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Subject: Zoology

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Paper : 08 Biology of Parasitism
Module : 18 Morphology, Life cycle, Pathogenecity, Diagnosis and Prophylaxis of *Entamoeba* Part 1

ज्ञान-विज्ञान-विमुक्तये

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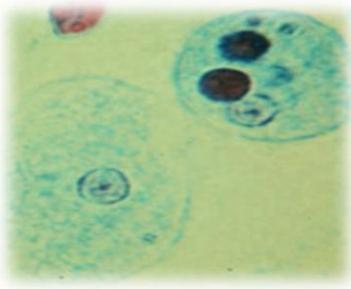
1. Learning Outcomes

After studying this unit you will be able to:

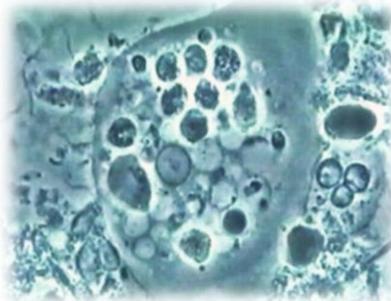
- Classify *Entamoeba*
- Understand the medical importance of *Entamoeba*
- Distinguish between the different species of *Entamoeba*
- Identify the pathogenic species of *Entamoeba*
- Describe the morphology of *Entamoeba histolytica*
- Explain the life cycle of *Entamoeba histolytica*
- Compare the life cycle of different species of *Entamoeba*

2. Introduction

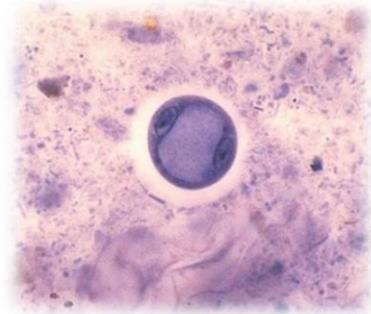
Genus *Entamoeba* was defined by Casagrandi and Barbagallo in 1895. *Entamoeba* is derived from the Greek words *Entos*: within and *Amoeba*: change. It is an acellular, anaerobic parasitic protistan found in vertebrates. There are different species of *Entamoeba*, *E. histolytica*, *E. coli*, *E. gingivalis*, *E. dispar*, *E. hartmanni*, *E. polecki*. Of these only *Entamoeba histolytica* is pathogenic to man and other vertebrates. It causes **amoebiasis, amoebic dysentery and other extraintestinal lesions (liver abscess)** in man. *E. gingivalis* is a harmless commensal found in large numbers in the mouth with poor oral hygiene. *E. coli* is a commensal of the lumen of the colon part of the large intestine. *E. hartmanni* is non-pathogenic and closely resembles *E. histolytica* in morphology except that it is smaller in size. *E. polecki* is a common naturally occurring parasite in monkeys and pigs, rarely found in man but if found can cause diarrhoea. It was Schaudinn in 1903 who differentiated the pathogenic form from the non-pathogenic form of *Entamoeba*.



Entamoeba histolytica



Entamoeba gingivalis



Entamoeba coli

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3. History of *Entamoeba*

Entamoeba gingivalis was the first amoeba to be described in man by Gros in 1849 in the tartar of teeth. The detail account of the parasite, *E. gingivalis* was given by Von Prowazak in 1904. Lewis described *Entamoeba coli* for the first time in 1870. Cunningham in 1871 and Grassi in 1878 reported its presence in the intestine of healthy persons. *Entamoeba histolytica* was first described by W.D. Lambl 1859 in the colonic autopsy of a child who died of diarrhoea. Fedor Loschin 1875 described the pathogenic nature of *Entamoeba histolytica*. He inoculated the parasite through the rectum of a dog and found that the dog had developed dysentery, thus proving the pathogenic nature of the parasite. Kartulis (1886) confirmed amoeba as a causative agent of amoebic dysentery and amoebic liver abscess. Dobell described the life cycle of the parasite in 1925. Brumpt in 1925 described *Entamoeba dispar*. Schaudinn in 1903 differentiated the pathogenic and non-pathogenic form of *Entamoeba*



Fedor Losch 1840-1903



Emile Brumpt 1877-1951



Fritz Schaudinn (1871-1906)



D.D. Cunningham (1843-1914)

