

# Intestinal protozoa

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9<sup>th</sup> of September 2020

# Intestinal Protozoa

Single-cell eukaryotes, neither animals, nor plants nor fungi

→ Protozoa: the „first animal“

→ outdated as a systematic category

- *Cryptosporidium*
- *Cyclospora cayentanensis*
- *Cystoisospora* (*Isospora*) *belli*
- *Entamoeba histolytica*
- *Giardia duodenalis*

**Cryptosporidiosis**

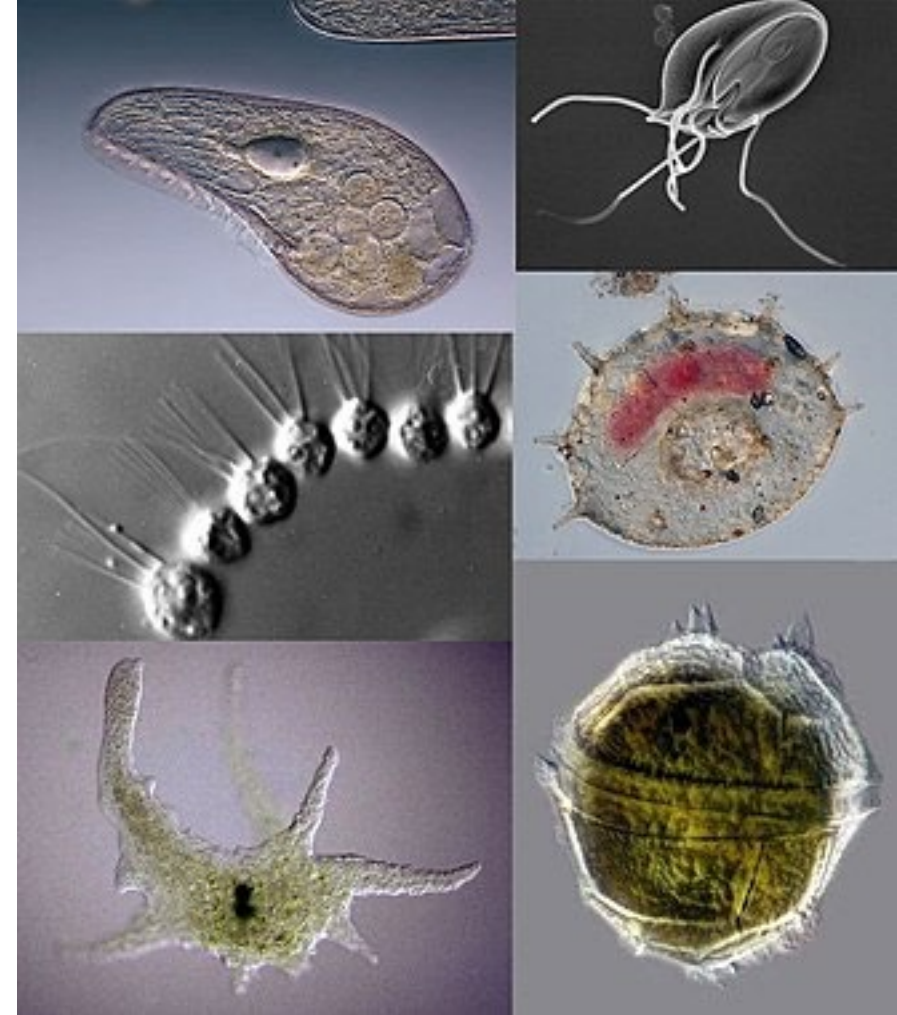
**Cyclosporiasis**

**Cystoisosporiasis**

**Amebiasis**

**Giardiasis**

Microsporidiae are fungi (or closely related to...)



Non-medical Protozoa

# Falling leave

[falling leave](#)

# The clinical case

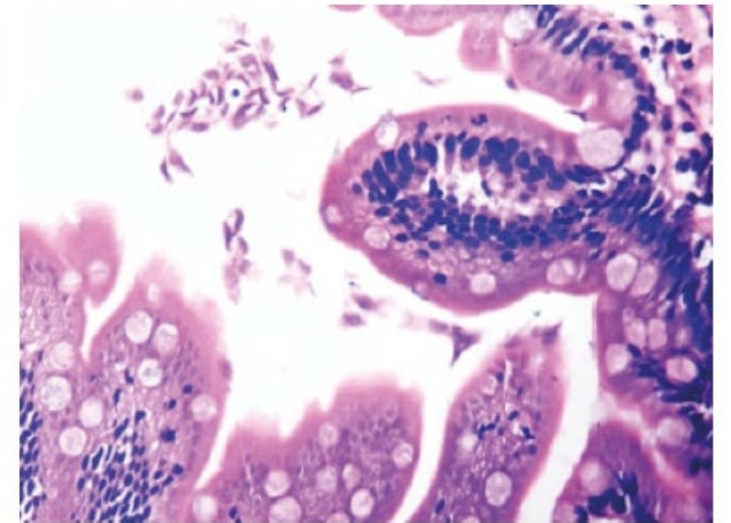
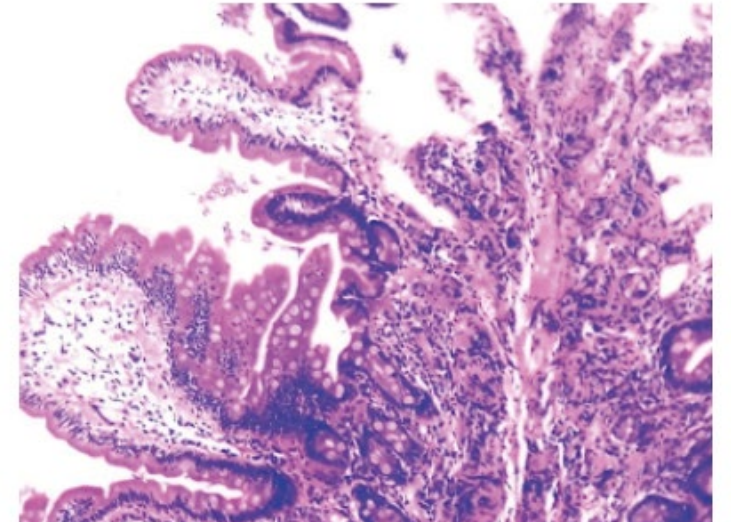
- 4-year old boy from Kathmandu, Nepal
- Chronic diarrhea since 2 years
- Normal developmental mile stones except low weight for age
- Bilateral pitting edema, ascites
- Normal hematological profile
- Albumin 12g/l (35-55)
- Anti-Tissue Transglutaminase, total IgA normal

Stool examination with *E. histolytica*, red blood cells

Gastroscopy: mild „gastritis“, normal duodenum

Histology: - Focal blunting of villi  
- Lymphocytic inflammation  
- ***Giardia duodenalis* trophozoites**

Metronidazol → full recovery



# *Giardia duodenalis*

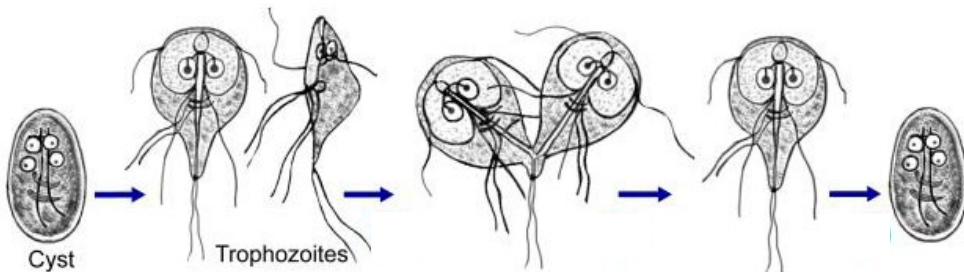
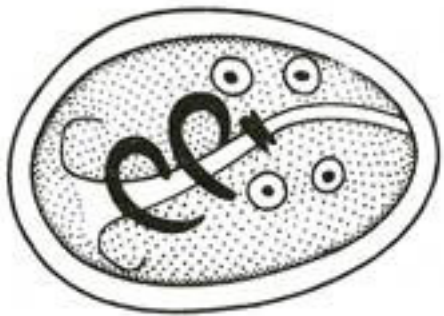
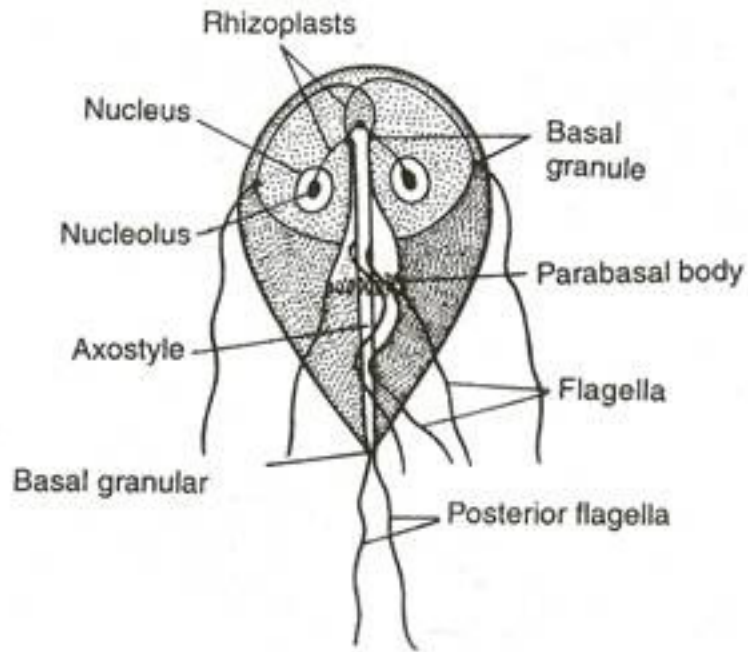
## Trophozoite

