Introduction to Protozoology
Protozoans are microscopic one-celled organisms that are categorized according to their method of movements.

- **Ciliates** – the only parasitic ciliate that causes disease in humans in *Balantidium coli*
- **Flagellates** – three of the most common and medically significant include: *Giardia lamblia*, *Trypanosome* sp. and *Trichomonas vaginalis*
- **Amoeba** – include the pathogenic amoeba *Entamoeba* and *Endolimax* which cause dysentery in humans
- **Apicomplexa** – no special organs for movement (*Toxoplasma*)
<table>
<thead>
<tr>
<th>Division</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sporozoa</td>
<td>All are intracellular parasites, e.g. <em>Plasmodium</em> in red blood cell</td>
</tr>
<tr>
<td>Flagellates</td>
<td>Move by beating of one or more flagella, e.g. <em>Trypanosoma</em></td>
</tr>
<tr>
<td>Amoebae</td>
<td>Move by extending pseudopodia, no fixed shape, e.g. <em>Entamoeba</em></td>
</tr>
<tr>
<td>Ciliates</td>
<td>Move by beating of many cilia, e.g. <em>Balantidium</em></td>
</tr>
</tbody>
</table>
Forms and reproduction

Cysts - infective forms, survive in the environment

Trophozoites - vegetative forms, capable for reproduction:
- Shizogony (asexual)
- Binary fission (asexual)
- Endodiogony
- Sporogony (sexual)
- Conjugation
The Protozoa

• Blood and tissue protozoa
  (e.g., *Plasmodium* spp.)

• Intestinal and urogenital protozoa
  (e.g. *Entamoeba histolytica*,
   *Cryptosporidium* spp.)
Intestinal and urogenital protozoa
Intestinal protozoa

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  - Second level
- Third level
  - Fourth level
  - Fifth level
Intestinal protozoa

Fecal-Oral Transmission Factors
- Poor personal hygiene
- Children (e.g., day-care centers)
- Institutions (e.g., prisons, mental hospitals, orphanages)
- Food handlers
- Developing countries
- Poor sanitation
- Lack of indoor plumbing
- Endemic travelers' diarrhea
- Water-borne epidemics
- Water treatment failures
- Male homosexuality
- Oral-anal contact
- Zoonosis?

Entamoeba = no
Cryptosporidium = yes
Giardia = controversial
Principle of stool sampling collection, handling and processing for parasites examination

Collection and handling:
• Minimum 3 samples
• Clean, water-tight container with a screw-cap lid
• The smallest acceptable amount of stool is 2-5g
• Urine should not be allowed to contaminate the specimen
• The specimen container should be labeled correctly (patients’ name, date and time of sample collection, test/tests requested, suspected diagnosis, clinical findings, travel history)
Preservation (fixation)

• The ideal specimen is a freshly collected stool sample
• 5-10% formalin
• PVA – polyvinyl alcohol
• MIF – merthiolate iodine formalin
Processing

- Macroscopic examination:
  - consistency
  - color
  - gross abnormalities
  - blood and mucus in feces

- Microscopic examination: standard procedures
Direct wet preparations

Saline wet preparations:
good for the recovery of the motile protozoan trophozoites

Iodine wet preparations:
study of the detailed morphology of protozoan cysts
Concentration methods

Reason for their use:
(a) removal of debris from the sample
(b) parasites are often present in low numbers and need to be condensed into one area of the sample

• Formalin-ether (or ethyl acetate) concentration procedure: after centrifugation of the sample the parasites present are heavier than solution and settle in the sediment of the tube

• Zinc sulfate flotation technique: after 15min parasites come out on the surface of the solution
Permanent stains

- Trichrome stain
- Giemsa stain
- Iron hematoxylin stain
- Modified acid-fast stain (modified Ziehl-Neelsen stain)

Immunologic diagnosis

- Detection of Ag from specific parasites in the stool
  (IF, ELISA)
Amebas of human beings

