**Entamoeba histolytica**

Causes: Amoebiasis.

Geog. Distribution: **cosmopolitan**

Habitat: **caecum and sigmoido-rectal region of man.**

Infective stage: **Quadrinucleate cyst.**

Mode of infection:
- Eating raw vegetables (salad)
- Drinking water
- Flies and food handlers (cyst passer)
- Faeco-oral

Heteroinfection

Autoinfection

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Entamoeba histolytica

Morphology of Trophozoite (vegetative form):

- 10-60 X 15-30 µ average (20-25 µ)
- Cytoplasm is clearly differentiated into:
  - **Ectoplasm**: is clear with well developed pseudopodia.
  - **Endoplasm**: dense & fine granular enclosing:
    - **Nucleus**: spherical containing central karyosome & peripheral evenly distributed small chromatin dots.
    - **Food vacuoles**: contain leucocytes-bacteria-may be RBCs.
Entamoeba histolytica

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**Entamoeba histolytica**

**Precyst stage:**
- 10-60 X 15-30 μ, average (15-20 μ)
- Round or oval with a blunt pseudopodia.
- Absent cyst wall
- Single nucleus present.

**Cyst stage:**
- 10-20 μ, average (15 μ)
- Four nuclei are present in mature quadrinucleated cyst
- Glycogen mass & chromatoid bodies are present in immature cysts – disappear in mature ones.

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Entamoeba histolytica

Morphology of cyst:

Precyst or unripe cyst
- Glycogen mass
- Cyst wall
- Nuclei
- Chromidial bodies and bars

Ripe cyst

Encystation when dehydrated in bowel lumen
- Passed in diarrhoea
  - Discharges undigested food
  - Precyst
    - Condenses to spherical mass
  - Cyst
    - Secretes tough cyst wall
    - Food inclusions
      - glycogen
      - chromidial bars
    - Two consecutive mitoses
      - produce 4 nuclei
      - less conspicuous
      - may disappear

Passed in semi-formed stool
- Glycogen and chromidial bars
- Passed in semi-formed or formed stool

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Life Cycle of *Entamoeba* inside human colon

In the lumen:
- Precyst
- Uninucleate cyst
- Binucleate cyst
- Pass out in stool

Attached to mucosa:
- Lumen (non-invasive) form
- Quadrinucleate cyst
- Enter with food
- Binary fission
- Trophozoite

Mucosa of large intestine

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Entamoeba histolytica

Life cycle of *E. histolytica*

- **Step 1:** Mature Cysts
- **Step 2:** Ingested
- **Step 3:** Excystation
- **Step 4:** Multiplication
- **Step 5:** Feces

**Legend:**
- ▲ = Infective Stage
- ▲ = Diagnostic Stage

**Abbreviations:**
- A = Non Invasive Colonization
- B = Intestinal Disease
- C = Extra-Intestinal Disease

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Pathogenesis

Depends on:

- Parasite virulence.
- Host resistance.
- Condition of the intestinal tract.

Non-pathogenic:
in the lumen.

OR Pathogenic:
trophozoites invade intestinal mucosa.

Trophozoites produce histolytic enzyme that produce necrosis of mucosa leading to the formation of flask-shaped ulcer.

Trophozoites exist in the base of the ulcer.
Factors determining Pathogenicity

1- Strain  
* E. dispar similar to E. histolytica differ in being non invasive

2- Virulence  
* Adherence and colonization  
* Enterotoxin production  
* Contact dependent cell lysis  
* Lytic enzyme secretion (proteolysis)  
* Phagocytic activity

3- Host factors

1- Immunity (secretory IgA)  
2- Nutrition: carbohydrate rich diet ↑, protein ↓, change diet habit
3- Drugs: immunosuppressive.  
4- Debilitating states (malignancy, pregnancy, etc).
5- Intestine: bacteria & intestinal flora – hypermotility or stasis of the bowel.
Pathogenesis & Complications

- Perforation
  - Haemorrhage (rare)

- Secondary infection

- Amoeboma (rare)
  - Clinically simulates neoplasm
  - Intussusception
  - Obstruction