- Tear drop shaped (dorso-lateral view)
- Spoon-shaped (side view)
- 10-20mm x 5-15mm x 2-4mm
- 4 pairs of flagella
- 2 identical nuclei
- Ventral disc (attachment)
- No Golgi, no peroxisome, no true mitochondria
- Falling leaf motility

## Cyst

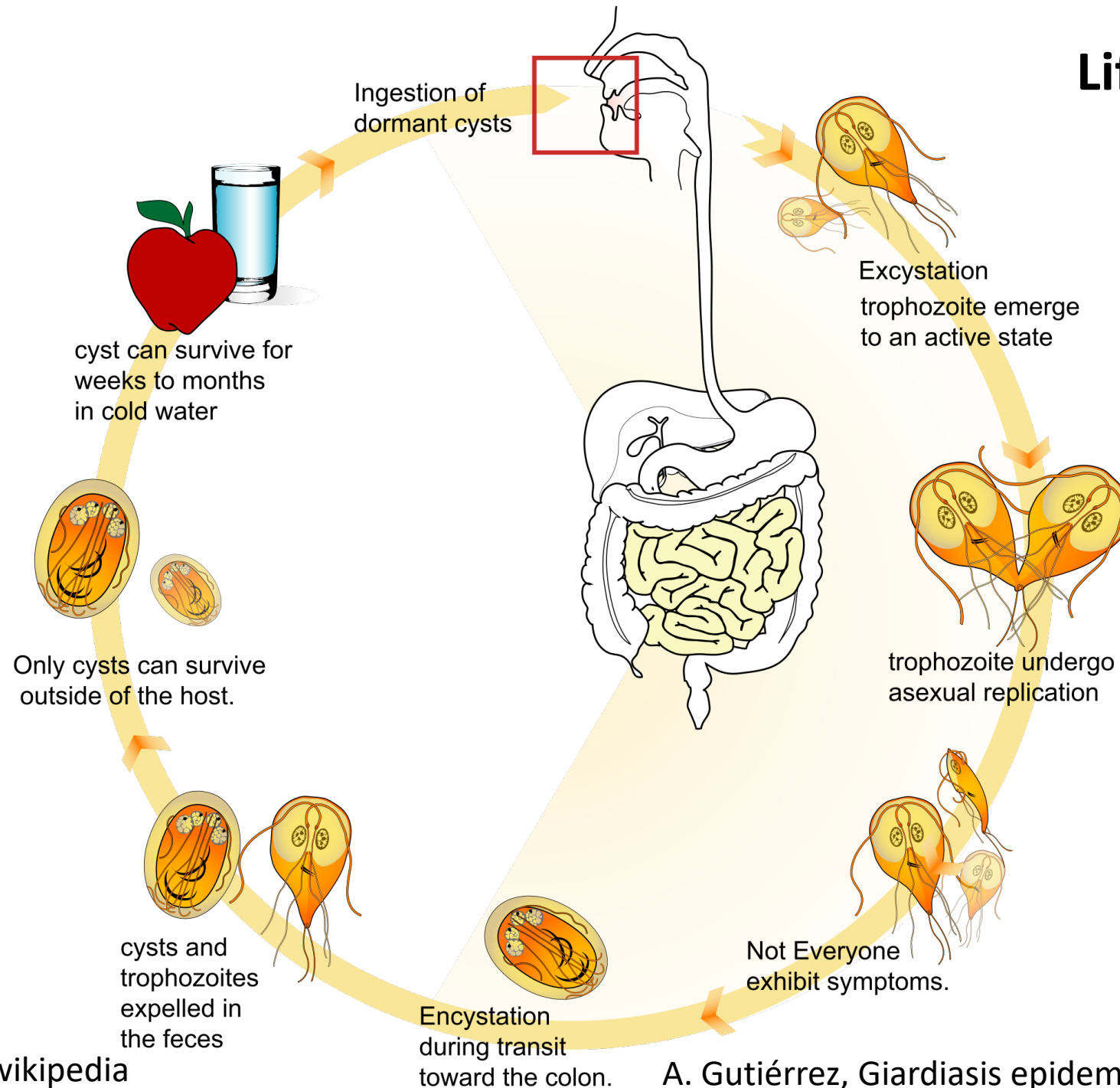
- Oval shaped
- 7-10 x 11-14 mm
- 4 nuclei
- Thick hyaline wall

→ Some resistance to mild chlorination, boiling water  
→ Survives up to 3 months in water





# Life cycle of *giardia duodenalis*



10-100 cysts are required

Signals excystation:

- Gastric acid (pH 1.3-2.7)
- Bile acid
- Proteases

Attachment by adhesive disc  
In small intestine

Encystation

- Shortening flagella, condensation cytoplasm, hyline cyst wall

Reservoir

- Humans
- Dogs
- Beaver

«reverse zoonotic transmission» to

- Beaver
- Muskrat
- Coyote
- Muskoxe
- Gorilla
- Painted dog
- Marsupial
- Seal



# Transmission

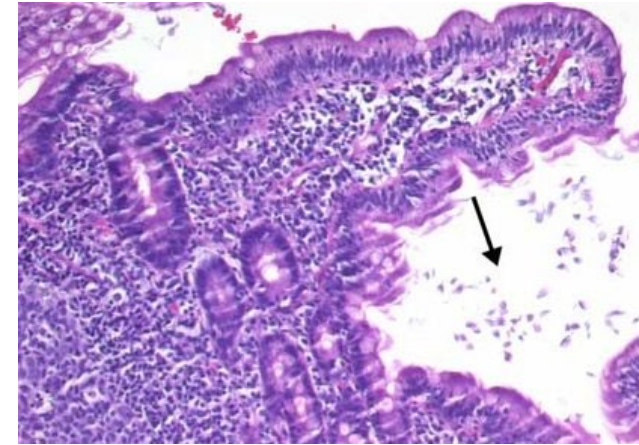
- Waterborn - swimming
  - Fresh water, pond
  - Hiker, camper, adventurer...
- Waterborn - drinking water
  - Drinking at-risk tap water, untreated surface water, shallow pond water
- Foodborn
  - Handling of food by carriers (10-100 cysts!)
- Fecal oral transmission
  - Diaper-age children in day-care centers
  - Adults working in day-care centers
  - Institutionalized individuals
  - MSM
  - Immunocompromised individuals  
CVID, hypogammaglobulinemia, HIV, CF, immunosuppression





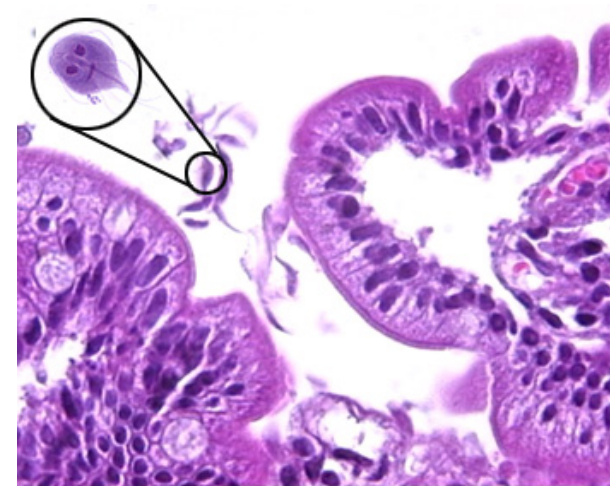
# Epidemiology

- 280 Million cases per year
- Prevalence 10-50% in developing countries  
poor health, hygiene, overcrowding, children, dogs
- Industrialized countries
  - Rare in some highly developed countries (D, CH, GB)
  - USA: prevalence approx. 1%, higher in counties with reliance on own wells



