Other Protozoan Pathogens

*Giardia lamblia*  
(Stiles 1915)  

*Trichomonas vaginalis*  
(Donné 1836)  

*Balamidium coli*  
(Malmsten 1857)  

*Entamoeba histolytica*  
(Schaudinn 1903)  

*Naegleria fowleri*  
(Culbertson, 1971)  

*Acanthamoeba spp.*  
(Culbertson, 1971)  

*Balamuthia mandrillaris*  
(Martinez, 1993)  

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**Giardia lamblia**  
(Stiles 1915)
Giardia lamblia

“...animalcules a-moving very prettily. Their bodies were somewhat longer than broad, & their belly, which was flat-like, furnisht with sundry little paws...yet for all that they made but slow progress.”

Anthony van Leeuwenhoek, letter to Robert Hooke, 1681

Giardia was first observed in 1681 by van Leeuwenhoek in his diarrhea

Giardia lamblia

flagellated protozoa
found throughout world
lacks mitochondria
aerotolerant but respires as anaerobe
lives in small intestine
glucose & arginine are energy sources
(obtains by degrading mucus)
unable to synthesize nucleic acid bases (salvages)
fecal-oral route of infection
contaminated drinking water
children in daycare centers
beavers are major reservoir hosts “beaver fever”
CDC reported 21,300 cases of Giardia in US in 2000
highly likely that many more occurred but were undiagnosed
*Giardia* exists in 2 forms

**trophozoite (active form)**
- pear-shaped
- motile (possesses 8 flagella)
- binucleate (*both nuclei are transcriptionally active*)
- no mitochondria, peroxisomes or hydrogenosomes
- disc for attachment to epithelial cell surface

**cyst (environmentally resistant form)**
- ingestion of a cyst initiates infection
- parasites excyst in response to being in new host

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### Giardia lamblia

- Infection acquired by ingestion of **cysts**
  - non-dividing, with rigid cell walls
  - survives in water or desiccation
- Cysts pass through stomach
  - low pH → signals that parasite is in host
  - excystation in upper small intestine
- Trophozoites (*disease-causing stage*)
  - colonize small intestine
  - adhere to intestinal epithelia
  - cause *malabsorption* & *diarrhea*
- Encystation
  - trophozoites in distal small intestine
  - triggered by high pH & bile salts
  - requires cell wall synthesis
- Cysts are passed in host feces, mature & survive until ingested by another host.
**Giardia lamblia Excystation**

Cyst passes through stomach & into small intestine
response to environmental cues
sequential exposure to HCl & digestive enzymes
→ 2 binuclear trophozoites emerge from cyst
attach to epithelial cells by ventral discs
lectins are on ventral disk surface

**Giardia lamblia Encystation**

encystation induced by exposure to bile & elevated pH
cysts shed with fecal mass
encysted parasites can survive outside of host
withstand mild chemical treatment (chlorinated H₂O)
boiling water at high-altitude may not kill
survive in cold water (weeks to months)
can be killed by freezing or desiccation (drying out)
**Giardia Pathogenesis**

*Caused by trophozoite form*
- adhere to columnar cell microvilli
- secretion of proteins at adhesion site
  - antibody production & immunity
  - flattening of villi
  - steatorrhea & malabsorption
  - rapid weight loss

*Steatorrhea: formation of non-solid feces with increased fat excretion. Stools float due to excess fat from malabsorption, have an oily appearance & be foul smelling.*

*Malabsorption: abnormality in digestion or absorption of food nutrients across the GI tract. This may lead to malnutrition & anemia.*

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Some individuals are at greater risk for acquiring giardiasis = chronic *Giardia* infection
- *protracted disease with more severe symptoms*
- HIV AIDS patients
- cancer chemotherapy
- cystic fibrosis
- children with underlying malnutrition
~50% of infected individuals do not become ill infected but asymptomatic can become chronic carriers (cyst passers) individuals who develop disease most prominent symptom is protracted diarrhea can be mild or acute if untreated, diarrhea can last weeks to months chronic infections characterized by steatorrhoea & malabsorption syndrome rapid & substantial weight loss general debility & fatigue

**Giardia Clinical disease**

Treatment:
- symptomatic patients should be treated
- metronidazole is drug of choice
- resistant strains in AIDS patients (nitazoxanide is alternative)