4. Classification of Entamoeba

Kingdom : Protista

Phylum : Sarcomastigophora

Sub phylum : Sarcodina

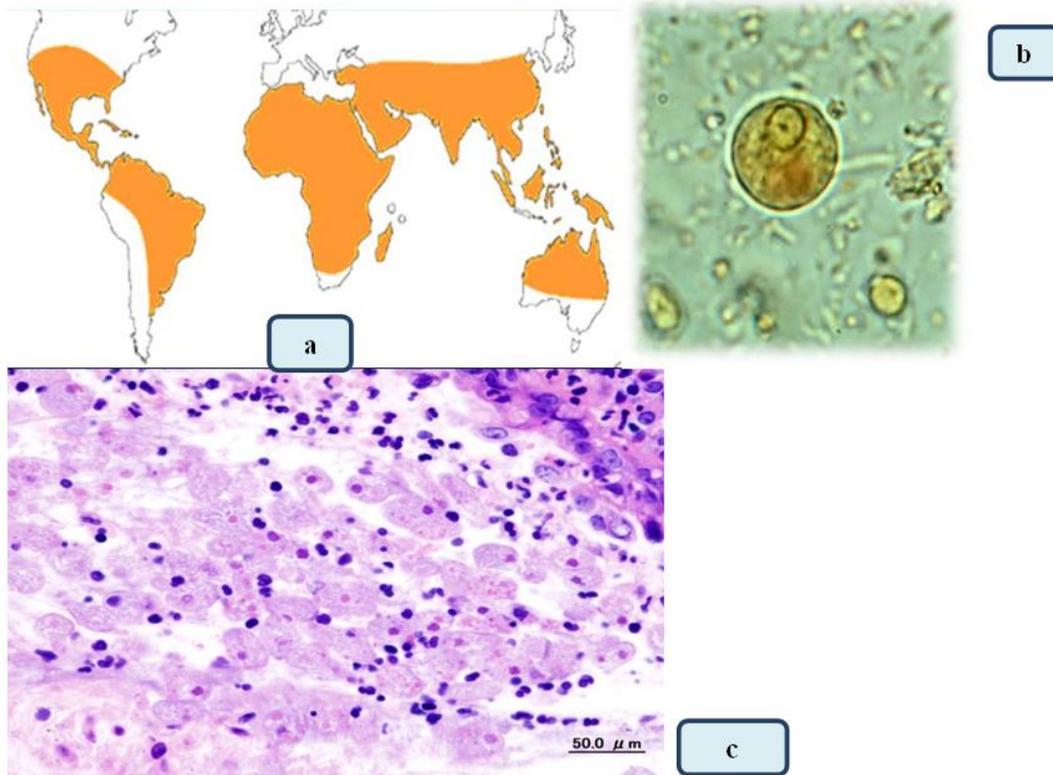
Super class : Rhizopoda

Class : Lobosa

Genus : *Entamoeba*

NOTE: *Vth ed. Barnes has been consulted for classification Entamoeba histolytica*

5. Geographical distribution of *Entamoeba histolytica*

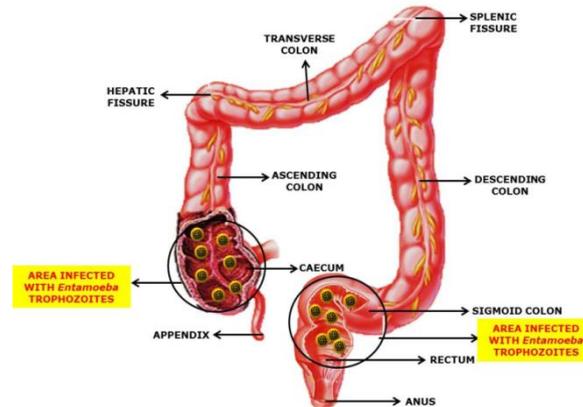


- a. Map showing distribution of amoebiasis (Source: <http://slideplayer.com/slide/8267391/>)
 b. *Entamoeba histolytica* (Source: <https://simple.wikipedia.org/wiki/Amoebiasis>)
 c. Amoebic dysentery in colon biopsy
 (Source: [https://commons.wikimedia.org/wiki/File:Amoebic_dysentery_in_colon_biopsy_\(1\).jpg](https://commons.wikimedia.org/wiki/File:Amoebic_dysentery_in_colon_biopsy_(1).jpg).)

Entamoeba histolytica infection is worldwide in distribution, more common in the tropics and the sub-tropics. It is the third leading cause of death in the developing countries of the world. About 50 million people are infected by *Entamoeba histolytica* and about 40,000–100,000 deaths occur in a year. (S.J.van Hal *et al.*, 2007).

6. Habit and Habitat

Entamoeba histolytica lives in the mucosa and sub-mucosa of the large intestine (caecum and sigmoidorectal region of man).



Source: <http://vle.du.ac.in/mod/book/print.php?id=9845&chapterid=15981>

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Entamoeba histolytica exists in two forms

- **Minuta** or non-pathogenic, non-invasive form
- **Virulent** or pathogenic or tissue invading form

Minuta form is smaller in size and lives in the lumen of the large intestine whereas the **virulent** form lives in the mucosa and sub-mucosa of the large intestine of man.

7. Host

Human, apes and monkeys. Also found in pigs, dogs, cats and rats

8. Reservoir

Human and monkeys. Asymptomatic human carriers act as reservoir for the spread of *Entamoeba histolytica*. As such there are no animal reservoirs.

9. Morphology

Entamoeba histolytica exists in three forms:

- **Trophozoite or feeding stage or free moving stage (Tropho: food/nourishment; zoite: zoon)** lives in the mucosa and sub-mucosa of large intestine (colon and cecum). It is the invasive form of the parasite.