<table>
<thead>
<tr>
<th>Trophozoite</th>
<th>Entamoeba histolytica</th>
<th>Entamoeba hartmanni</th>
<th>Entamoeba polecki*</th>
<th>Endolimax nana</th>
<th>Iodamoeba bütschlii</th>
</tr>
</thead>
</table>

*Rare, probably of animal origin

*Second Level

*Third Level

*Fourth Level

*Fifth Level
# Amebas of human beings

<table>
<thead>
<tr>
<th>Organism</th>
<th>Size (μm)</th>
<th>Motility</th>
<th>Nuclei (Stained)</th>
<th>(Numbers)</th>
<th>Nuclei Chromatoidals</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Entamoeba histolytica</em></td>
<td>10-60</td>
<td>Active</td>
<td>Karyosome small and central; chromatin, fine and peripheral</td>
<td>1-6</td>
<td>Ends rounded or square</td>
<td>Pathogenic</td>
</tr>
<tr>
<td><em>Entamoeba hartmanni</em></td>
<td>4-12</td>
<td>Active</td>
<td>Karyosome small central; chromatin fine and peripheral</td>
<td>1-4</td>
<td>Ends rounded or square</td>
<td>Nonpathogenic</td>
</tr>
<tr>
<td><em>Entamoeba gingivalis</em></td>
<td>5-35</td>
<td>Sluggish</td>
<td>Karyosome small central; chromatin fine and peripheral</td>
<td>—</td>
<td>—</td>
<td>Mouth-dwelling nonpathogenic</td>
</tr>
<tr>
<td><em>Entamoeba polecki</em></td>
<td>10-20</td>
<td>Round</td>
<td>Karyosome small and central; chromatin variable</td>
<td>1</td>
<td>Ends pointed</td>
<td>Rare in humans nonpathogenic</td>
</tr>
<tr>
<td><em>Entamoeba moshkovskii</em></td>
<td>10-60</td>
<td>Round</td>
<td>Karyosome small and central; chromatin fine and peripheral</td>
<td>1-4</td>
<td>Ends rounded</td>
<td>Nonpathogenic</td>
</tr>
<tr>
<td><em>Entamoeba coli</em></td>
<td>10-50</td>
<td>Sluggish</td>
<td>Karyosome large and eccentric; chromatin clumpy and peripheral</td>
<td>1-8</td>
<td>Ends jagged</td>
<td>Nonpathogenic</td>
</tr>
<tr>
<td><em>Endolimax nana</em></td>
<td>6-15</td>
<td>Sluggish</td>
<td>Karyosome large and variable; little or no chromatin</td>
<td>1-4</td>
<td>None</td>
<td>Nonpathogenic</td>
</tr>
<tr>
<td><em>Iodamoeba bütschlii</em></td>
<td>6-25</td>
<td>Active</td>
<td>Karyosome large and central; chromatin absent</td>
<td>1</td>
<td>None</td>
<td>Nonpathogenic</td>
</tr>
</tbody>
</table>
Epidemiology and incidence

• worldwide in distribution, more prevalent in underdeveloped nations with poor sanitation
• the source for infection in humans: contaminated water or vegetables
• cysts are not eradicated with chlorine
• boiling of water is necessary for econtamination
• disease is seen at all ages
• equally distributed in men and women
• invasive disease occurs in 50 million people worldwide each year
Entamoeba histolytica s. dysenteriae

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    - Third level
      - Fourth level
        » Fifth level

Pseudopodia
Trophozoite (vegetative) - Fecal or uterine cyst
Pseudocyst

Endoplasm with inclusions - Ectoplasm
Cytoplasm

Nucleus
Membrane - Chromatin lining membrane - Fibril network - Karyosome

Glycogen mass - Cyst wall - Nuclei
Chromidal bodies and bars

Important note
E. dispar is morphologically identical to E. histolytica but the trophozoites are not haematophagous
Entamoeba histolytica - life cycle

