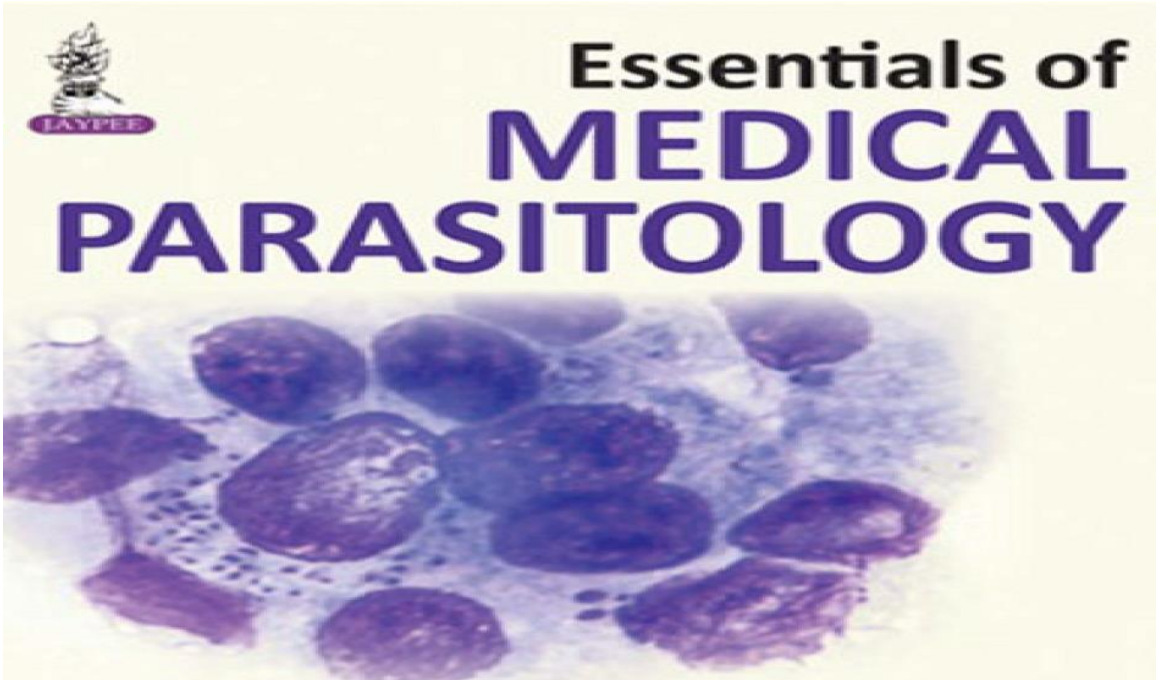


References

. Essentials of MEDICAL PARASITOLOGY

1. Apurba Sankar Sastry MD (JIPMER),DNB, MNAMS, PDCR,Assistant Professor Department of Microbiology,Jawaharlal Institute of Postgraduate Medical Education & Research
2. Sandhya Bhat K MD, DNB, MNAMS, PDCR,Assistant Professor,Department of Microbiology,Pondicherry Institute of Medical SciencesA Unit of Madras Medical Mission(Pondicherry, India)

ENTERIC AMOEBAE

Intestinal **amoebiasis** is a potentially serious infection, although trophozoites may inhabit the intestines for years without causing damage or symptoms, during which time the person infected is an asymptomatic carrier.

These two organisms are found worldwide, especially in tropical countries and in those with low sanitation standards. As many as 50 million new cases of amoebiasis appear per year in the world, which result in the death of possibly up to 100,000 people .

Table 3.1: Taxonomy of Amoeba

Kingdom	*Subkingdom*	*Phylum*	*Class*	*Order*	*Genus*
Protozoa	Neozoa	Amoebozoa	Entamoebidea	Euamoebida	*Entamoeba* *Endolimax* *Iodamoeba*
			Amoebaea	Acanthopodida	*Acanthamoeba*
		Percolozoa	Heterolobosea (flagellated amoeba)	Schizopyrenida	*Naegleria*

ENTAMOEBA HISTOLYTICA

In cases where amoebic **dysentery** is suspected, a fresh fecal sample is necessary. If a rectal ulcer is present, a swab from either the stool or the site of the ulcer should be examined with the use of a microscope, via a wet mount. A fresh stool while still warm should be examined quickly in order to see the colorless and motile trophozoites.