# Pathophysiology

- Attachment, epithelial damage by Giardia surface lectins
- Enterocyte damage, villi atrophy, crypt hyperplasia, brush border damage
- Malabsorption of water and nutrients
- Not invasive!!  
disease restricted to the intestinal lumen



# Clinical course

- Most individuals are asymptomatic
  - Asymptomatic shedding for up to 6 months
- Acute Giardiasis
  - Start of symptoms: 2-4 weeks after exposure
  - Mild diarrhea (2-5 stools per day), foul smelling...
  - Upper GI symptoms
  - Weight loss
  - Self-limiting after 5-7 days

Diarrhea	90%
Malaise	86%
Steatorrhea	75%
Abdominal cramps, bloating	71%
Flatulence	75%
Nausea	69%
Weight loss	66%
Vomiting	23%
Fever	15%
Constipation	13%
Urticaria	10%

# Chronic Giardiasis

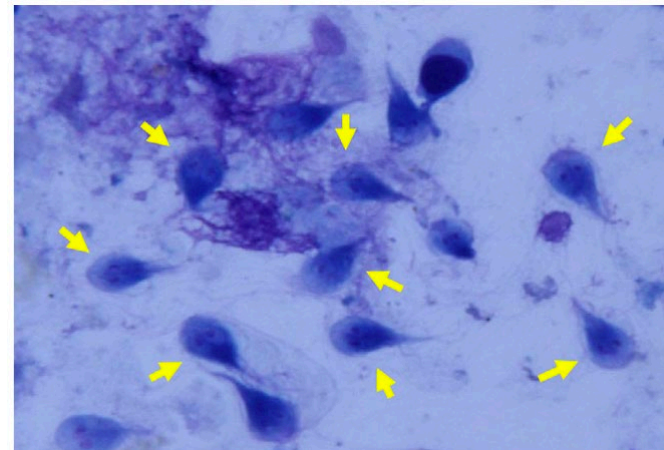
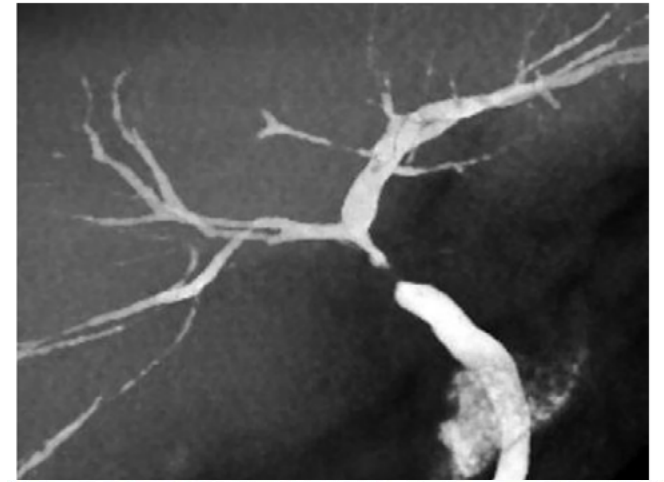
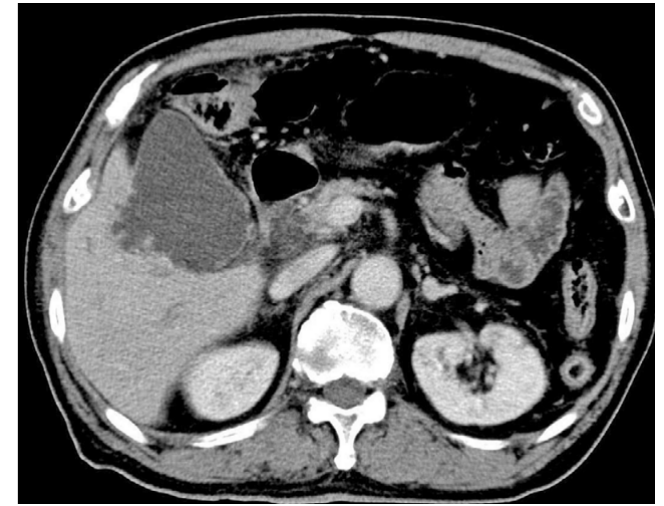
## **Chronic giardiasis** (50% of symptomatic individuals)

- Loose stools (usually no diarrhea)
- Steatorrhea, Malabsorption
- Profound weight loss (10-20% of body weight)
- Depression, Fatigue
- Abdominal cramping, Borborygmi, Flatulence, Burping
- Malabsorption (hypoalbuminemia, vitamin A, B12, folate deficiency)

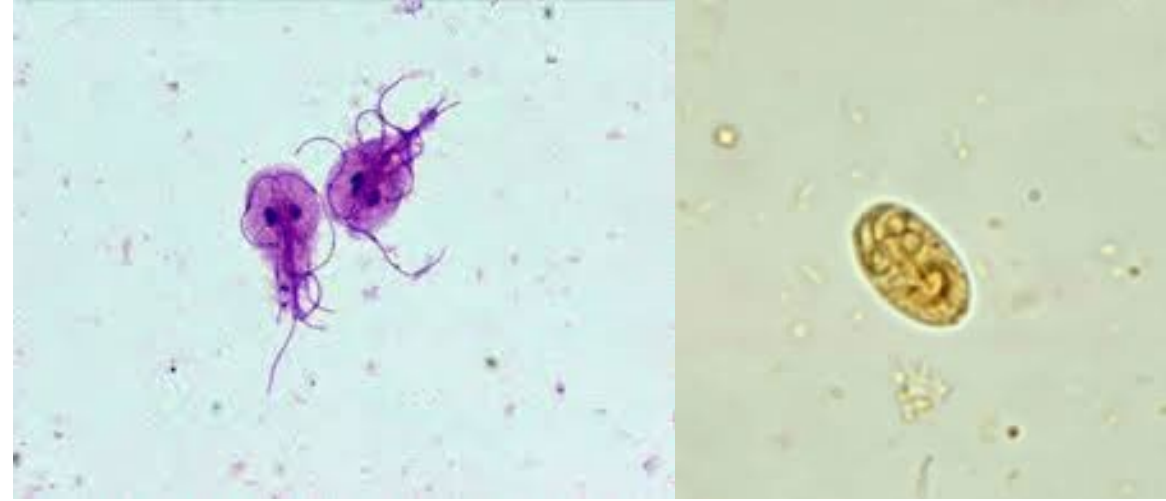
Children: Protuberance of abdomen, stunted growth, delayed development

## **Complications**

- Cholecystitis, Cholangitis
- Granulomatous hepatitis
- 39% IBS
- 31% chronic fatigue



# Diagnosis



- Stool microscopy (three samples)
  - Direct wet mount (saline suspension): trophozoites identified by falling leaf motility
  - Fixation, staining for observation of cysts
  - Labor intensive, expertise required
- Antigen detection assays
  - Direct immune fluorescence
  - 12-30% higher sensitivity
- Nucleic acid amplification assays



# Treatment

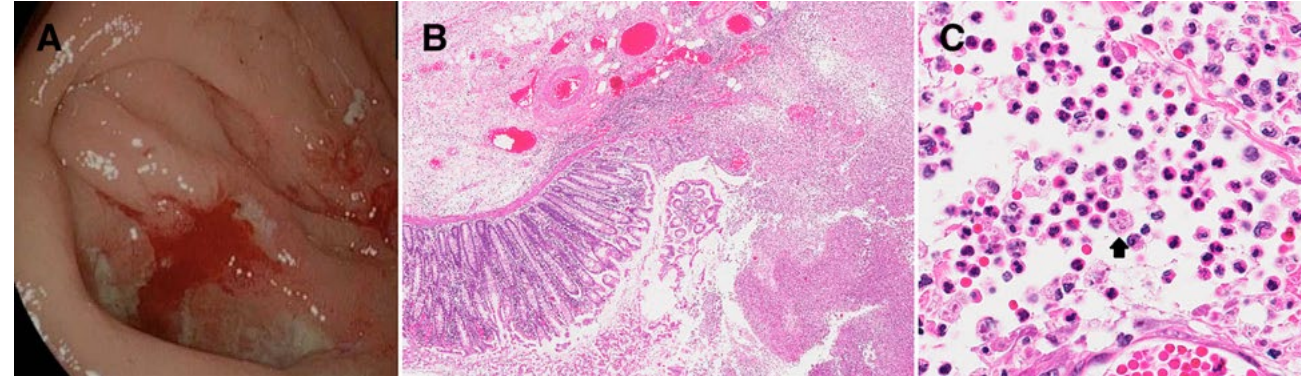
- |   |                           |
|---|---------------------------|
| • <b>Tinidazol</b> 2g, once                             | CH: needs to be imported) |
| • <b>Nitazoxanide</b> 500mg, 2x per day, for three days | CH, EU: not approved      |
| • <b>Metronidazole</b> 500mg, 2x per day for 5.7 days   | e.g. Flagyl®              |
| • <b>Albendazol</b> 400mg 1x per day for 5 days         | Zentel®                   |
| • <b>Mebendazol</b> 200mg 3x per day for 5 days         | Vermox®                   |
| • <b>Paromomycine</b> 10mg/kg 3x per day, 7-10 days     | Humatin®                  |

Within 5-7 days resolution of symptoms and parasitological cure expected...