- **Pre-cyst:** It is the transient stage, between the trophozoite and cyst, formed in the lumen of the large intestine
- **Cyst:** It is the non-feeding infective stage of the parasite found in the lumen of the large intestine

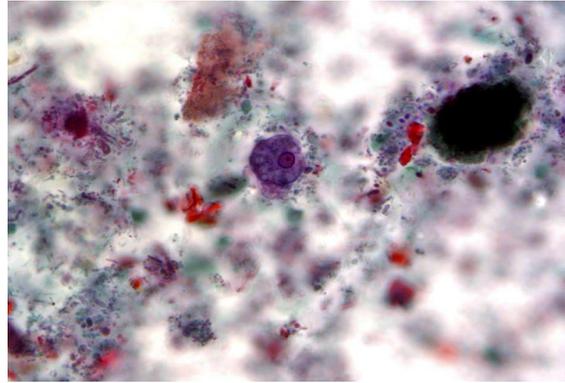
Trophozoite:

Size: Varies in size from 8µm- 40µm with an average size of about 20µm -25µm.

Shape: Not fixed, constantly changing its shape by thrusting out pseudopodia.

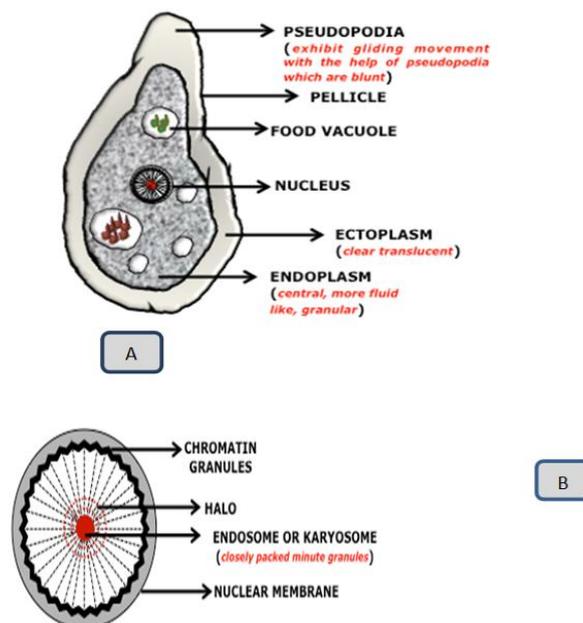
Cytoplasm: The cytoplasm is differentiated into a clear, thin translucent ectoplasm and a central fluid like granular endoplasm. Endoplasm contains nucleus, numerous food vacuoles, erythrocytes, granules and tissue debris. The trophozoite is an anaerobic parasite. It lacks mitochondria, golgi bodies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

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



Trichrome stained trophozoite of *Entamoeba histolytica*

Source: <http://www.pixnio.com/science/microscopy-images/amebiasis-entamoeba-histolytica/using-a-trichrome-stain-trophozoite-of-the-single-celled-parasite-entamoeba-histolytica>



A. Diagrammatic sketch of Trophozoite of *Entamoeba histolytica*

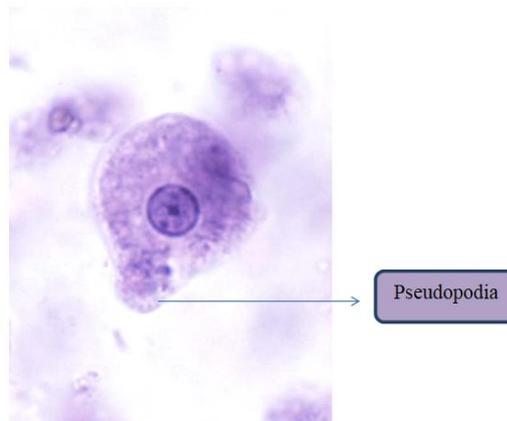
B. Detailed structure of nucleus of Trophozoite of *Entamoebahistolytica*

Source: <http://vle.du.ac.in/mod/book/print.php?id=9845&chapterid=15981>

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Locomotion: The trophozoite shows slow, gliding movement in one direction. They move about with the help of pseudopodia. Pseudopodia are finger like flowing extensions of the cytoplasm which may be short and wide or long and narrow. Locomotion is brought about by the forward extension of the ectoplasm followed by the granular endoplasm which then flows

into the finger like extensions. The direction of movement may change suddenly by giving out pseudopodia at some other site. The movement of the trophozoite is dependent on the consistency of the surrounding medium, age of the parasite and the temperature of the host.



Source: <http://www.k-state.edu/parasitology/625tutorials/Ehistolytica.html>

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

Trophozoites are exclusively parasitic forms.

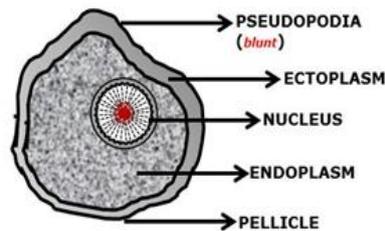


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PRE CYST: Smaller in size (10-20 μ m) than the trophozoite but larger than cyst, round or oval with a blunt pseudopodia. It has a relatively large nucleus that retains all the characteristics of the nucleus of a trophozoite. The trophozoite extrudes food vacuoles before

encystment so that the endoplasm is free from red blood cells and other ingested food particles.



