in which larval stage of the parasite remains viable without further development is referred as a paratenic host . Such host transmits the infection to another host, e.g. fish for plerocercoid larva of *D.lalum*.

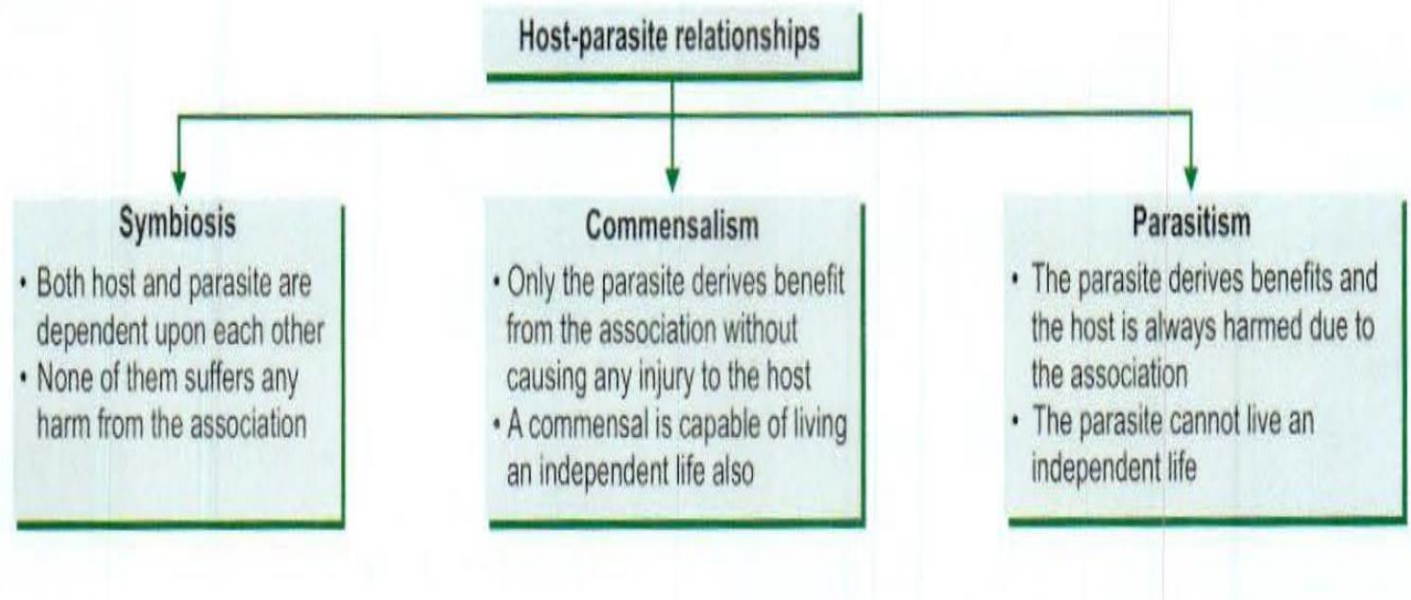
- Reservoir host : In an endemic area, a parasitic infection is continuously kept up by the presence of a host, which harbors the parasite and acts as an important source of infection to other susceptible hosts, e.g. dog is the reservoir host of hydatid disease.

- Accidental host: The host, in which the parasite is not usually found, e.g. man is an accidental host for cystic echinococcosis.

HOST-PARASITE RELATIONSHIPS

Host-parasite relationships are of following types

- Symbiosis
- Commensalism
- Parasitism.



LIFE CYCLE OF PARASITES

- Direct life cycle: When a parasite requires only single host to complete its development, it is called as direct life cycle, e.g. *Entamoeba histolytica* requires only a human host to complete its life cycle .

- Indirect life cycle: When a parasite requires two or more species of host to complete its development, the life cycle is called as indirect life cycle, e.g. malarial parasite requires both human host and mosquito to complete its life cycle.

SOURCES OF INFECTION

- **Contaminated soil and water:**

- Soil polluted with embryonated eggs (roundworm,whipworm) may be ingested or infected larvae in soil, may penetrate exposed skin (hookworm).

- Infective forms of parasites present in water may be ingested (cyst of ameba and *Giardia*).

- Water containing the intermediate host may be swallowed (guinea worm larva).

- Infected larvae in water may enter by penetrating exposed skin (cercariae of schisotosomes).

- Free-living parasites in water may directly enter through vulnerable sites (*Naegleria* may enter through nasopharynx).

- **Food :**

- Ingestion of contaminated food or vegetables containing infective stage of parasite (amebic cysts, *Toxoplasma* oocysts, Echinococcus eggs).

- Ingestion of raw or undercooked meat harboring infective larvae(cysticercus , the larval stage of *Taenia solium*).

- **Vectors :**

A vector is an agent, usually an arthropod that transmits an infection from man to man or from other animals to man, e.g. female *Anopheles* is the vector of malarial parasite.

Vectors can be:

- Biological vectors: The term biological vector refers to a vector, which not only assists in the transfer of parasites but the parasites undergo development or multiplication in their body as well. They are also called as true vectors. Example of true vectors are:
  - Mosquito: Malaria, filariasis
  - Sandflies: Kala-azar
  - Tsetse flies: Sleeping sickness
  - Reduviid bugs: Chagas disease
  - Ticks: Babesiosis.
- Mechanical vectors: The term mechanical vector refers to a vector, which assists in the transfer of parasitic form between hosts but is not essential in the life cycle of the parasite. Example of mechanical vectors is:
  - Housefly: Amebiasis

- **Self (autoinfection) :**

Finger-to-mouth transmission, e.g. pinworm Internal reinfection, e.g.*Strongyloides*.

MODES OF INFECTION

- Oral transmission: The most common method of transmission is through oral route by contaminated food, water, soiled fingers, or fomites. Many intestinal parasites enter the body in this manner; the infective stages being cysts, embryonated eggs, or larval forms. Infection with E. histolytica and other intestinal protozoa occurs when the infective cysts are swallowed.

- Skin transmission: Entry through skin is another important mode of transmission. Hookworm infection is acquired, when the larvae enter the skin of persons walking barefooted on contaminated soil. Schistosomiasis is acquired when the cercarial larvae in water penetrate the skin.

- Vector transmission: Many parasitic diseases are transmitted by insect bite, e.g. malaria is transmitted by bite of female Anopheles mosquito, filariasis is transmitted by bite of *Culex* mosquito. A vector could be a biological vector or a mechanical vector.

- Direct transmission: Parasitic infection may be transmitted by person-to person contact in some cases, e.g. by kissing in the case of gingival amebae and by sexual intercourse in trichomoniasis.

- Vertical transmission: Mother to fetus transmission may take place in malaria and toxoplasmosis.

- Iatrogenic transmission: It is seen in case of transfusion malaria and toxoplasmosis after organ transplantation.

PATHOGENESIS

Parasitic infections may remain inapparent or give rise to clinical disease. A few organisms, such as *E. histolytica* may live as surface commensals , without invading the tissue.

- Clinical infection produced by parasite may take many forms: acute, subacute, chronic, latent, or recurrent.

- Pathogenic mechanisms, which can occur in parasitic infections are:

- Lytic necrosis: Enzymes produced by some parasite can cause lyric necrosis. *E. histolylica* lyses intestinal cells and produces amebic ulcers.

- Trauma: Attachment of hookworms on jejunal mucosa leads to trauma tic damage of villi and bleeding at the site of attachment.

- Allergic manifestations: Clinical illness may be caused by host immune response to parasitic infection, e.g. eosinophilic pneumonia in *Ascaris* infection and anaphylactic shock in rupture of hydatid cyst.

- Physical obstruction: Masses of roundworm cause intestinal obstruction. Plasmodium falciparum malaria may produce blockage of brain capillaries in

cerebral malaria.

- inflammatory reaction: Clinical illness may be caused by inflammatory changes and consequent fibrosis, e.g. lymphadenitis in filariasis and urinary bladder granuloma in *Schistosoma haematobium* infection.

- Neoplasia: A few parasitic infection have been shown to lead to malignancy. The liver fluke , and *S. haematobium* may cause urinary bladder cancer.

- Space occupying lesions: Some parasites produce cystic lesion that may compress the surrounding tissue or organ, e.g. hydatid cyst.

Lec. : 2

Parasitology : Is the science that deals with parasites , their hosts and the relationship between them .

Zoonosis : defined zoonosis as those diseases and infections , which are naturally transmitted between vertebrate animals and man.

SCIENTIFIC NOMENCLATURE & CLASSIFICATION OF PARASITES

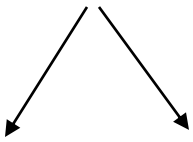
According to the binomial nomenclature as suggested by Linnaeus , each parasite has two names : a genus and a species name. These names are either derived from: names of their discoverers, Greek or Latin words of the geographical area where they are found, habitat of the parasite, or hosts in which parasites are found and its size and shape. All parasites are classified under the following taxonomic units—the kingdom, subkingdom, phylum, subphylum, super class, class, subclass, order, suborder, super family, family, genus and species. The generic name of the parasite always begins with an initial capital letter and species name with an initial small letter, e.g., *Entamoeba histolytica* .

Kingdom : 1. Protista (Unicellular)

Phylum : Protozoa

Subphylum : 1. Sarcomastigophora 2.Sporozoa 3.Ciliate

Apicomplexa Ciliophora



1.Amoeba 2. Flagellate

Sarcodina Mastigophora

PROTOZOA

Single-celled eukaryotic microorganisms belonging to kingdom Protista are classified as Protozoa (Greek protos: first ; zoon: animal).

- Parasitic protozoa are adapted to different host species.
- Out of 10,000 species of parasitic protozoa, man harbours only about 70 species.

GENERAL FEATURES

- The single protozoa , cell performs all functions.
- Most of the protozoa are completely nonpathogenic but few may cause major diseases such as malaria, leishmaniasis and sleeping sickness.
- Protozoa like *Cryptosporidium parvum* and *Toxoplasma gondii* are being recognized as opportunistic pathogens in patients affected with human immunodeficiency virus (lllV) and in those undergoing immunosuppressive therapy.
- Protozoa exhibit wide range of size (1 - 150 μm), shape and structure; yet all possess essential common feature

STRUCTURE

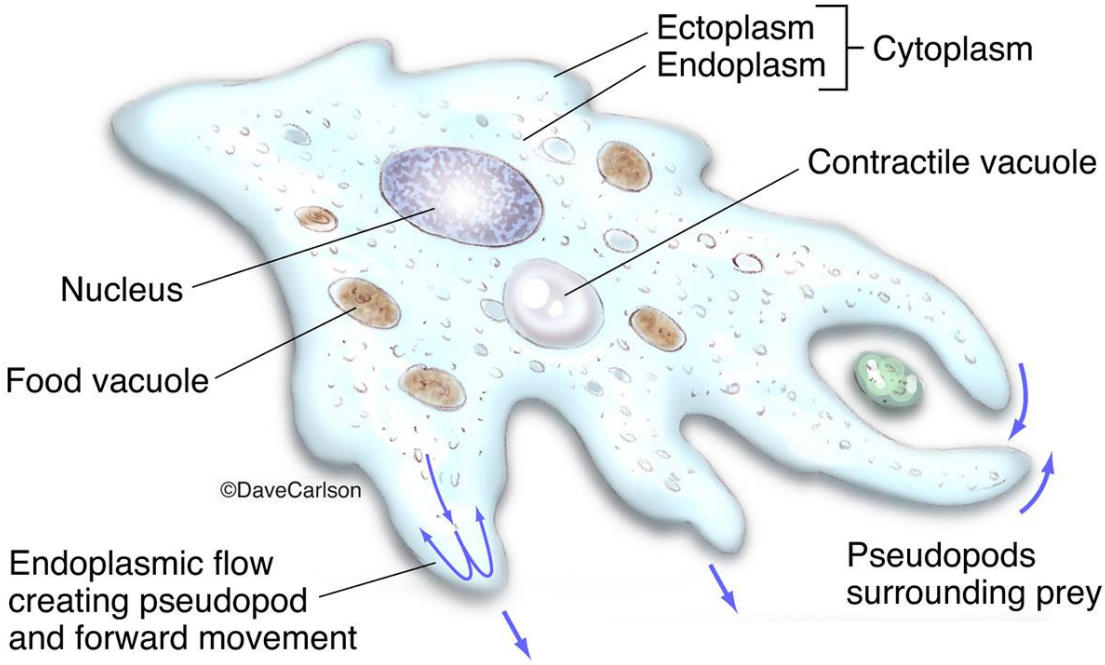
The typical protozoan cell is bounded by a trilaminar unit membrane, supported by a sheet of contractile fibrils enabling the cell to move and change in shape.

CYTOPLASM

It has two portions:

1. Ectoplasm: Outer homogeneous part that serves as the organ for locomotion and for engulfment of food by producing pseudopodia is called as the ectoplasm . It also helps in respiration , discharging waste material and in providing a protective covering of cell.

2. Endoplasm The inner granular portion of cytoplasm that contains nucleus is called endoplasm. The endoplasm shows number of structures : the Golgi bodies, endoplasmic reticulum , food vacuoles and contractile vacuoles. Contractile vacuoles serve to regulate the osmotic pressure .



