

جامعة الانبار

كلية : الصيدلة

قسم : الصيدلة

اسم المادة باللغة العربية: الطفيليات الطبية

اسم المادة باللغة الإنكليزية: **Medical Parasitology**

المرحلة: الثانية

التدريسي: أ.م.د. مهند عبدالمجيد محمد

عنوان المحاضرة باللغة العربية: النسيج العصبي

عنوان المحاضرة باللغة الإنكليزية: **Intestinal protozoa (Amoeba)**

محتوى المحاضرة:

Intestinal protozoa (Amoeba)

Features of protozoa:

- ✓ Protozoa (from the Greek words *proto*, meaning "first", and *zoa*, meaning "animals") is a grouping of eukaryotes many of which are motile.
- ✓ The most important protozoans range usually from 10 to 52 micrometers, but can grow as large as 1 mm, and are seen easily by microscope
- ✓ They move by a variety of organs pseudopodia, flagella and cilia.
- ✓ They are found in different parts of the body intestine, blood and tissues, cavities like mouth, Uro-genital system.
- ✓ Most intestinal protozoa have direct life cycle. They have either: Trophozoit and cyst or Trophozoit only.

Life stages of intestinal Protozoa

A- Cyst stage: It is non motile, none feeding, non active stage, it is the infective stage if the parasite has trophozoite and cyst stage in the life cycle.

It is diagnostic stage in case of chronic infection. Transmitted to the human by contamination of food and water.

B- Trophozoite stage: It is active, motile, feeding stage of parasite. *it is the pathogenic stage of parasite. *it is the diagnostic stage in case of acute infection.

C- Or It has only Trophozoite without cyst stage:

(Trophozoite stage will be the pathogenic, diagnostic and infective stage if the parasite has no cyst stage).

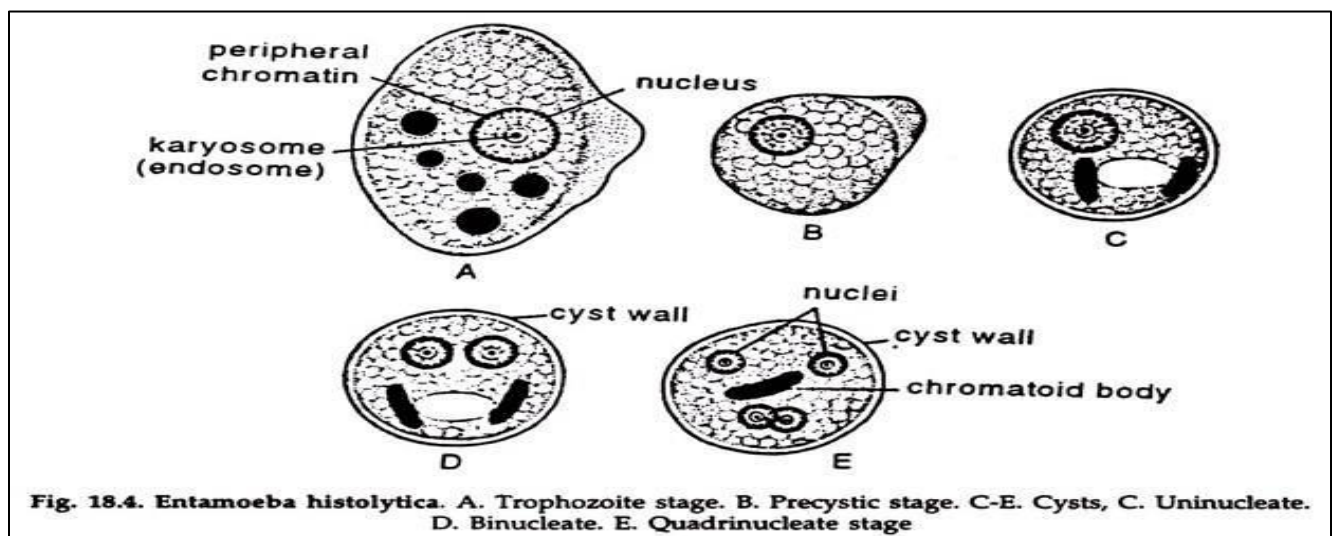


Fig. 18.4. *Entamoeba histolytica*. A. Trophozoite stage. B. Precystic stage. C-E. Cysts, C. Uninucleate. D. Binucleate. E. Quadrinucleate stage

1.1 Pathogenic Intestinal Amoeba: AMOEBIASIS

Intestinal Protozoa Amoeba: *Entamoeba histolytica*

Diseases: *Entamoeba histolytica* causes amebic dysentery and liver abscess.