→ Check stool cultures again, resistencies against tinidazol, metronidazole emerging

# The clinical case

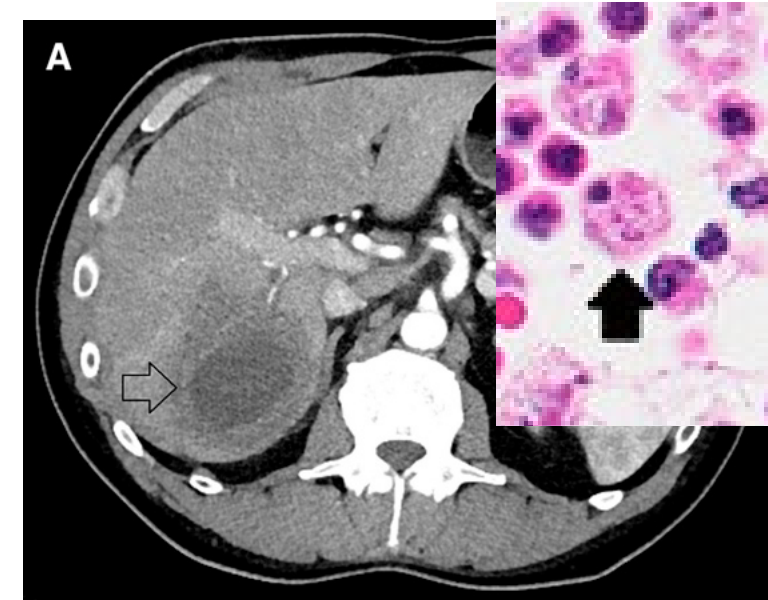
- 57-year old man from Cape York, Australia
- Worked at a sewage treatment facility
- Small bowel obstruction, conservative management
- Colonoscopy with ulcerations



1 year later

- 4-week history of weight loss, abdominal pain
- At presentation afebrile, right upper quadrant tenderness
- CT scan: large mass, right lobe of the liver
- Purulent aspirate, sterile cultures
- Serology: Indirect hemagglutination (IHA) assay for *E. histolytica*: titer 1:16,384

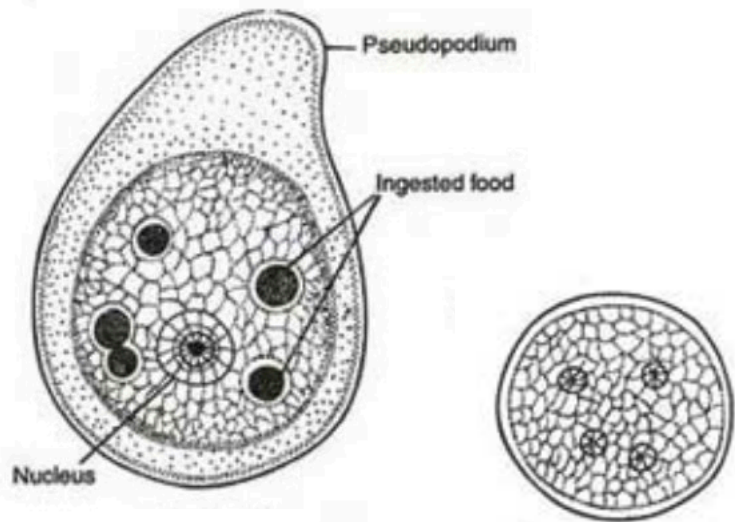
Metronidazole, paromomycin → full recovery



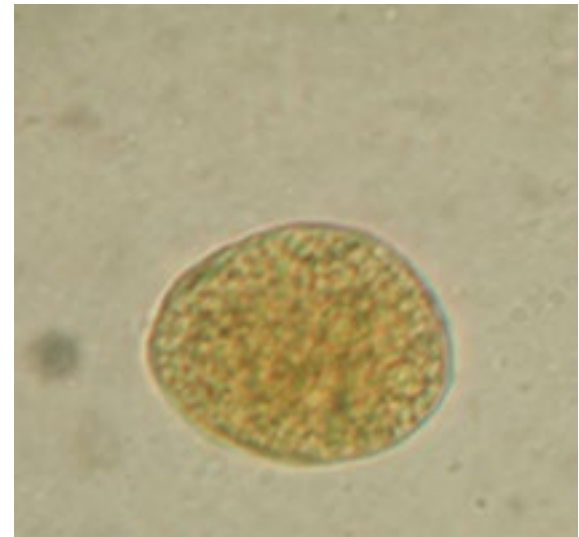
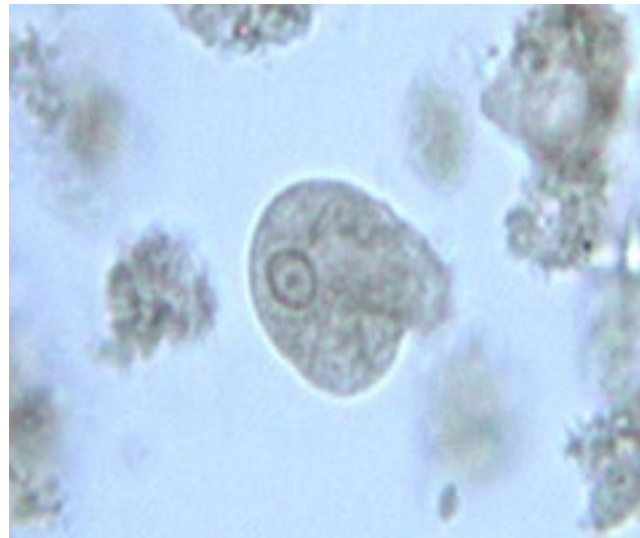
# Infectious cycle

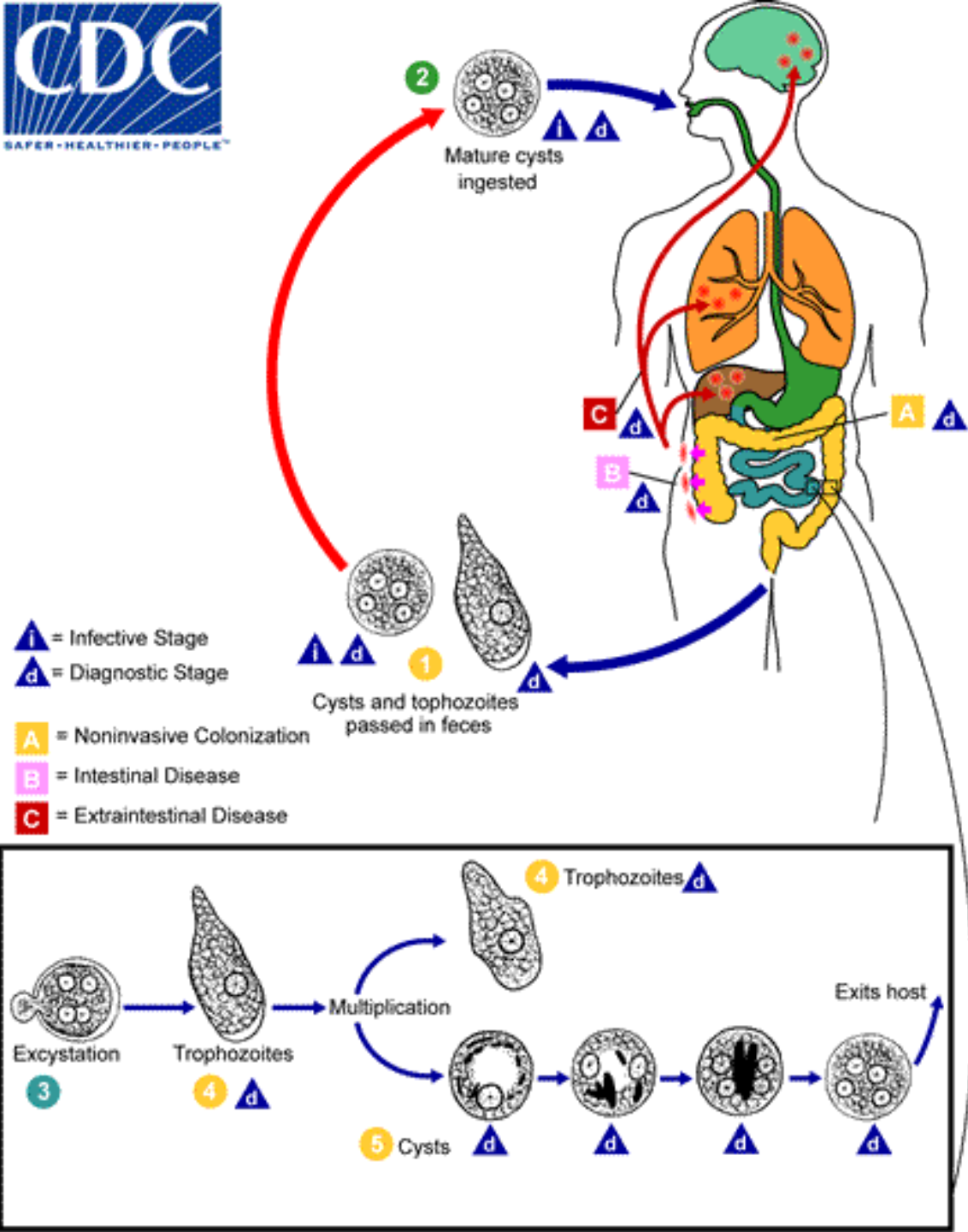
- *Entamoeba histolytica*, *E. dispar* (not pathogenic)  
*E. dispar* has less capacity for erythrophagocytosis
- Non-pathogenic amebae: *Endolimax nana*, *Iodamoeba buetschlii*...  
cave: confusion with pathogenic *Entamoebae*