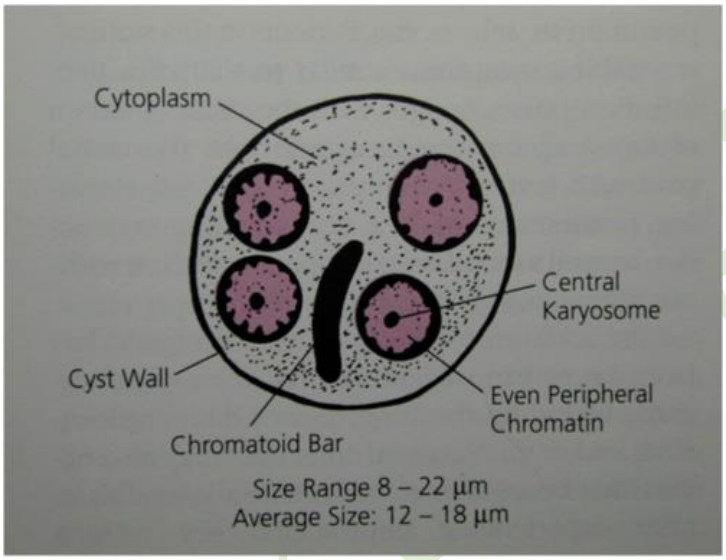
Amoeba as an example of typical organelles for protozoa

NUCLEUS

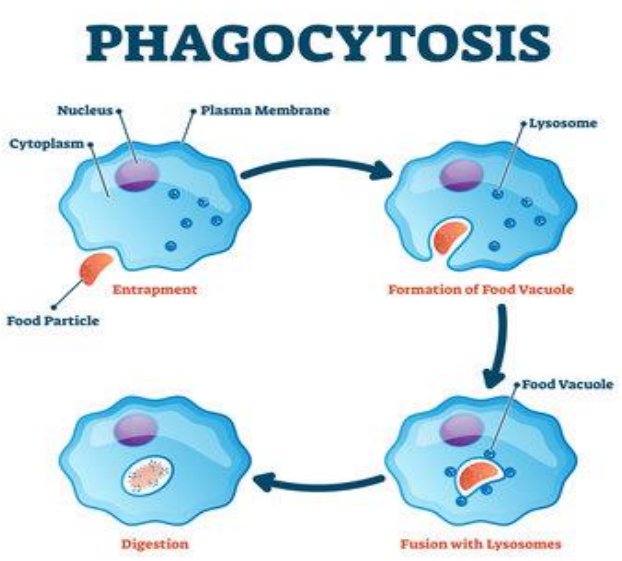
The nucleus is usually single but may be double or multiple ; some species having as many as 100 nuclei in a single cell.

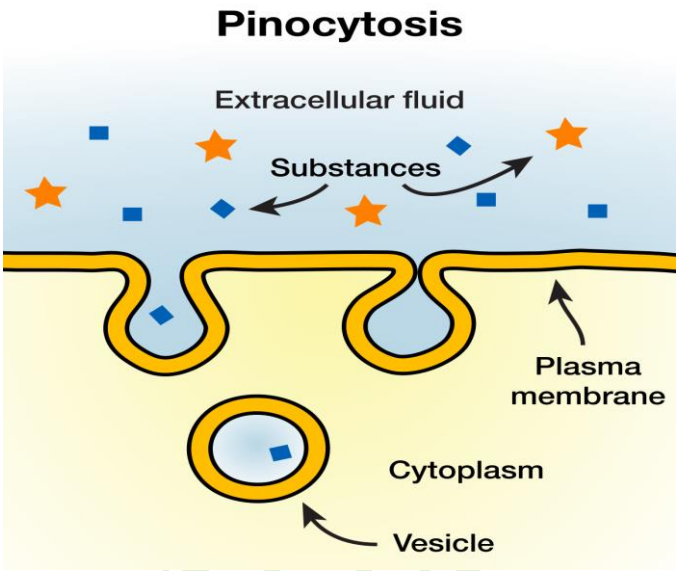
- The nucleus contains one or more nucleoli or a central karyosome .
- The chromatin may be distributed along periphery (peripheral chromatin) or as condensed mass around the karyosome.

- **Karyosome** : It is a deoxyribonucleic acid (DNA) containing body, situated peripherally or centrally within the nucleus and found in intestinal ameba, e.g. *E. histolytica* & E.coli.
- **Chromatoid body:** (aggregations of ribosomes) is called chromatoid body e.g. as found in *E. histolytica* cyst .



- **Kinetoplast** : Nonnuclear DNA present in addition to nucleus is called kinetoplast. It is seen in trypanosomes. Flagellum originates near the kinetoplast. Point of origin of flagellum is called as basal body.
- **Cyst :** inactive , nonfeeding , nonmotile , resistant & infective stage of protozoa . usually passed in feces & provided with highly condenced cytoplasm & resistant cell wall .
- **Trophozoite** : (trophos: nourishment) : Active , feeding , motile , growing & pathogenic stage of the protozoa . It derives nutrition from the environment by diffusion , pinocytosis and phagocytosis .





REPRODUCTION

Reproduction can be:

- Asexual reproduction
- Sexual reproduction.

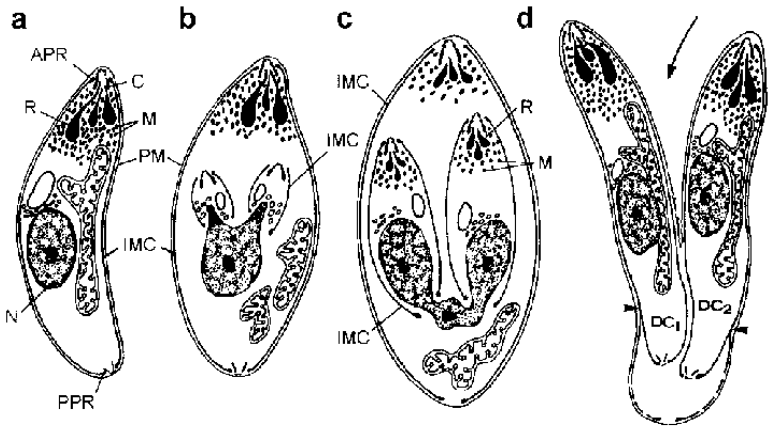
Reproduction usually occurs asexually in protozoan ; however, sexual reproduction occurs in ciliates and sporozoans.

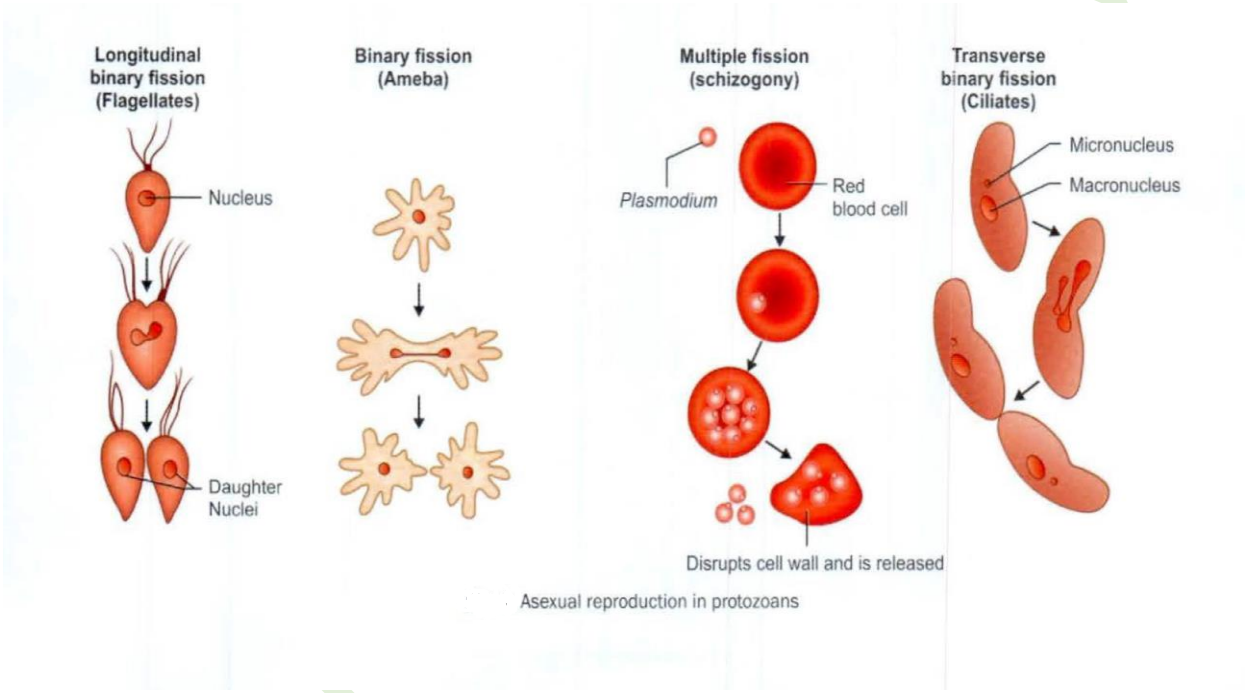
Asexual Reproduction

- Binary fission: It is a method of asexual reproduction, by which a single parasite divides either longitudinally or transversally into two or more equal number of parasites. Mitotic division of nucleus is followed by division of the cytoplasm. In amebae, division occurs along any plane, but in flagellates, division is along longitudinal axis and in ciliates, in the transverse plane .

- Multiple fission or schizogony: *Plasmodium* exhibits schizogony, in which nucleus undergoes several successive divisions within the schizont to produce large number of merozoites .

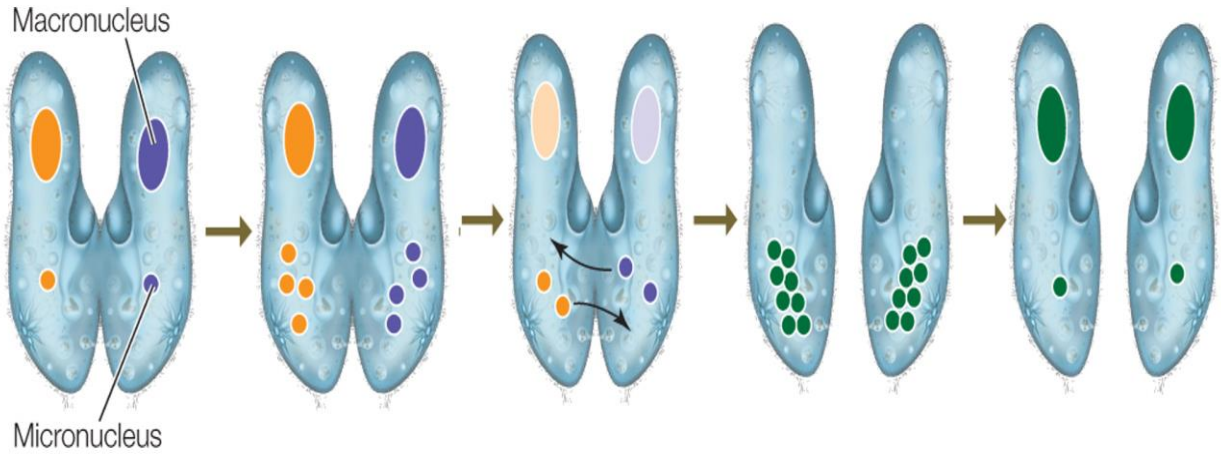
- Endodyogeny: Some protozoa like *Toxoplasma*, multiply by internal budding, resulting in the formation of two daughter cells.



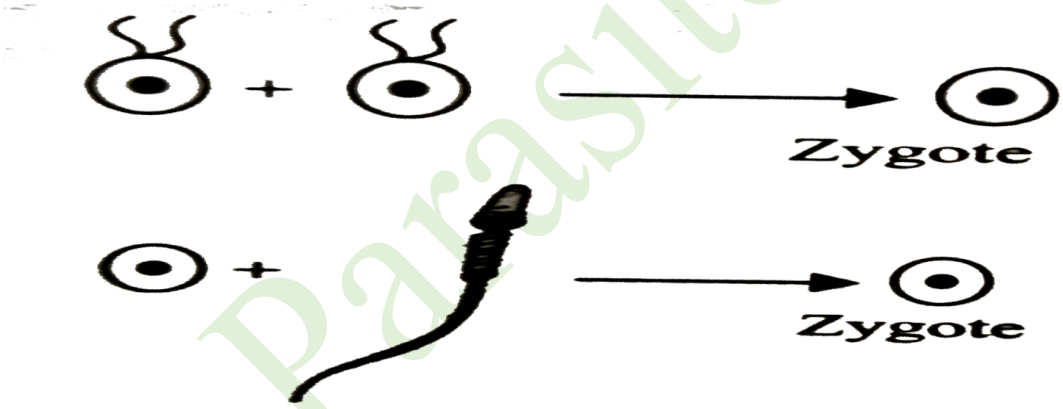


Sexual Reproduction

- Conjugation: In ciliates, the sexual process is conjugation, in which two organisms join together and reciprocally exchange nuclear material e.g. *Balanlidium coli* .



- Gametogony or Syngamy: In Sporozoa , male and female gametocytes are produced, which after fertilization form the zygote, which gives rise to numerous sporozoites by sporogony e.g. *Plasmodium* .



Lec. : 3

****Free living protozoa :** feed on bacteria, algae, fungi, other protozoa, and organic detritus , Some free-living protozoa resemble plants containing green plastids that enable them to perform photosynthesis .

Free-living protozoa are found in all habitats—in the deep ocean or in shallow freshwaters, in hot springs or in ice, under the soil, or in the snow on mountain tops . Parasitic protozoa have however adapted to different host species, with more restricted physicochemical requirements .

A cytostome : (from cyto-, cell and stome-, mouth) or mouth like structure is a part of a cell specialized for phagocytosis , pinocytosis .

SubPhylum : Sarcomastigophora

1. Amoeba (Sarcodina) :

Simple protozoa that have no fixed shape. They are classified under the Phylum .