Prevention & Control:
- screen food handlers & children in daycare
- proper disposal of human waste
- maintenance of buffer zones around watersheds
- filtration of drinking water supplies
- no vaccine
Trichomonas vaginalis
(Donné 1836)

flagellated, anaerobic protozoa
transmitted person-to-person by sexual contact
worldwide distribution
no reservoir hosts (other than humans)
exposure does not lead to permanent immunity
reinfection after treatment is common
infests both males & females
females: vaginal itching, inflammation, purulent discharge
males: infection is usually asymptomatic
exists only in *trophozoite* stage (*no cyst stage*)
motile (4 flagella)
infection by sexual intercourse with infected person
*trophozoites* must adhere to epithelial cells to infect
uses adhesins & ligand-carbohydrate interactions
lysosomal hydrolases secreted
  parasite enzymes (acid phosphatase) are cytotoxic
  cause target cells to lyse & release contents
  cell debris is ingested by parasite
parasite uses carbohydrases to detach from target cell
detachment allows it to move on to next cell
fragile outside of infected individual -- if they dry out, they die
**Trichomonas Cell & Molecular Pathogenesis**

hydrogenosome:
- unusual organelle derived from an ancient mitochondrion
- functions in anaerobic metabolism
- enzymes process glucose $\rightarrow$ acetate $\rightarrow$ $H_2$
- contains enzymes for putrescine biosynthesis
- essential for parasite growth
- analog inhibitors kill parasites
- lacks some enzymes to process glucose acetate to $H_2$
- rest of glycolytic cycle is cytosolic

secretes proteases at the site of attachment
- cell death in heavy infection
- sloughing of sheets of epithelium
- intense inflammation of infected area

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**Trichomonas Clinical Disease in Women**

20% of infected women are asymptomatic
80% suffer from range of symptoms
- mild discomfort & dyspareunia to incapacitating illness
- signs & symptoms are exacerbated during menstruation
- vaginal itching or burning
- thick yellow blood tinged discharges
- burning on urination & urethral discharge
- (rare) urticaria complication of heavy infection in women
- colpitis macularis ("strawberry cervix")
- vaginal & vulvar erythema

**Dyspareunia**: painful sexual intercourse for women.
**Urticaria**: itchy, raised, swollen areas on skin or mucous membranes.
**Erythema**: reddening of the skin.
**Trichomonas Clinical Disease in Men**

Infection usually *asymptomatic* in men

source of infection since they are unaware of infection

if prostate is infected -- pain in groin & upon urination

*infection increases transmission of HIV due to erosion of vaginal wall & bleeding*

women with *T. vaginalis* can infect newborn

passing through birth canal of infected mother

ectopic infection: *urinary tract infections & (rare)*

*involvement of the lungs resulting in pneumonia-like syndrome*

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**Trichomonas Clinical disease**

**Treatment:**

drug of choice is metronidazole (a *pro-drug*)

*metronidazole is converted to active intermediates by hydrogenosome-associated pyruvate ferreredoxin oxidoreductase & hydrogenase under anaerobic conditions*

parasite is inhibited by intermediates (unknown mechanism)

~2-5% of individuals infected with resistant strains

inactive forms of enzymes

derive energy from glucose by alternate pathways

tinidazole is alternative drug for resistant forms

**Prevention & Control:**

condom use during sexual intercourse

treating both sexual partners
Balantidium coli
(Malmsten 1857)

*This organism is an alveolate, a grouping that also includes the Apicomplexa*

- pigs are a common reservoir host
- occurs throughout world
- only ciliated protozoan to regularly infect humans
- endemic in Japan, New Guinea, Micronesia, Seychelles Islands, Thailand, South Africa, Central & South America & Europe
- sporadic epidemics in institutionalized populations
- locates to the large intestine
- causes dysentery ("balantidiasis")
- occasional fatalities

**Reservoir Hosts:**
- non-human primates
- horses & cattle
- pigs & boars
- rats & guinea pigs
**Balantidium coli Stages**

**Trophozoites:** (invasive stage)
- Two nuclei (macronucleus & micronucleus)
- Resides in tissues of large intestine
- Ingests living cells
- Causes ulcerations at infection site

**Cysts:** (infectious stage)
- Found in fecal mass
- Encystation
  - Triggered by dehydration of intestinal contents
  - Usually occurs in the distal large intestine
  - Can also occur outside of the host in feces