Important Properties: The life cycle has two stages: the motile (trophozoite) the nonmotile (cyst)

These cysts are killed by boiling but not by chlorination of water supplies.

They are removed by filtration of water.

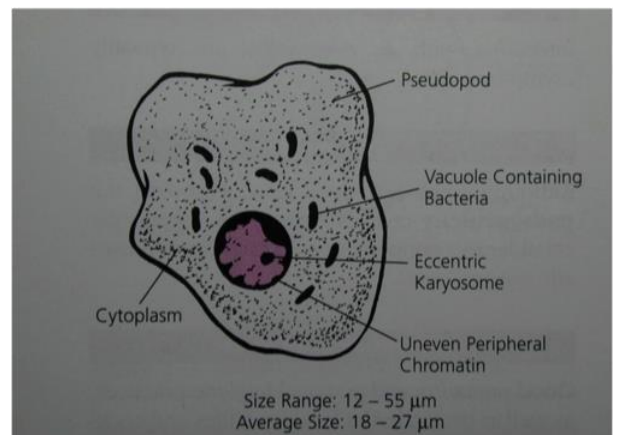
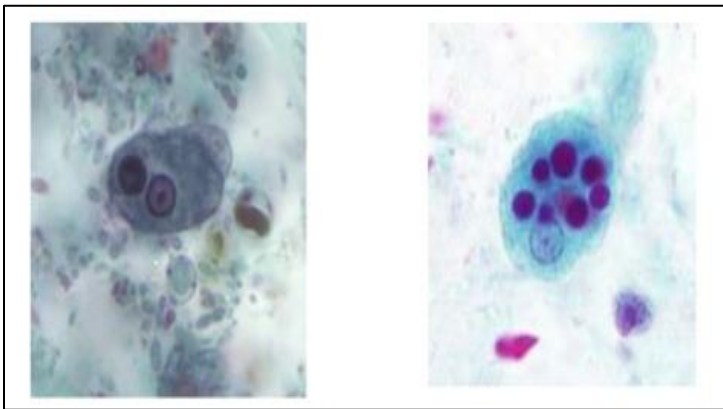
The mature trophozoite has a single nucleus with lining of peripheral chromatin and a central nucleolus (karyosome).

The cyst has four nuclei, an important diagnostic criterion

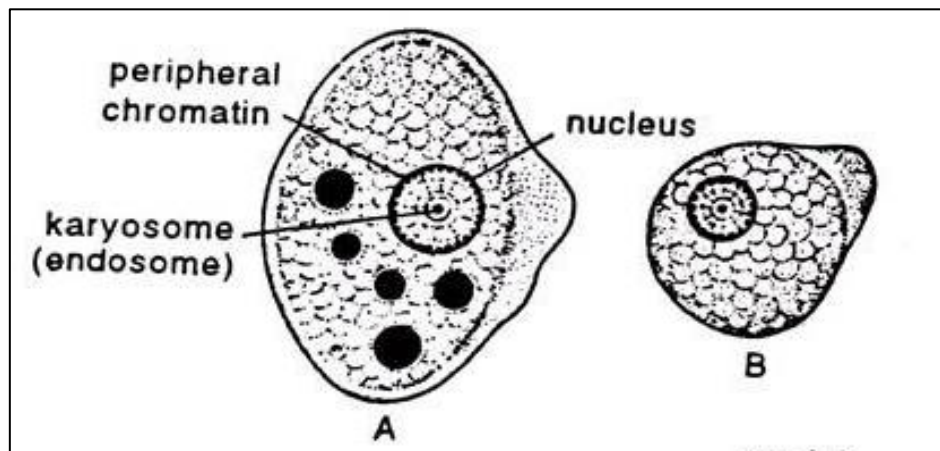
Entamoeba histolytica:

Morphological features

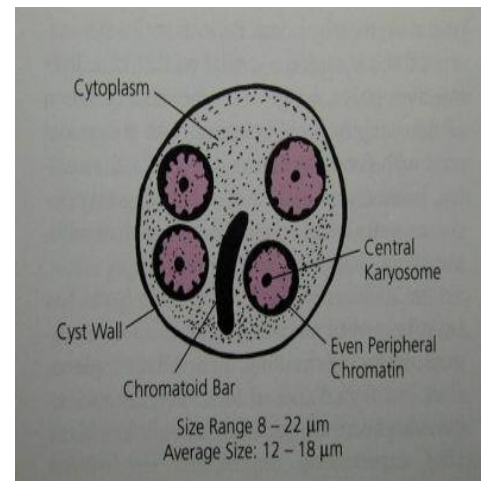
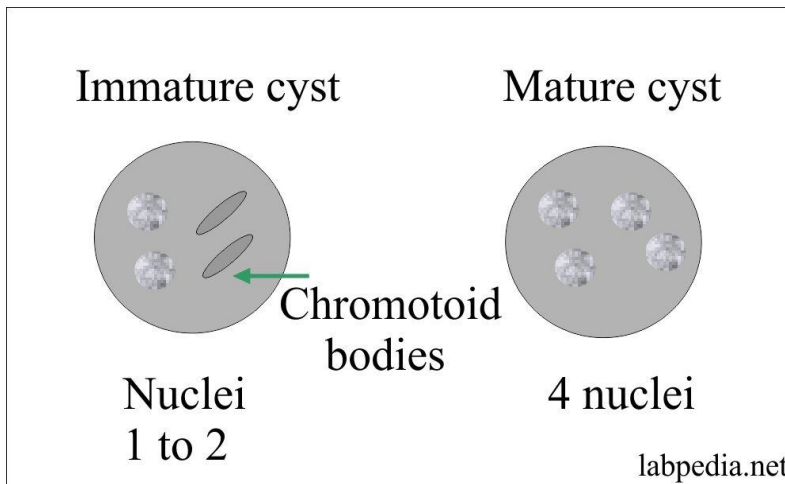
a- Trophozoites: Viable trophozoites vary in size from about 10-60 μ m in diameter. Motility is rapid, progressive, and unidirectional, through pseudopods. The nucleus is characterized by evenly arranged chromatin on the nuclear membrane and the presence of a small, compact, centrally located karyosome.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



(b)- Pre Cyst: Smaller to trophozoite but larger to cyst (10-20 μ m), oval with blunt pseudopodia. Food vacuoles and RBCs are disappear. Single rounded nucleus, absence of digested materials and lack of a cyst wall.



(c)- **Cyst:** Cysts range in size from 10-20µm. The immature cyst has inclusions namely; glycogen mass and chromatoidal bars. As the cyst matures, the glycogen completely disappears; the chromatoidals may also be absent in the mature cyst. Mature *Entamoeba histolytica* cysts have 4 nuclei that characteristically have centrally-located karyosomes and fine, uniformly distributed peripheral chromatin.



Info before the life

cycle:

- ✓ **Habitat:** Trophozoite in:
 - 1-Large intestine
 - 2- Extraintestinal infection. Cyst only in large intestine Life cycle: direct no intermediate host.
- ✓ **Infective stage:** Is mature quadrinucleated cyst.
- ✓ **Pathogenic stage:** Only Trophozoite which seen in diarrheic acute dysentery stool.
- ✓ **Diagnostic stage:** Cyst in chronic infection and trophozoite in acute diarrhic infection.
- ✓ **Mode of infection:** Contamination of food and water. Humans are the principal host, although dogs, cats and rodents may be infected

