Univ. of Mustansiriya
College of Pharmacy
Branch of Clinical Laboratory Sciences

Medical Parasitology
For Higher Diploma Students

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Lec. 2 Medical Parasitology

Parasitology is a dynamic field because the relationships between the parasites and their hosts are constantly changing. Parasites are often causing important diseases to humans and animals. Consequently, the host suffers from various **illnesses**, **infections** and **discomforts**. However, in some cases, the host may show **no signs** of infection at all.

Parasitic diseases may be presented by a wide variety of **clinical manifestations** depending on the invaded tissue or part in the host body. Medically important parasites affect billions of people, kill millions annually and causes massive injuries such as blindness and disfiguration on additional millions. Several aspects concerning these parasites are highlighted in the study of **medical parasitology** such as their classification, general characteristics, their biology, the ecological factors that affect their transmission, the immune response of the host body to these parasites as well as the diagnosis and the control of the diseases that developed by these parasites. Therefore, it is briefly the branch of medical sciences dealing with the **parasites** which live temporarily or permanently, on or within the human body (the **host**).

Types of Parasites

According to the nature of the host-parasite interactions and the environmental factors, the parasite may be one of the following types:

1. The **obligatory** parasite: it is completely dependent on its host and can't survive without it, e.g. hookworms.

2. The **facultative** parasite: is the parasite that can change its lifestyle between free-living in the environment and parasitic according to the surrounding conditions, e.g. *Strongyloides stercoralis*.

3. The **accidental** parasite: is that affects an unusual host, e.g. *Toxocara canis* (a dog parasite) in man.

4. The **temporary** parasite: is the parasite that visits the host only for feeding and then leaves it, e.g. Bed bug visiting man for a blood meal.

5. The **permanent** parasite: is that one that is live in or on its host without leaving it, e.g. Lice.

6. The **opportunistic** parasite: is the parasite that can produce disease in an immunodeficient host (like AIDS and cancer patients). Whereas in the immunocompetent host, it is either found in a latent form or causes a self-limiting disease, e.g. *Toxoplasma gondii*.

7. The **zoonotic** parasite: primarily infects animals and is transmittable to humans, e.g. *Fasciola* species.

8. The **erratic** parasite: is one that found in an organ in which it is not usually found, e.g. *Entamoeba histolytica* in the liver or lung of humans.

Parasites from the animal kingdom comprises about 800,000 identified species categorized into 33 phyla. The most acceptable taxonomic classification of human parasites includes **Endoparasites** and **Ectoparasites**. Endoparasites are sub-classified into **Protozoan** parasites (unicellular organisms) and **Helminthic** parasites (multicellular organisms). Helminthic parasites are either **flat worms** (**Trematodes**), segmented **tape** like worms (**Cestodes**) or **cylindrical** worms (**Nematodes or round worms**).

Endoparasites:

Most human's parasites live inside the body. These are helminths (worms of various types), protozoa, or sometimes larval stages of arthropods (insects, mites, etc.). Both helminthic and protozoan parasites can infect different tissues and organs of the human body. A great number of endoparasites lives in the intestines, or at least passes through the intestines, having been swallowed in food or water. Practically, any organ can be affected, however some parasites are targeting certain organs in the body like *Trichinella spp*. and *Toxoplasma gondii* which live in the muscles, the larvae of *Echinococcus spp.* and the liver flukes which occupy the liver, *Schistosoma haematobium* targets the urinary bladder.

Ectoparasites:

Human ectoparasites live on the host. They include the fleas, lice, mosquitoes, flies, bugs, mites, ticks …etc. In general, ectoparasites attach to the skin to feed and do not remain

on the host for their entire lives. Some of these organisms lie in a grey area between endoparasites and ectoparasites. Scabies mites, for example, are generally considered ectoparasites although the female mite hide into the skin. Fly larvae may feed on dead tissue in a wound, but some species never invade healthy tissue.