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**Balantidium coli Life Cycle**

Cysts are responsible for transmission
- Acquired by ingestion
- Excystation in small intestine

Trophozoites colonize large intestine
- Proliferate to cause pathology

Encyst $\rightarrow$ infective cysts
- Mature cysts passed in feces
**Balantidium coli**

*infection begins by ingestion of the cyst in contaminated food or water*
*trophozoite excysts in small intestine*
*relocates to large intestine*
*transverse & ascending colon epithelium is preferred site*
*usually limited to the bowel*
*HIV patients can have infection outside G.I. tract.*
 liver abscess has been reported
*infection in lungs and heart*

**Clinical Disease**

- trophozoites cause extensive destruction to surrounding tissue
- some trophozoites form cyst stage
- cysts exit from host in fecal mass & are immediately infectious
- pigs are presumed reservoirs in many cases -- infection is common when pigs live in close association with humans

- most infections are asymptomatic
- diarrhea & dysentery are chief complaints
- fever, nausea & vomiting
- rare ulcerative & granulomatous disease in colon & appendix
- AIDS patients more severe disease & ectopic infection

*Ulcer*: healing wounds on skin, mucous membranes or eye
*Granuloma*: a lesion of epitheloid macrophages surrounded by lymphocytes.
*Ectopic*: occurring in an abnormal place.
**Diagnosis:**
- identification in stool sample or histology section

**Treatment:**
- tetracycline is drug of choice
- metronidazole, paromomycin, chloroquine & iodoquinol also used successfully
- severe cases -- surgical bowel resection sometimes necessary

**Prevention & Control:**
- good sanitation
- clean source of drinking water

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**Entamoeba histolytica**
(Schaudinn 1903)

![Diagram of Entamoeba histolytica phylogeny]
**Entamoeba histolytica**

- transmitted from person to person by *fecal-oral route*
- resides in wall of large intestine
- one of leading causes of diarrheal disease throughout world
- protracted infection
- progresses watery diarrhea to *dysentery (bloody diarrhea)*
- may be fatal if left untreated
- can spread to extra-intestinal sites
- causes serious disease wherever it invades

Schaudinn (1903) described *Entamoeba histolytica* trophozoites & cysts. He died at age 35 of overwhelming amoebiasis – the outcome of self experimentation!

**Entamoeba histolytica**

histo-lytic = tissue destroying
lack mitochondria
lives as a *trophozoite* in the tissues of host
lives as a *resident cyst* in outside environment
food & water-borne disease
some animals can be infected
  (nonhuman primates & dogs)
animals not important reservoirs for human infection
estimated to infect ~50 million people worldwide
**Entamoeba histolytica**

**Trophozoite:** note nucleus (arrow) & numerous ingested red cells, 35 μm.

**Cyst:** two nuclei (arrows) & a smooth-ended chromatoidal bar can be seen, 15 μm.

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**Entamoeba histolytica Infection**

- **Ingestion in contaminated food and water**
- **Excystation:** one trophozoite with four nuclei emerges. It divides three times and each nucleus divides once to produce eight trophozoites from each cyst.
- **Trophozoites migrate to the large intestine**
- **Trophozoites multiply by binary fission**
- **Immature cyst**
- **Quadrinucleate cyst**
- **Mature cyst**
- **Noninvasive infection:** cysts exit host in the stool.
- **Invasive infection:** through the bloodstream, infecting sites such as the liver, brain, and lungs.
- **Encystation:** one trophozoite with four nuclei emerges, divides three times and each nucleus divides once to produce eight trophozoites from each cyst.
Entamoeba histolytica life cycle

trophozoite form

*facultative anaerobe*
metabolizes glucose as main energy source
remnant mitochondrion = “crypton” or “mitosome”
may contain ER & Golgi apparatus
invisible by electron microscopy

cyst form smaller
contains 4 round Entamoeba nuclei
each nucleus gives rise to trophozoite
ingestion of 1 cyst sufficient to initiate infection
one of most efficient pathogenic protozoa to infect humans

Facultative anaerobe: an organism that makes ATP by aerobic respiration if oxygen is present, but can switch to anaerobic fermentation.