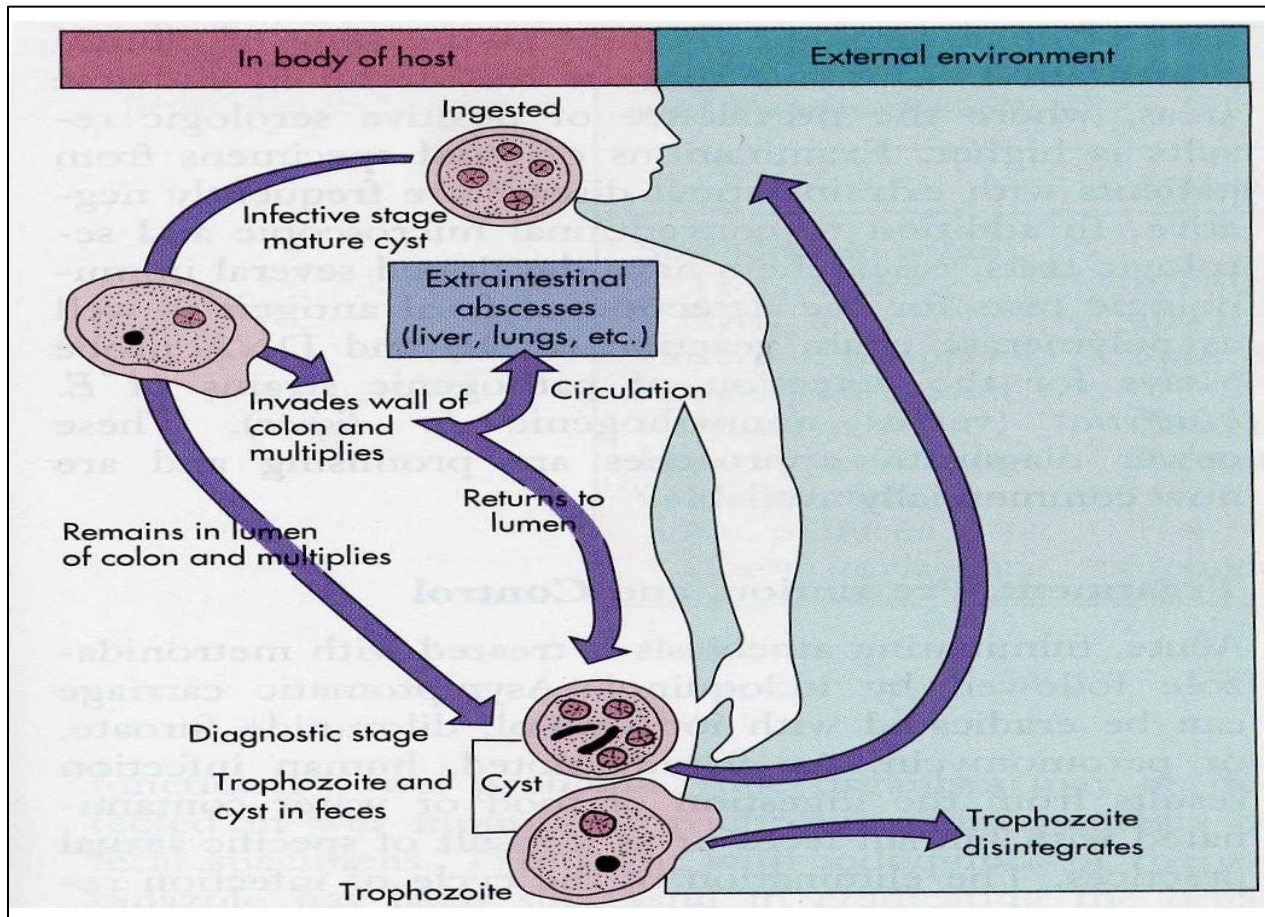
Life cycle of *Entamoeba histolytica*:

Intestinal infections occur through the ingestion of a mature quadrinucleate infective cyst, contaminated food or drink and also by hand to mouth contact. It is then passed unaltered through the stomach, as the cyst wall is resistant to gastric juice.

In terminal ileum (with alkaline pH), excystation takes place. Trophozoites being actively motile invade the tissues and ultimately lodge in the submucous layer of the large bowel. Here they grow and multiply by binary fission.

Trophozoites are responsible for producing lesions in amoebiasis. Invasion of blood vessels leads to secondary extra intestinal lesions. Gradually the effect of the parasite on the host is toned down together with concomitant increase in host tolerance, making it difficult for the parasite to continue its life cycle in the trophozoite phase.

A certain number of trophozoites come from tissues into lumen of bowel and are first transformed into pre-cyst forms. Pre-cysts secrete a cyst wall and become a uninucleate cyst. Eventually, mature quadrinucleate cysts form. These are the infective forms. Both mature and immature cysts may be passed in faeces. Immature cysts can mature in external environments and become infective.