Diagnosis of Parasitic diseases

Diagnosis of parasitic diseases depends on several laboratory methods like direct microscopy, imaging techniques, endoscopy and many other developed techniques, as well as the clinical picture and geographic location.

Diagnosis using direct microscopy is based on detecting the parasite by the examination of different specimens such as the **stool**, **urine**, **blood**, **CSF** and **tissue biopsies**. Whereas immunodiagnostic techniques are depending on **antigen and antibody-detection** assays. In addition, molecular-based diagnostic approaches offer a great sensitivity and specificity, and recently, the nanotechnology can also be applied as diagnostic procedures utilizing nanodevices.

The detection of enteric protozoa is a commonly requested test, particularly with increasing travel to and migration from endemic countries. Unfortunately, microscopy is slow and labor intensive and requires a high level of technical expertise. It also lacks both sensitivity and specificity, but recently developed nucleic acid amplification tests are automated and rapid and show superior accuracy. Additionally, proteomics shows promise for both the diagnosis of infections where parasite detection is difficult, and the potential for accurate assessment of cure in these cases.

The groups of parasites

Protozoa

Protozoa are unicellular eukaryotic organisms, have all the essential organelles that help them in their essential activities. All of them are microscopic, most of them live singly but many others living in colonies. Each cell unit performs all the necessary functions of life.

Thousands of species of protozoa have been described, the majority of which are free-living; yet many representatives of the subgroups **Sarcomastigophora** and **Ciliophora** are **parasitic**, and all the species of the subgroups **Apicomplexa** and **Microsporidia**. The main groups of parasitic protozoa are **Amebae**, the **Flagellates** and **Ciliates**.

Amebae

The members of this group are move by cytoplasmic extensions that are projected and retracted in response to external stimuli called **pseudopodia** (single **pseudopodium**). All amebae have a trophozoite stage in which they multiply by binary fission as long as the environmental conditions are favorable. Many species have an encysted stage that is more resistant to unfavorable conditions and provides an opportunity to transfer from one host to the next. All species in this subphylum that are parasitic in humans are, for the most part, nonpathogenic or produce only minor diseases with the exception of *Entamoeba histolytica*. At least eight species of amoebae belonging to three genera are known to parasitize humans. These are *Entamoeba histolytica*, *E. hartmanni*, *E. dispar*, *E. coli*, *E. polecki*, *E. gingivalis*, *Endolimax nana* and *Iodamoeba butschlii*. All inhabit the large intestine except *E. gingivalis* which is found in the mouth. In addition, amoebae belonging to at least three genera of the phylum Percolozoa: *Naegleria*, *Acanthamoeba*, and *Balamuthia*, normally free-living, have been shown on occasion to parasitize humans by accident.

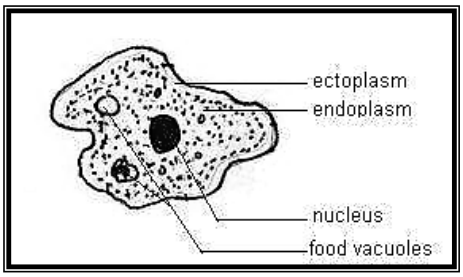
1. *Entamoeba histolytica*

Undoubtedly, the best-known species of amoebae parasitizing humans is *E. histolytica*, the causative agent of amoebic dysentery or amoebiasis, therefore it is called dysentery ameba. The parasite has a cosmopolitan distribution (worldwide distribution) especially the warm area. It is, nevertheless, important to remember that amoebiasis is not restricted to the tropics and subtropics where it is found also in temperate and even in arctic and antarctic zones.

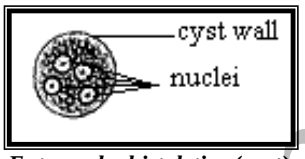
This parasite infects the human as well as cats, pigs and monkeys. *E. histolytica* can produce extreme illness and even death. Furthermore, since side effects from chemotherapy may be pronounced, it is of great importance that diagnosis of the condition be precise and accurate in order to assure treatment only when absolutely necessary, not merely to eliminate a protozoan that resembles *E. histolytica*.