- Invasion of blood vessels

- Direct extension outside bowel

- Peritonitis
  - Haemorrhage

- Surrounding inflammatory reaction and fibroblastic proliferation

- A mass under oedematous mucosa with
  - Internal abscesses of necrotic tissue and amoebae
  - Surrounding granulomatous tissue zone with eosinophils, lymphocytes and fibroblasts
  - Outer firm nodular fibrous tissue

Extraintestinal lesions - page 52

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This is followed by:

- Proliferation of connective tissue.
- Intensive ulcerations.
- Extra-intestinal invasion to brain, liver, lung or skin.
Asymptomatic: parasite in lumen and cysts pass in stool. (healthy cyst passer – most common – more than 75%)

Symptomatic: (gradual onset), fever (low grade), diarrhea, dysentery, abdominal pain, localized abdominal tenderness, tenesmus & strain, painful spasm of anal sphincter (indicates rectal ulceration). **Acute intestinal amoebiasis (colitis)**

Recurrent attacks of dysentery with intervening periods of constipation, abdominal distension & Flatulence, weight loss and cachexia. **Chronic intestinal amoebiasis**.

Rare progressive disease of high mortality (high fever- severe bloody diarrhea – diffuse tenderness – peritonitis) (fulminating colitis)

Amoebic hepatitis or amoebic abscess, lung abscess, brain abscess or skin abscess.

**Extra-intestinal amoebiasis**

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Complications

- **Amoeboma.**
  (localized granulomatous mass misdiagnosed with carcinoma)
- **Hemorrhage.**
- **Perforation of ulcer.**
  (secondary peritonitis --- rare but fatal)
- **Stricture of colon.**
  (secondary to fibrosis)
- **Appendicitis.**
Diagnosis (Intestinal amoebiasis)

- **Clinically:** **Dysentery:** painful frequent evacuation of small quantities of stool containing mucus tinged with blood.

- **Laboratory:**
  1. **Direct stool examination:** Trophozoites are found in diarrhoeic stool. Cysts are found in formed stool.
     - Wet preparation.
     - Iodine stained.
     - Permanent stain with iron haematoxylin or trichrome.

  2. **Concentration techniques** for cysts.
### In the Lab

<table>
<thead>
<tr>
<th>Typical amoebic dysentery stool</th>
<th>Typical bacillary dysentery stool</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulky.</td>
<td>Scanty.</td>
</tr>
<tr>
<td>Acidic.</td>
<td>Alkaline.</td>
</tr>
<tr>
<td>Scanty exudate.</td>
<td>Massive exudate.</td>
</tr>
<tr>
<td>Pus cells +</td>
<td>Pus cells +++</td>
</tr>
<tr>
<td>Blood +</td>
<td>Blood +</td>
</tr>
</tbody>
</table>

Amoebae trophozoites: present.  
Amoebae trophozoites: absent.
Diagnosis (intestinal amoebiasis)

3- Indirect diagnosis:

Serological tests **in chronic amoebiasis**.
Detection of copro-antigen using monoclonal antibodies.

Coating the well with MAb and add patient’s stool to detect *Entamoeba Ag*

Molecular techniques.

- **Radiological examination:** using barium enema.

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• Sigmoidoscopy: to visualize the ulcer, scrap, aspirate or take biopsy to see the trophozoites.
Diagnosis (Extraintestinal amoebiasis)

- **Clinical:** according to the organ affected.

- **Laboratory:**
  1. Examination of aspirate from lung or liver abscesses for trophozoites.
  2. Liver scanning.
  4. Serology.

5. Leucocytosis due to 2ry bacterial infection.

Coat with Ag and look for Ab in patient’s serum

Coat with Ab and look for Ag in patient’s serum

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Treatment

• **Metronidazole, Tinidazole.**  
  Tissue amoebicide
  Very effective in killing amoebas in the wall of the intestine, in blood and in liver abscesses.

• **Diluxanide furoate.**  
  Luminal amoebicide
  kills trophozoites and cysts in the lumen of the intestine.