Pseudopodia are formed by the ectoplasm thrusting out, being followed by the endoplasm flowing in , to produce blunt projections , Pseudopodial processes appear and disappear , producing quick changes in the shape of the cell , These are employed for locomotion and engulfment of food by phagocytosis .

Amoebae may be free-living or parasitic . A few of the free-living amoebae can , on occasion act as human pathogens , producing meningoencephalitis and other infections . Some of them can act as carriers of pathogenic bacteria. The parasitic amoebae inhabit the alimentary canal.

1. *Entamoeba* :

- *Entamoeba histolytica , Entamoeba hartmanni , Entamoeba polecki , Entamoeba dispar , Entamoeba coli , , , Entamoeba gingivalis.*

- *Entamoeba histolytica*

Is an important human pathogen, causing amoebic dysentery as well as hepatic amoebiasis and other extraintestinal lesions .

History

History *Entamoeba histolytica* was discovered in 1875 by Losch in the dysenteric feces of a patient in St Petersburg , Russia. He also observed it in colonic ulcers at autopsy and produced dysentery in a dog by inoculation through the rectum . In 1890 , William Osier reported the case of a young man with dysentery who later died of liver abscess. Councilman and Lafleur in 1891 established the pathogenesis of intestinal and hepatic amoebiasis and introduced the terms ‘amoebic dysentery’ and ‘amoebic liver abscess.

Geographical Distribution E. histolytica is world-wide in prevalence. It is much more common in the tropics than elsewhere, but it has been found wherever sanitation is poor, in all climatic zones, from Alaska (61° N) to the Straits of Magellan (52°S). It has been reported that about 10 per cent of the world’s population and 50 per cent of the inhabitants of some developing countries may be infected with the parasite. The infection is not uncommon even in affluent countries, about 1 per cent of Americans being reported to be infected. While the large majority of the infected are asymptomatic, invasive amoebiasis causes disabling illness in an estimated 50 million persons and death in 50,000 annually, mostly in the tropical belt of Asia. Africa and Latin America. It is the third leading parasitic cause of mortality, after malaria and schistosomiasis. E. histolytica is found in the human colon. Natural infection also occurs in monkeys, dogs and probably in pigs also but these animals do not appear to be relevant as sources of human infection. Infection is mostly asymptomatic. It commonly occurs in the lumen of the colon as a commensal, but sometimes invades the intestinal tissues to become a pathogen .

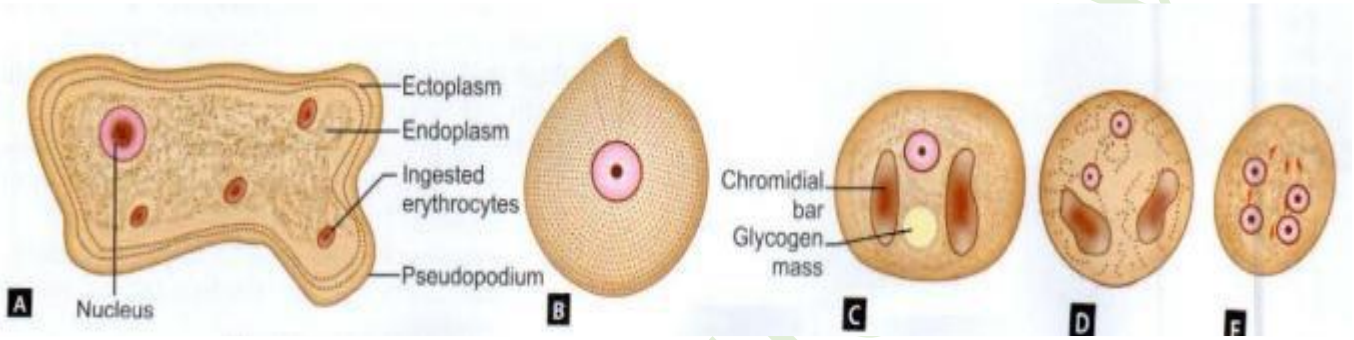
Morphology

E. histolytica occurs in three forms :

a. Trophozoite.

b. Precystic.

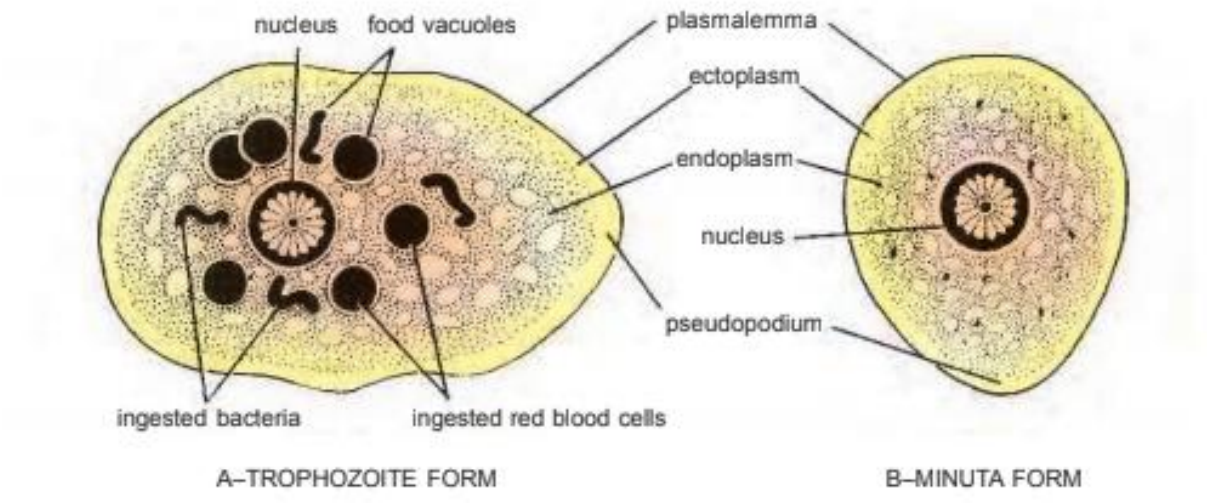
c. Cystic stages.



Entamoeba histolytica: (A) Trophozoite; (B) Precystic stage; (C) Uninucleate. (D) Binucleate cyst and (E) Mature quadrinucleate cyst.

Trophozoite

Trophozoite is the vegetative or growing stage of the parasite. It is the only form present in tissues. It is irregular in shape and varies in size from 12- 60 µm; average being 20 µrn. It is large and actively motile in freshly-passed dysenteric stool, while smaller in convalescents and carriers. The parasite, as it occurs free in the lumen as a commensal is generally smaller in size, about 15-20 µm and has been called the **Minuta form** .



Cytoplasm: Outer ectoplasm is clear, transparent and refractile. Inner endoplasm is finely granular, having a ground glass appearance. The endoplasm contains nucleus, food vacuoles, erythrocytes, occasionally leukocytes and tissue debris.

Pseudopodia are finger-like projections formed by sudden jerky movements of ectoplasm in one direction, followed by the streaming in of the whole endoplasm.

Typical ameboid motility is a crawling or gliding movement and not a free swimming one. The direction of movement may be changed suddenly, with another pseudopodium being formed at a different site, when the whole cytoplasm flows in the direction of the new pseudopodium. The cell has to be attached to some surface or particle for it to move. In culture tubes, the trophozoites may be seen crawling up the side of the glass tube.

***Pseudopod formation and motility are inhibited at low temperatures.**

Nucleus is spherical 4- 6 μm in size and contains central karyosome, surrounded by clear halo and anchored to the nuclear membrane by fine radiating fibrils called the Linin network, giving a cartwheel appearance. The nucleus is not clearly seen in the living trophozoites, but can be clearly demonstrated in preparations stained with iron hematoxylin. Nuclear membrane is lined by a rim of chromatin distributed evenly as small granules. The trophozoites from acute dysenteric stools often contain phagocytosed erythrocytes. This feature is diagnostic as phagocytosed red cells are not found in any other commensal intestinal amebae. The trophozoites divide by binary fission in every 8 hours . Trophozoiles survive up to 5 hours at 37°C and are killed by drying, heat and chemical sterilization. Therefore, the infection is not transmitted by trophozoites. Even if live trophozoites from freshly-passed stools are ingested, they are rapidly destroyed in stomach and cannot initiate infection.

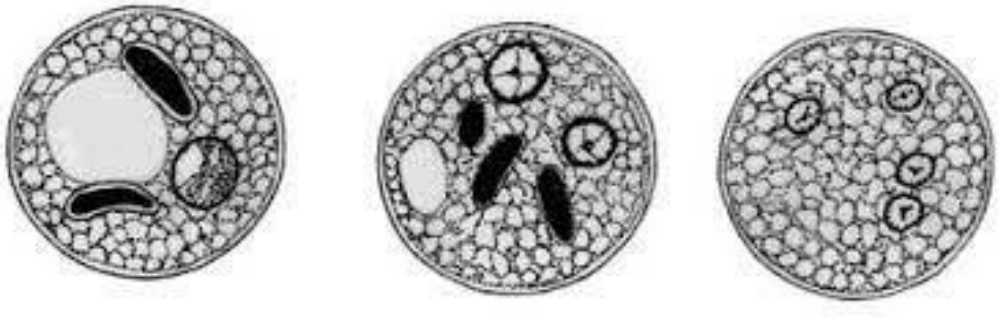
Precystic Stage

Trophozoites undergo encystment in the intestinal lumen. Encystment does not occur in the tissues nor in feces outside the body. Before encystment, the trophozoite extrudes its food vacuoles and becomes round or oval, about 10- 20 μmin size. This is the precystic stage of the parasite. It contains a large glycogen vacuole and two chromatid bars. It then secretes a highly retractile cyst wall around it and becomes cyst.

Cystic Stage

The cyst is spherical in shape about 10-20 μmin in size. The early cyst contains a single nucleus and two other structures a mass of glycogen, and 1- 4 chromatoid bodies or chromatoid bars, which are cigar-shaped. The chromatoid bodies are so-called because they stain with hematoxylin, like chromatin. As the cyst matures, the glycogen mass and chromidial bars disappear and the nucleus undergoes two successive mitotic divisions to form two and then four nuclei. 1he mature cyst is, thus quadrinucleate. The cyst wall is a highly refractile membrane, which makes it highly resistant to gastric juice and unfavorable environmental conditions.The nuclei and chromidial bodies can be made out in unstained films, but they appear more prominently in stained preparations. With iron hematoxylin stain, nuclear chromatin and chromaroid bodies appear deep blue or black, while the glycogen mass appears unstained. When stained with iodine, the glycogen mass appears

golden brown, the nuclear chromatin and karyosome bright yellow, and the chromatoid bodies appear as clear space, being unstained.



Cystic stage ⟶

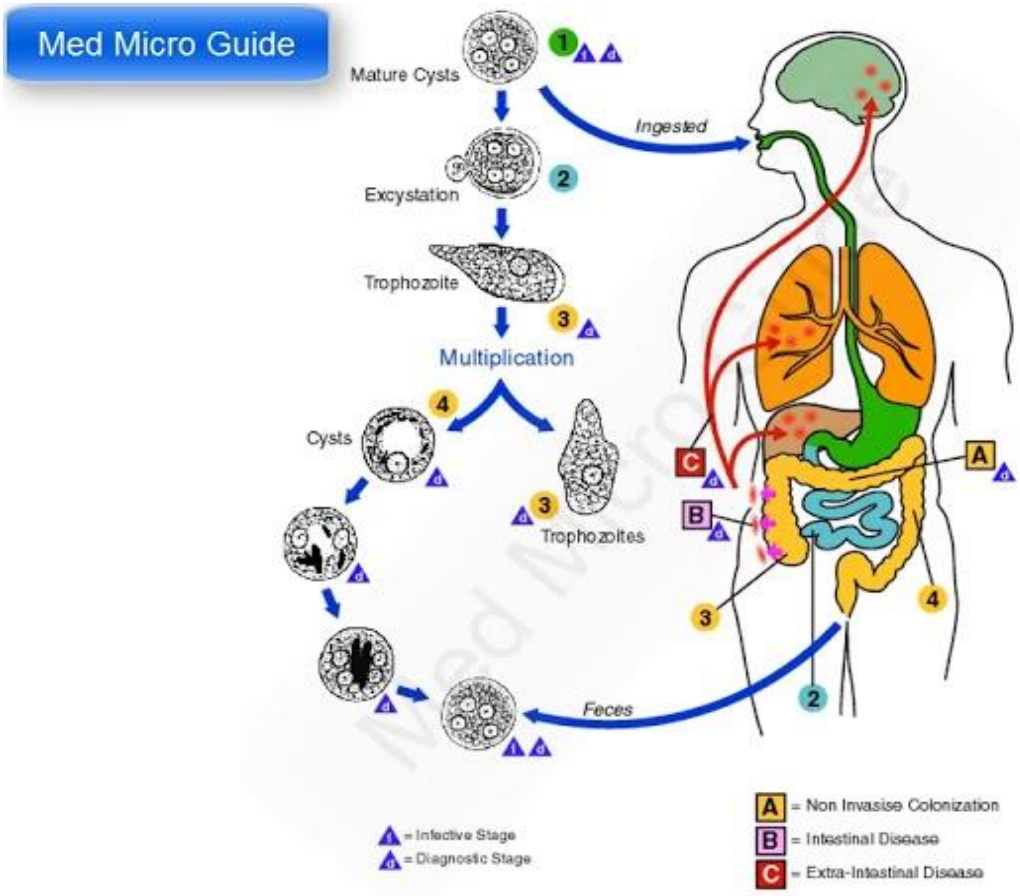
Life Cycle

Entamoeba histolytica passes its life cycle only in one host (man).