Dysentery is a general term that is used to describe a serious inflammatory disorder affecting the intestines that results in intense diarrhoea and is often accompanied by pain and fever. It can result from a variety of causes and amoebic and bacterial dysentery occur in both temperate and tropical regions.

There are two stages in the life cycle of *Entamoeba histolytica*: the actively growing and feeding stage referred to as the trophozoite form and the transmission stage called the cyst form (Figure 2.1). Like all other parasitic protozoa (but unlike the free-living forms), *Entamoeba histolytica* has no contractile vacuole. It also lacks mitochondria and this led some workers to suspect that it diverged from the path of evolution of other eukaryotes before these organelles were acquired. Molecular analysis has since demonstrated the presence of genes that code for proteins of mitochondrial origin within the *Entamoeba histolytica* genome and the presence of an organelle called a ‘mitosome’. Mitosomes are double-walled structures that lack DNA

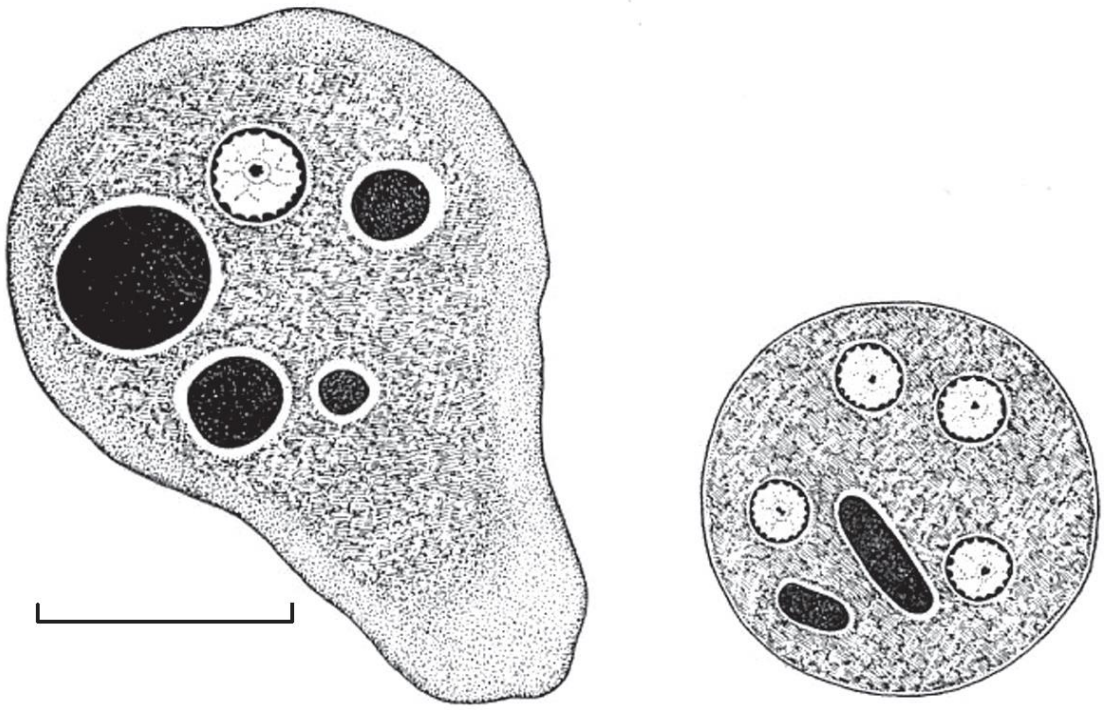


Figure 2.1 *Entamoeba histolytica* trophozoite (a) and cyst (b).

The trophozoite is 12–60 m in size and has a clear granular outer cytoplasm, a more densely granular inner cytoplasm, and there is an aggregated region of chromatin referred to as a karyosome centrally located within the nucleus. Reproduction takes place asexually by cell division and through cyst formation. The stimuli that cause the trophozoites to transform into cysts are uncertain but it is an essential part of the life cycle. This has led to the suggestion that if drugs could be developed that would prevent cyst formation, it might be possible to reduce parasite transmission.

