Intestinal Protozoa

Amebae
Flagellate protozoa
Coccidia
and others
Protozoa: numbers in millions

*Entamoeba histolytica*: 500; 48 severe; 0.07 deaths.

Malaria: 300-500; 2-3 deaths (mainly children).

*Giardia lamblia*: 500.

Eosinophilia > 10%

With helminths,
insects (myasis),
not with protozoa excepting
Isospora belli and Dientamoeba fragilis (with pinworms?)
Protozoa in faeces

• Cysts and trophozoites from amebae, flagellates, and coccidia.

• The size is essential for identification (to be measured with a calibrated micrometer).

• The aspect of the nucleus is also important for the identification.
Ocular micrometer disk

- Each objective must be calibrated with reference material.

- Can be roughly checked with a counting chamber, with RBCs ...
Direct examination in saline

• Standard-preparation, containing approximately 2 mg faeces.

• In fresh faeces it is possible to observe trophozoites (*Entamoeba histolytica*, ...).

• Cysts of protozoa are difficult to see, because they are colourless.
Ritchie-enrichment and Lugol stain

• Screen the entire preparation with objective 10x.

• Suspect elements (cysts, eggs,…) are checked with higher enlargement (40x, 50x, 100x).

• Amebae and flagellates stain brown-yellow with Lugol.

• The identification of cysts from protozoa is based on the size and the aspect of the nucleus.
Specific gravities

- S.G. of Zn-sulfate 33 %: 1.180
- S.G. of formol-solution 10 %: 1.019
- S.G. of ether: 0.714
- S.G. of parasites: *Ancylostoma* 1.055; *Giardia* 1.060; *Entamoeba histolytica (coli)* and *Endolimax nana* 1.065 - 1.070; *Ascaris* 1.110; *Trichuris* 1.150, *Chilomastix mesnili* 1.180; *Ascaris* (unfertilized) 1.200 (Bailenger, 1965).
Protozoa in faeces

- Visible unstained: (mobile) trophozoites, *Blastocystis hominis, Cyclospora caeytanensis*.

- Visible with Lugol-stain: cysts of amebae and flagellates.

- With special stains: iron-hematoxylin (trophozoites and cysts); safranine stain (*Cryptosporidium parvum*).

- Sometimes the elements are already visible in the Gram stain: *Blastocystis hominis*, trophozoites and cysts of *Giardia lamblia*.
<table>
<thead>
<tr>
<th>AMEBAE</th>
<th>Entamoeba histolytica</th>
<th>Entamoeba hartmanni</th>
<th>Entamoeba coli</th>
<th>Entamoeba polecki&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Endolimax nana</th>
<th>Iodamoeba bütschlii</th>
<th>Dientamoeba fragilis&lt;sup&gt;2&lt;/sup&gt;</th>
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<tbody>
<tr>
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<sup>1</sup>Rare, probably of animal origin  
<sup>2</sup>Flagellate

*Scale:* 0 5 10 μm

Courtesy ASM

**FIGURE 1** Amebae and flagellate (*Dientamoeba fragilis*) found in human stool specimens. (From reference 4.)
Nuclei of intestinal protozoa

Entamoeba histolytica

Entamoeba hartmanni

Entamoeba coli

Endolimax nana

Dientamoeba fragilis

Iodamoeba bütschlii

Courtesy ASM
Fig. 5-3. – Répartition géographique de l’amibiase (d’après R. Deschiens). $I^1$: isotherme 25 °C de juillet; $I^2$: isotherme 25 °C de janvier. En grisé, les zones d’endémie de l’amibiase; les flèches indiquent les zones de débordement les plus fréquentes de l’amibiase en zone tempérée.