?



?





- 1: Cysts are found in solid stool  
Trophozoites are found in liquid stool
- 2: Cysts from fecally contaminated water or hands  
1 cyst is sufficient  
or during sexual encounters
- 3: Excystation in the small intestine
- 4: Release of Trophozoites  
Migration to large intestine
  - A: non-invasive disease
  - B: intestinal invasion
  - C: invasion of blood vessels → extraintestinal disease
- 5: Production of infectious cysts



# Transmission

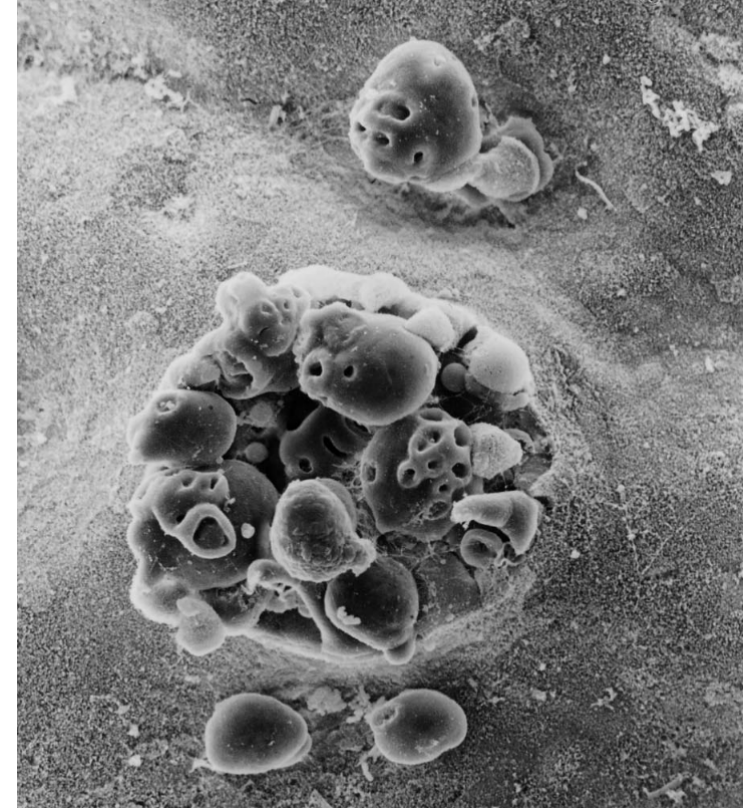
- Transmitted by the fecal-oral route (70-80%). **5-F:**
  - Finger (contaminated hands)
  - Fomites («Keimträger», public places, door handles)
  - Fly
  - Feaces
  - Food
- Developed countries: travelers (>1 month)
- Sexually transmitted disease (MSM)

**High prevalence regions:** India, Africa, Mexico, Central America, South America

- Prevalence up to 50%!!!
- 50 million cases of colitis per year
- 100,000 deaths per year

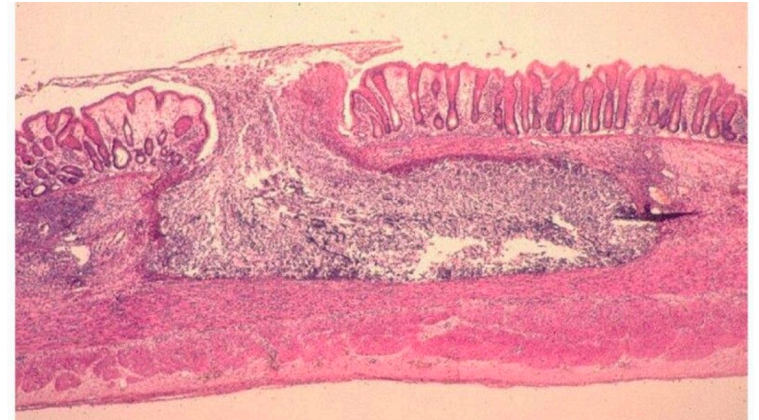
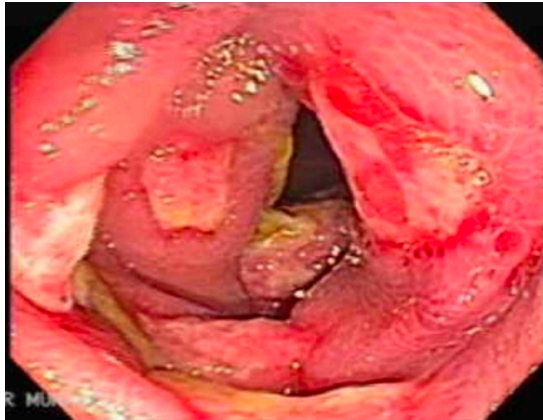
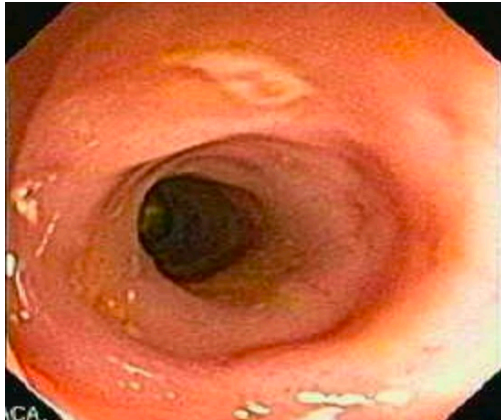
# Clinical course

- Asymptomatic 90%
- Invasive disease:
  - Subacute onset (1-3 weeks)
  - Diarrhea (94-100%)
  - Bloody stool (94-100%)
  - Abdominal pain (12-80%)
  - Fever: 38%
  - Fulminant amebic colitis, e.g. toxic megacolon
    - 0.5% of cases, mortality 40%
- Risk factors: steroids (25% mortality), young age, pregnancy, alcoholism, malnutrition