Diagrammatic sketch of pre-cyst of *Entamoeba histolytica*

Source: <http://vle.du.ac.in/mod/book/print.php?id=9845&chapterid=15981>

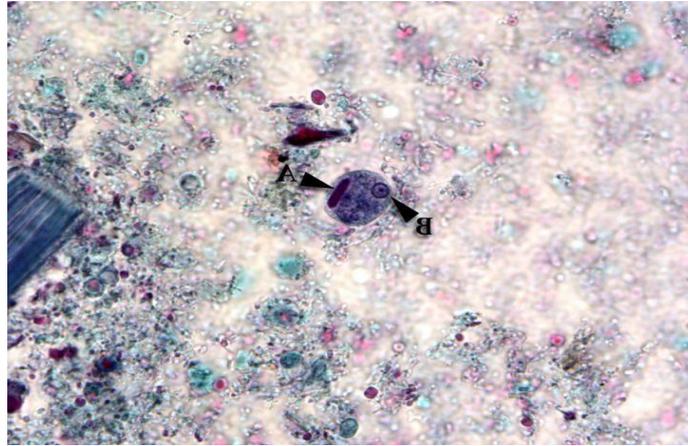
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CYST: Cyst exists in three forms:

- Immature uninucleate cyst
- Binucleate cyst
- Mature quadrinucleate cyst

The trophozoite becomes completely round and is surrounded by a transparent, highly retractile double walled resistant cyst wall. The cyst wall varies greatly in size from 6-9 μm (small race) to 12-15 μm (large race). The cytoplasm is clear and hyaline (transparent/glassy). Before encystment, the parasite eliminates food vacuoles and accumulates considerable amount of food in the form of **glycogen mass** and black bodies called **chromatoidal bodies**. These chromatoidal bodies are large, smooth, oblong rods with rounded ends. They may be one to several in number. They are called **chromatoidal bars** or **chromatoidal bodies** because they stain as chromatin with haematoxylin stain. It is believed that the chromatoidal bodies contain DNA and phosphates and serve as storage for ribosomes. Cyst when formed is uninucleate but the nucleus within the cyst divides twice to form a quadrinucleate cyst. **It is this mature quadrinucleate cyst (cyst containing four nuclei) which is the infective stage.** Occasionally, additional division may result in aberrant form with more nuclei. Although the nucleus retains all the characteristics of the trophozoite, it is smaller in size due to successive mitotic division. The mature quadrinucleate cyst lacks chromatoidal bodies and glycogen mass as they are absorbed by the cytoplasm.

The chitinous wall around the cyst makes it resistant to the gastric contents of the stomach, adverse environmental conditions and to the chlorine concentration found in the potable water.