Courtesy Gentilini M. & Duflo B.
Entamoeba histolytica

Entamoeba histolytica

Trophozoite (*magna* variety) in faeces. Diameter approximately 30 μm. Nucleus with typical fine chromatin picture and central karyosome (Lugol stain).
Entamoeba histolytica

Entamoeba histolytica

Courtesy Tulane
Entamoeba histolytica

Cyst in faeces with three visible nuclei. In the nucleus at the right we clearly see the central karyosome (Lugol stain).
Entamoeba histolytica

Cyst in faeces with two visible nuclei. In the nucleus at the left we clearly see the central karyosome (Lugol stain).
*Entamoeba histolytica*

Cyst in faeces with two visible nuclei. We clearly see the central karyosomes (Lugol stain).
Entamoeba histolytica

Cyst in faeces with one visible nucleus with a central karyosome (Lugol stain).
Entamoeba histolytica

Cyst in faeces with one visible nucleus with a central karyosome (Lugol stain).
*Entamoeba histolytica*

Cyst in faeces with one visible nucleus with a central karyosome (Lugol stain).
Entamoeba histolytica

Cyst in faeces with one visible nucleus with a central karyosome (Lugol stain).
Entamoeba histolytica

Cyst in faeces with one visible nucleus with a central karyosome (Lugol stain).
Entamoeba histolytica

Cyst with one visible nucleus and one cylindrical chromatoidal body (Lugol stain).
Entamoeba histolytica

Cyst with two visible nuclei and one cylindrical chromatoidal body (Lugol stain).
Entamoeba histolytica

Cyst with one visible nucleus and one cylindrical chromatoidal body (Lugol stain).
**Entamoeba histolytica - dispar**

- *Entamoeba polecki*
  *Entamoeba hartmanni* (small race *E. histolytica*)

- *Entamoeba histolytica* Laredo strain
  *Entamoeba dispar*
  non pathogenic strains grow between 20 and 37°C, pathogenic only at 37°C
  isoenzyme analysis: only 9 zymodemes are pathogenic
Entamoeba histolytica - dispar

- PCR, isoenzyme analysis, and antigen detection (JCM, 1998, 449).
- ITM-Antwerp: PCR on fecal material.
Entamoeba hartmanni

Small cyst with one visible nucleus. It has also been named Entamoeba histolytica small race. The nucleus shows the peripheral chromatin and a central karyosome (Lugol stain).
**Entamoeba hartmanni**

Small oval cyst with one visible nucleus. It has also been named *Entamoeba histolytica* small race. The nucleus shows the peripheral chromatin and a central karyosome (Lugol stain).
Entamoeba coli

Mature cyst with spongy cytoplasm in faeces. Four of the eight nuclei are visible in this plane. Note the coarse peripheral chromatin and the central karyosome of the nuclei (Lugol stain).
Entamoeba coli

Large cyst (> 15 μm) in faeces with four (to six) visible nuclei (Lugol stain).
**Entamoeba coli**

Large cyst (> 15μm) in faeces with three nuclei visible (Lugol stain).
Entamoeba coli

Large cyst (> 20µm) in faeces with four visible nuclei (Lugol stain).
Entamoeba coli

Large cyst in faeces with five nuclei and at the right a bundle of sharp chromatin bodies (Lugol stain).
**Endolimax nana**

Many cysts are visible, each with one to four nuclei. The cysts are smaller than 10 μm and contain four nuclei with a massive central karyosome. The two larger and darker cysts are *Giardia lamblia* (Lugol stain).
Endolimax nana

Many cysts are visible with several nuclei. The cysts are smaller than 10 μm and contain nuclei with a massive central karyosome (Lugol stain).
Endolimax nana

Three cysts are visible with two to four nuclei. The cysts are smaller than 10 μm and contain nuclei with a massive central karyosome (Lugol stain).
Endolimax nana

Cyst with three visible nuclei. The cysts are smaller than 10 μm and contain four nuclei with a massive central karyosome (Lugol stain).
Iodamoeba bütschlii

Mononucleate cyst with glycogen vacuole (dark brown with this stain) in faeces (Lugol stain).
Iodamoeba bütschlii

Cyst with glycogen vacuole (dark brown with this stain) and one massive nucleus in faeces (Lugol stain).
**Iodamoeba bütschlii**

Cyst with glycogen vacuole (dark brown with this stain) and one massive nucleus in faeces (Lugol stain).
Dientamoeba fragilis