The parasite has 2 distinct stages (trophozoite and cyst) which are commonly recognized in the feces of the patient, but only the trophozoite is present in the tissue. The trophozoite lives in the last part of the small intestine and in the large intestine stuck with mucosa, especially in the caecum and sigmoidorectal area; it varies from (15-60µ) in diameter, however, trophozoite up to (90µ) in diameter have been observed in the dysenteric stool. It has finely granular, somewhat viscous endoplasm and a clear ectoplasm, pseudopodia are broadly finger-like (lobopodia) extends from the ectoplasm. In addition, there are many food vacuoles containing parts of epithelial cells, Bacteria and sometimes many

R.B.Cs. and leukocytes. The nucleus rounded, vesicular, surrounded by a delicate nuclear membrane which is studded on its inner surface with minute regular chromatin granules. In the center of the nucleus, there is a single dense bead-like chromatin body, the karyosome (centric karyosome). Unfortunately, other species of *Entamoeba*, notably *E. dispar*, show similar nuclear morphologies.



***Entamoeba histolytica* (trophozoite).**



***Entamoeba histolytica* (cyst).**

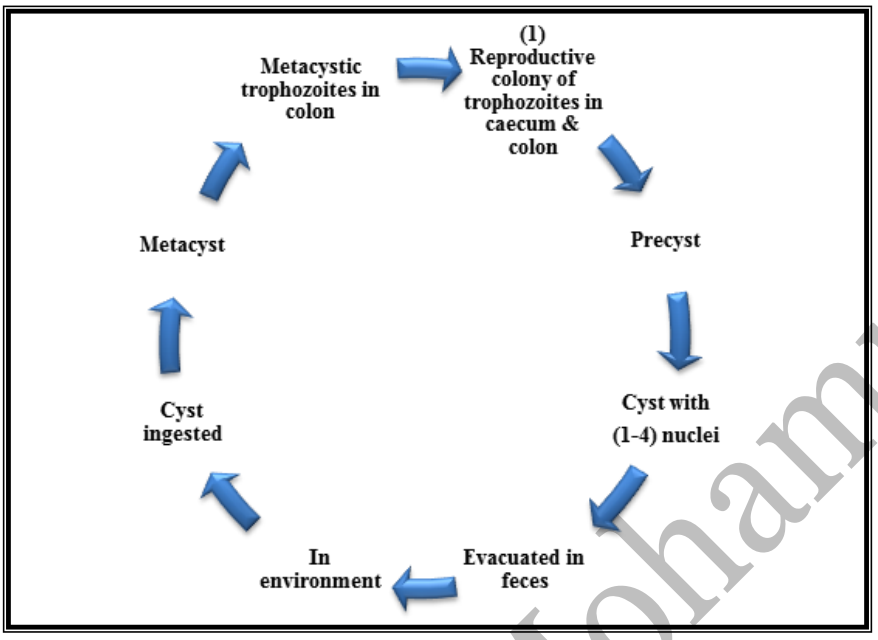
The trophozoite grow and multiply continuously in the intestine, but sometimes it is encysting in the intestine, whenever, the trophozoite will discharge the undigested food and become spherical, then it secretes a delicate, solid membrane. The cyst contains a nucleus (the same one of the trophozoite), glycogen mass and some chromatoid bars or bodies with hazy margin and rounded ends. The latter structures are considered to be deposits of nucleic acids such as RNA and may vary in shape but always have smoothly rounded ends in *E. histolytica*. The nucleus will firstly divide into 2 nuclei then each of the two daughter nuclei divides once again, so, the mature cyst typically has 4 nuclei. The cyst ranges between (10-20µ) in diameter, spherical or may have an oval shape.

A viable cyst is highly resistant to dryness and freezing and even to certain chemicals e.g. chlorinated compounds, and fluorides. Cysts in water can survive for a month, while those in feces on dry land can survive for more than 12 days; they tolerate temperatures up to a thermal death point of 50˚C. However, it is affected by bacterial putrefaction of the medium, hypertonicity, direct sunlight and heat. Greater intestinal motility and/or large volumes of ingested food reduce the potential for establishment of the amoebae.