Entamoeba histolytica

excystation in small intestine (host environmental cues)
sequential exposure to acidic & basic pH environment
4 trophozoites/cyst in small intestine divide once → 8
peristalsis carries to large intestine
trophozoites penetrate perimucosal space
trophozoites engulf & kill living cells
occupy increasingly large areas of tissue
flask-like ulcers develop
some trophozoite encyst in lumen of ulcer
cysts continuously produced & exit host in feces
survive in warm, moist conditions for weeks

Hematogenous or lymphatic spread possible (no role in lifecycle)

Hematogenous: originating in the blood or spread through bloodstream
Lymphatic (vessels): a network of thin tubes that carry lymph & white blood cells; they branch (like blood vessels) into all tissues of the body.
Entamoeba Cellular & Molecular Pathogenesis

Attachment is necessary for parasite mediated cytotoxicity. Lectin-carbohydrate interaction

Lectins: sugar-binding proteins, highly specific for sugar

Trophozoite surface membrane contains
- Phospholipase A (converts phospholipids into fatty acids)
- Neuraminidase (removes sialic acid from proteins & lipids)
- Metalocollagenase (breaks collagen peptide bonds)
- 4 secreted cysteine proteases (break peptide bonds)

Enzymes may aid parasite in moving through extracellular matrix.

Attachment triggers secretion of pore forming peptide (role in host cell lysis).

Trophozoites in liver abscess (arrows). Note ingested host cells inside parasites.

Low-magnification histologic section of amoebic ulcer in small intestine. Organisms can be seen at margin of ulcer.

Portion of transverse colon showing extensive ulceration due to intestinal infection with E. histolytica.
Entamoeba Clinical Disease

- many people are asymptomatic
  (some become carriers)
- wide range of clinical manifestations
  diarrhea is common
    lasts few days
    entire bowel involved
  dysentery (bloody stools)
  colicky pain
  flatulence
  abdominal tenderness

Entamoeba Clinical Disease

dysentery can worsen (life-threatening)

or

chronic ill health

bouts of diarrhea, abdominal cramping & discomfort
amoeboma: granuloma of eosinophils, amoebae & necrotic colonic tissue, frequently misdiagnosed as cancer
disease worsens
colon may become atonic
perforation at point(s) of ulceration
  peritonitis may develop (more common in children)
  perforated bowel adheres to abdominal wall
  perforation may extend to skin
cutaneous amoebiasis
  can progress rapidly
  also occurs in perianal area (trophozoites from rectum)
**Entamoeba histolytica in HIV patients**

extraintestinal amoebiasis -- serious pathological consequences
most common extraintestinal site is liver (hepatic amoebiasis)
slowly progressive disease
fever & pain
tenderness in right quadrant of abdomen
next most common extraintestinal infection site are lungs
  effusion, pleurisy, empyema or lung abscess
rupture into the pericardium -- usually fatal
cerebral amoebiasis (rare)
  abrupt onset
  high mortality unless diagnosed early

Effusion: an abnormal collection of fluid in a body cavity or space
Pleurisy: inflammation of the pleural cavity lining surrounding the lungs
Empyema: a collection of pus within a naturally existing body cavity

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**Entamoeba histolytica**

**Diagnosis:**
detection of antigens in stool
PCR on stool or tissue samples

**Treatment:** metronidazole
  for both intestinal & extraintestinal infections
  no naturally occurring resistant strains
  resistant strains can be induced in laboratory
  *treatment does not affect cyst stage*
cystidal agent is also required
  diloxanide furoate
  iodoquinol (diidohydroxyquin)

**Prevention & Control:**
safe drinking water (watershed management)
screening of food handlers (asymptomatic cyst passers)
Naegleria fowleri
Acanthamoeba spp.
Balamuthia mandrillaris

• free living amoeba
• can cause serious disease in humans
• all species have worldwide distribution
• isolated from all types of freshwater habitats & soils
**Naegleria fowleri**

thermophilic -- thrives in standing freshwater
*(hot springs, heated swimming pools & hot tubs)*
flagellated, trophozoite & cyst forms
ubiquitous in distribution
US, Europe, Australia, South & Central America & Southeast Asia
most prevalent in natural water sources in spring & fall
can cause a serious often fatal infection
primary amoebic meningoencephalitis (PAM)
infection in central nervous system (CNS)
acquired by swimming/bathing in water above 37°C
environment selects for an abundance of thermally tolerant organisms

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**Naegleria fowleri Infection**

splashing/diving forces water up nose
*either flagellated form or trophozoite (amoeboid form) can infect*
migration to brain
trophozoites lyse tissues → extensive destruction
Symptoms
severe frontal headache
vomiting
confusion
fever
coma
*rapid death*

*In August 2008, a 9-year-old boy was killed after infection while swimming in Lake Elsinore. The boy was the first ever confirmed case in Riverside County.*
Cysts, trophozoites & flagellated forms occur in the environment (water). Both the trophozoite & flagellated forms can infect humans.