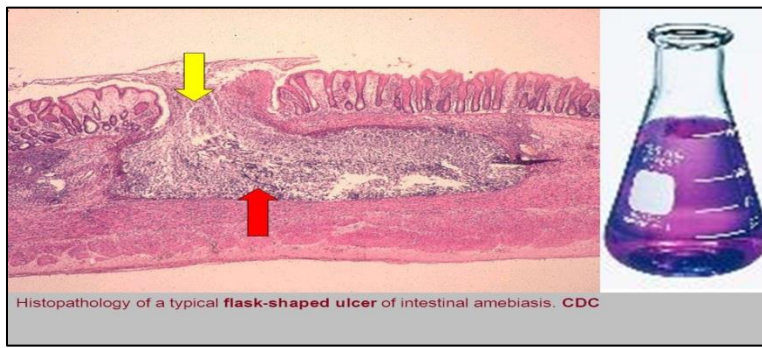
Reproduction of *E. histolytica*

The Reproduction occurs in three stages:

- **Excystation**
 - **Encystation**
 - **Multiplication**
- **Excystation:** This is the process of transformation of cyst to trophozoites. During excystation a quadrinucleate cyst give rise to eight amoebulae each one of which is being capable of developing into trophozoites.
 - **Encystation:** This is the process of transformation of trophozoite to cyst and occurs inside the lumen of the intestine of an infected individual.
 - **Multiplication:** This occurs only in the trophozoite forms of the entamoeba histolytica , growing and multiplication takes place inside the tissue. Reproduction of trophozoites occurs by simple binary fission.

Pathogenesis & Epidemiology:

1. The organism is acquired by ingestion of cysts that are transmitted by the fecal-oral route in contaminated food and water.
2. The ingested cysts differentiate into trophozoites in the ileum but tend to colonize the cecum and colon.
3. The trophozoites invade the colonic epithelium and secrete enzymes that cause localized necrosis. As the lesion reaches the muscularis layer, a typical "**flask-shaped**" ulcer forms.
4. Progression into the submucosa leads to invasion of the portal circulation by the trophozoites. By far the most frequent site of systemic disease is the liver, where abscesses containing trophozoites form.



Clinical Findings:

1. Acute amebiasis presents as dysentery (i.e., bloody, mucus-containing diarrhea) accompanied by lower abdominal discomfort, flatulence, and tenesmus.
2. Chronic amebiasis: diarrhea, weight loss, and fatigue also occur. Roughly 90% of those infected have asymptomatic infections, but they may be carriers, whose feces contain cysts that can be transmitted to others. In some patients, a granulomatous lesion called an ameboma may form in the cecal or rectosigmoid areas of the colon. These lesions can resemble an adenocarcinoma of the colon.
3. Amebic abscess of the liver is characterized by right-upper-quadrant pain, weight loss, fever, and a tender, enlarged liver. Right-lobe abscesses can penetrate the diaphragm and cause lung disease.

Immunity:

E.histolytica elicits both the humeral and cellular immune responses, but it is not yet clearly defined whether it modulates the initial infection or prevents reinfection.

Laboratory Diagnosis:

1. Intestinal amebiasis: finding either trophozoites in diarrheal stools or cysts in formed stools. Diarrheal stools should be examined within 1 hour of collection. Trophozoites contain ingested red blood cells, because cysts are passed intermittently.

-E. histolytica can be distinguished by two criteria:

(1) The nature of the nucleus of the trophozoite (has a small central nucleolus and fine chromatin granules along the border of the nuclear membrane). (2) The second is cyst size and number of its nuclei.

2. A complete examination for cysts includes a wet mount in saline, an iodine-stained wet mount, and a fixed, trichrome-stained preparation. These preparations are also helpful in distinguishing amebic from bacillary dysentery.

3. Serologic testing ex. indirect hemagglutination test.

4. Detects nucleic acids of the organism in a PCR-based assay.

Treatment:

Acute, fulminating amebiasis is treated with metronidazole followed by iodoquinol, and asymptomatic carriage can be eradicated with iodoquinol, diloxanide furoate, or paromomycin. The cysticidal agents are commonly recommended for asymptomatic carriers who handle food for public use.

Metronidazole, chloroquine, and diloxanide furoate can be used for the treatment of extra intestinal amoebiasis.

Prevention:

1. Avoiding fecal contamination of food and water. **2.** Good personal hygiene such as hand washing. **3.** Purification of water supplies. **4.** In areas of endemic infection, vegetables should be cooked.