The cysts are 10–15 m in diameter and (when mature) contain four nuclei and characteristic bar-shaped chromatoidal bodies that serve as a store of nucleoprotein. The cell wall contains chitin which provides protection and enables the cyst to survive in the outside environment for prolonged periods.

Mode of transmission :

Feco-oral route(most common): By ingestion of contaminated food or water with mature quadrinucleated cysts

Sexual contact.

Vector: Very rarely, flies and cockroaches may mechanically transmit the cysts from feces, and contaminate food and water.

Stage 4

Development in man (small intestine)

Excystation: In small intestine, the cyst wall gets lysed by trypsin and a single tetranucleated trophozoite (metacyst) is liberated which eventually undergoes a series of nuclear and cytoplasmic divisions to produce eight small **metacystic trophozoites**

Metacystic trophozoites are carried by the peristalsis to ileocecal region of large intestine and multiply by binary fission, and then colonize on the mucosal surfaces and crypts of the large intestine

After colonization, trophozoites show different courses depending on various factors like host susceptibility, age, sex, nutritional status, host immunity, intestinal motility, transit time and intestinal flora.
Amoebic dysentery: Trophozoites of *E. histolytica* secrete proteolytic enzymes that cause destruction and necrosis of tissue, and produces flask shaped ulcers on the intestinal mucosa.

Amoebic liver abscess: In few cases, erosion and necrosis of small intestine are so extensive that the trophozoites gain entrance into the radicals of portal veins and are carried away to the liver where they
multiply causing amoebic liver abscess.