**Naegleria fowleri**

**Diagnosis:**
- amoeba can be isolated from cerebrospinal fluid
- rapid diagnosis is essential — *death within 5 days of infection*
- no serological or PCR-based tests

**Treatment & Prevention:**
- little clinical experience with this usually fatal disease
- no recommended drugs of choice
- Amphotericin B is only agent with known efficacy
- diagnosis usually made too late for effective treatment

In 2007, 6 children in Texas, Arizona & Florida died from Naegleria infection acquired while swimming. Before 2007, the CDC had only documented 24 cases in the US.
**Acanthamoeba Spp.**

Found in
- soil (one of most common soil protozoa)
- water (fresh, brackish & ocean), sewage, swimming pools
- contact lens equipment
- dental treatment units
- dialysis machines
- heating, ventilating & air conditioning systems
- humans & animals
  - nostrils & throats
  - brain, skin & lung

2 stages
- no flagellated stage (unlike *N. fowleri* )
- cysts & trophozoites
- trophozoites are infective form

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**Acanthamoeba Ulcerative Keratitis**

- contact lenses washed in tap water or contaminated solution
- amoeba invade cornea & begin to erode surface
- creates burning sensation
- perceived gritty consistency under lid when eye is closed
- a ring-enhancing lesion develops & impairs vision
- partial or total blindness may ensue if left untreated

*2007 recall of contact lens solution by manufacturer Advanced Medical Optics, Inc. (Santa Ana, CA). The recall due to a link between Acanthamoeba keratitis & use of this lens solution.*
**Acanthamoeba** in Respiratory system & Skin

opportunistic pathogen
rarely causes disease in humans
~400 cases have reported worldwide
portal of entry:
lesions in the skin or respiratory tract
via inhalation of airborne cysts
individuals with compromised immune systems
(HIV-AIDS)
diabetes, malignancies, malnutrition, lupus
invades CNS by hematogenous dissemination (bloodstream)
granulomatous amebic encephalitis (GAE)
disseminated disease -- skin lesions

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**Acanthamoeba Infection**

1. Cysts
2. Trophozoite
3. Mitosis

Cysts and trophozoites in tissue

1. Results in severe keratitis of the eye.
2. Results in granulomatous amebic encephalitis (GAE) and/or disseminated disease in individuals with compromised immune systems.
3. Results in skin lesions.

[Source: CDC](http://www.dpd.cdc.gov/dpdfax)

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1. Infective Stage
2. Diagnostic Stage
**Acanthamoeba Infection**

**Diagnosis:**
microscopy of amoeba in biopsy tissue, CSF or tear secretions

**Treatment & Prevention:**
• few cases
• reliable treatment unclear due to lack of clinical experience
• few patients survive CNS infection
• keratitis also difficult to treat
• topical miconazole, propamidine & Neosporin show promise
• prevention of keratitis by sterile contact lens solution

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**Balamuthia Infection**

*Balamuthia mandrillaris*

- infects immuno-compromised or -competent individuals
- cyst and trophozoite stages
- infection via lungs or skin
- multiple foci of infection
- can invade CNS
- slow developing *ulcerative, granulomatous* disease
- diplopia
- frontal headache
- seizures
- occasional death

*AIDS patients may experience overwhelming disseminated infection*
Balamuthia Infection

1. Cyst
2. Trophozoite
3. Mitosis

Amebae (cysts and trophozoites) can enter humans in various ways:

4. Through nasal passages to the lower respiratory tract
5. Through unbroken or broken skin

Cysts and trophozoites in tissue

= Infective Stage
= Diagnostic Stage

Results in granulomatous amebic encephalitis (GAE), disseminated disease, or skin lesions in individuals who are immune competent as well as those with compromised immune systems.