  **Asymptomatic patients:** are given luminal amoebicide as *Diluxanide furoate.*

  **Symptomatic patients:** are given tissue amoebicide as *Metronidazole* followed by luminal amoebicide as *Diluxanide furoate.*
Treatment of Amoebic abscesses by aspiration or open surgical drainage

Aspiration of abscess

Surgical drainage of abscess

Thick chocolate-coloured or anchovy-sauce pus with trophozoites

Aspiration of abscess

Surgical drainage of abscess

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Epidemiology

• **Cyst passers** are the main source of infection.
• Cysts remain **viable** in faeces for few days, in water for longer periods.
• Cysts are **killed** by dryness, heat (over 55ºC) and by chlorine.

Control

- Treatment of patients.
- Examination and treatment of food handlers.
- Environmental sanitation.
- Personal prophylaxis.
- Human faeces should not be used as fertilizers.
Check for understanding

M.C.Q.

1- *Entamoeba histolytica* trophozoites are found in:
   a- Duodenum of infected human.
   b- Jejunum of infected human.
   c- Caecum of infected human.
   d- All of the above.

2- Infection with *Entamoeba histolytica* occurs through eating green salad contaminated with:
   a- Trophozoites of *Entamoeba histolytica*.
   b- Cysts of *Entamoeba histolytica*.
   c- Both trophozoites and cysts of *Entamoeba histolytica*.
3- Pathogenicity of *Entamoeba histolytica* depends on:
   a- Parasite virulence.
   b- Host resistance.
   c- Condition of intestinal tract.
   d- All of the above.

4- Patients with **chronic** intestinal amoebiasis suffer from:
   a- Dysentery, tenesmus with painful spasm of anal sphincter.
   b- Recurrent attacks of dysentery alternating with constipation.
   c- Fever, diarrhoea and tenesmus.
   d- Non of the above.

5- The following findings in stool suggest **amoebic** not bacillary dysentery infection:
   a- Acidic pH and presence of Charcot Leyden Crystals.
   b- Alkaine pH and presence of Charcot Leyden Crystals.
   c- Massive exudate with presence of many pus cells.
State True Or False

• Cyst passers are the main source of *Entamoeba histolytica* infection.  
  True

• Trophozoites of *Entamoeba histolytica* produce ulcers with indurated margin in intestinal mucosa.  
  False

• Examination and treatment of food handlers is very important to control *Entamoeba histolytica* infection.  
  True

• Infection with *Entamoeba histolytica* is totally localized to the gastrointestinal tract.  
  False

• Both trophozoites and cysts of *Entamoeba histolytica* are infective to man.  
  False
M.C.Q.

Rounded quadrinucleate cysts (20µ) seen in stool of patients should be reported:

1- *Entamoeba histolytica* cyst
2- *Entamoeba dispar* cyst
3- *Entamoeba histolytica / dispar* cyst
4- *Entamoeba coli* cyst

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CASE STUDY

• A 28 year old male presented with low grade fever, loss of appetite and pain in right upper quadrant since 15 days.

• Three months back, he suffered from dysentery.

• Physical examination revealed an enlarged liver.

• Blood picture showed leucocytosis with raised eosinophils.

• CT scan showed the presence of cavitative lesion in the right side of the liver.

• Aspirate from liver showed motile trophozoites in wet mount preparation.
**Entamoeba dispar**

- *E. dispar* - formerly designated as non-pathogenic *E. histolytica*
- 9x more prevalent than *E. histolytica*
- Morphologically identical with *E. histolytica*
- their DNA and ribosomal RNA are different
- microscopic identification unreliable
- **Dx:** - negative serologic tests
  - Trophozoite will have no ingested RBC

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• Morphologically indistinguishable from *E. histolytica/E. dispar*

  **trophozoite**

  » similar to *E. histolytica* except that it is much smaller (5-12 µm)
  » ingest bacteria but does not ingest RBCs
  » 1 nucleus w/ small and compact, central karyosome
  » more sluggish, non-progressive motility

  **cyst**

  » 5-10µm, spherical in shape
  » Mature: 4 nucleus with a coarse cytoplasm
  » immature cysts - Usually have chromatoidal bars
    (short with tapered ends, or thin and bar-like)
Entamoeba hartmanni

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Entamoeba coli

*most common endocommensal of humans
* has a worldwide distribution
* 10-50% of the population can be infected worldwide
* Feeds on bacteria and any other cells available to it
* does not invade tissues
* common inhabitant of the lumen of the cecum and colon of man and other animals
* Has the typical Entamoeba nucleus
**Entamoeba coli**