1.2 Nonpathogenic Intestinal Amoeba:

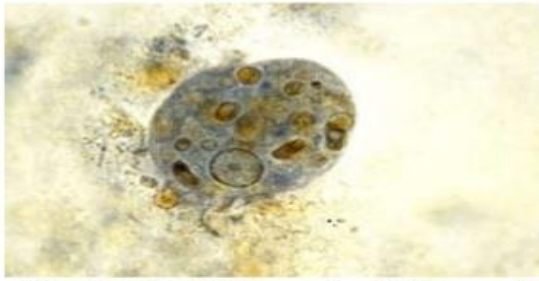
Most of these amoebae are commensal organisms that can parasitize the human gastrointestinal tract.

Entamoeba coli the life cycle stages include; trophozoite, precyst, cyst, metacyst, and metacystic trophozoite. Typically the movements of

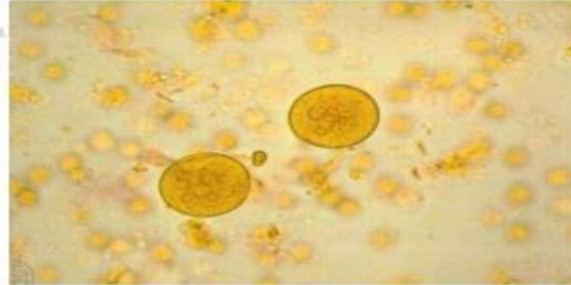
trophozoites are sluggish, with broad short pseudopodia and little locomotion, but at a focus the living specimen cannot be distinguished from the active trophozoite of *E.histolytica*. However, the cysts are remarkably variable in size. *Entamoeba coli* are transmitted in its viable cystic stage through faecal contamination. *E.coli* as a lumen parasite is non-pathogenic and produces no symptoms. The mature cyst (with more than four nuclei 8-nuclei) is the distinctive stage to differentiate *E.coli* from the pathogenic *E.histolytica*. Specific treatment is not indicated since this amoeba is non-pathogenic. The presence of *E.coli* in stool specimen is evidence for faecal contamination. Prevention depends on better personal hygiene and sanitary disposal of human excreta.

Trophozoites of *Entamoeba coli* usually measure 15–50 µm. The **trophozoites** have a single nucleus with a characteristically large, eccentric karyosome and coarse, irregular peripheral chromatin. The cytoplasm is usually coarsely granular and vacuolated (often described as “dirty” cytoplasm).

Cysts of *Entamoeba coli* are usually spherical but may be elongated and measure 10–35 µm. Mature **cysts** typically have 8 nuclei but may have as many as 16 or more. *Entamoeba coli* is the only *Entamoeba* species found in humans that has more than four nuclei in the **cyst** stage.



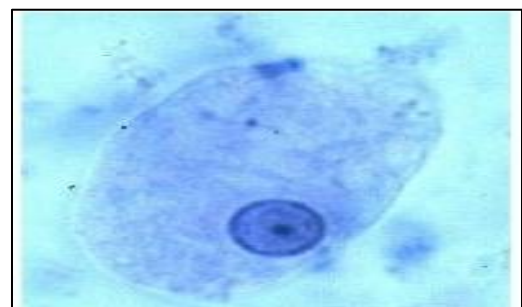
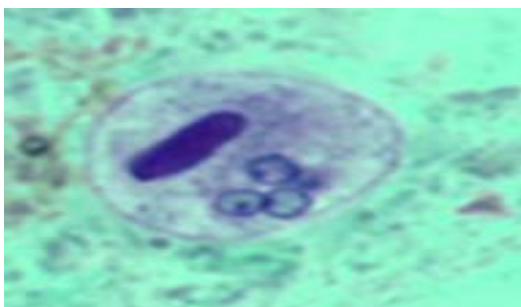
Trophozoite of *Entamoeba Coli* :
(iron hematoxylin stain)



Cysts of *Entamoeba Coli* :
(iodine mount)

Entamoeba dispar: A common noninvasive parasite, is indistinguishable in its cysts and trophozoite forms from *Entamoeba histolytica*, the cause of invasive amebiasis, by microscopy.

Trophozoites in trichrome stained smears usually measure 15 to 20 μm . Presence of one nucleus with evenly arranged chromatin on the nuclear membrane and a small, centrally located karyosome are morphological features of trophozoites. The cytoplasm is finely granular and few ingested bacteria or debris may be present. Presence of red blood cells within the cytoplasm of trophozoites is a diagnostic feature for the identification of *E. histolytica*. Ingested RBCs are not frequently seen; in the absence of this diagnostic characteristic *E. histolytica*/*E. dispar* should be reported. Cysts usually measure 12 to 15 μm and have 1 to 4 nuclei. Chromatoid bodies with bluntly rounded ends may also be present.