- Two nuclei.
- “The unflagellated human flagellate “.
- Only (very labile) trophozoites, no cysts.
- Questionable enteric pathogen.
- Doxycycline, paromomycin, metronidazole (Sanford et al., 2010).
Dientamoeba fragilis Infection

(Dientamoeba fragilis)

1. Trophozoites in feces
2. Transmission via fecal/oral route.
3. It has been postulated that transmission occurs via helminth eggs, such as Ascaris and Enterobius.
4. Trophozoites ingested

i = Infective Stage

ı = Diagnostic Stage

- No cyst stage has been identified.
- Binary fission
- Trophozoites in lumen of colon

Courtesy CDC
Dientamoeba fragilis

SAF fixative (sodium acetate acetic acid formalin) and iron hematoxylin stain have replaced the PVA (polyvinyl alcohol fixative with HgCl$_2$) and trichrome stain.
Preservatives

(PVA)
- Polyvinyl alcohol
- “gold standard”
- Contains mercuric chloride = waste problem
- Trichrome or iron hematoxylin

SAF
- Sodium acetate formalin
- Alternative, no waste problem
- Iron hematoxylin
Sodium acetate acetic acid formalin (SAF)

- Sodium acetate: 1.5 g
- Acetic acid, glacial: 2.0 ml
- Formaldehyde, 37-40 %: 4.0 ml
- Distilled water: 92.0 ml
Dientamoeba fragilis
In saline (Unstained).
Dientamoeba fragilis

In saline (Unstained).
Dientamoeba fragilis
In saline (Unstained).
Dientamoeba fragilis

- From The Netherlands.
- In 247 unpreserved stool specimens: none.
- In 247 SAF-preserved stool specimens: 24.


Stained with hematoxylin.
Dientamoeba fragilis

- In Brussels, Belgium.
- SAF-preserved stool specimens used.
- *D. fragilis* (6.3%) and *G. lamblia* (7.1%) in 448 patients.

Stained with hematoxylin.

<table>
<thead>
<tr>
<th></th>
<th>Trichomonas hominis</th>
<th>Chilomastix mesnili</th>
<th>Giardia lamblia</th>
<th>Enteromonas hominis</th>
<th>Retortamonas intestinalis</th>
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*FIGURE 8* Flagellates found in human stool specimens. (From reference 4.)
Antoon van Leeuwenhoek

*Giardia lamblia*

1681
Giardiasis
(Giardia intestinalis)

Contamination of water, food, or hands/fomites with infective cysts.

Trophozoites are also passed in stool but they do not survive in the environment.

Courtesy CDC
Trophozoite of *Giardia lamblia*

- Pear shape
- 12-15 x 5-10 x 2-4 μm
- 2 nuclei
  - large karyosome, no peripheral chromatin
- Fibrils (axonemes) evident
  - bilateral symmetry
- Pair of median bodies
- Adhesive disk (not always evident)
- 4 pair flagella
  - motility likened to a falling leaf

Courtesy Tulane
Giardia lamblia

Trophozoite with two nuclei and several flagella in faeces (Gram stain).
Giardia lamblia

Trophozoites with several flagella in faeces (Gram stain).
Giardia lamblia

Trophozoite with adhesive (sucking) disk in faeces (Unstained).
Giardia lamblia

Trophozoite with two nuclei and several flagella
(May-Grünwald-Giemsa stain)
**Giardia lamblia**

Trophozoite in faeces with two nuclei and several flagella. Length: 10 to 20 µm  
(May-Grünwald-Giemsa stain)
Cyst of *Giardia lamblia*

- Oval shape
- 11-14 x 6-10 μm
- Distinct cell wall set apart from cytoplasm
- 4 nuclei at anterior end
  - large karyosome, no peripheral chromatin
- Fibrils (axonemes) evident
- Median bodies

*Courtesy Tulane*
*Giardia lamblia*

Cyst with two visible nuclei and flagella (Lugol stain).
Giardia lamblia

Cyst with two visible nuclei and flagella (Lugol stain).
**Giardia lamblia**

Cyst with two visible nuclei and flagella in faeces collected with SAF (Unstained).
**Giardia lamblia**

Two cysts with visible nuclei and flagella (Lugol stain).
Giardia lamblia

Many cysts, round or oval, some with visible nuclei and flagella (Lugol stain).
Giardia lamblia

Older shrunked cyst with two visible nuclei (Lugol stain).