# Colonoscopy??

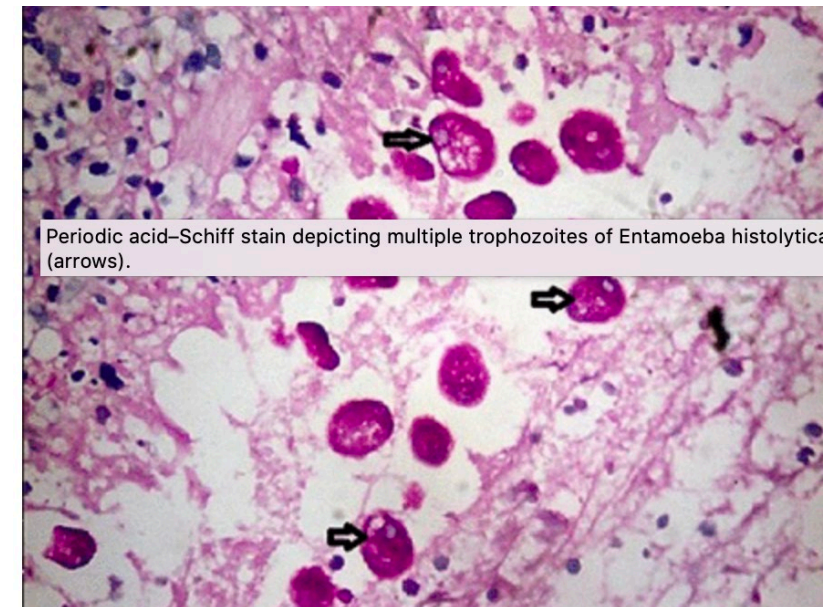
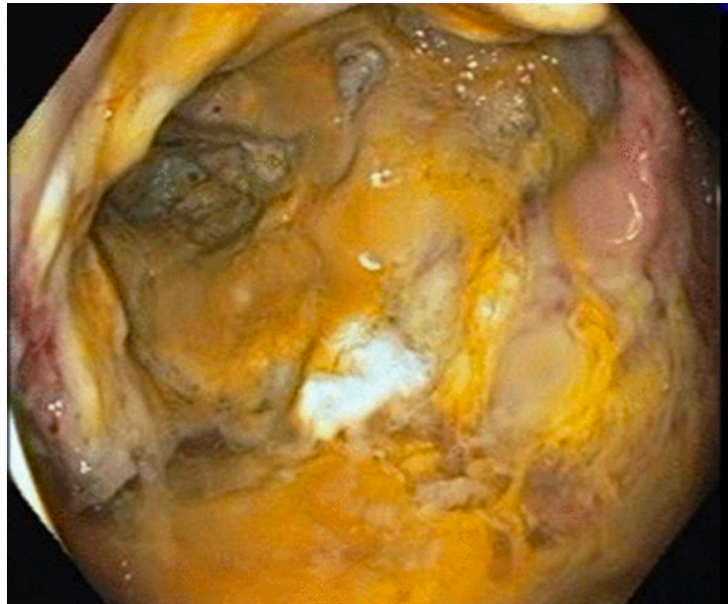
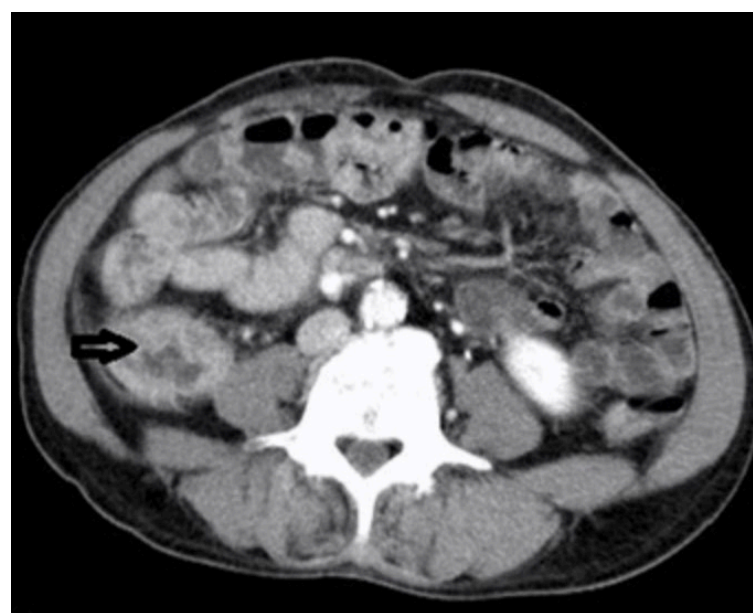
- Not for routine use → perforation
- Ulcers/ erosions
  - Right-sided: cecum: 93%,
  - Distal: rectum 45%
- Flask shaped ulcers?
- Histological hallmark: lateral extension into submukosa





# Clinical case

- 60-year old man, 4 days fever, lower quadrant pain, no diarrhea, no blood
- 4x3cm lump palpable
- Elevated inflammatory markers
- CT-scan: thickening wall of cecum wall
- Colonoscopy: ulcerations in right colon
- Histology of colon biopsy: multiple trophozoites
- Metronidazole i.v.: resolution of symptoms in 2 days, disappearance of mass in 1 week
- Diagnosis: **Ameboma** („tumor like mass, involving whole thickness of the colon“)



Periodic acid–Schiff stain depicting multiple trophozoites of *Entamoeba histolytica* (arrows).



# Diagnosis

- Stool microscopy
  - Cannot differentiate between *E. histolytica*, *E. dispar*  
phagocytosis of erythrocytes is not pathognomonic
  - Needs 3 specimen on separate days to detect 85-95% of cases
  - Experienced microscopist
- Antigen testing
  - ELISA, RIA, IF
  - Easier, greater sensitivity
- PCR tests
  - 100x more sensitive than antigen testing
  - Expensive (not routine)
- Serology
  - Specific: only against *E. histolytica*, not against *E. dispar*
  - 90% sensitivity after 5-7 days, lasts for 6-12 months

# Extraintestinal manifestations

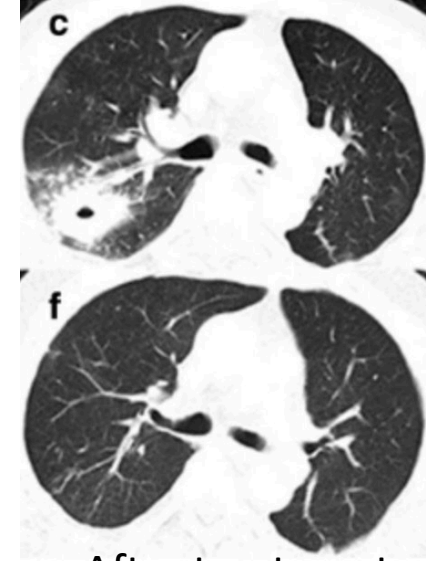
## Liver abscess

- Men, 30-50 years (due to alcohol?)
- 8-20 weeks, median 12 weeks after return from endemic area
  - Late presentations after years is possible
  - Chronic conditions possible
- Fever, right upper quadrant pain (epigastrium, chest, shoulders)
- Imaging: 70-80% solitary lesions (multiple lesions possible)
- Drainage: „anchovy paste“: brown fluid – necrotic hepatocytes
  - Trophocyoits only in peripheral regions (20% diagnostic)
  - Antigen testing (or PCR) can be helpful
- Diagnosis: Serology (92-97% at time of presentation)
  - Stool microscopy typically negative

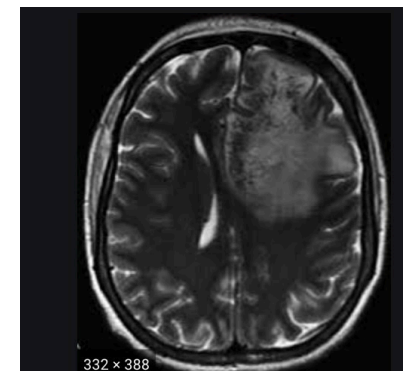
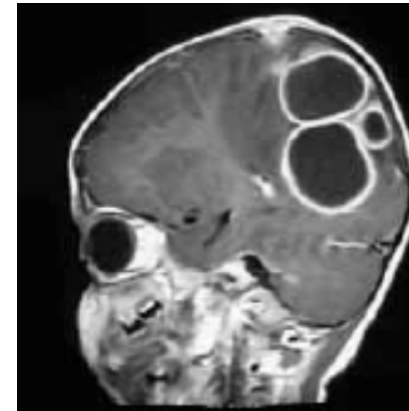
## Pleuropulmonary infection

## Cardiac infection

## Brain abscess



After treatment



# Treatment

- *E. histolytica*: always... *E. dispar*: not required
- Systemic drug
  - Metronidazole 500-750mg 3 x per day for 7-10 days OR
  - Tinidazol 2g for 3 days (less clinical failures, less side effects)  
approved in CH since 1973, currently not available
  - Ornidazol (Tiberal):
    - 1.5g 3 days – intestinal amebiasis
    - 1g for 10 days – extraintestinal amebiasis
- Luminal agent:
  - e.g. Paromomycin (Humatin), 25-30 mg/kg/d in 3 doses for 7 days