Cyst

Trophozoites

Entamoeba hartmanni in all of its life-cycle stage, *E.hartmanni* resembles *E.histolytica* except in size, yet there is a slight overlap in the size range. The trophozoites do not ingest red blood cells, and their motility is generally less vigorous than that of *E.histolytica*. As in other amebae, infection is acquired by ingestion of food or water contaminated with cyst-bearing faeces. Identification is based on examination of small amebae in unstained or iodine-stained preparations. Usually no treatment is indicated, measures generally effective against faecal-borne infections will control this amoebic infection.

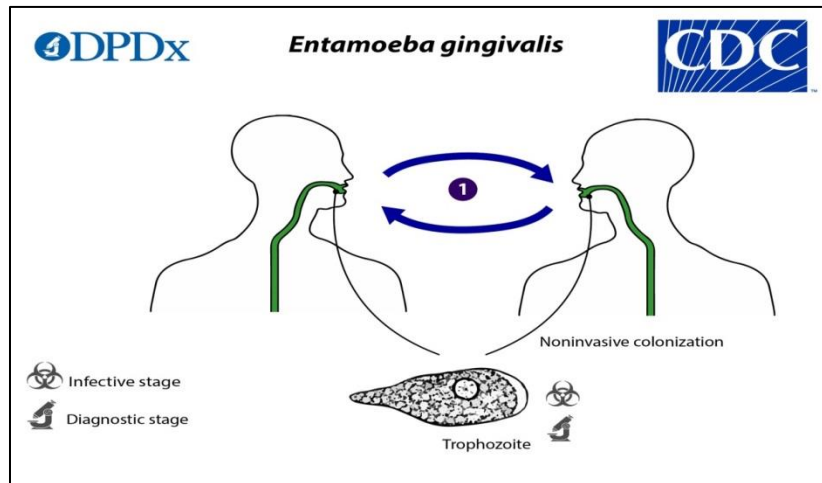
Endolimax nana: Is a lumen dweller in the large intestine, primarily at the cecal level, where it feeds on bacteria. The life cycle is similar to *E.histolytica*. Motility is typically sluggish (slug-like) with blunt hyaline pseudopodia, Projects shortly. Human infection results from ingestion of viable cysts in polluted water or contaminated food. Typical ovoid cysts of *E.nana* are confirmative. Rounded cysts and living trophozoites are often confused with *E.hartmanni* and *E.histolytica*. No treatment is indicated for this nonpathogenic infection. Prevention can be achieved through personal cleanliness and community sanitation.

Iodamoeba buetschlii: The natural habitat is the lumen of the large intestine, the principal site probably being the caecum. The trophozoite feeds on enteric bacteria; it is a natural parasite of man and lower primates. It is generally regarded as a nonpathogenic lumen parasite. No treatment is

ordinarily indicated. Prevention is based on good personal hygiene and sanitation in the community.

Entamoeba gingivalis: Only the trophozoite stage presents, and encystation probably does not occur. *E.gingivalis* is a commensal, living primarily on exudate from the margins of the gums, and thrives best on unhealthy gums. No specific treatment is indicated. However the presence of *E.giingivalis* suggests a need for better oral hygiene. The infection can be prevented by proper care of the teeth and gums.

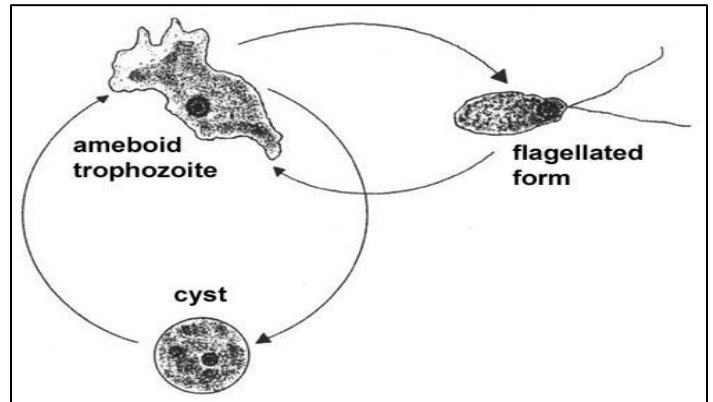
1.3.