20 µm
**Giardia lamblia**: antigen detection by IF and ELISA

- Monoclonal antibodies: Merifluor (MERIDIAN) (*Cryptosporidium* and *Giardia*).
- 8/9 *Giardia* ELISAs are OK. (JCM, 1998, 1338).
- Triage parasite panel (BIOSITE) useful. (JCM, 2000, 3337; JCM, 2001, 334).
- One ELISA almost as sensitive as two microscopic examinations. (Mank T. 1997).
Antigen detection

- Good sensitivity and specificity
- In combination with conventional tests
- High cost (Triage = $ 19.44/test)
Chilomastix mesnili

Pearshaped cyst with one nucleus (Lugol stain).
*Chilomastix mesnili*

Pearshaped cyst with one nucleus (Lugol stain).
*Chilomastix mesnili*

Pearshaped cyst with one nucleus (Lugol stain).
Chilomastix mesnili

Two pearshaped small cysts with one nucleus (Lugol stain).
Chilomastix mesnili

Trophozoite with one nucleus and flagella (Lugol stain).
*Pentatrichomonas hominis*

In blood containing stool. Shown here are the long undulating membrane and the axostyle (8 to 15μm) (Osmic acid-Giemsa stain).
<table>
<thead>
<tr>
<th>CILIATE</th>
<th>COCCIDIA</th>
<th>BLASTOCYSTIS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Balantidium coli</strong></td>
<td><strong>Isospora belli</strong></td>
<td><strong>Sarcocystis spp.</strong></td>
</tr>
<tr>
<td>Trophozoite</td>
<td>immature oocyst</td>
<td>mature oocyst</td>
</tr>
<tr>
<td>Cyst</td>
<td>mature oocyst</td>
<td>single sporocyst</td>
</tr>
</tbody>
</table>

**Scale:** 0 10 20 30 μm

**FIGURE 7** Ciliate, coccidia, and *Blastocystis hominis* found in human stool specimens. (From reference 4.)

*Courtesy ASM*
Balantidium coli

In stool. An ovoid ciliate, here as trophozoite, with clearly visible macro- and micronucleus, cystosome, and cilia. Size 60 to 80 μm (Unstained).
Isospora belli

Continued development of the oocyst occurs outside the body, to form two mature sporocysts, each containing four sporozoites, resembling Sarcocystis spp. Two immature ovoid oocysts in faeces. The oocyst contains one immature sporont (at the left), occasionally a dividing sporont (at the right) or two sporonts (not seen) (Unstained).
**Isospora belli**

The oocyst contains a dividing sporont (Unstained).
**Isospora belli**

- Human to human transmission.
- Eosinophilia may be present.
- Worldwide.
- Oocyst very pale and transparent.
- Wet-preparation examination preferred over the stained smear.
- Cotrimoxazole (HIV).

Two sporocysts with each four sporozoites.

Isosporiasis
(Isospora belli)

1. Oocysts in feces
2. Mature oocysts with sporozoites
3. Immature oocysts with sporoblasts
4. Immature oocysts with sporozoites
5. Oocysts in feces

1 = Infective Stage
4 = Diagnostic Stage

Sporozoites
Mature oocysts
Asexual
Merozoites
Microgamete
Fertilization
Macrogamete
Sexual

Courtesy of CDC
**Sarcocystis** spp.

Human contamination is mainly due to consumption of improperly cooked pork or beef meat. Mature sporocyst containing four sporozoites in faeces (Unstained).

**The Life Cycle of Sarcocystis cruzi**

The parasites infect the intestinal tissues of the host, reproduce asexually, and finally produce oocysts.

The definitive host is infected when it ingests bradyzoites in the tissue.

Zoites, sarcocysts, or Miescher's tubules, filled with bradyzoites, form in the host's tissues.

