ZOOLOGY

Biology of Parasitism
Morphology, Life cycle, Pathogenicity, Diagnosis and Prophylaxis of Entamoeba Part 1
### Description of Module

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<th>ZOOLOGY</th>
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<td>Paper Name</td>
<td>Biology of Parasitism; Zool 008</td>
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<td>Keywords</td>
<td>Trophozoite, precyst, cyst, chromatoidal bars, excystation, encystation, metacystictrophozoites, amoebiasis, amoebic dysentery, extraintestinalinvasion.</td>
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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. hartmannii* 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*. 
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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
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*

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).
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 protistans 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 up to the nuclear membrane.
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 host's 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 (10-20µm) 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*


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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  

**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 Bailenger’s solution. The nuclei of binucleate cyst of *Entamoeba histolytica* are often paired

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

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**Quadrinucleate mature cyst fixed smear**
Source: [https://commons.wikimedia.org/wiki/File:Entamoeba_histolytica_on_fixed_smear.jpg](https://commons.wikimedia.org/wiki/File:Entamoeba_histolytica_on_fixed_smear.jpg)
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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 ileocelecal 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 **encystations** 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 up to 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.

**Source:** http://vle.du.ac.in/mod/book/view.php?id=9845&chapterid=15983 (CC)

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**Mode of Infection:** faecal-oral route

**Infected 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](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)

<table>
<thead>
<tr>
<th>Stages</th>
<th>Entamoeba histolytica</th>
<th>Entamoeba coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trophozoite Form</td>
<td>8µm-40 µm</td>
<td>18µm-50 µm</td>
</tr>
<tr>
<td>Fresh preparation</td>
<td>Very active</td>
<td>Sluggish</td>
</tr>
<tr>
<td>Size</td>
<td>Finger shaped, hyaline</td>
<td>Granular, short and blunt</td>
</tr>
<tr>
<td>Motility</td>
<td>Finely granular, ectoplasm and endoplasm clearly marked</td>
<td>Granular, ectoplasm and endoplasm not defined, ectoplasm scarcely seen</td>
</tr>
<tr>
<td>Pseudopodia</td>
<td>RBC, leucocytes and tissue debris</td>
<td>Bacteria and other food material but never RBC</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytoplasmic inclusions</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Nuclear character

<table>
<thead>
<tr>
<th></th>
<th>Small and central karyosome, delicate nuclear membrane lined with minute chromatin granules</th>
<th>Large and eccentric karyosome, thick nuclear membrane lined with coarse chromatin granules</th>
</tr>
</thead>
</table>

### Cyst Form

<table>
<thead>
<tr>
<th>Size</th>
<th>10µm-15µm</th>
<th>15µm-20µm</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Nucleus</th>
<th>1-4 in number, central karyosome</th>
<th>1-8 in number, eccentric karyosome</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Chromatoidal bars</th>
<th>Thick bars with rounded ends</th>
<th>Thread-like with square or pointed ends</th>
</tr>
</thead>
</table>

### Source:

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- [Trophozoite of Entamoeba coli with ingested bacteria](http://www.publicdomainfiles.com/show_file.php?id=13523762418607)
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 4µm-12µm while the cyst varies from 5µm-10µm. 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.
16. Comparison between the various *Entamoeba*

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Size</th>
<th>Movement</th>
<th>Inclusions</th>
<th>Nuclear characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Karyosome</td>
</tr>
<tr>
<td><em>Entamoeba histolytica</em></td>
<td>8µm-40 µm</td>
<td>Active, unidirectional</td>
<td>RBC, leucocytes and tissue debris</td>
<td>Small and central</td>
</tr>
<tr>
<td><em>Entamoeba gingivalis</em></td>
<td>20µm-150 µm</td>
<td>Active</td>
<td>cellular debris, bacteria and ingested leucocytes, no RBC</td>
<td>Small and central</td>
</tr>
<tr>
<td><em>Entamoeba hartmanni</em></td>
<td>4µm-12µm</td>
<td>Sluggish</td>
<td>Bacteria, No RBC</td>
<td>Small and central</td>
</tr>
<tr>
<td><em>Entamoeba coli</em></td>
<td>18µm-50 µm</td>
<td>Sluggish</td>
<td>Bacteria and other food material but no RBC</td>
<td>Large and eccentric</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Size</th>
<th>Nuclei</th>
<th>Cytoplasmic inclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chromatoid Body</td>
</tr>
<tr>
<td><em>Entamoeba histolytica</em></td>
<td>10µm-15µm</td>
<td>1-4 in number with central karyosome. Fine, symmetrical peripheral granules</td>
<td>Thick bars with rounded ends</td>
</tr>
<tr>
<td><em>Entamoeba gingivalis</em></td>
<td></td>
<td>Cyst form is absent in <em>Entamoeba gingivalis</em></td>
<td></td>
</tr>
<tr>
<td><em>Entamoeba hartmanni</em></td>
<td>5µm-12µm</td>
<td>1-4 in number with small, central karyosome. Fine, symmetrical peripheral granules</td>
<td>Rounded ends</td>
</tr>
<tr>
<td><em>Entamoeba coli</em></td>
<td>15µm-20 µm</td>
<td>1-8 in number with eccentric karyosome. Coarse and asymmetrical granules</td>
<td>Thread-like with square or pointed ends</td>
</tr>
</tbody>
</table>
### 17. Summary of *Entamoeba histolytica*

<table>
<thead>
<tr>
<th><strong>Entamoeba histolytica</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Causative agent /etiological agent of</strong></td>
<td>Amoebiasis, acute amoebic dysentery, chronic amoebic dysentery, liver abscess, splenic abscess, pulmonary amoebiasis, cerebral amoebiasis</td>
</tr>
<tr>
<td><strong>Definitive host</strong></td>
<td>Human, monogenetic parasite</td>
</tr>
<tr>
<td><strong>Habitat</strong></td>
<td>Caecum and colon</td>
</tr>
<tr>
<td><strong>Infective stage</strong></td>
<td>Quadrinucleate/ tetranucleate mature cyst</td>
</tr>
<tr>
<td><strong>Port of entry</strong></td>
<td>Faecal-oral route</td>
</tr>
<tr>
<td><strong>Method of transmission</strong></td>
<td>Contaminated fruits and raw vegetables, Contaminated water, Unhygienic habits, Mechanical vectors like houseflies and cockroaches</td>
</tr>
<tr>
<td><strong>Pathogenic stage</strong></td>
<td>Trophozoite</td>
</tr>
<tr>
<td><strong>Distinctive feature of trophozoite</strong></td>
<td>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</td>
</tr>
<tr>
<td><strong>Distinctive features of immature cyst:</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Uninucleate cyst</strong></td>
<td>Parasite is enclosed in a cyst wall, single nucleus(nucleus retains all the characteristics of the trophozoite), chromatoidal bars and glycogen mass present</td>
</tr>
<tr>
<td><strong>Binucleate cyst</strong></td>
<td>Two nuclei, all other characteristics are same as uninucleate cyst</td>
</tr>
<tr>
<td><strong>Quadrinucleate cyst</strong></td>
<td>Parasite enclosed in a cyst wall with four nuclei, chromatoidal bars and glycogen mass absent</td>
</tr>
</tbody>
</table>
Summary of life cycle of *Entamoeba histolytica*