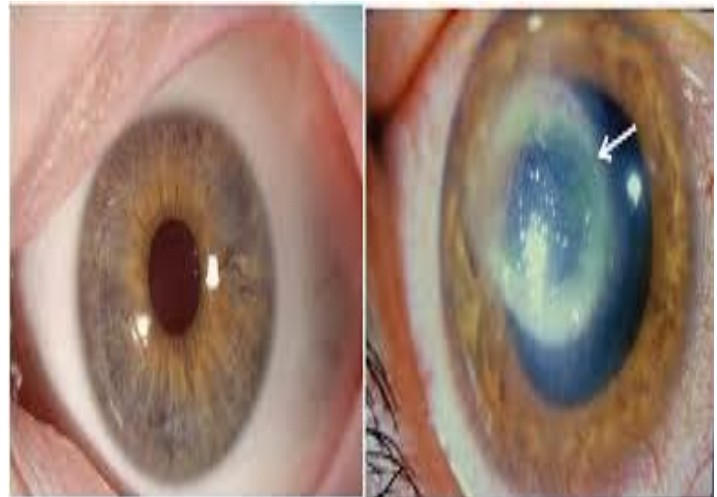
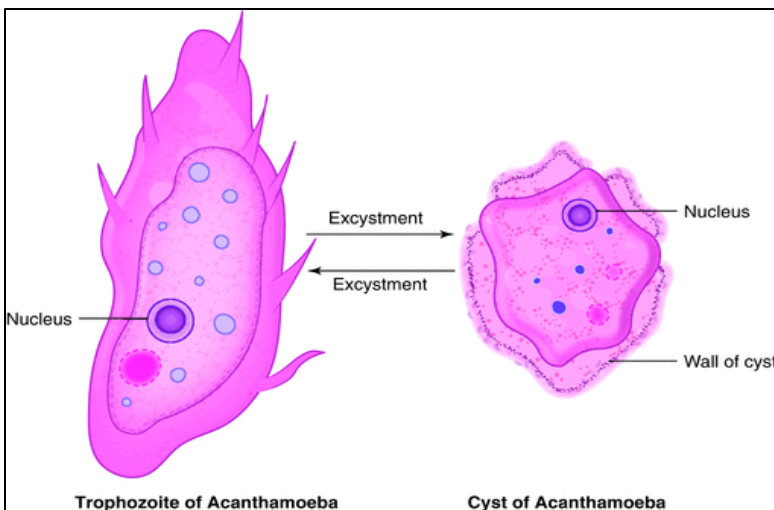
PATHOGENIC FREE-LIVING AMOEBAE:

Among the numerous free-living amoebae of soil and water habitats, certain species of Naegleria, Acanthamoeba and Balamuthia are facultative parasites of man. Most human infections of these amoebae are acquired by exposure to contaminated water while swimming. Inhalation of cysts from dust may account for some infections.

Naegleria fowleri: The trophozoites occur in two forms. Amoeboid forms with single pseudopodia and flagella forms with two flagella which usually appear a few hours after flooding water or in CSF.



Acanthamoeba species: Are free-living amoebae that inhabit a variety of air, soil, and water environments. However, these amoebae can also act as opportunistic as well as nonopportunistic pathogens. *Acanthamoeba* has two forms, the metabolically active trophozoite and a dormant, stress-resistant cyst. The trophozoites have an irregular appearance with spine-like



pseudopodia. Acanthamoeba causes three main types of illness involving **the eye** (**Acanthamoeba keratitis**), the **brain** and **spinal cord** (Granulomatous Encephalitis), and **infections** that can spread throughout the entire body (disseminated **infection**). Naegleria fowleri causes acute primary amoebic meningoencephalitis.

Balamuthia species: The trophozoite may be bi-nucleated. Unlike most amoebae the nuclear envelope breaks down during mitosis. Naegleria, Acanthamoeba, Balamuthia organisms are opportunistic pathogens.

Acanthamoeba & Balamuthia organisms are responsible for granulomatous amoebic encephalitis and single or multiple brain abscesses, primarily in immunocompromised individuals. For the diagnosis of Naegleria, canthamoeba, and Balamuthia infections, specimens of nasal discharge and cerebrospinal fluid; and in cases of eye infections corneal scraping should be collected. The clinical specimen can be examined with saline wet preparation and Iodine stained smear. Treatment of free-living amoebic infections is largely ineffective.