Cysts and trophozoites are typically found in diarrheal stool. Infection by *Entamoeba histolytica* occurs by ingestion of mature cysts in fecally contaminated food, and water. Excitation occurs in the small intestine and trophozoites are released, which migrate to the large intestine. The trophozoites multiply by binary fission and produce cysts, and both stages are passed in the feces. Because of the protection conferred by their walls, the cysts can survive days to weeks in the external environment and are responsible for transmission. Trophozoites passed in the stool are rapidly destroyed once outside the body, and if ingested would not survive exposure to the gastric environment. The cysts passing in stool of infected individuals. In some patients, the trophozoites invade the intestinal mucosa or, through the bloodstream, extraintestinal sites such as the liver, brain, and lungs, with resultant pathologic manifestations.

Excystation : The stage in the life cycle of a parasite in which it escapes from a cyst (after being swallowed by its host) .

Encystation is the formation of a three-layered hard crust or a cyst around an amoeba to protect itself from unfavorable conditions . The amoebae lose their motility and pseudopodial movements.


Med Micro Guide
Mature Cysts
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Ingested
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Cysts
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Trophozoites
Feces
2
A
B
C
3
4
= Infective Stage
= Diagnostic Stage
A = Non Invasise Colonization
B = Intestinal Disease
C = Extra-Intestinal Disease

Infective Form Mature quadrinucleate cyst passed in feces of convalescents and carriers. The cysts can remain viable under moist conditions for about I0 days.

Mode of Transmission
Man acquires infection by swallowing food and water contaminated with cysts.

Excystation: It happened when the cyst reaches cecum or lower part of the ileum, due to the alkaline medium, the cyst wall is damaged by trypsin.

Metacyst mean the cytoplasm gets detached from the cyst wall and ameboid movements appear causing a tear in the cyst wall, through which quadrinucleate ameba is liberated.

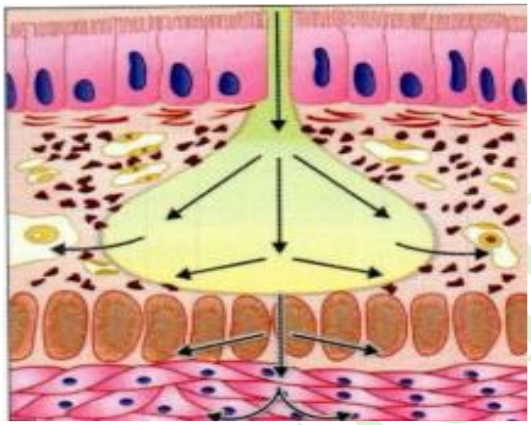
Metacystic Trophozoites mean the nuclei in the metacyst immediately undergo division to form eight nuclei, each of which gets surrounded by its own cytoplasm to become eight small amebulae or metacystic trophozoites.

Pathogenesis and Clinical Features

E. histolytica causes intestinal and extraintestinal amebiasis. Incubation period is highly variable. On an average, it ranges from 4 days to 4 months. Amebiasis can present in different forms and degree of severity , depending on the organ affected and the extent of damage caused.

Intestinal Amebiasis
The lumen-dwelling amebae do not cause any illness. They cause disease only when they invade the intestinal tissues. This happens only in about 10% of cases of infection, the remaining 90% being **Asymptomatic.**
Not all strains of *E. hislolylica* are pathogenic or invasive. Differentiation between pathogenic and nonpathogenic strains can be made by susceptibility to complement mediated lysis and phagocytic activity or by the use of genetic markers or monoclonal antibodies and zymodeme analysis.

Amebic ulcer is the typical lesion seen in intestinal amebiasis . The ulcers are multiple and are confined to the colon, being most numerous in the cecum and next in the sigmoido rectal region. The intervening mucous membrane between the ulcers remains healthy. Ulcers appear initially on the mucosa as raised nodules with pouting edges measuring pinhead to l inch. They later break down discharging brownish necrotic material containing large numbers of trophozoites.



The typical amebic ulcer is **flask-shaped** in cross section, with mouth and neck being narrow and base large and rounded. Multiple ulcers may coalesce to form large necrotic lesions with ragged and undermined edges and are covered with brownish slough.

Ameboma Occasionally, a granulomatous pseudotumoral growth may develop on the intestinal wall by rapid invasion from a chronic ulcer. This amebic granuloma or ameboma may be mistaken for are malignant tumor. Amebomas are most frequent at cecum and rectosigmoid junction.

Lec. : 4

Treatment

Both luminal and tissue Amebicides : **Metronidazole** and related compounds like **Tinidazole** and **Imidazole** act on both sites and are the drug of choice for treating amebic colitis and amebic liver abscess .

NONPATHOGENIC INTESTINAL AMEBA

Entamoeba coli

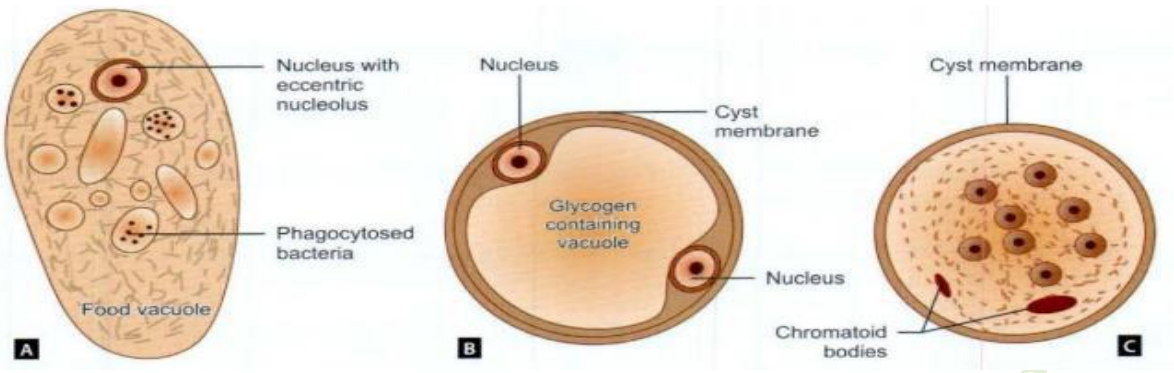
E. coli was first described by Lewis (1870) and Cunningham (1871) in Kolkata and its presence in healthy persons was reported by Grassi (1878). It is worldwide in distribution and a nonpathogenic commensal intestinal amoeba .

o It is a larger than *E. histolytica* about 20-50 μm with sluggish motility and contains ingested bacteria but no red cells . The nucleus is clearly visible in unstained films and has a large eccentric karyosome and thick nuclear membrane lined with coarse granules of chromatin.

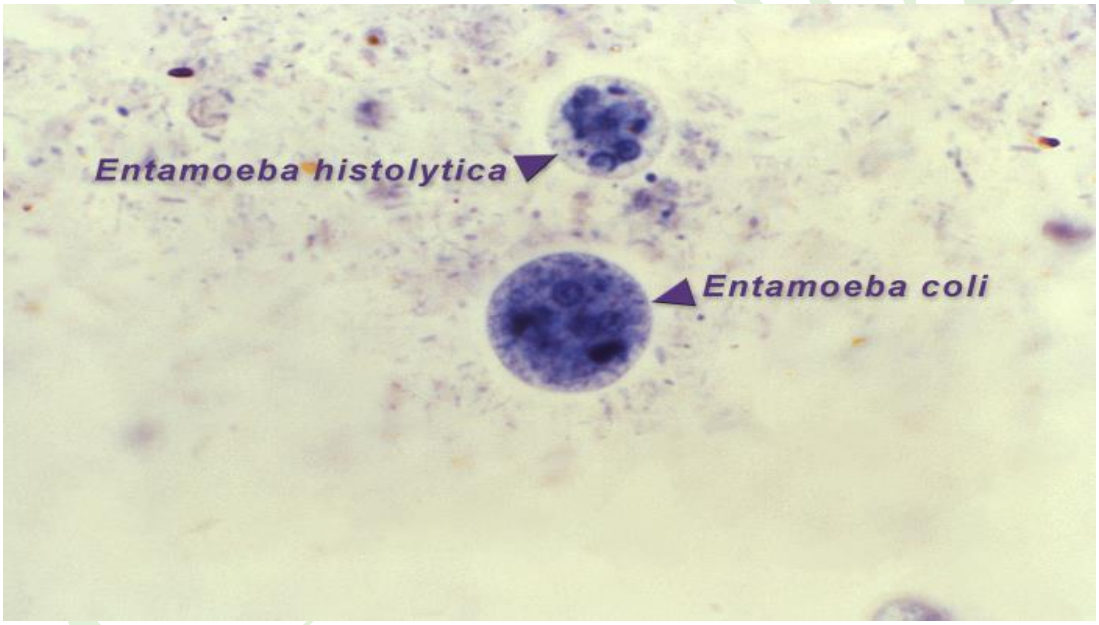
o Cysts are large, 10- 30 μm in size, with a prominent glycogen mass in the early stage. The chromatoid bodies are splinter-like and irregular. The mature cyst has eight nuclei.

Life cycle

The life cycle is the same as in *E. histolytica* except that it remains a luminal commensal without tissue invasion and is nonpathogenic.



***E. coli* (A) Trophozoite (B) Binucleate cyst , (C) Eight-nucleate cyst**



Entamoeba dispar

E. dispar is morphologically indistinguishable (both cyst and trophozoite) from *E. histolytica* , so it may be considered as a subspecies of E. histolytica.

Morphology and Life Cycle

The life cycle is essentially identical to that of *E. coli* or any of the other nonpathogenic intestinal protozoa, and the cyst form is the infective form for humans.

- Living trophozoites vary in diameter from about 12 to 60 μm. Motility has been described as rapid and unidirectional, with pseudopods forming quickly in response to the conditions around the organism; it may appear to be sporadic. Although this characteristic is often described, it is rare to diagnose these organisms on the basis of motility seen in a direct wet mount. The cytoplasm is differentiated into a clear outer ectoplasm and a more granular inner endoplasm.
- When the organism is examined on a permanent stained smear (trichrome or iron hematoxylin), the morphological characteristics are easily seen.

- The nucleus generally has evenly arranged chromatin on the nuclear membrane and has a small, compact, centrally located karyosome.
- The cytoplasm is described as finely granular with few ingested bacteria or debris in vacuoles. Ingested RBCs are never seen in the trophozoites ; if ingested RBCs are seen, this finding identifies the organism as *E. histolytica*, not *E. dispar* .

- *E. dispar* parasite is nonpathogenic, usually colonizes in the large intestine (10 times more than *E. histolytica*) but doesn't invade intestinal mucosa .
- It can be distinguished from *E. histolytica* by :

- Light and electron microscopy studies (differences would not be recognized by routine diagnostic methods such as the permanent stained smear)
- RBC inside trophozoites—present only in *E. histolytica*.
- Zymodeme study (hexokinase isoenzyme pattern-with *E.histolytica*) .
- Molecular methods , PCR amplifying
- Detection of lectin antigen in stool (with *E.histolytica*)
- It grows well in polyxenic media, poorly grows on axenic media.
- *E. dispar* doesn't induce antibody production.

Entamoeba moshkovskii

E. moshkovskii is also morphologically indistinguishable from *E. histolytica* and *E. dispar* (may be the third subspecies of *E. histolytica*).

- This species was first described from Moscow sewage by Tshalaia in 1941 and was thereafter reported to occur in many different countries including India. It can be distinguished from *E. histolytica* by:
  - Isoenzyme analysis
  - Molecular methods
  - Detection of lectin antigen.
- It is a non-pathogen harboring in the intestine recent studies from Bangladesh and India have reported *E. moshkovskii* as a sole potential pathogen in patients presenting with gastrointestinal symptoms and/or dysentery, highlighting the need for further study to investigate the pathogenic potential of this organism.
- *E. moshkovskii* is found worldwide and is generally considered to be a free-living amoeba . Although first isolated from sewage, *E. moshkovskii* can also be found in clean riverine sediments to brackish coastal pools. Apparently, there are some differences that separate this organism from *E. histolytic* and *E. dispar* . However, these differences pertain to physiology rather than morphology ; *E. moshkovskii* is osmotolerant, can be cultured at room temperature, and is resistant to **Emetine** .
- Based on microscopic morphology, this organism is indistinguishable from *E. histolytica* and *E. dispar*, except in cases of invasive disease when *E. histolytica* contains ingested RBCs.

Morphology and Life Cycle

The life cycle is essentially identical to that of *E. dispar* and morphological differences are minimal to none. In wet preparations, trophozoites usually range in size from 15 to 20 μm and cysts normally range in size from 12 to 15 μm. It is important to remember that on the permanent stained smear there is a certain amount of artificial shrinkage due to dehydration; therefore, all of the organisms,

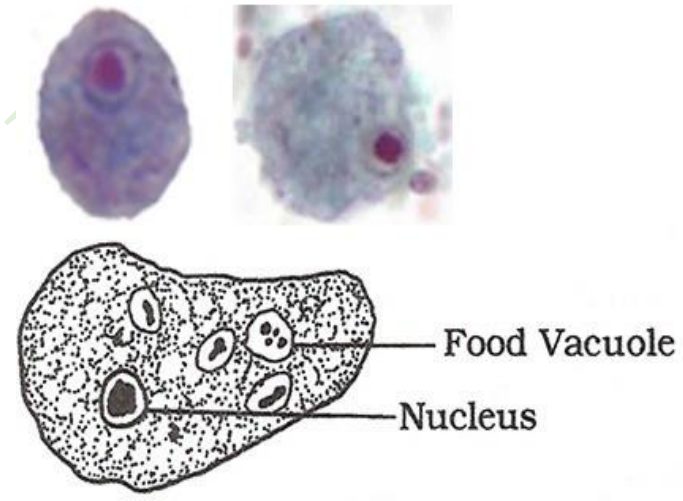
including pathogenic *E. histolytica* , may be somewhat smaller (from 1 to 1.5 µm) than the sizes quoted for the wet-preparation measurements

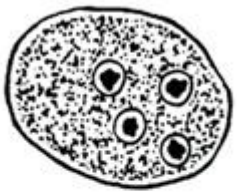
- Trophozoites do not ingest RBCs, and the motility is similar to that of both *E. histolytica* and *E. dispar* . Nuclear and cytoplasmic characteristics are very similar to those seen in E. histolytica; however, trophozoites of *E. moshkovskii* do not contain ingested RBCs.
- Cysts **:** Nuclear characteristics and chromatoidal bars are similar to those in *E. histolytica* and *E. dispar* .