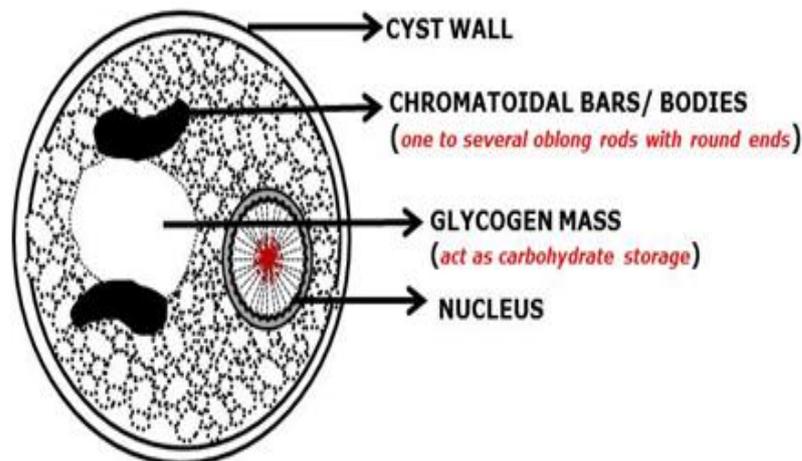


Trichrome stained uni-nucleate cyst of *Entamoeba histolytica*

A: Chromatoidal body

B: Nucleus

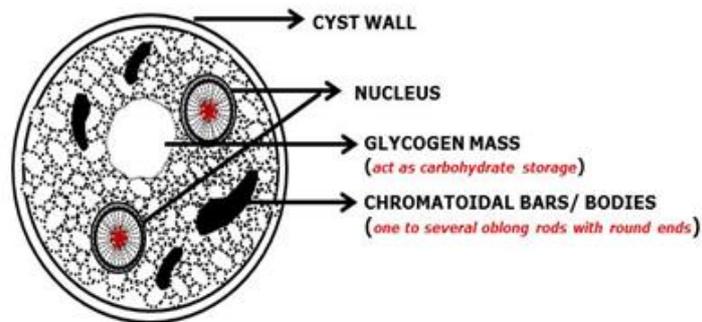
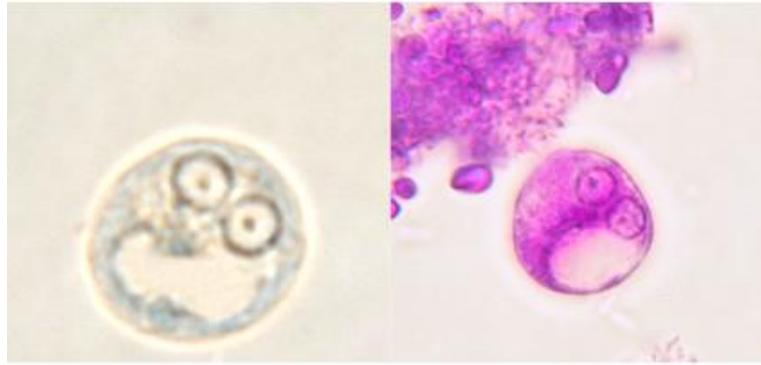
Source: <http://www.pixnio.com/science/microscopy-images/amebiasis-entamoeba-histolytica/cyst-of-the-single-celled-parasite-entamoeba-histolytica>



Diagrammatic sketch of uninucleate cyst of *Entamoeba histolytica*

Source: <http://vle.du.ac.in/mod/book/print.php?id=9845&chapterid=15981>

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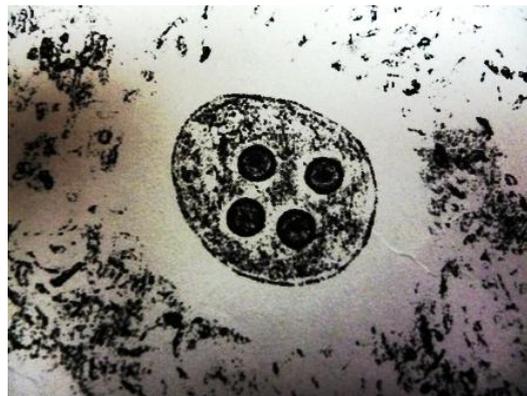
A: Left, an unstained immature binucleate cyst. Right, binucleate cyst stained with Baileger's solution. The nuclei of binucleate cyst of *Entamoeba histolytica* are often paired

Source: <http://www.atlas-protozoa.com/gallery.php>

B. Diagrammatic sketch of binucleate cyst of *Entamoeba histolytica*

Source: <http://vle.du.ac.in/mod/book/print.php?id=9845&chapterid=15981>

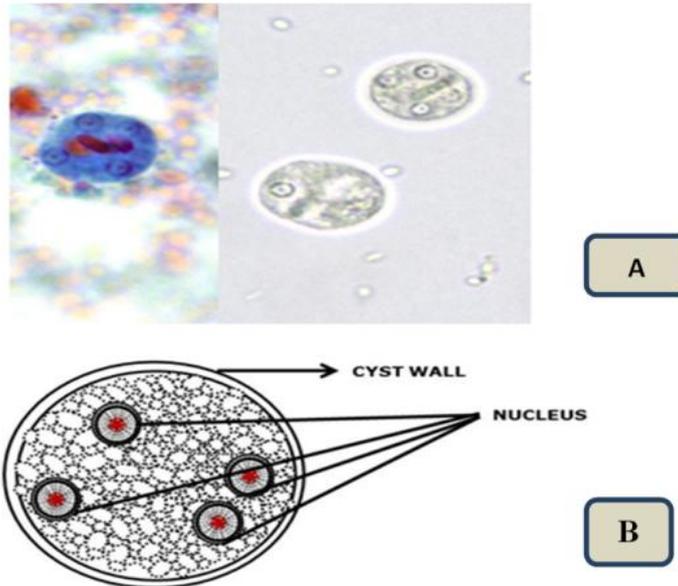
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Quadrinucleate mature cyst fixed smear

Source: https://commons.wikimedia.org/wiki/File:Entamoeba_histolytica_on_fixed_smear.jpg

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A: Left, Trichrome stain: Mature cyst with four nuclei. Right, Formalin-fixed specimen.

B: Diagrammatic sketch of quadrinucleate cyst of *Entamoeba histolytica*

A. Source: <http://www.atlas-protozoa.com/gallery.php>

B. Source: <http://vle.du.ac.in/mod/book/print.php?id=9845&chapterid=15981>

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10. Life cycle

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

The infection begins when the host swallows the mature quadrinucleate cyst along with the contaminated food or water. As the cyst wall is resistant to the acidic content of the stomach, the quadrinucleate cyst passes unaltered into the small intestine where **excystation** takes place. In the intestine, the cyst wall is digested by the action of trypsin in an alkaline medium at a temperature of 37°C. During this process, the cytoplasmic body retracts and loosens from the cyst wall. Pseudopodia are formed at various points and vigorous amoeboid movements occur within the cyst. Frequently, the pseudopodia press against the wall at certain spots as though the imprisoned organism were searching for exit. Eventually, a tetranucleate amoeba known as **metacyst** (amoeba with four nuclei) emerges out. Immediately on emergence, the four nuclei of the metacyst undergo division to form eight nuclei. Each nuclei gets