E. histolytica exhibits a typical fecal-oral life cycle consisting of infectious cysts passed in the feces and trophozoites which replicate within the large intestine. Trophozoites colonize the large intestine, especially the cecal and sigmoidorectal regions, where they feed on bacteria and cellular debris.
Entamoeba histolytica – life cycle

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        - Fifth level
Possible Virulence Factors

Host factors
• ineffective innate immunity
• inflammatory response

Parasite factors
• resistance to host response
  (eg, complement resistance)
• adherence properties
• cytolytic properties
• ability to breakdown tissues (e.g. secreted proteases)
Entamoeba histolytica - trophozoite

*Entamoeba histolytica*. Time lapse photography under phase contrast. The point at C is a marker against which the movement of the trophozoite can be judged; at point B are particles towards which the trophozoite progresses and eventually engulfs. D indicates a recently ingested red blood cell and at A the characteristic nucleus is evident. ×750

The cells in both of the above series were harvested from a culture in Jones’ medium.
Entamoeba histolytica – colon ulcers

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Entamoeba histolytica - intestinal amebiasis

Trophozoites can invade the colonic epithelium and produce ulcers and dysentery

Colon ulcer HP

Blood and mucus in feces (dysentery)

Trophozoites can invade the colonic epithelium and produce ulcers and dysentery.

Colon ulcer HP

Blood and mucus in feces (dysentery)
Extraintestinal amebiasis

E. histolytica is found primarily in the colon where it can live as a non-pathogenic commensal or invade the intestinal mucosa (green).

The ameba can metastasize to other organs via a hematogenous route (purple), primarily involving the portal vein and liver. The ameba can also spread via a direct expansion (blue) causing a pulmonary infection, cutaneous lesions or perianal ulcers.
Extraintestinal amebiasis

- Abscess in liver
- Aspirate from liver abscess
- Cutaneous amebiasis
- Ano-rectal amebiasis
- Genital amebiasis
Incubation period

- patients develop symptoms with invasive disease within 3 weeks of ingestion of the cysts
- amebic liver abscess formation takes about 3 months to develop
- some patients apparently carry the organisms for prolonged periods before developing significant clinical manifestations
Clinical Syndromes
Associated with Amebiasis

Intestinal Disease
- asymptomatic cyst passer
- symptomatic nondysenteric infection
- amebic dysentery (acute)
- fulminant colitis + perforation (peritonitis)
- ameboma (amebic granuloma)
- perianal ulceration

Extraintestinal Disease
- liver abscess
- pleuropulmonary amebiasis
- brain and other organs
- cutaneous and genital diseases
Diagnosis of intestinal amebiasis

**Intestinal Disease**
- stool examination
  - cysts and/or trophozoites
- sigmoidoscopy
  - lesions, aspirate, biopsy
- antigen detection
  - histolytica/dispar
E. histolytica - trophozoites

Heidenhein stain

Wet mount
E. histolytica – cyst

E. coli cyst (Iodine wet preparation)  E. histolytica cyst (wet mount)
Diagnosis of intestinal amebiasis

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      - Fourth level
        - Fifth level
Amebiasis - diagnosis

• all patients with invasive disease have blood in the stools
• cysts or trophozoites should be visible on microscopic evaluation of the stool
• colonic biopsy specimens reveal organisms
• antiamoebic antibodies are positive in patients with invasive disease only
• leukocytosis without eosinophilia is often seen in patients with invasive amebic disease
• elevated liver function tests can be seen in cases of liver involvement
Diagnosis of extraintestinal amebiasis

Extraintestinal (hepatic) Disease

- serology
  - current or past?
- imaging
  - CT, MRI, ultrasound
- abscess aspiration
  - only select cases
  - reddish brown liquid
  - trophozoites at abscess wall