Endolimax nana

This common commensal ameba is widely distributed.

- It lives in the human intestine.
- The trophozoite is small (nana: small), less than 10 µmin size with a sluggish motility (one nucleus) .
- The nucleus has karyosome connected to nuclear membrane by one or none coarse strands.
- The cyst is small, oval and quadrinucleate (4 nucleus) with glycogen mass and chromidial bars , which are inconspicuous or absent .
- It is nonpathogenic.

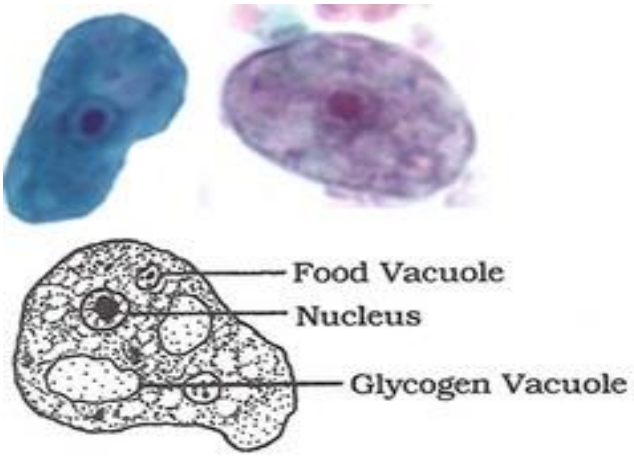


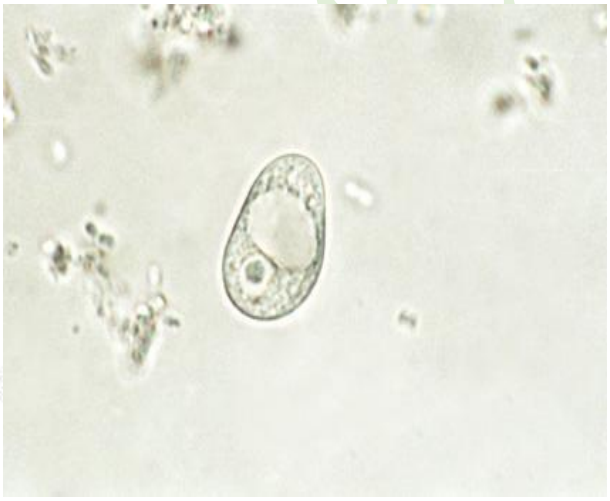


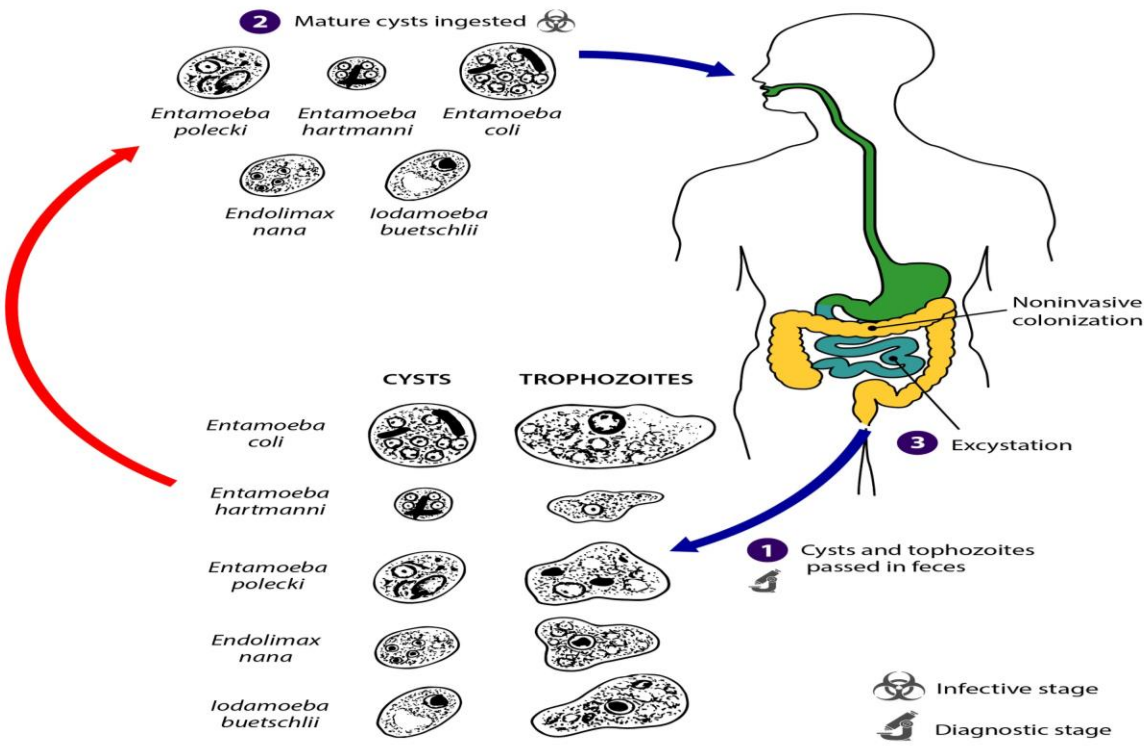
6 to 8 µm (range 5 to 10 µm)

lodamoeba butschlii

- This is widely distributed, though less common than *E. coli* and *E. nana* The trophozoice is small, 6- 12 μm, with conspicuous nucleus .
- The prominent karyosome is half the size of the nucleus, having bull 's eye appearance. The cyst is oval, uninucleate and has a prominent iodine staining glycogen mass .
- It is nonpathogenic.





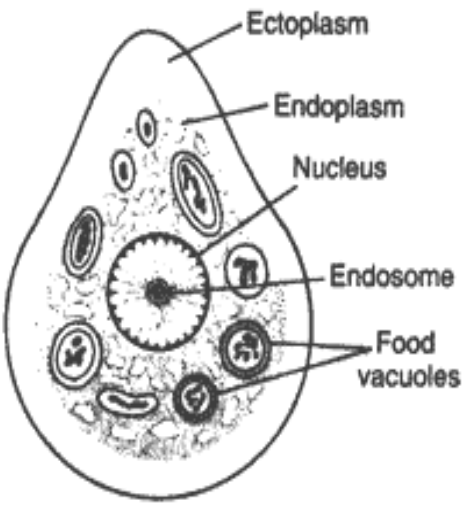


NONPATHOGENIC MOUTH AMOEBA :

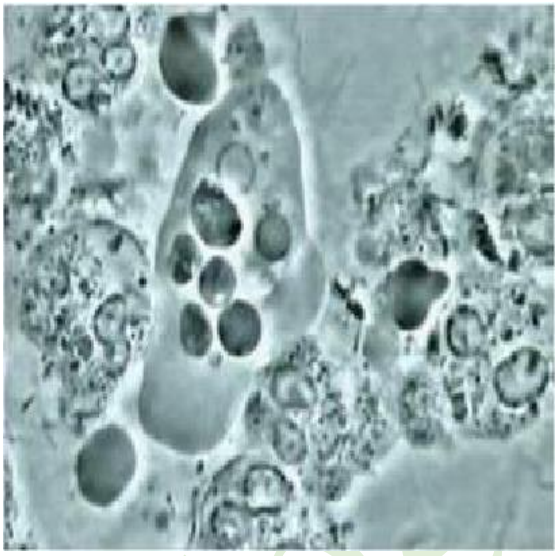
Entamoeba gingivalis

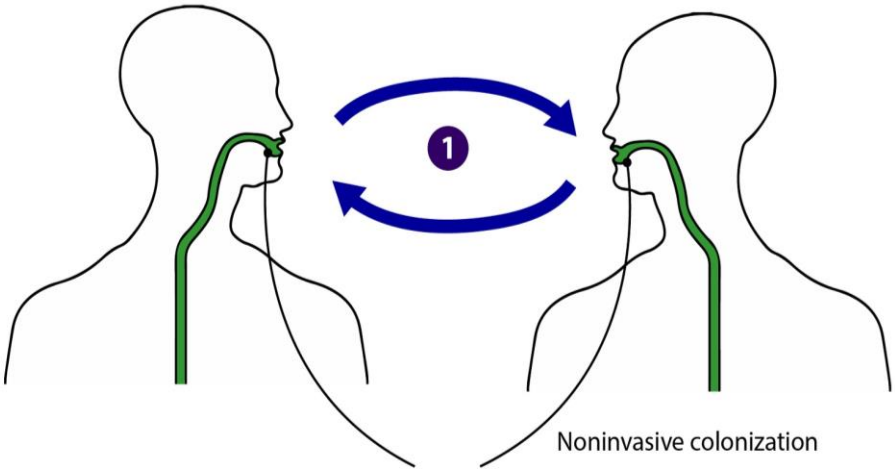
E. gingivalis was the first ameba of humans, discovered by Gros in 1849.

- It is global in distribution.
- Only the trophozoite is found ; the cystic stage being apparently absent. The trophozoite is about 10-20 μm, actively motile with multiple pseudopodia.
- The cytoplasm contains food vacuoles with ingested bacteria, leukocytes and epithelial cells.
- Nucleus is round with central karyosome lined by coarse chromatin granules.
- The ameba lives in gingival tissues and is abundant in unhygienic mouths. [it is a commensal and is not considered to cause any disease.
- It is transmitted by direct oral contact.



Entamoeba gingivalis





Noninvasive colonization

Infective stage

Diagnostic stage

Trophozoite

Lec. : 5

Flagellates

Parasitic protozoa , which possess whip-like flagella as their organs of locomotion are called as flagellates and classified as:

- SubPhylum: Sarcomastigophora
- Class : Zoomastigophora

Depending on their habitat, they can be considered under :

- Lumen-dwelling flagellates: Flagellates found in the alimentary tract and urogenital tract.
- Hemoflagellates : Flagellates found in blood and tissues.
- Most luminal flagellates are nonpathogenic commensals . Two of them cause clinical diseases :
  - *Giardia lamblia* , which can cause diarrhea
  - *Trichomonas vaginalis* , which can produce vaginitis and urethritis.

Giardia Lamblia

History and Distribution

- It is one of the earliest protozoan parasites to have been recorded.
- The flagellate was first observed by Dutch scientist Antonie van Leeuwenhoek (1681) in his own stools.
- It is named *Giardia* after Professor Giard of Paris and *lamblia* after Professor Lambie of Prague, who gave a detailed description of the parasite.

- It is the most common protozoan pathogen and is worldwide in distribution.
- Endemicity is very high in areas with low sanitation, especially tropics and subtropics. Visitors to such places frequently develop traveller's diarrhea caused by Giardiasis through contaminated water.

Habitat

G. lamblia lives in the duodenum and upper jejunum and is the only protozoan parasite found in the lumen of the human small intestine.

Protozoan's found in small intestine

- *Giardia lamblia*
- *Cryptosporidium parvum*
- *Isospora belli*
- *Cyclospora caytenensis*
- *Sarcocystis hominis and suihominis*

Morphology

It exists in 2 forms :

* Trophozoite (or vegetative form)

* Cyst (or cystic form) .

Trophozoite

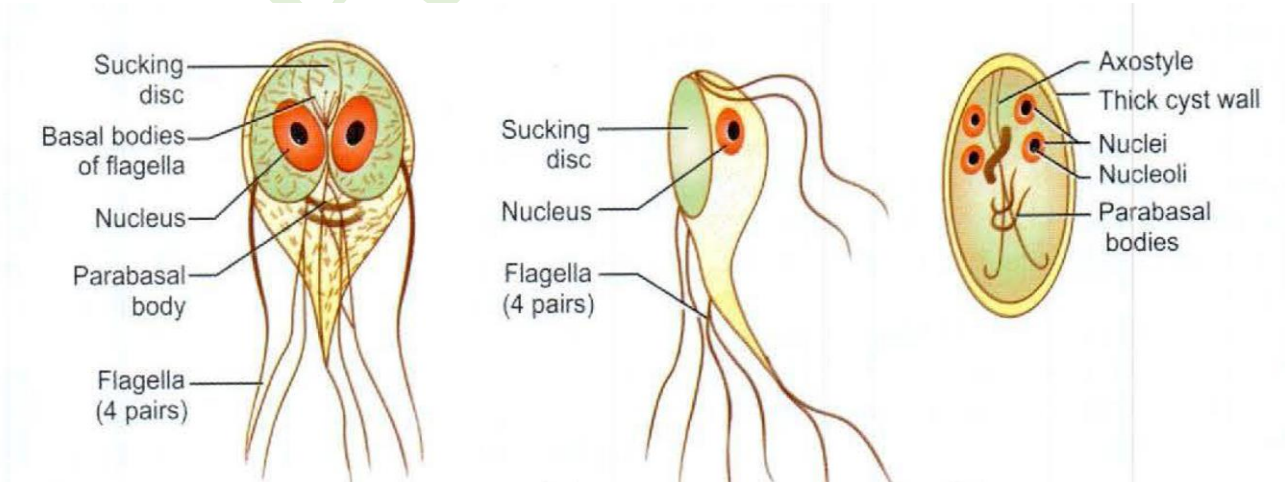
The trophozoite is in the shape of a tennis racket (**heartshaped** or **pyriform shaped**) and is rounded anteriorly and pointed posteriorly. It measures 15 µm x 9 µm wide and 4 µm thick. Dorsally, it is convex and ventrally, it has a concave sucking disc, which helps in its attachment to the intestinal mucosa. It is bilaterally symmetrical and possesses :

- 1 pair of nuclei .
- 4 pairs of flagella Blepharoplast (basal bodies) , from which the flagella arise (4 pairs) .
- Pair of axostyles , running along the midline .
- Two sausageshaped parabasal or median bodies , lying transversely posterior to the sucking disc.
- The trophozoite is motile , with a slow oscillation about its long axis , often resembling **falling leaf**.