surrounded by a bit of its own cytoplasm and leads an independent existence. Thus, eight amoebulae are formed. These are known as **metacystic or metacyclic trophozoites** which are actively motile. The metacystic trophozoites move down to the caecum and ileocolic region of the intestine. The young amoebulae being actively motile invade the tissues and finally lodge themselves in the mucosa and submucosa of the large intestine-its final abode. They prefer this site as the organic material (food), pH and gases in this part of the large intestine are more stable and ideal for the existence of *E. histolytica* trophozoites. Here, the trophozoites grow at the expense of living tissues and multiply by simple binary fission. The trophozoites secrete histolysin which causes necrosis and destruction of the host's tissue and helps the parasite to derive nourishment from the dissolved dead tissues.

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

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

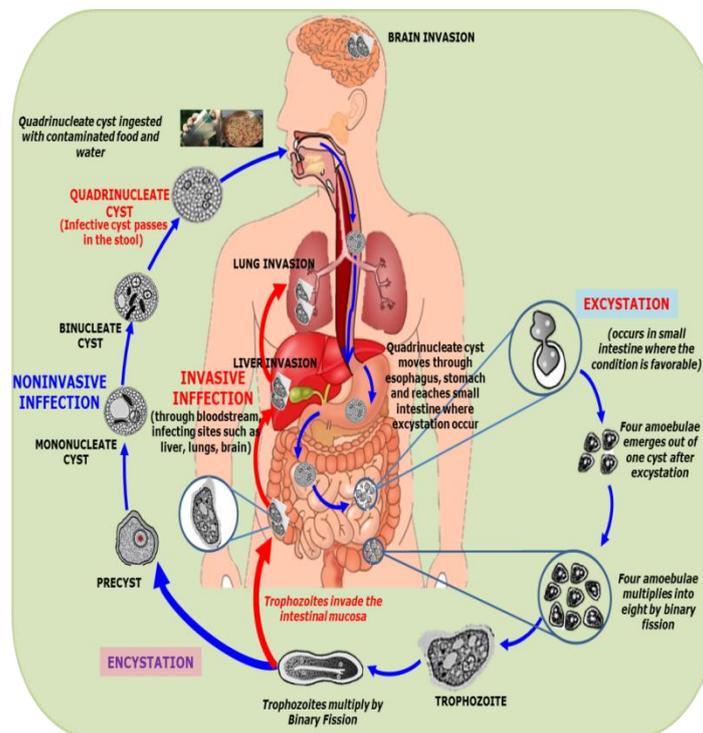
Encystation takes only a few hours and the mature quadrinucleate cyst can remain viable in the lumen of the large intestine for only two days. Mature quadrinucleate cysts are passed out along with the faeces of the host. About 45 million cysts may be voided out from one infective person in a day. The cysts are resistant to the environmental conditions and can live for a few weeks to a few months depending on the temperature (thermal death occurs at

50°C). Moisture is essential for the long existence of the cyst. They can live upto 10 days in a moist stool. The cysts are however, susceptible to dessication.

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

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

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



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Mode of Infection: faecal-oral route

Infective Stage: Mature Quadrinucleate cyst

Source of Infection:

Carriers of *Entamoeba histolytica* are of two types:

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

11. Transmission

- Fruits, raw vegetables and food contaminated by faeces containing quadrinucleate cyst
- Contaminated water
- Unhygienic habits
- Mechanical vectors like houseflies and cockroaches. Houseflies may act as carrier of the cysts from faeces to unprotected food and water. The droppings of cockroaches too have been found to contain mature cysts and thus they also serve as a source of infection.
- Sexual transmission of *Entamoeba histolytica* has been reported in about 20-30% homosexuals.

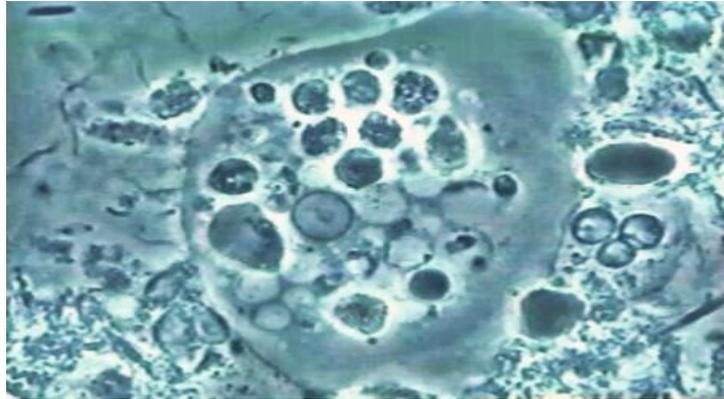
12. *Entamoeba dispar*

Non pathogenic, morphologically similar to *Entamoeba histolytica*. It lives in the lumen of the large intestine and lacks the capability to invade the intestinal mucosa. The trophozoites lack the RBC. Non-invasive parasite that does not evoke production of antibodies in the serum of man.