Stage 4

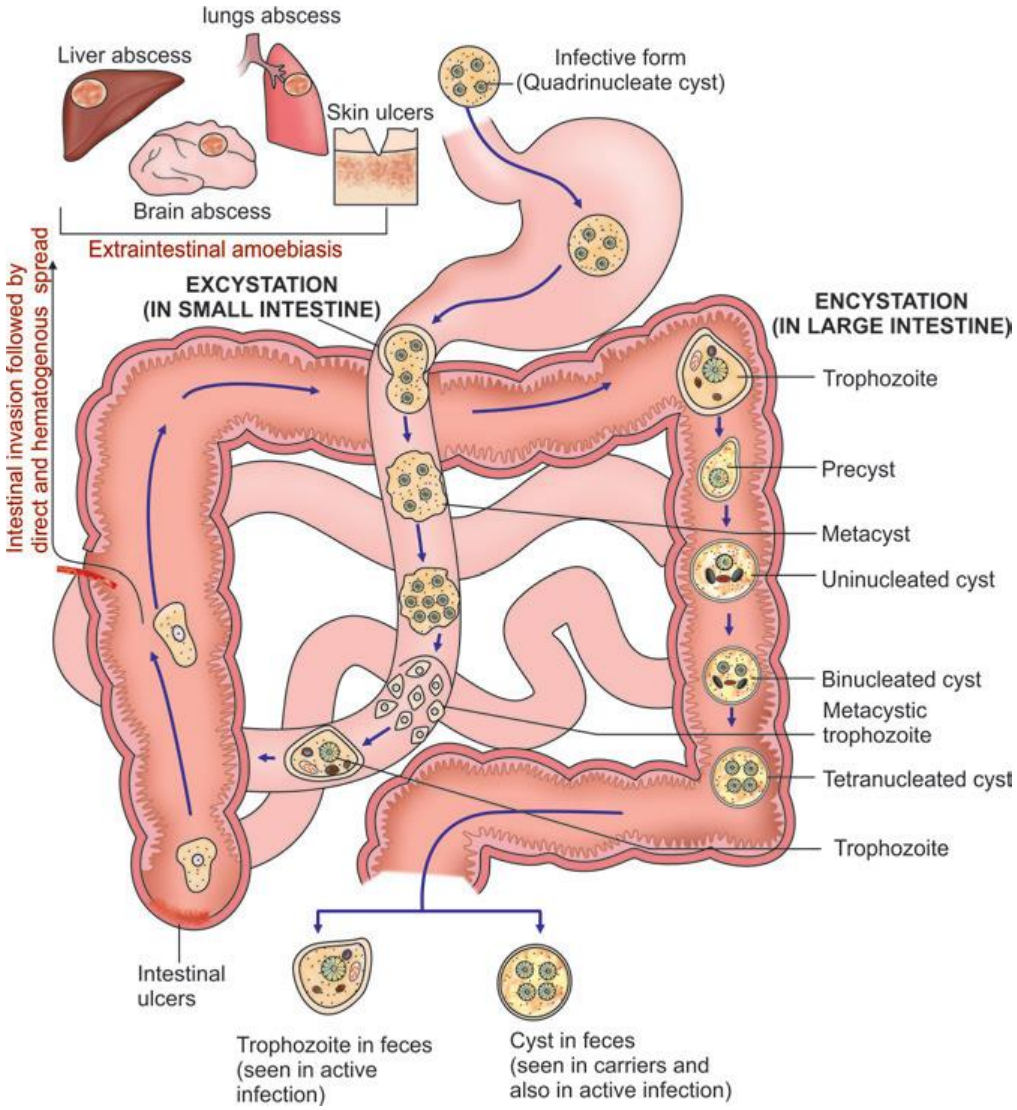


Fig. 3.2: Life cycle of *Entamoeba histolytica*

Development in man (large intestine)

Encystation: After some days, when the intestinal lesion starts healing and patient improves, the trophozoites transform into precysts then into quadrinucleated cysts which are liberated in feces Encystation occurs only in the large gut. Cysts are never formed once the trophozoites are excreted in stool .

Factors that induce cyst formation include food deprivation, overcrowding, desiccation, accumulation of waste products, and cold temperatures

Mature quadrinucleated cysts released in feces can survive in the enviornment and become the infective form. Immature cysts and trophozoites are some times excreted, but get disintegrated in the environement. (Fig.3.2).

Pathogenesis of Intestinal amoebiasis

Stage 4

Trophozoites invade the colonic mucosa producing characteristic ulcerative lesions and profuse bloody diarrhea (amoebic dysentery). Males and females are affected equally with a ratio of 1:1.

Amoebic ulcer

The classical ulcer is **flask-shaped** (broad base with a narrow neck).

- It may be localized to ileocecal region (most common site) or sigmoidorectal region or may be generalized involving the whole length of the large intestine
- Ulcers are usually scattered with intervening normal mucosa
- It may be superficial (confined to muscularis mucosa and heal without scar) or deep ulcer (beyond muscularis mucosa and heals with scar formation) .

.

❖ ***Complications of intestinal amoebiasis***

(fig. 3.3) There are following types of complications:

- **Fulminant amoebic colitis:** Resulting from generalized necrotic involvement of entire large intestine, occurs more commonly in immunocompromised patients and in pregnancy

- **Amoebic appendicitis:** Results when the infection involves appendix

- **Intestinal perforation and amoebic peritonitis:** Occurs when the ulcer progresses beyond the serosa

- **Toxic megacolon and intussusception :**(segment of intestine invaginates into the adjoining intestinal lumen, causing bowel obstruction)
- **Perianal skin ulcers:** By direct extension of ulcers to perianal skin
- **Amoeboma (amoebic granuloma)**
- **Chronic amoebiasis:** It is characterized by thickening, fibrosis, stricture formation with scarring and amoeboma formation.