- aspiration of a liver abscess often fails to recover the organism, since it lives in the walls of the abscess
Diagnosis of extraintestinal amebiasis

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    - Third level
      - Fourth level
        - Fifth level

```
If positive for abscess
  Serology for amebas
    If positive
      Treat
      If positive
        Treat
        If positive
          Search for other etiologies
          If negative
            Treat
            If positive
              Search for other etiologies
              If negative
                Search for other etiologies
      If negative
        Aspirate
        If positive
          Search for other etiologies
          If negative
            Search for other etiologies
            If negative
```

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Entamoeba coli

- nonpathogenic comensal
- trophozoites do not ingest erythrocytes and do not invade tissues
- the cyst has 8 nuclei (versus 4 nuclei of *E. histolytica*)
E. dispar

- *E. dispar* is morphologically identical to *E. histolytica*, but does not produce an invasive disease.
Free-living amoebas - morphology

- Intamoeba histolytica
- Naegleri a fowleri

Acanthamoeba castellanii

- Trophozoite
- Acantopodia
- Ectocyst
- Zyste
- Endocyst
Free-living amoebas

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Free-living amoebas:
_Acanthamoeba_ and _Naegleria_ sp.

**Acanthamoeba sp.**
- found in soil and lakes
- inhabit immunologically privileged sites as eye or brain
- eye infection by contaminated contact lenses

**Disease**
- ulcerative keratitis – if untreated leads to loss of the eye
- granulomatous amoebic encephalitis (GAE)
Free-living amoebas: 
*Acanthamoeba* and *Naegleria* sp.

**Naegleria fowleri**
- in worm water, mud, lakes
- the infection is acquired by accidental inhalation of contaminated water while swimming or playing

Disease
- primary amoebic meningoencephalitis (PAM) – rapidly turn into a deep coma, almost always fatal
Free-living amoebas - life cycles

- Acanthamoeba spp.
- Naegleria fowleri
Acanthamoeba spp. - Keratitis

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    - Third level
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- Encystment or cyst hatch
- Promotive or proliferative form
- Vegetative form (trophozoite)
- External environment (water, soil, dust)
- Contaminated contact lenses
- Contaminated cleaning solution
- Corneal injury
- Acanthamoeba keratitis
  (can occur in immunologically competent host)
Acanthamoeba spp. - Keratitis

Acanthamoeba culbertsoni
Trophozoite

Eye infection
(Acanthamoeba keratitis)
Acanthamoeba spp.

**Acanthamoeba spp. - Keratitis**

Cysts in corneal scraping (H&E)

**Acanthamoeba spp. - GAE**

Trophozoites in brain (H&E)
Acanthamoeba spp. – skin infection
Naegleria spp.

Naegleria fowleri trophozoite

N. fowleri in brain (PAM)
Balantidium coli

- large ciliate, common parasite of pigs, rarely causes disease in humans
- transmission by fecal-oral route

Disease
- most infections are asymptomatic
- may develop dysentery and colitis with nausea, vomiting and fever
Balantidium coli – trophozoite

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  - Second level
    - Third level
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        - Fifth level
**Balantidium coli - diagnosis**

- Trophozoites in colon (HP)

- Trophozoites in stool specimen

- Cyst in stool specimen
Giardia lamblia – etiology

- a flagellate protozoon: *G. lamblia* (*Giardia intestinalis*)
- exists in trophozoite and cyst forms
- the infective form is the cyst of the parasite
- cysts remain infective in water for a few months
- when ingested by a new host, they excyst in the upper gastrointestinal tract and liberate trophozoites, which attach with their suckers to the surface of the duodenal or jejunal mucosa and multiply by binary fission
- when trophozoites drop off the duodenal and jejunal mucosa, they are carried on with the contents in the gut and encyst
Giardia lamblia – life cycle

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    - Third level
      - Fourth level
        » Fifth level
Giardia lamblia - epidemiology