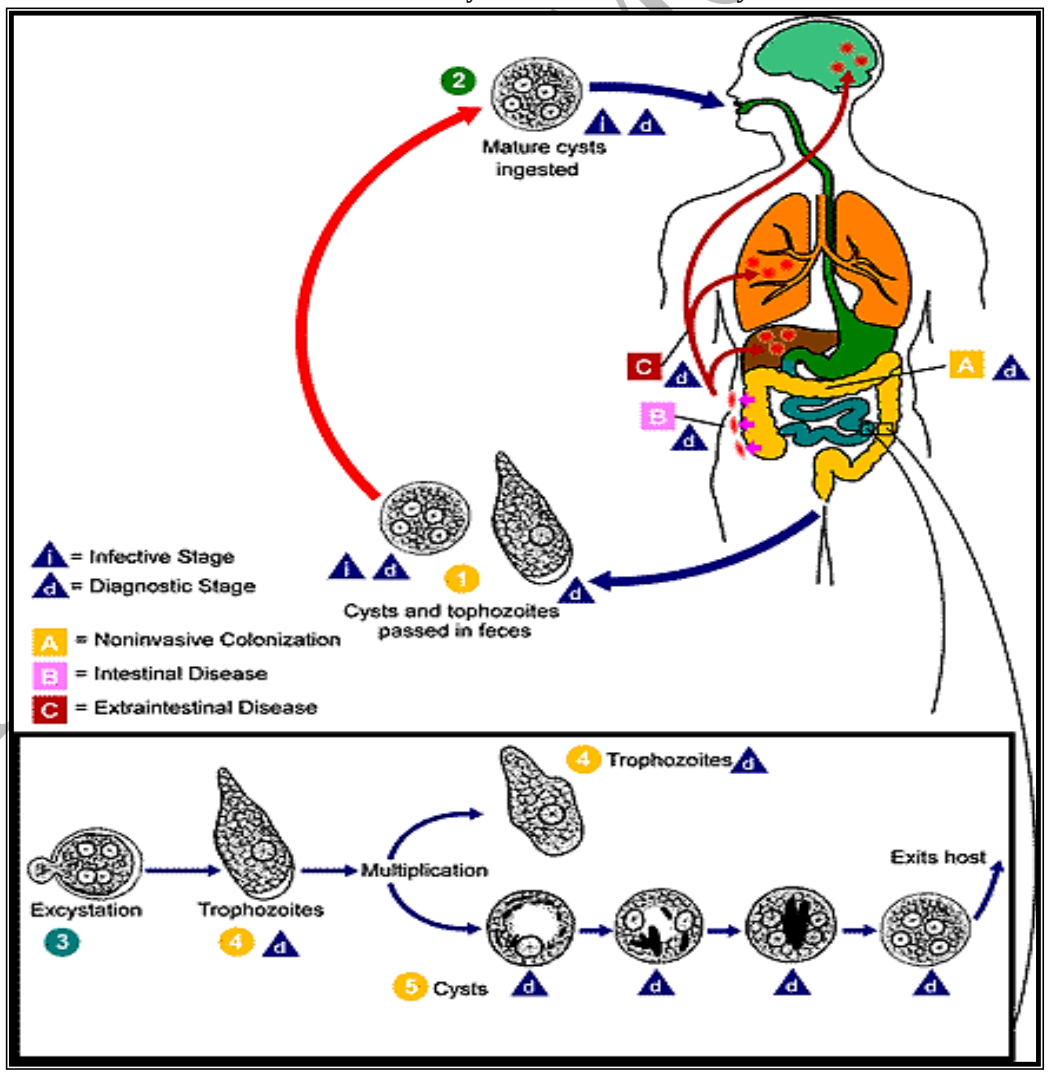
Life cycle

When the cyst swallowed with the foods or drinks, excystation occurs and the freeing of the young trophozoites will occur in the duodenum where the pH is neutral or weakly alkaline, as well as the effects of the digestive enzymes, which will destroy the cyst wall. These young freeing trophozoites will arrive then to the large intestine and some of them will be in contact with the mucosa. When these cysts evacuated in the feces of the infected patient it will arrive to the environment and the cycle would be repeated again, see the figure.