# The clinical case

- A 60-year old otherwise healthy woman resident of Natal (Northeast Brazil)
- Real estate administrator
- Diarrhea for the past six months.
  - 6 months before presentation: intermittent aqueous diarrhea with abdominal cramps in the lower abdomen, single episode of fecal incontinence.
  - 3 months before presentation: alternating liquid feces and separate, hard lumps.
  - At presentation: increase in the volume of feces and number of evacuations (up to six per day), flatulence, abdominal cramps in the lower abdomen that were alleviated after evacuation. abdominal distension. Symptoms aggravated upon ingestion of fatty or dairy foods. Weight loss of 4kg.
  - History of depression but ceased psychiatric treatment, trazodone stopped after six months.
- Normal blood count. Negative HIV test

Acid fast stain of well of condominium

→ Alternate source of drinking water

→ Asymptomatic





Cryptosporidiosis

Cystoisosporidiosis (*Isospora belli*)

Cyclosporidiosis

# Cryptosporidiosis

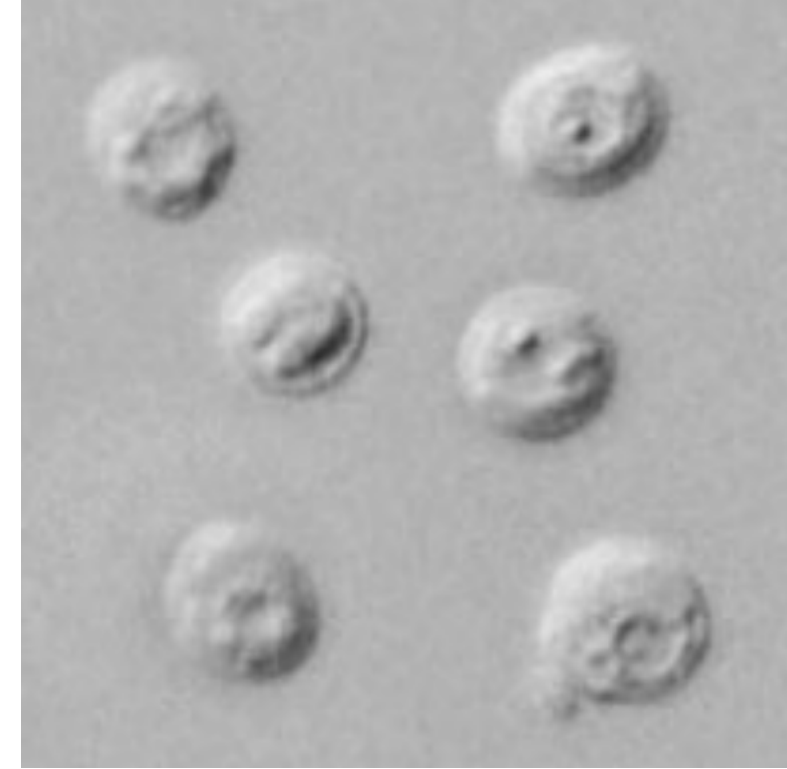
- *Cryptosporidium parvum* (animals and humans)
- *Cryptosporidium hominis* (mainly humans)

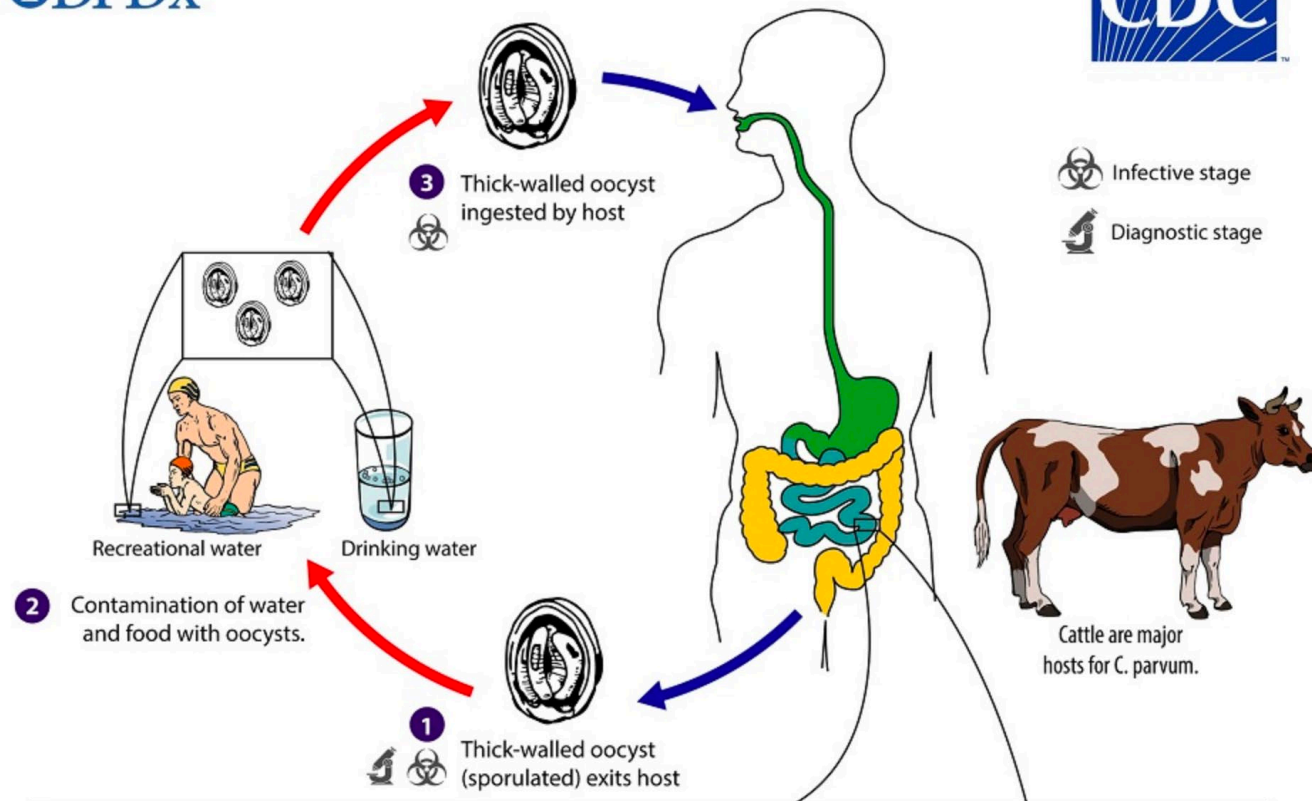
Worldwide occurrence (mainly children: #2 cause of diarrhea )

- Seroprevalence USA: 25-60%
- Seroprevalence developing countries: 65-95%
  - Risk factors: overcrowding, household diarrhea, animal contacts, open defecation

Transmission (excretion of oocysts)

- Waterborn (recreational water, drinking water)
- Foodborn outbreaks (cafeteria, raw milk, cider)
- Person-person transmission (e.g. daycare centers)
- Possibly inhalation





1: Sporulated oocysts are excreted by the host and contain 4 sporozoites

2: Transmission via contaminated water food (e.g. raw milk), fecal-oral...