- globally distributed parasitosis
- infection is usually sporadic and spreads from person to person directly by the fecal-oral route or indirectly by ingestion of fecally contaminated water or food
- humans are the principal reservoir of infection
- overland travelers to the Far East are at high risk for infection
Giardia lamblia – incubation period

- infection may be asymptomatic or symptomatic
- the ratio of asymptomatic to symptomatic cases is high
- children usually acquire the infection but exhibit a high degree of tolerance
- symptoms develop a few days to several weeks (average, 9 days) after ingestion of cysts
- severe infection may develop in immunodeficient hosts
- infection may become chronic
**Giardia lamblia** – clinical manifestations

- The main symptom is diarrhea (may continue for weeks or months if untreated), steatorrhea.
- Lead to malabsorption, particularly of lipids and lipid-soluble vitamins (may be difficult in children), loss of weight.
- Do not penetrate the mucosa.
- Crampy abdominal pain, urgent call to stool.
- Stool: pale, offensive, bulky, with much flatus but no blood or mucus.
- Anorexia and possibly vomiting in each stage of symptomatic infection.
- In immunocompetent self-limited infection in 4 weeks.
Giardia lamblia

- Trophozoites adhere to mucosa

Trophozoites - EM
Giardiasis - diagnosis

**Duodenal aspiration**

- Enterotest ("string test"): a string is taped to the patient’s face and a gelatin capsule attached to the string is swallowed. After the capsule has dissolved and the string has reached the duodenum (4 hours later), the string is retrieved and examined for parasites.

**Duodenal biopsy**

- Intestinal biopsy reveals partial villous atrophy
- trophozoites may be seen on the surface of the bowel
Giardiasis - diagnosis

- direct saline smear of stool for characteristic cysts
- repeat three times for up to 90% success of identifying the cysts versus 50% to 70% on single stool specimen examination
- trophozoites are found in fresh diarrheal stools
  - trophozoite: pear-shaped, 15 µm long, 9 µm wide, 3 µm thick; possesses four pairs of flagella
- cysts are found in form stools
  - cyst: oval, 8 to 14 µm long, 5 to 10 µm wide; contains four small nuclei and a central refractile axostyle
Giardia lamblia

- Trophozoite in stool specimen

- Cyst in stool specimen – wet mount
Giardiasis - diagnosis

Indirect diagnosis

• serology as useful diagnostic aids
• antigen-capture ELISA can be used to demonstrate submicroscopic infections in faeces
• ELISA to detect IgM in serum provides evidence of current infection
• IgA-based ELISA can detect specific antibodies in saliva
Giardiasis - treatment

• Metronidazole (with efficacy up to 80%-95%)
  – in adults: either 250 to 500 mg for 5 days
  – avoid alcohol intake, as it may produce side effects such as headache and flushing.
  – in children, dosage modified: 5 mg/kg for 7 days
Trichomonas vaginalis

✓ Exist only as a trophozoite!!! (no cyst form)

✓ Cosmopolitan, strictly human, sexually transmitted flagellate

✓ Disease: trichomoniasis

Trophozoite adhere to epithelial cells
Trichomonas vaginalis – epidemiology

- Trichomoniasis is a sexually transmitted disease that accounts for 25% of vaginitis
- usually women have symptoms, while males are asymptomatic but may act as reservoirs of infection
- 30% women will develop symptoms within 6 months
- *T. vaginalis* is isolated from prostatic secretions of 70% of male consorts of infected women
Trichomonas vaginalis – life cycle

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        » Fifth level
*Trichomonas vaginalis* – clinical manifestations

**Females:** vulvar erythema, pruritus, edema
- Vaginal discharge
  - Purulent: 60%
  - Frothy: 10% to 35%
  - Gray: 45%
  - Yellow-green: 35%
- Strawberry or “flea-bitten” cervix, which can be seen by colposcopy

**Males:** asymptomatic, urethral discharge, dysuria
Trichomonas vaginalis – diagnosis

Specimen: vaginal secretions, urethral discharge, urine sediment, prostatic secretions