Multiplication of this species is thus seen to occur at two stages during the life cycle: by binary fission in the intestine-dwelling mature trophozoite stage and by nuclear division followed by binary fission in the metacystic stage.



Scheme for the life cycle of *Entamoeba histolytica*".

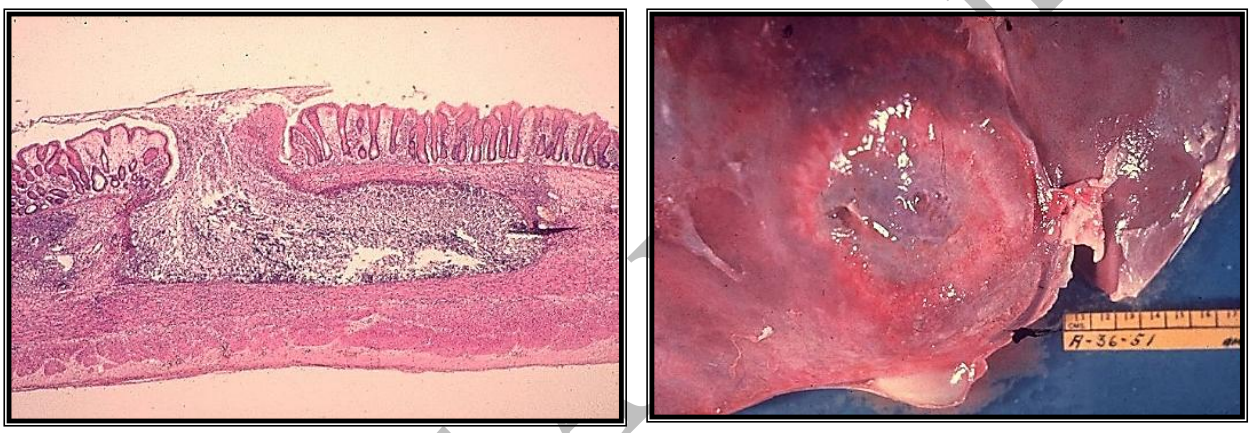


"Diagram for the life cycle of *Entamoeba histolytica*".

Pathology

The trophozoite has the ability to destroy the host epithelial cells causes their lysis (the cause of the name). It may be reach to the submucosa, start in feeding and attack the blood capillaries and feed on the R.B.Cs., the blood will then flow to the lumen of the intestine and exit with the stool, which is the first important symptom of the infection (the bloody stool).

After a period, the parasite may invade other body organs where it produces abscesses. For instance, they may reach the liver and causes abscesses called **hepatic amebiasis** and liver dysfunction, or to the lungs and causes **pulmonary amebiasis** and pneumonitis, or it can reach to the brain and causes encephalitis, or to the spleen, heart, joints, bones, muscles, urogenital system and even the skin.



Entamoeba histolytica **(flask shaped ulcer in the intestine).** ***Entamoeba histolytica*** **(amebic-abscess).**

Individual who develops dysentery, the mucosal ulceration may penetrate deeper into the intestinal tissue, causing vast areas of tissue to be destroyed. The overlying mucosal epithelium then may be sloughed off, exposing these necrotic areas. This destructive process is usually followed by a regenerative period, resulting in a thickening of the intestinal wall as a result of the deposition of fibrous connective tissue.

Symptoms

A wide spectrum from asymptomatic infection "luminal amebiasis" to invasive intestinal amebiasis (dysentery, colitis, appendicitis, toxic megacolon, amebomas), to invasive extraintestinal amebiasis (liver abscess, peritonitis, pleuropulmonary abscess, cutaneous and genital amebic lesions).