Cyst

It is the infective form of the parasite . The cyst is small and oval (Ellipsoid) , measuring 12 µm x 8 µm and is surrounded by a hyaline cyst wall . Its internal structure includes :

- 2 pairs of nuclei grouped at one end .
- A young cyst contains 1 pair of nuclei .
- The axostyle lies diagonally , forming a dividing line within cyst wall.
- Remnants of the flagella and the sucking disc may be seen in the young cyst.



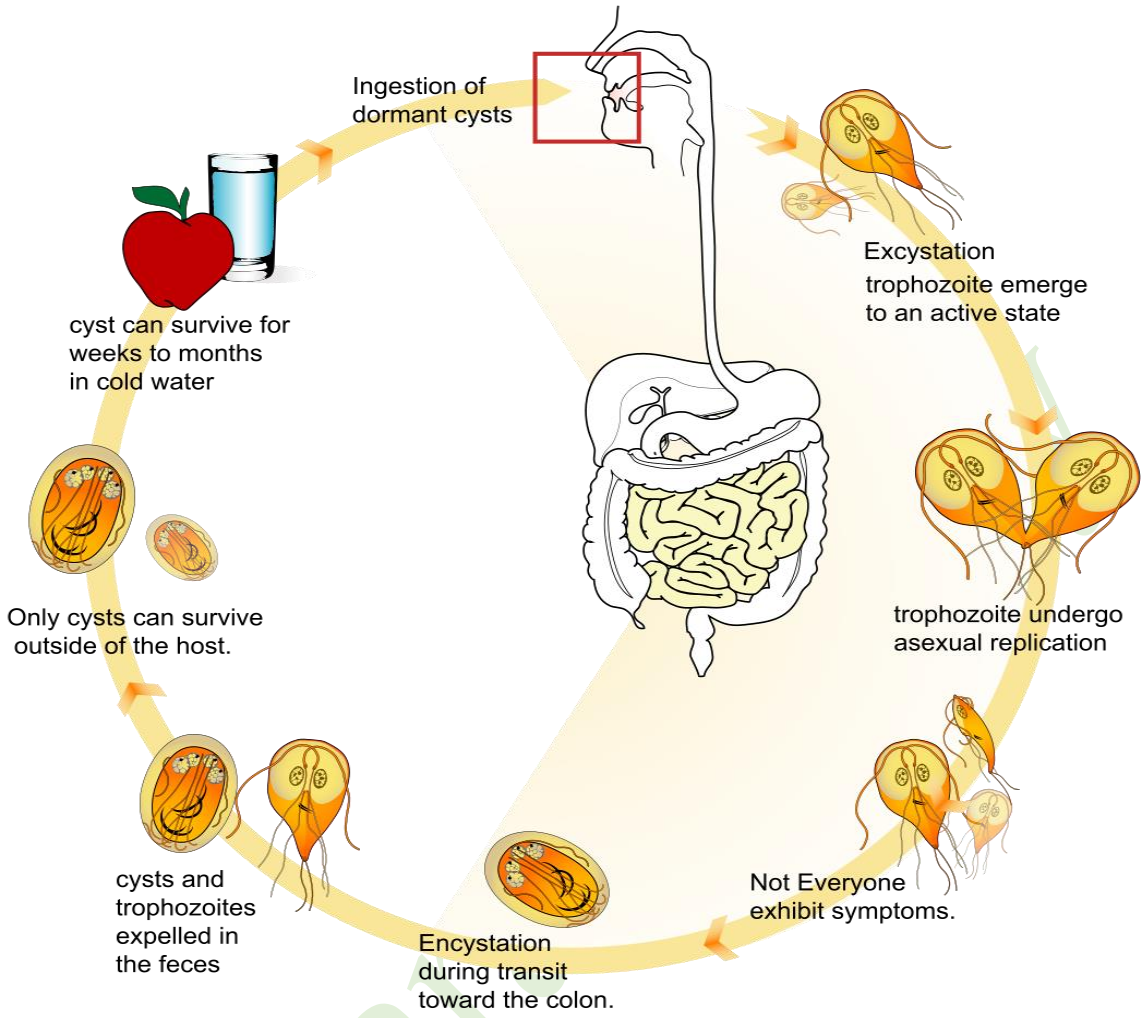
Mode of transmission:

Man acquires infection by ingestion of cysts in contaminated water and food . Direct person to person transmission may also occur in children and mentally ill persons.

Enhanced susceptibility to Giardiasis is associated with blood group A , achlorhydria , use of cannabis , chronic pancreatitis , malnutrition , immune defects such as 19A deficiency and hypogammaglobulinemia.

****Achlorhydria** and **hypochlorhydria** refer to states where the production of hydrochloric acid in gastric secretions of the stomach and other digestive organs is absent or low, respectively . It is associated with various other medical problems.

- Within half an hour of ingestion, the cyst hatches out into two trophozoites, which multiply successively by binary fission and colonize in the duodenum
- The trophozoites live in the duodenum and upper part of jejunum , feeding by pinocytosis .
- During unfavorable conditions, encystment occurs usually in colon .
- Cysts are passed in stool and remain viable in soil and water for several weeks .There may be 200,000 cysts passed per gram of feces.
- Infective dose is 10–100 cysts.



Pathogenicity and Clinical Features

G. lamblia is typically seen within the crypts of duodenal and jejunal mucosa . It does not invade the tissue , but remains tightly adhered to intestinal epithelium by means of the sucking disc.

They may cause abnormalities of villous architecture by cell apoptosis and increased lymphatic infiltration of lamina propria . *Variant specific surface proteins (VSSP) of *Giardia* play an important role in virulence and infectivity of the parasite. * Often they are asymptomatic, but in some cases, *Giardia* may lead

to mucus diarrhea , fat malabsorption (steatorrhea) , epigastric pain, and flatulence . The stool contains excess mucus and fat but no blood . *Children may develop chronic diarrhea , malabsorption of fat , vitamin A , protein , sugars like xylose disaccharides , weight loss, and **Sprue-like syndrome** (Symptoms of sprue-like enteropathy include severe, chronic diarrhea with substantial weight loss, as well as abdominal pain , fatigue , bloating , nausea , vomiting and anemia) . *Occassionally, Giardia may colonize the gall bladder , causing biliary colic and jaundice. *Incubation period is variable, but is usually about 2 weeks.

Protozoan's parasites causing diarrhea

- *Giardia lamblia*
- *Entamoeba histolytica*
- *Cryptosporidium parvum*
- *Cyclospora cayetanensis*
- *Isospora belli*

Treatment

Metronidazole (250 mg, thrice daily for 5–7 days) and **Tinidazole** (2 g single dose) are the drugs of choice.

*Cure rates with Metronidazole are more than 90%.

*Tinidazole is more effective than Metronidazole.

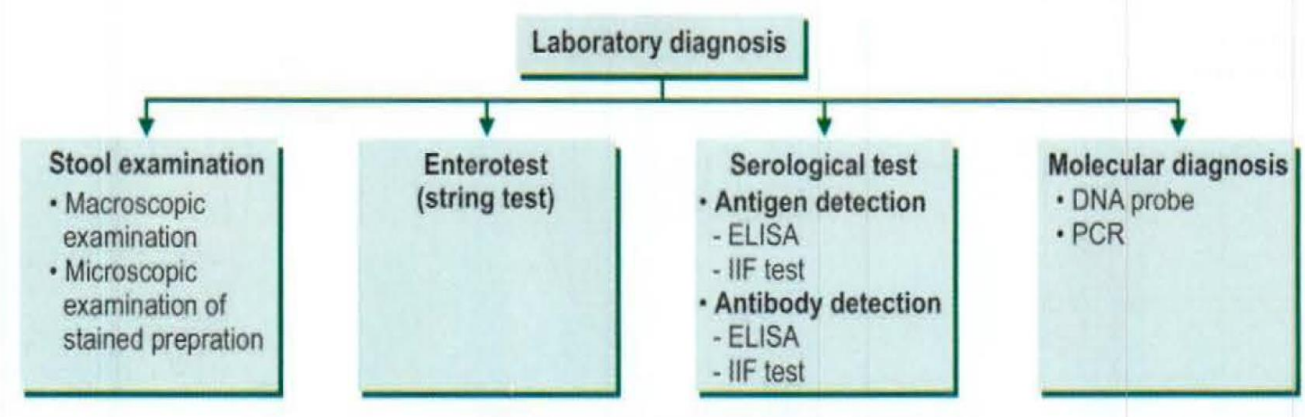
***Furuzolidone** and **Nitazoxamide** are preferred in children , as they have fewer adverse effects.

*__Parmomycin__ , an oral aminoglycoside can be given to symptomatic pregnant females.

Notes:

*****Only symptomatic cases need treatment.

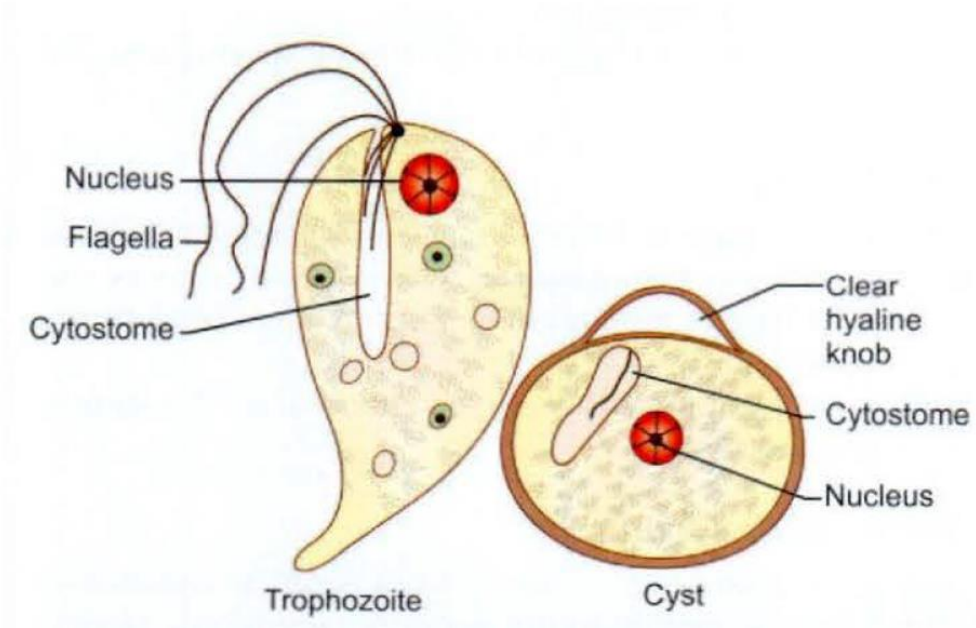
*****_Giardia_ is the only protozoan parasite found in the lumen of the human small intestine (duodenum and jejunum).