Methods:
- Microscopic examination
  - direct wet mount
  - Giemsa stain
  - Acridine orange fluorescent stain
- Culture – Diamond’s medium
- PCR

Findings: trophozoite!!!
Trichomoniasis – diagnosis

• examination of a wet saline mount of vaginal discharge under a microscope shows motile, flagellated protozoa in a background of many polymorphonuclear leukocytes
• the pH of vaginal discharge is greater than 4.5
• culture for *Trichomonas* in special medium (Diamond medium) has a high yield of positives
• direct examination and culture of urine sediment is the test of choice for diagnosing males
Trichomoniasis – treatment

• the preferred treatment is a single 2-g dose of metronidazole. Alternately, 500 mg bid for 7 days can be used
• coitus should be avoided until treatment is complete and both partners are asymptomatic
• a single dose of 2 g of metronidazole may be given to pregnant women only after the first trimester
• treatment failure occurs in up to 30% when the male partner is not treated
Intestinal Coccidia: oocyst morphology
Cryptosporidium parvum

- Infection usually occurs by ingestion of oocysts from fecally contaminated water.

- Oocysts can survive as long as 18 months in the environment.

- Studies have shown that ingestion of less than 1,000 oocysts can lead to disease.
Cryptosporidium parvum

• intracellular protozoan that is responsible for self-limited diarrhea in children and adults and protracted and even fatal diarrhea in patients with HIV infection

• the entire life cycle occurs within one person
Cryptosporidium parvum

Incubation
• Incubation is between 7 and 10 days

Clinical Manifestations
• diarrhea in normal persons occurs at various degrees of severity from 2 days to 1 month
• patients may have crampy abdominal pains
• low-grade fevers may occur
• in patients with immunosuppression, such as HIV infection, voluminous diarrhea with as much as 15 L/d can occur
Cryptosporidiosis - diagnosis

- stool specimens reveal oocysts with Giemsa stains or modified acid-fast stains
- fluorescent antibody stains for stool or tissue specimens are available
- fecal leukocytes are not present
- fat absorption is impaired
- vitamin B12 levels may become low

Modified Ziehl-Neelsen stain
Cryptosporidiosis - treatment

- there is no effective treatment for this illness
- patients who are immunocompetent are likely to run a self-limited illness of several days to 6 weeks, for which supportive care is given
- in HIV-infected individuals, supportive care is critical
Isospora belli – life cycle

1. Immature, unsporulated oocyst is excreted through feces.

2. Sporoblast divides into two.

3. Each sporoblast develops into a sporocyst with 4 sporozoites, resulting in mature oocysts. The time spent in stages 1 through 3 is 2–3 days.

4. Mature oocyst is ingested.

5. Oocyst bursts. Sporozoites are released and lodge into the intestinal lining. Sporozoites undergo asexual reproduction to form merozoites. The merozoites mature into gametes which undergo fertilization to produce a new oocyst.
*Isospora belli*

Isosporiasis:
- diarrhea, malabsorption, eosinophilia,
- particularly in patients with AIDS

**Diagnosis:**
- examination of concentrated stools
- Kinyoun stain
Cyclospora cayetanensis

Epidemiology: contaminated water, fruits and vegetables
Manifestation: diarrhea
Diagnosis: oocysts in stool samples
Treatment: Bactrim®
Occasionally humans can act as intermediate hosts for *Sarcocystis* of other animals.
Sarcocystis spp.: S. suihominis and S. bovihominis

Miescher’s tubes

Sarcocystis suihominis (oocyst)
Unclassified protozoa *Blastocystis hominis*

- Large central body (vacuole)
  - worldwide
  - commonly found in stool specimens
  - pathogenicity is unclear (not to cause any disease in most cases of isolation)
  - self-limited, acute diarrhea

Diagnosis: identification in stool specimen