Stage 4

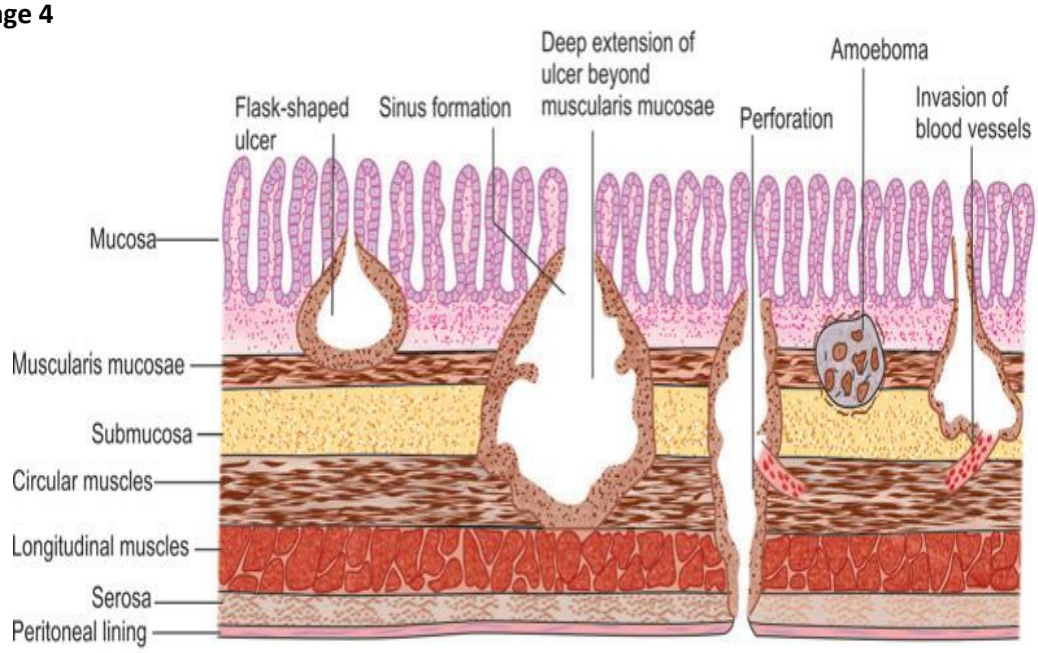


Fig. 3.3: Complications of intestinal amoebiasis (cross section of intestinal wall)

Pathogenesis of Extraintestinal amoebiasis

Following 1–3 months of intestinal amoebiasis, about 5–10% of patients develop extraintestinal amoebiasis. Liver is the most common site (because of the carriage of trophozoites through the portal vein) followed by lungs, brain, genitourinary tract and spleen.

Amoebic liver abscess

The most common group affected: Adult males (male and female ratio is 9:1).
The most common affected site is the posteriorsuperior surface of the right lobe of liver. Abscess is usually single or rarely multiple (Fig. 3.4).

- Amoebic trophozoites occlude the hepatic venules; which leads to anoxic necrosis of the hepatocytes. Inflammatory response surrounding the hepatocytes leads to the formation of abscesses

- Microscopically the abscess wall is comprised of:

  - ✓ **Inner central zone** of necrotic hepatocytes without amoeba
  - ✓ **Middle zone** of degenerative hepatocytes, RBC, few leucocytes and occasionally amoebic trophozoites
  - ✓ **Outer zone:** comprised of healthy hepatocytes invaded with amoebic trophozoites.

Fig. 3.4: Cross section of liver showing amoebic liver abscess (right side)

Laboratory Diagnosis — Intestinal amoebiasis

- Stool microscopy by wet mount, permanent stains, etc—detects cysts and trophozoites
- Stool culture
  - Polyxenic and axenic culture
- Stool antigen detection (copro-antigen)—CIEP, ELISA, ICT
- Serology
  - Amoebic antigen—ELISA
  - Amoeboic antibody—IHA, ELISA and IFA
- Isoenzyme (zymodene) analysis
- Molecular diagnosis
  - Nested multiplex PCR and real time PCR

Treatment	Amoebiasis
• Metronidazole or tinidazole is the drug of choice for intestinal amoebiasis and amoebic liver abscess (Table 3.5) • Other measures include fluid and electrolyte replacement and symptomatic treatment.	

Entamoeba coli

- *E. coli* is a nonpathogenic amoeba that colonizes the large intestine. Th e life cycle is similar to *E. histolytica* It has also three forms—trophozoites, precyst and cyst (Table 3.6, Figs 3.7, 3.8 and 3.9)
- It is frequently found in the stool samples of healthy individuals and should be differentiated from that of *E. histolytica* (Table 3.6).