Oocysts are passed in the host's feces.

The oocysts excyst, and the parasites infect the host's tissues.

Oocysts are ingested by the intermediate host.

(Parasites and Parasitological Resources)

Courtesy The Ohio State University
Cryptosporidium parvum

Several oocysts are seen as clear white holes in the densely coloured fecal mass (Gram stain).
Cryptosporidium parvum

Several oocysts are seen as clear white holes in the densely coloured fecal mass (Gram stain).
*Cryptosporidium parvum*

Numerous oocysts containing four sporozoites are present. The bacteria are stained in blue by methyleneblue (Safranin stain).
*Cryptosporidium parvum*

Numerous oocysts. The bacteria are stained blue with methyleneblue (Safranin stain).
Cryptosporidium parvum

Numerous oocysts. The bacteria are stained blue with methyleneblue (Safranin stain).
Cryptosporidium parvum

Four oocysts containing four sporozoites are present. The bacteria are stained in blue by methyleneblue (Safranin stain).
Cryptosporidium parvum

Oocyst containing four sporozoites (Modified Ziehl-Neelsen stain).
**Blastocystis hominis**

- Contains a central-body form
- 5 - 30 μm
- 81 / 247 patients; 5 x more in SAF (The Netherlands)

(Gram stain)

Blastocystis hominis

- Belongs to the stramenoiles.
- Pathogenicity uncertain (HIV).
- Most prevalent human “protozoon”.
- Relatively labile (fixative recommended).

(Lugol stain)
Blastocystis hominis

Several cysts in a smear from faeces (Gram stain).
Blastocystis hominis

Several cysts in a smear from faeces (Gram stain).
Blastocystis hominis

Cysts in faeces (Hematoxylin stain).
Blastocystis hominis

Cyst in faeces (Lugol stain).
Blastocystis hominis

Two cysts in faeces. At right the cyst reproduces by binary fission (Lugol stain).
Blastocystis hominis

Cyst in faeces collected with SAF (Unstained).
Blastocystis hominis

Several cysts of Blastocystis hominis and of Giardia lamblia in faeces (Lugol stain).
**Cyclospora cayetanensis**

- Blue-green algae, cyanobacterium-like bodies (CLB).
- Spherical oocyst (8-10 μm) containing small granules with a greenish shade, showing fluorescence under UV illumination.
- Do not stain with Lugol.
- Are acid fast with the Ziehl-Neelsen stain.

Cyclospora cayetanensis

Oocyst in faeces. Spherical oocyst (8-10 μm) containing small granules with a greenish shade, giving the aspect of a morula (Unstained).
Cyclospora cayetanensis

Two spherical oocysts in faeces (8-10 μm) containing small granules with a greenish shade, giving the aspect of a morula (Unstained).
Cyclospora cayetanensis

Spherical oocysts (8-10 μm) containing small granules in faeces (Ziehl-Neelsen stain).
Microsporidia

Immunosuppression (AIDS)

Intestine: *Enterocytozoon* spp., *Encephalitozoon (Septata)* spp.

Tissues: *Nosema* spp., *Encephalitozoon* spp., *Pleistophora* spp., ...

Albendazole (GSK)
**Microsporidia: laboratory diagnosis**

Very small spores (1 - 2 µm)
Modified trichrome stain, Uvitex 2B, …
Transmission electron microscopy
Immunofluorescence assays
Molecular methods
Microsporidia

Spore containing a polar tubule, an extrusion mechanism for injecting the infecting spore content into host cells.

Courtesy Garcia L. 1999.
Microsporidia

Small round to oval elements in faeces. They are only slightly larger than the light green bacteria (Trichrome stain).
**Geotrichum candidum**

The round (blasto-)spores and the rectangular arthrospores of this fungus are sometimes mistaken in stool for protozoal cysts. *G. candidum* stains dark violet with the Gram stain and yellow-brown with Lugol (Lugol stain).
Geotrichum candidum

*G. candidum* in culture. The round (blasto-)spores and the rectangular arthrospores of this fungus are sometimes mistaken in stool for protozoal cysts (Unstained).