Chronic: Abdominal discomfort or soft stool for variable periods, may be suddenly developed to dysentery or acute abdominal pain. Recurrent episodes of dysentery with blood and mucus in the feces. Interfering gastrointestinal disturbances and constipation. Cysts can be found in the stool.

Acute: Frequent dysentery with necrotic mucosa and abdominal pain. In the former type, severe diarrhea (i.e., blood and mucus in liquid feces) usually develops after an incubation period of 1 to 4 weeks and is commonly accompanied by a fever.

Diagnosis

The typical stool in amebic dysentery consists of exudates, mucous, blood and may be little fecal material and we are mainly looking for the cyst stage. In liquid stool, trophozoite may also be found, but only cyst stage is present in the solid stool.

For the diagnosis procedure, the fresh stool sample is required to prepare the wet mounts. Concentrates from fresh stool can be used for the wet mounts, with or without iodine stain. For permanently stained preparations, we use trichrome stain. Concentration procedures, however, are not useful for demonstrating trophozoites. In addition, *E. histolytica* trophozoites can also be identified in aspirates or biopsy samples obtained during colonoscopy or surgery. Monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA) is currently a popular alternative method. Serological tests and X-ray scans may prove useful in revealing abscesses of the liver.

Treatment:

Appropriate chemotherapy should be employed to destroy the trophozoites, relieve the symptoms and control secondary bacterial infections. The drug of choice for the entire spectrum of symptoms is metronidazole (Flagyll) or tinidazole. In addition, a bland diet, low in carbohydrates such as sugar and high in liquids and proteins, is recommended. To combat secondary bacterial infections, antibiotics such as tetracycline are used in combination with either metronidazole or tinidazole. Hepatic amoebiasis also responds well to metronidazole, although the treatment is not totally effective.

Host Immune Response

Entamoeba histolytica infection in the colon can initiate an intense post inflammatory response, both acute and chronic.

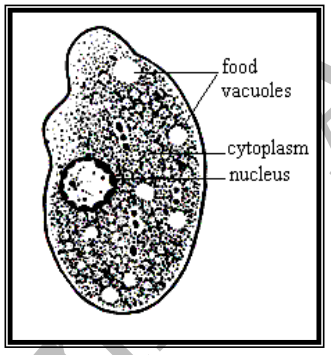
There are reports that some of *E. histolytica* excretory and secretory products (e.g., proteins) may alter the macrophage metabolism and thus reduce the efficacy of the patient's immune response, especially in the case of invasive amoebiasis. For instance, it has been shown that *E. histolytica* trophozoites produce a small peptide called **monocyte locomotion inhibitory factor (MLIF)**, which inhibits the motility of host monocytes

and macrophages and also suppresses the monocyte and neutrophil nitric oxide production. It is probable that these factors contribute to the ability of the trophozoites to survive within the host and even establish prolonged infections.

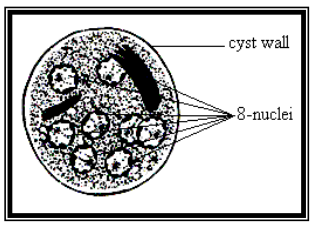
A degree of naturally acquired immunity to *E. histolytica* has been reported in humans. This immunity has been linked to a mucosal anti-adherence lectin IgA response.

2. *Entamoeba coli*

Generally considered nonpathogenic in humans (commensal). The trophozoite does not ingest or invade host tissues. It has a cosmopolitan distribution; its presence is evidence that the host has ingested fecal material. There is two stages trophozoite and cyst. The trophozoite has a spherical shape with a diameter of (15-50µ) and the ectoplasm couldn't recognize from the endoplasm. The food vacuoles contain bacteria, yeast and other enteric microbes as well as and fragments of intestinal debris. The nuclear membrane studded from the inner surface with large irregular chromatin granules with eccentric and large karyosome. The trophozoite has a sluggish movement, shortly extended pseudopodia.