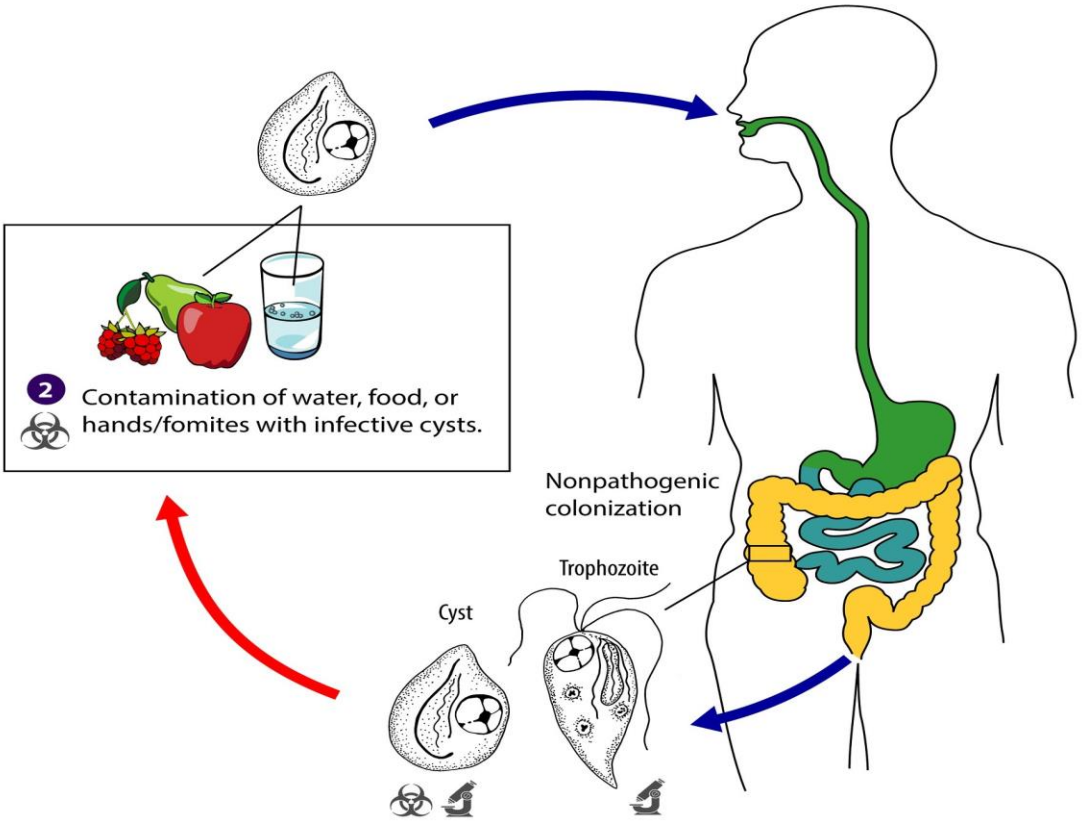


Lec. : 6

➢ ***Chilomastix mesnili***

- This occurs as trophozoites and cysts
- The trophozoite is pear-shaped measuring 5-20 μm in length and 5- 10 μm in breadth . At the anterior end, it has a spherical nucleus. A distinct spiral groove is seen on one side of the nucleus.
- The cysts are lemom-shaped having a spiral projection at the anterior end. lt measures 5-10 μm in length and 4-6 μm in breadth and is surrounded by a thick cyst wall.
- Both trophozoites and cysts are demonstrated in the semi-formed stool.
- It is a **harmless** commensal of **cecum** where the organism feeds on bacteria and food debris . Since infection is acquired through ingestion of cysts , prevention depends on improved personal hygiene .



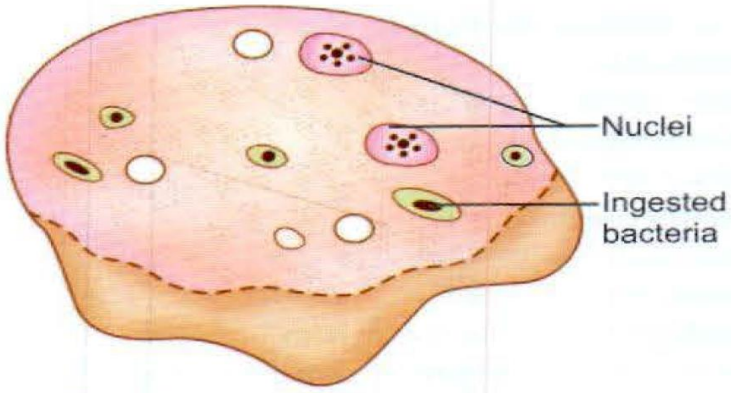


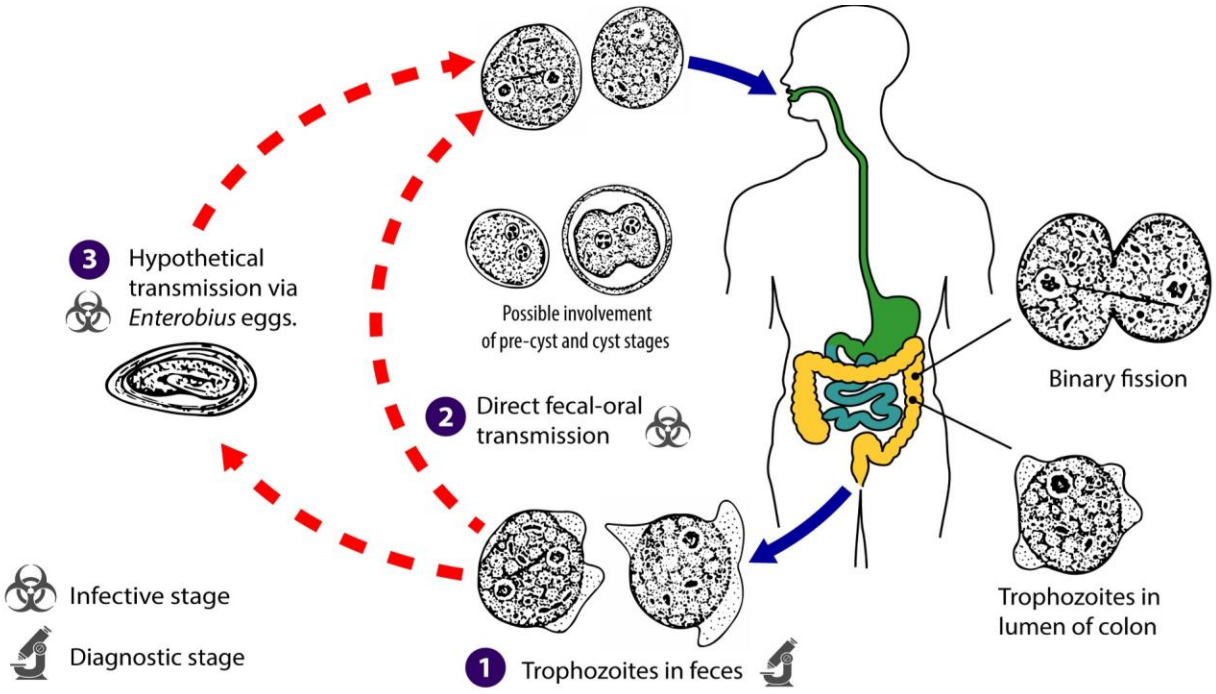
➢ ***Dientamoeba fragilis***

D. fragilis was previously considered as an amoeba but has now been reclassified as an amoeboflagellate, based one electron microscopic study and antigenic similarity to *Trichomonas* .

- It is unique as it has only trophozoite stage but no cyst stage.
- The name *Dientamoeba fragilis* is derived from the binucleate nature of trophozoite (*Dientamoeba*) and the fragmented appearance (*fragilis*) of its nuclear chromatin.
- It is seen worldwide and is reported to be the most common intestinal protozoan parasite in Canada.
- It lives in **colonic** mucosal crypts, feeding on bacteria. It does not invade tissues, but may rarely ingest red blood cells (RBCs).

- The trophozoite is 7- 12 μm in diameter. It is motile with broad hyaline leaf-like pseudopodia . **They have 1-4 nuclei ; the binucleate form being the most common . The nuclear chromatin is present as 3-5 granules in the center, with no peripheral chromatin on the nuclear membrane.**
- In the absence of cyst stage, its mode of transmission is not clear. Possibly, it is transmitted from person to person by the fecal-oral route or by the eggs of *Enterobius vermicularis* and other nematodes, which may serve as a vector.
- Formerly believed to be nonpathogenic, it has now been associated with a variety of symptoms like intermittent diarrhea, abdominal pain, flatulence, anorexia, nausea, malaise and fatigue.
- High incidence is seen among children between 2 years and 10 years of age.
- Laboratory diagnosis is made by demonstration of trophozoites in s tool. At least three s tool specimens should be collected over a period of 7 days.
- Metronidazole, Iodoquinol , Paromomycin and Tetracycline have been used for treatment .



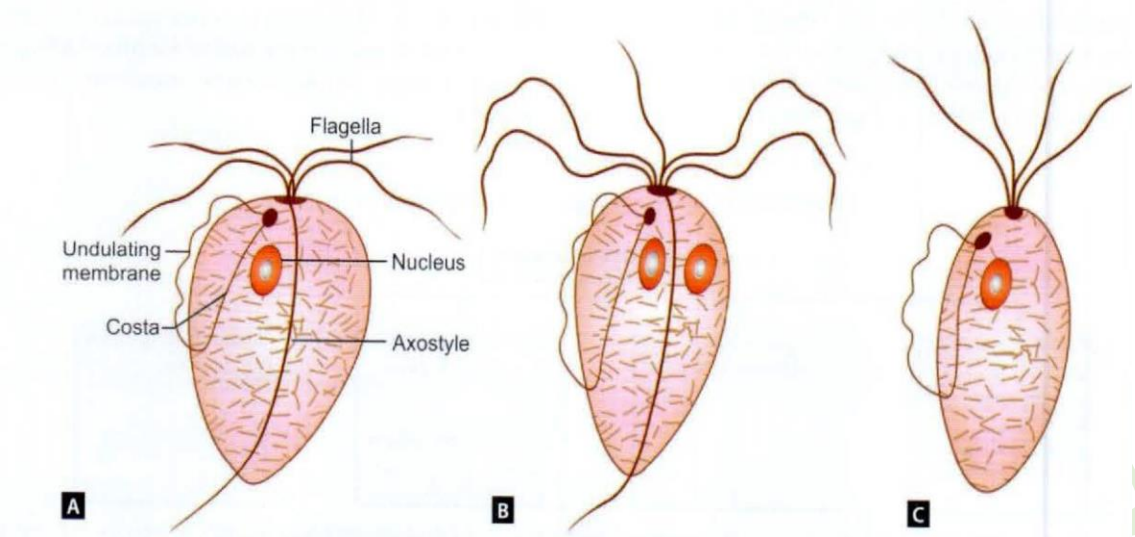


Urogenital flagellates

➢ ***Trichomonas***

Trichomonas differs from other flagellates, as they exist only in trophozoite stage. Cystic stage is not seen. Genus *Trichomonas* has three species , which occur in humans :

1. *T. vaginalis* (A)
2. *T. hominis* (B)
3. *T. tenax* (C)



➢ ***Trichomonas vaginalis***

History and distribution

T. vaginalis was first observed by Donne (1836) in vaginal secretion. Prevalence of Trichomoniasis varies from 5% patients at hospitals to 75% in sexual workers.

Morphology

It is pear-shaped or ovoid and measures 10-30 μm in length and 5-10 μm in breadth with a short undulating membrane reaching up to the middle of the body .

- It has four anterior flagella and fifth running along the outer margin of the undulating membrane, which is supported at its base by a flexible rod (costa)
- A prominent axostyle runs throughout the length of the body and projects posteriorly like a tail.
- the cytoplasm shows prominent siderophilic granules, which are most numerous alongside the axostyle and costa.
- It is motile with a rapid jerky or twitching type movement.

Habitat

In females, it lives in vagina and cervix and may also be found in Bartholin 's glands , urethra and urinary bladder . in males, it occurs mainly in the anterior urethra , but may also be found in the prostate and preputial sac .

Life cycle

Life cycle of *T. vaginalis* is completed in a single host either male or female.

Mode of transmission

- The trophozoite cannot survive outside and so infection has to be transmitted directly from person-to-person . Sexual transmission is the usual mode of infection .
- Babies may get infected during birth.
- **Trichomoniasis** often coexists with other sexually transmitted diseases like Candidiasis , Gonorrhea , Syphilis or human immunodeficiency virus (HIV).
- Vaginal pH of more than 4.5 facilitates infection.
- Fomites such as towels have been implicated in transmission.
- Trophozoites divide by binary fission.
- As cysts are not formed, the trophozoite itself is the infective form.
- Incubation period is roughly 10 days.

Pathogenesis

T. vaginalis particularly infects squamous epithelium and not columnar epithelium. It secretes cysteine proteases, adhesins , lactic acid and acetic acid, which disrupt the glycogen levels and lower the pH of the vaginal fluid .

- It is an obligate parasite and cannot live without close association with the vaginal, urethral, or prostatic tissues.

- Parasite causes petechial hemorrhage and mucosal capillary dilation (strawberry mucosa), metaplastic changes and desquamation of the vaginal epithelium .

- Intracellular edema and so called chicken-like epithelium, is the characteristic feature of Trichomoniasis.

Clinical features

Infection is often asymptomatic, particularly in males, although some may develop urethritis, epididymitis and prostatitis.

- In females, it may produce severe pruritic vaginitis with an offensive, yellowish green, often frothy discharge, dysuria and dyspareunia . Cervical erosion is common . Endometritis and pyosalpingitis are infrequent complications.

- Rarely, neonatal pneumonia and conjunctivitis have been reported in infants born to infected mothers.

- The incubation period of trichomoniasis is 4 days to 4 weeks.

Treatment

Simultaneous treatment of both partners is recommended as it is an STD.

- Metronidazole 2 g orally as a single dose or 500 mg orally twice a day for 7 days is the drug of choice.

- In patients not responding to treatment with standard regime, the dose of metronidazole may be increased or it may be administered parenterally.

- In pregnancy, metronidazole is safe in 2^{nd} and 3^{rd} trimesters.

➢ ***Trichomonas hominis***

T. hominis measures 8- 12 μm, pyriform-shaped, and carries five anterior flagella and an undulating membrane that extends the full length of the body.

- It is a very harmless commensal of the cecum.

- Microscopic examination of stool will reveal motile trophozoite of *T. hominis* .

- Transmission occurs in trophic form by fecal-oral route .

➢ ***Trichomonas tenax***

T. tenax , also known as *T. buccalis* , is a harmless commensal which lives in mouth, in the periodontal pockets , carious tooth cavities and, less often, in tonsillar crypts.

- It is smaller(5-10 μm) than *T. vaginalis* .
- It is transmitted by kissing , through salivary droplets and fomites. There are sporadic reports of its involvement in respiratory infections and thoracic abscesses .
- Better oral hygiene rapidly eliminates the infection and no therapy is indicated.

Lec. : 7

Hemoflagellate

Including genus : *Leishmania* and *Trypanosoma* (blood tissue species) : There are four morphological forms of clinical significance associated with thehemoflagellates:

- Amastigote .
- Promastigote .
- Epimastigote .
- Trypomastigote .