13. *Entamoeba gingivalis*

This is the first amoeba to be described in man by Gros in 1849 in the soft tartar collected from the teeth of human. It has only the trophozoite stage, cystic stage is absent in them. The trophozoite structure is same as that of *Entamoeba histolytica* except that the food vacuole does not contain RBC. The food vacuole however contains cellular debris, bacteria and ingested leucocytes. It is a commensal present in the margins of the gum and sometimes in

the crypts of the tonsils. The infection is transmitted from the mouth of an infected person during the act of kissing (oral-oral contact). Trophozoite moves rapidly with the help of numerous blunt pseudopodia.



Source: https://commons.wikimedia.org/wiki/File:Entamoeba_gingivalis.jpg
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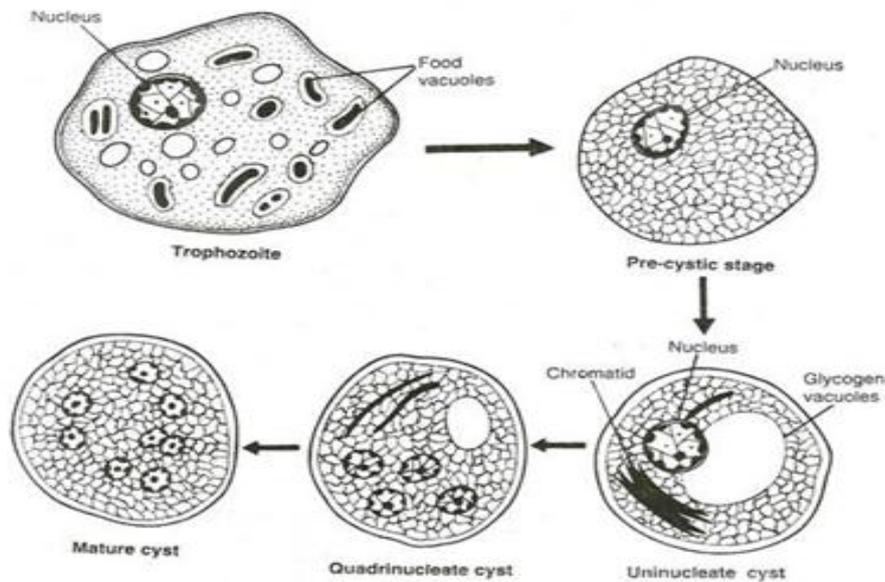
14. *Entamoeba coli*

A commensal present in the lumen of the large intestine. A non-invasive *Entamoeba* that exists in all the three forms, trophozoite, pre-cyst and cyst. The life cycle of *Entamoeba coli* is same as *Entamoeba histolytica*. The morphological difference between *Entamoeba histolytica* and *Entamoeba coli* is given below.

The infective stage of *Entamoeba coli* is a mature cyst containing 8 nuclei (octanucleate cyst)

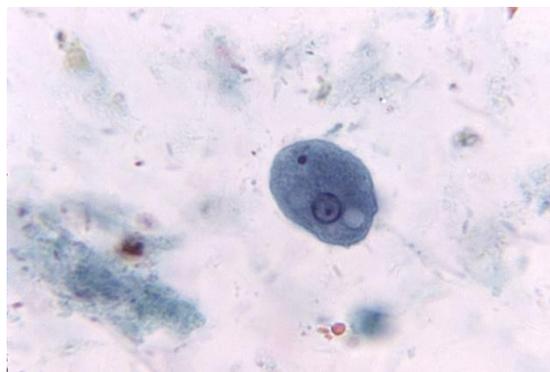
Morphological difference between <i>Entamoeba histolytica</i> and <i>Entamoeba coli</i>		
Stage	<i>Entamoeba histolytica</i>	<i>Entamoeba coli</i>
Trophozoite Form		
Fresh preparation		
Size	8µm-40 µm	18µm-50 µm
Motility	Very active	Sluggish
Pseudopodia	Finger shaped, hyaline	Granular, short and blunt
Cytoplasm	Finely granular, ectoplasm and endoplasm clearly marked	Granular, ectoplasm and endoplasm not defined, ectoplasm scarcely seen
Cytoplasmic inclusions	RBC, leucocytes and tissue debris	Bacteria and other food material but never RBC

Nuclear character	Small and central karyosome, delicate nuclear membrane lined with minute chromatin granules	Large and eccentric karyosome, thick nuclear membrane lined with coarse chromatin granules
Cyst Form		
Size	10µm-15µm	15µm-20µm
Nucleus	1-4 in number, central karyosome	1-8 in number, eccentric karyosome
Chromatoidal bars	Thick bars with rounded ends	Thread-like with square or pointed ends



Source: https://gl.wikipedia.org/wiki/Ficheiro:Entamoeba_coli_Stages.png

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Trophozoite of *Entamoeba coli* with ingested bacteria