***Entamoeba coli* (trophozoite).**



***Entamoeba coli* (cyst).**

The mature cyst has a diameter of (10-35µ), 8 nuclei, the chromatoid bodies have an irregular sharp ended (splinter-like). It lives in the lumen of the caecum and lower level of the large intestine.

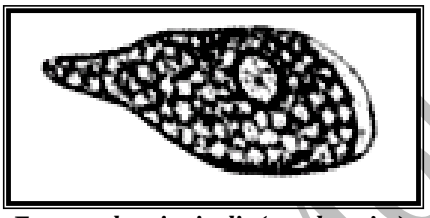
The life cycle is similar to that of ***E. histolytica***, except that the trophozoite in this example doesn't attack the mucosa of the intestine.

3. *Entamoeba gingivalis*

It is a parasite of the mouth of man and other mammals, including several species of monkeys, dogs and cats. It is cosmopolitan in distribution, commonly found in the tartar and debris associated with the gingival tissues of the mouth. It lives in/on the teeth, gums

and sometimes tonsils. There is little indication that it is pathogenic, and, while it abounds in people with unhealthy oral conditions (i.e., gingivitis or periodontitis), a cause and effect relationship has not been established.

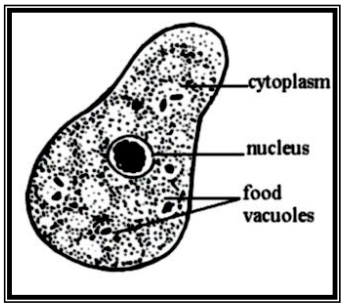
Only trophozoite stage has been described in this parasite, which is measure (5-35µ) in diameter. In most respects, it closely resembles ***E. histolytica***, with a few to several fingerlike pseudopodia, finely granular endoplasm and clear ectoplasm. The nucleus contains a small karyosome that is central or slightly eccentric in position. Endocytotic vacuoles are often numerous and the parasite may contain oral epithelial cells, leukocytes, occasionally erythrocytes and various microbial organisms although it is not itself invasive. No cysts are formed and transmission is either directly by oral to oral contact (kissing) or indirectly via trophozoite-contaminated food, chewing gum, toothpicks, etc.



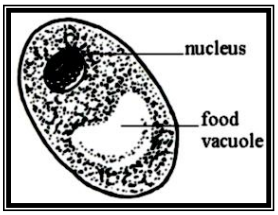
Entamoeba gingivalis **(trophozoite).**

4. *Iodamoeba butschlii*

The parasite has a cosmopolitan distribution, but it is seldom as common as *E. coli* and *E. nana*. It is commensal, lives in the lumen of the large intestine especially the caecum. It has 2 stages, trophozoite and cyst. The trophozoite movement is sluggish, the ectoplasm not easily distinguished from the endoplasm. It measures (8-20µ) in diameter, the vacuoles contain bacteria feeding on bacteria and yeast, as is evident from the contents of their food vacuoles. The nucleus is spherical, vesicular and has rather a thick membrane and large karyosome (centric or somewhat eccentric in position).