Table 3.6: Differences between *Entamoeba histolytica* and *Entamoeba coli*

	Entamoeba histolytica	*Entamoeba coli*
Trophozoite		
Size	15–20 µm	20–25 µm
Motility	• Very active and unidirectional purposeful motility • Pseudopodia with finger like projection	• Sluggish, nonpurposeful and aimless motility in any direction • Blunt pseudopodia
Cytoplasm	Clearly differentiated to ectoplasm and endoplasm	Not differentiated
Cytoplasmic inclusions	RBC, leucocytes, tissue debris and bacteria	Same except it doesn't contain RBC
Nucleus	• Karyosome is small and central • Nuclear membrane is thin and lined by fine chromatin granules	• Karyosome is large and eccentric • Nuclear membrane is thick and lined by coarse chromatin granules
Precyst		
	10–20 µm size, oval with blunt pseudopodium, no food vacuoles and nucleus same as trophozoite	Same as *E. histolytica* except size is 20 µm
Cyst		
Size	12–15 µm	15–25 µm
Nucleus	Same as trophozoite	Same as trophozoite
Number of nuclei	1–4	1–8
Chromatoid body	Thick bars with rounded ends	Filamentous and thread like ends

Fig. 3.8: Trophozoite of *Entamoeba coli* (Iron hematoxylin stain) shows nucleus with coarse peripheral chromatin and abundant food vacuoles in the cytoplasm containing fecal debris
Source: Giovanni Swierczynski, Bruno Milanesi. "Atlas of human intestinal protozoa Microscopic diagnosis" (*with permission*)

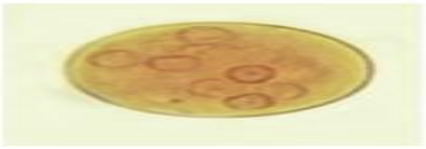


Fig. 3.9: Cyst of *Entamoeba coli* (Iodine mount) shows seven nuclei
Source: Giovanni Swierczynski, Bruno Milanesi. "Atlas of human intestinal protozoa Microscopic diagnosis" (*with permission*)

Entamoeba gingivalis

It is the first parasitic amoeba of humans to be described; recovered from the soft tarter between the teeth.

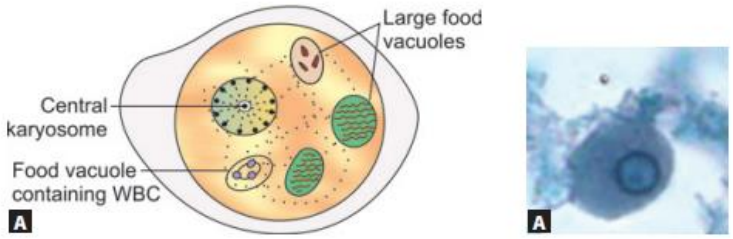
- **It is unusual in two respects:**

1. It inhabits in the mouth rather than in the large intestine
2. Only trophozoite stage exists; no cystic stage

Trophozoite is similar to that of *E. histolytica* trophozoite except (Fig. 3.11 A and B):

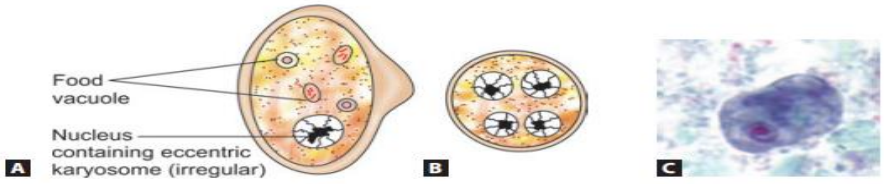
Stage 4

- ✓ Smaller in size (10–15 µm)
- ✓ Larger food vacuoles containing WBCs
- ✓ Nucleus similar to that of *E. histolytica*
- **It is recovered from:**
  - ✓ Vaginal secretions of women
  - ✓ Oral cavities of patients on radiation therapy and human immunodeficiency virus (HIV) infection
  - ✓ Patients with pyorrhea alveolaris



Figs 3.11 A and B: Trophozoite of *Entamoeba gingivalis* (A) schematic diagram; (B) trichrome stain
Source: B- DPDx Image Library, Centre for Disease Control and prevention (CDC), Atlanta (*with permission*)

Figs 3.12 A to C: *Entamoeba polecki* (A) cyst (schematic diagram); (B) cyst (trichrome stain); (C) trophozoite (trichrome stain)
Source: B- and C- DPDx Image Library, Centre for Disease Control and prevention (CDC), Atlanta (*with permission*)



Figs 3.13 A to C: *Endolimax nana* (A and B) trophozoite and cyst (schematic diagram); (C) trophozoite (trichrome stain)
Source: C- DPDx Image Library, Center for Disease Control and Prevention (CDC), Atlanta (*with permission*)