Source: http://www.publicdomainfiles.com/show_file.php?id=13523762418607



Cyst of *Entamoeba coli*

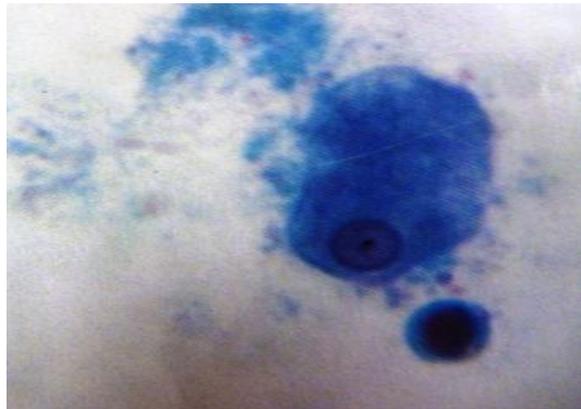
Source: https://commons.wikimedia.org/wiki/File:Entamoeba_coli_cyst.jpg

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15. *Entamoeba hartmanni*

A non-pathogenic commensal found in the lumen of the large intestine of human. It is cosmopolitan in distribution. Exists both in trophozoite and cyst stage. Morphologically similar to *E. histolytica* except that it is smaller in size. The trophozoites vary in size from 4 μ m-12 μ m while the cyst varies from 5 μ m-10 μ m. The trophozoites do not ingest erythrocytes.

The life cycle is similar to *E. histolytica* except that it is a non-invasive *Entamoeba* and the trophozoites are found only in the lumen of the large intestine.



Trophozoite of *Entamoeba hartmanni*

Source: https://commons.wikimedia.org/wiki/File:Entamoeba_hartmani.jpg

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16. Comparison between the various *Entamoeba*

Table showing the characteristics of trophozoite of various *Entamoeba* species

Species	Size	Movement	Inclusions	Nuclear characteristics	
				Karyosome	Peripheral chromatin
<i>Entamoeba histolytica</i>	8µm-40 µm	Active, unidirectional	RBC, leucocytes and tissue debris	Small and central	Delicate nuclear membrane lined with minute chromatin granules
<i>Entamoeba gingivalis</i>	20µm-150 µm	Active	cellular debris, bacteria and ingested leucocytes, no RBC	Small and central	Fine, minute chromatin granules
<i>Entamoeba hartmanni</i>	4µm-12µm	Sluggish	Bacteria, No RBC	Small and central	Fine chromatin granules
<i>Entamoeba coli</i>	18µm-50 µm	Sluggish	Bacteria and other food material but no RBC	Large and eccentric	thick nuclear membrane lined with coarse chromatin granules

Table showing the characteristics of cyst of various *Entamoeba* species

Species	Size	Nuclei	Cytoplasmic inclusions	
			Chromatoid Body	Glycogen
<i>Entamoeba histolytica</i>	10µm-15µm	1-4 in number with central karyosome. Fine, symmetrical peripheral granules	Thick bars with rounded ends	Diffuse
<i>Entamoeba gingivalis</i>	Cyst form is absent in <i>Entamoeba gingivalis</i>			
<i>Entamoeba hartmanni</i>	5µm-12µm	1-4 in number with small, central karyosome. Fine, symmetrical peripheral granules	Rounded ends	Diffuse
<i>Entamoeba coli</i>	15µm-20 µm	1-8 in number with eccentric karyosome. Coarse and asymmetrical granules	Thread-like with square or pointed ends	Diffuse

17. Summary of *Entamoeba histolytica*

<i>Entamoeba histolytica</i>		
Causative agent /etiological agent of	Amoebiasis, acute amoebic dysentery, chronic amoebic dysentery, liver abscess, splenic abscess, pulmonary amoebiasis, cerebral amoebiasis	
Definitive host	Human, monogenetic parasite	
Habitat	Caecum and colon	
Infective stage	Quadrinucleate/ tetranucleate mature cyst	
Port of entry	Faecal-oral route	
Method of transmission	Contaminated fruits and raw vegetables, Contaminated water, Unhygienic habits, Mechanical vectors like houseflies and cockroaches	
Pathogenic stage	Trophozoite	
Distinctive feature of trophozoite	Distinctive nucleus (chromatin granules, centrally located karyosome, spikes radiating out from the karyosome to the periphery), food vacuole containing RBC, contractile vacuole wanting. Mitochondria, golgi bodies and rough endoplasmic absent absent	
Distinctive features of immature cyst:	Parasite is enclosed in a cyst wall, single nucleus(nucleus retains all the characteristics of the trophozoite), chromatoidal bars and glycogen mass present	
Uninucleate cyst		
Binucleate cyst		Two nuclei, all other characteristics are same as uninucleate cyst
Quadrinucleate cyst		Parasite enclosed in a cyst wall with four nuclei, chromatoidal bars and glycogen mass absent

Summary of life cycle of *Entamoeba histolytica*