Iodamoeba butschlii **(trophozoite).**



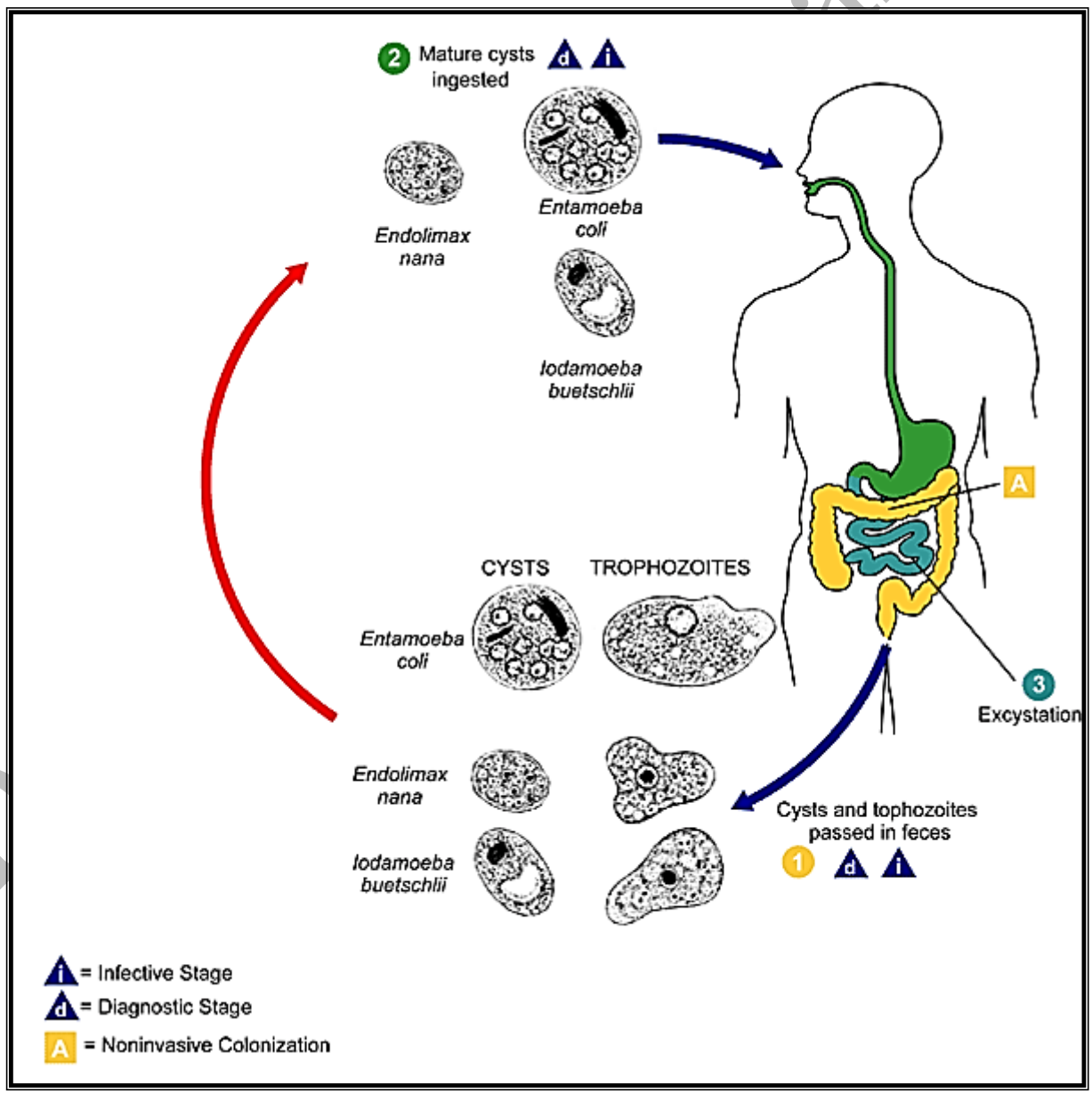
Iodamoeba butschlii **(cyst).**

It is transmitted by a cyst that is very distinctive, facilitating identification. The cyst is variable in shape, usually irregular rounded (ovoid), and (5-18µ) in diameter, usually contain one nucleus. There is a relatively big mass of glycogen that stains deep brown

with iodine [the cause of the name ***Ioda.***], and also help in the differentiation of this parasite from other intestinal amebae.

5. *Endolimax nana*

It is the smallest of the intestinal amoebae infecting humans, its trophozoite range (6-15µ). The trophozoite lives in the host's colon and is generally considered to be nonpathogenic. The life cycle is identical to that of other cyst-forming amoebae, with the cyst being the infective stage. *E. nana* cysts can be identified and distinguished from other cysts by their smaller size (5-14µ) ovoid shape, and one to four vesicular nuclei, each usually containing a large, eccentric karyosome. The nuclear envelope is very thin and is difficult to see even in stained preparations. Trophozoites actively feed upon bacteria and multiply rapidly by binary fission.



Life cycle of intestinal amebae