**Trophozoites**

- Usually 15-25 µm in diameter (range 10-50 µm)
- Cytoplasm:
  - More vacuolated or granular endoplasm with bacteria and debris but no RBCs
  - Dirty-looking or honeycomb appearance
  - Narrower, less differentiated ectoplasm
- Pseudopodia: broader and blunter
  - function more to ingest food
  - sluggish, non-directional motility
- Nucleus
  - 1 nucleus
  - Thicker, irregular, coarsely granular peripheral chromatin with a large eccentric karyosome (not compact, may/may not be eccentric)
Entamoeba coli

Cysts
- size: 10-35 μm
- Nucleus
  - Usually spherical
  - mature cyst: 8 nuclei
  - Immature cyst: 2 or more nuclei
  - Karyosome is large, may/may not be compact and/or eccentric
- Cytoplasm: coarsely granular
- Chromatoidal bodies: Splinter-shaped or broom-shaped with rough, pointed end
Entamoeba coli

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Entamoeba coli

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Entamoeba gingivalis

- a common inhabitant of the mouth of man
- lives on the surface of teeth and gums, in gum pockets and sometimes in the tonsillar crypts
- Organisms are more common in persons with pyorrhea (gum disease) but they are not the cause of the condition
- Hosts: Humans, other primates, dogs and cats
- Prevalence is from 50 to 95%
- Transmission:
  - kissing
  - droplet spray
  - sharing eating utensils

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Entamoeba gingivalis

**Trophozoite**
- 10-20µm
- Moves quickly
- Has numerous blunt pseudopodia
- Has numerous food vacuoles that contain cellular debris and bacteria and ingested leukocytes

- **no cyst stage**

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Entamoeba gingivalis
Endolimax nana

- Second most common endocommensal of humans
- Worldwide distribution 30%
- Lives in the large intestine mainly near the cecum
- Feed on bacteria
- Non pathogenic
- Outstanding feature:
  - Larger karyosome than those of the genus Entamoeba
  - Absent peripheral chromatin

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**Endolimax nana**

**Trophozoites**
- small size of 6 to 15 µm
- Cytoplasm is granular and vacuolated
- nucleus exhibits a large irregularly shaped karyosome (may appear “blot-like”) with no peripheral chromatin on the nuclear membrane
- Pseudopodia are blunt and hyaline
- Sluggish, non-progressive motility

**Cyst**
- Spherical or ovoid in shape
- Mature cyst
  - 5 – 14 µm in diameter
  - 4 nuclei when mature
  - A refractile cyst wall present
  - Chromatoidal bodies are not usually found
Endolimax nana

Trophozoite

Cyst

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Iodamoeba bütschlii

- Not very common endocommensal in people
- Non-pathogenic
- Lives in the large intestine, predominantly in the cecal areas

- Has a very high prevalence in pigs
  - 50% of pigs are infected with this ameba in France and Egypt
  - Pigs are probably its normal host
**Iodamoeba bütschlii**

**Trophozoites**
- Usually 9-14 µm long (range 6-20 µm)
- Single large vesicular nucleus with large chromatin-rich karyosome, surrounded by a layer of achromatic granules globules and anchored to nuclear membrane by achromatic fibrils (described as “basket nuclei”)
- Cytoplasm appears granular, containing vacuoles with ingested bacteria and debris
- Hyaline pseudopodia with sluggish progressive motility

**Cysts**
- 5-20 µm, oval-round in shape
- Uninucleated “basket nuclei”
- Prominent characteristic: large glycogen vacuole which stains deeply w/ iodine
Iodamoeba butschlii

Quiste
Iodamoeba butschlii

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**Dientamoeba fragilis**

**LIFE CYCLE** - it does not form cysts and trophozoites cannot survive passage through the small intestine.

Humans probably get infected by this endocommensal when they ingest pinworm eggs!

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Nuclear Morphology

Entamoeba histolytica

Entamoeba hartmanni

Entamoeba coli

Endolimax nana

Dientamoeba fragilis

Iodamoeba butschlii

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Intestinal Amebae

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<th>Iodamoeba butschlii</th>
<th>Dientamoeba fragilis</th>
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<tbody>
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