General characteristics

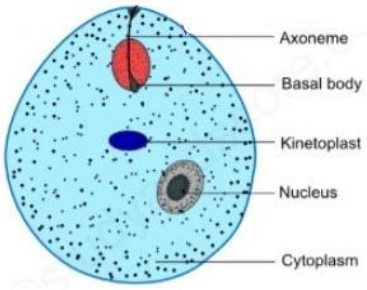
1. They live in the blood and tissues of man and other vertebrate hosts and in the gut of the insect vector.
2. Members of this family have a single nucleus, a kinetoplast and a single flagellum.
3. Nucleus is round or oval and is situated in the central part of the body.
4. Kineloplast consists of a deeply staining parabasal body and adjacent dot-Like blepharoplast (pasal body) .
5. The parabasal body and blepharoplast are connected by one or more thin fiber .
6. Flagellum is a thin, hair-like structure, which originate from the blepharoplast.

7. The portion of the flagellum ,which is inside the body of the parasite and extends from the blepharoplast to surface of the body is known as axoneme .
8. A free flagellum at the anterior end traverses on the surface of the parasite as a narrow undulating membrane.
9. Hemoflagellates exist in two or more of four morphological stages.

The transmission of hemoflagellates

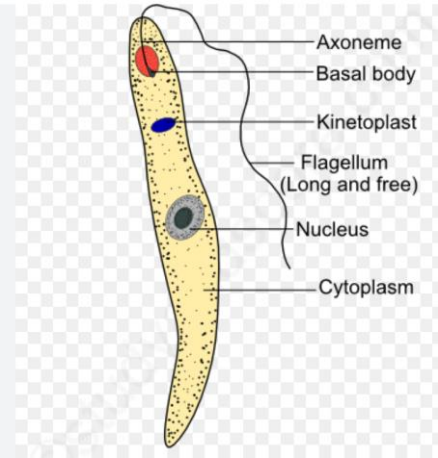
Is accomplished by the bite of an arthropod vector. Flagellate protozoa found in blood or tissues of human and there are two genera of medical importance (*Leishmania* & *Trypanosoma*) . The major difference between these two genera is that primary diagnostic form found in *Leishmania* is the Amastigote , whereas that of *Trypanosoma* is the Trypomastigote

1. Amastigotes: It is Roundish to oval in shape, Consist of a nucleus and kinetoplast. The large single nucleus is typically located off-center . The dotlike blepharoplast is attached to a small axoneme, this axoneme extends to the edge of the organism .The single parabasal body is located adjacent to the blepharoplast.



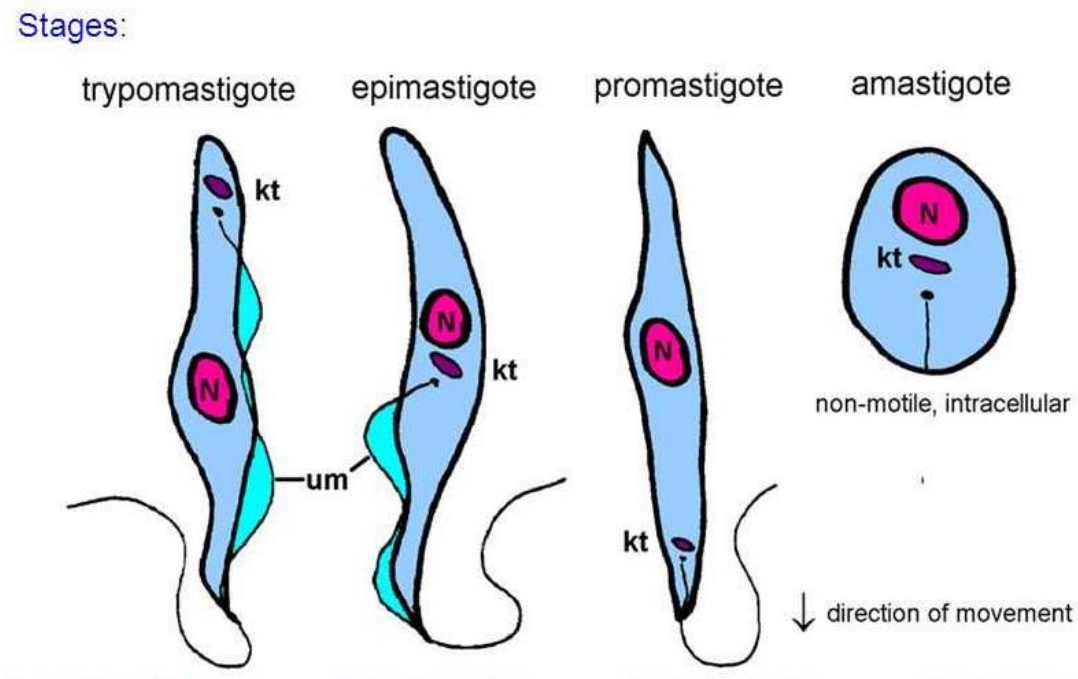
2. Promastigotes: It is Long and slender in appearance. The large single nucleus is located in or near the center .The kinetoplast is located in the

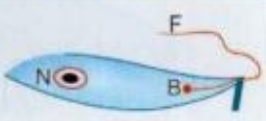
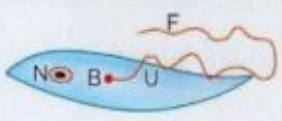
anterior end of the organism .A single free flagellum extends anteriorly from the axoneme .



3. Epimastigotes : It is long and slightly wider than promastigote form . The large single nucleus is located in posterior end . The kinetoplast located anterior to the nucleus . Undulating membrane extending half of the body length .A single free flagellum extends anteriorly from the axoneme.

4. Trypomastigotes: It is C or U shape in stained blood films . Long and slender in appearance .One nucleus located anterior to the kinetoplast .The kinetoplast is located in the posterior end of the organism .Undulating membrane extending entire body length .A single free flagellum extends anteriorly from the axoneme when present



	Amastigote	*Promastigote*	*Epimastigote*	*Trypomastigote*
Morphological characteristics	Rounded or ovoid, without any external flagellum. The nucleus, kinetoplast and axial filaments can be seen. The axoneme extends up to the anterior end of the cell	Lanceolate in shape. Kinetoplast is anterior to the nucleus (antinuclear kinetoplast) near the anterior end of the cell, from which flagellum emerges. There is no undulating membrane	Elongated, with the kinetoplast placed more posteriorly, though close to and in front of the nucleus (juxtanuclear kinetoplast). The flagellum runs alongside the body as a short undulating membrane, before emerging from the anterior end	This stage is elongated, spindle-shaped with a central nucleus. The kinetoplast is posterior to the nucleus (postnuclear kinetoplast) and situated at the posterior end of the body. The flagellum runs alongside the entire length of the cell to form a long undulating membrane before emerging as a free flagellum from the anterior end
Seen in	*Trypanosoma cruzi* and *Leishmania* as intracellular form in vertebrate host	It is the infective stage of *Leishmania*, found in the insect vector as well as in cultures *in vitro*	It is the form in which *Trypanosoma brucei* occur in salivary gland of the vector tsetse fly and *Trypanosoma cruzi* in the midgut of the vector reduviid bug. *Note:* This stage is lacking in *Leishmania*	This is the infective stage of trypanosomes found in arthropod vector and in the blood of infected vertebrate. *Note:* This stage is lacking in *Leishmania*
Schematic illustration				

Morphological form of Hemofagellate

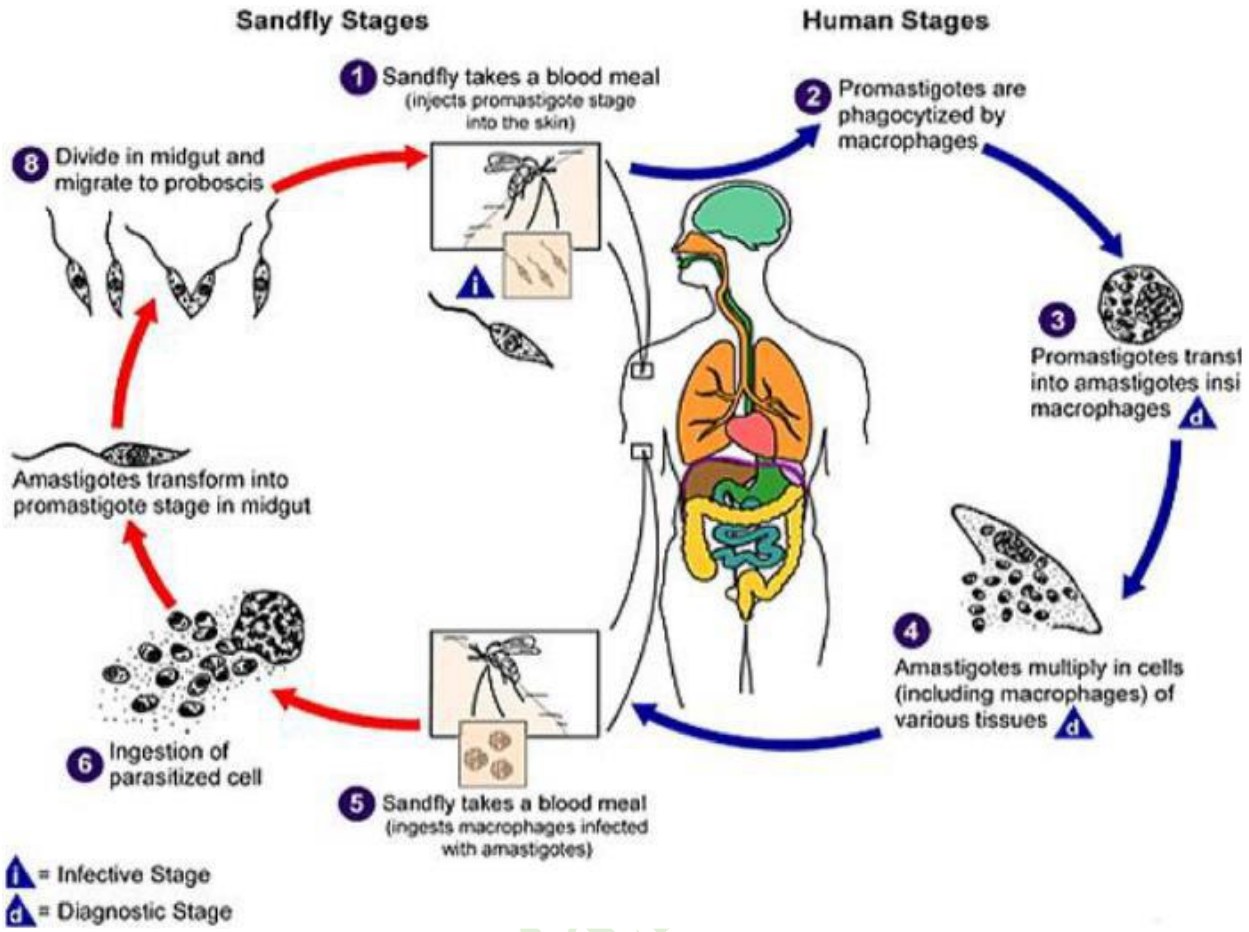
Genus *Leishmania*

Leishmaniasis : There are many different species of *Leishmania* and the disease that they cause . species of *Leishmania* which are pathogenic for man :

- *L. donovani* causes Visceral Leishmaniasis (Kala-Azar, Black fever , Dum Dum fever)
- *L. tropica* cause Cutaneous Leishmaniasis (Oriental sore , Delhi ulcer , Aleppo , Delhi or Baghdad boil)
- *L. braziliensis* (also, *L. mexicana* & *L. peruviana*) are etiologic agents of Mucocutaneous Leishmaniasis .

Life Cycle

Leishmania are transmitted by arthropod . In this case it is a small biting fly known as a **Sand Fly** . *Leishmania* spend part of their life cycle in the gut of the sand fly , but their life cycle is completed in a vertebrate host. Within the sand fly gut, the protozoa are carried as extracellular promastigotes , these parasites multiply in the gut and migrate toward the pharynx . Sandflies transferred promastigotes to the vertebrate host when the sand fly takes a meal blood by expelling *Leishmania* into the bite wound of the mammalian host. From where they pass into the blood and tissues of the human host.



Life Cycle of *Leishmania* spp.

Pathogenesis and clinical features

Leishmaniasis is a parasitic disease caused by several species of genus . Different species of *Leishmania* cause different disease.

A. ***L. donovani*** causes Visceral Leishmaniasis also called Kala-Azar & Dum Dum fever . cause spleenomegaly & hepatomegaly the infection is generalized and the parasite is distributed in the internal organs . The parasite may also cause a variety of skin lesions (Dermal Leishmaniasis) without any visceral manifestations.

B. ***L. tropica*** : causes Cutaneous Leishmianiasis , tropic sore or baghdad boil, oriental sore . The infection is limited to a local lesion of the skin and subcutaneous tissues.

C. ***L. brasiliensis*** causes Mucocutaneous Leishmaniasis or Espundia. The infection is limited to a local lesion of the skin but may metastasis to other areas of skin and oro-nasal mucosa .The primary lesion often disappears spontaneously, followed by mucocutaneous lesions that destroy the mucosal surface of the nose, pharynx, and larynx. If the condition is untreated, potentially fatal secondary bacterial infections and disfigurement may occur.

Treatment

Pharmacologic therapies include the following:

- Pentavalent antimony (sodium stibogluconate or meglumine antimonate): Used in Cutaneous Leishmaniasis
- Liposomal amphotericin B (AmBisome): Effective against pentavalent antimony−resistant Mucocutaneous disease and Visceral Leishmaniasis