3: Excystation, parasitize the epithelium → Brush border

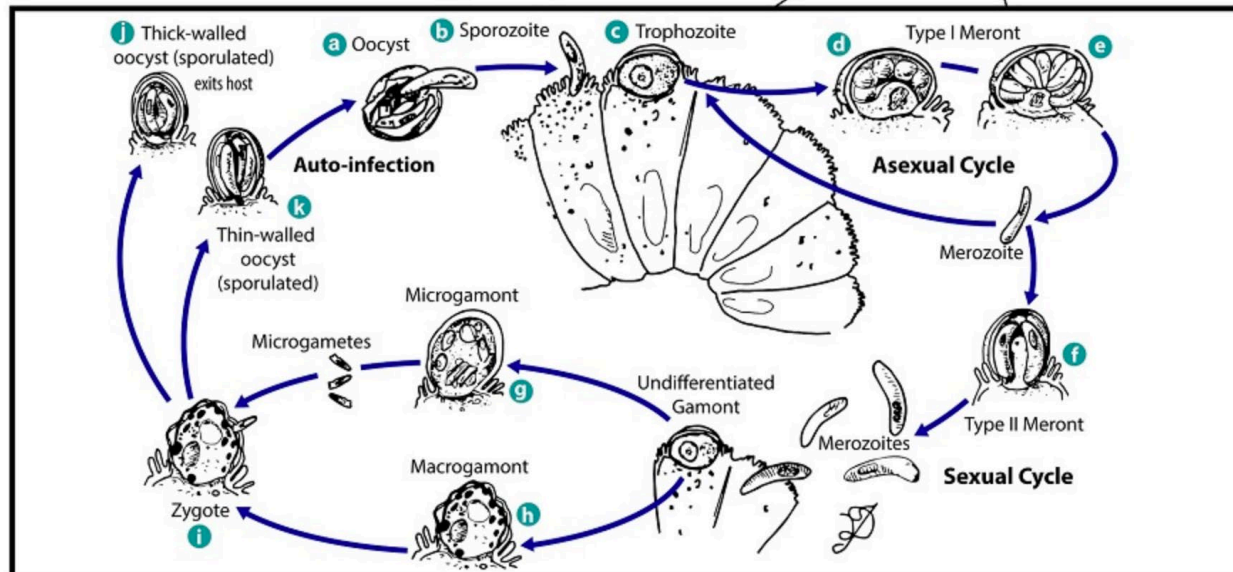
Asexual cycle – Meront, Merozoites

Sexual cycle

- Microgamont (male) → Microgametes
- Macrogamont (female) → Zygotes

→ Thin walled oocysts → autoinfection

→ Thick-walled oocysts → excretion



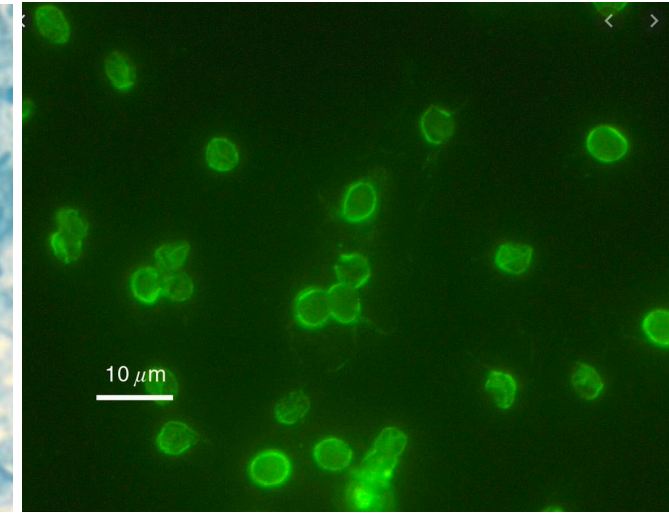
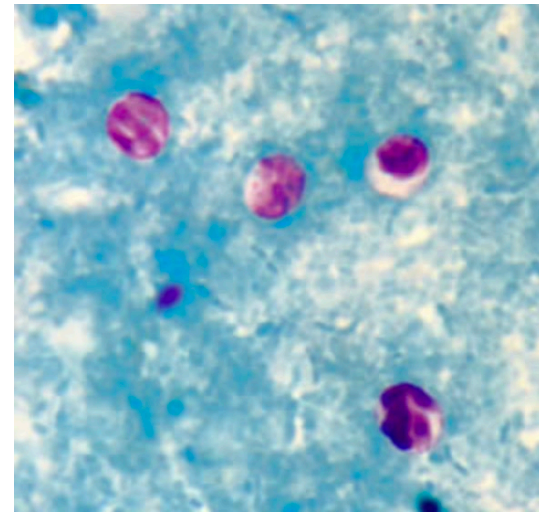
# Clinical course

- Incubation time 7 days (range 2-10)
- Immunocompetent host:
  - Self-limiting diarrhea after 3 weeks
  - Occasionally persistent, recurrent disease
  - After 12 months: 38% abdominal pain, 33% diarrhea, 33% joint pain, 31% weight loss, 28% IBS, 22% fatigue
- Immunocompromised host (HIV, CD4 counts  $<100$  cells/ $\mu$ l)
  - Severe diarrhea
  - Fever, fatigue
  - Malabsorption, wasting
  - Extraintestinal manifestations in lungs, pancreas biliary tract: 10-30% with HIV
- Young children in developing countries
  - Diarrhea and malnutrition



# Diagnosis

- Microscopy (3 specimen)
  - Modified acid-fast stain (ZN)
  - Immunofluorescence (IF)
- PCR
- Serology

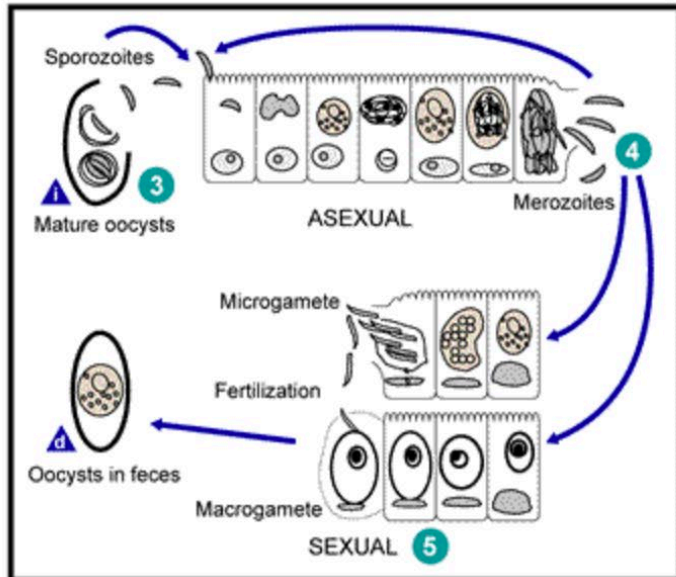
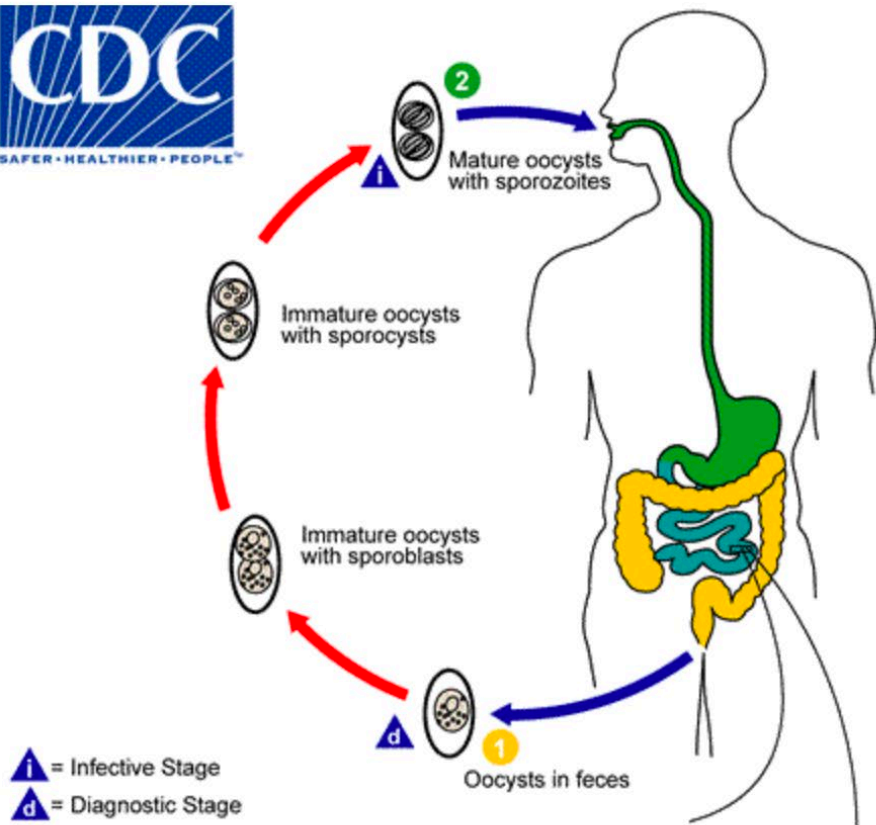


# Treatment

- Immunocompetent host
  - Nitazoxanid (not approved in CH)
  - Paromomycin (Humatin)
  - Azithromycin, Rifaximin
- Immunosuppressed host
  - HIV: HAART +/- antimicrobial therapy

# *Cystoisospora belli* (former *Isospora belli*)

- Tropical/ subtropical countries
- Waterborn disease
- Individuals at risks
  - Travelers
  - Immunocompromized individuals (HIV, CD4 count <50 cell/ $\mu$ l)
    - Trimethoprim/ sulfamethoxazol prophylaxis decreases risk
- Symptoms: profuse watery diarrhea of sudden onset, fever, weight loss
- Rare: Cholangitis, cholecystitis
- Diagnosis: stool smear
- Treatment
  - Trimethoprim/ sulfamethoxazol
  - Ciprofloxacin



1: Immature oocysts contains 1 sporoblasts

- Sporoblasts divide, produce cyst wall
- Each sporoblast produces 4 sporozoites

2: Transmission via contaminated water  
cysts must reside outside the body for 1-2 days

3: Excystation  
invade the epithelium

Asexual cycle – Schizont, Merozoites

Sexual cycle

- Microgametes (male)
- Macrogametes (female)

→ Oocysts in feces

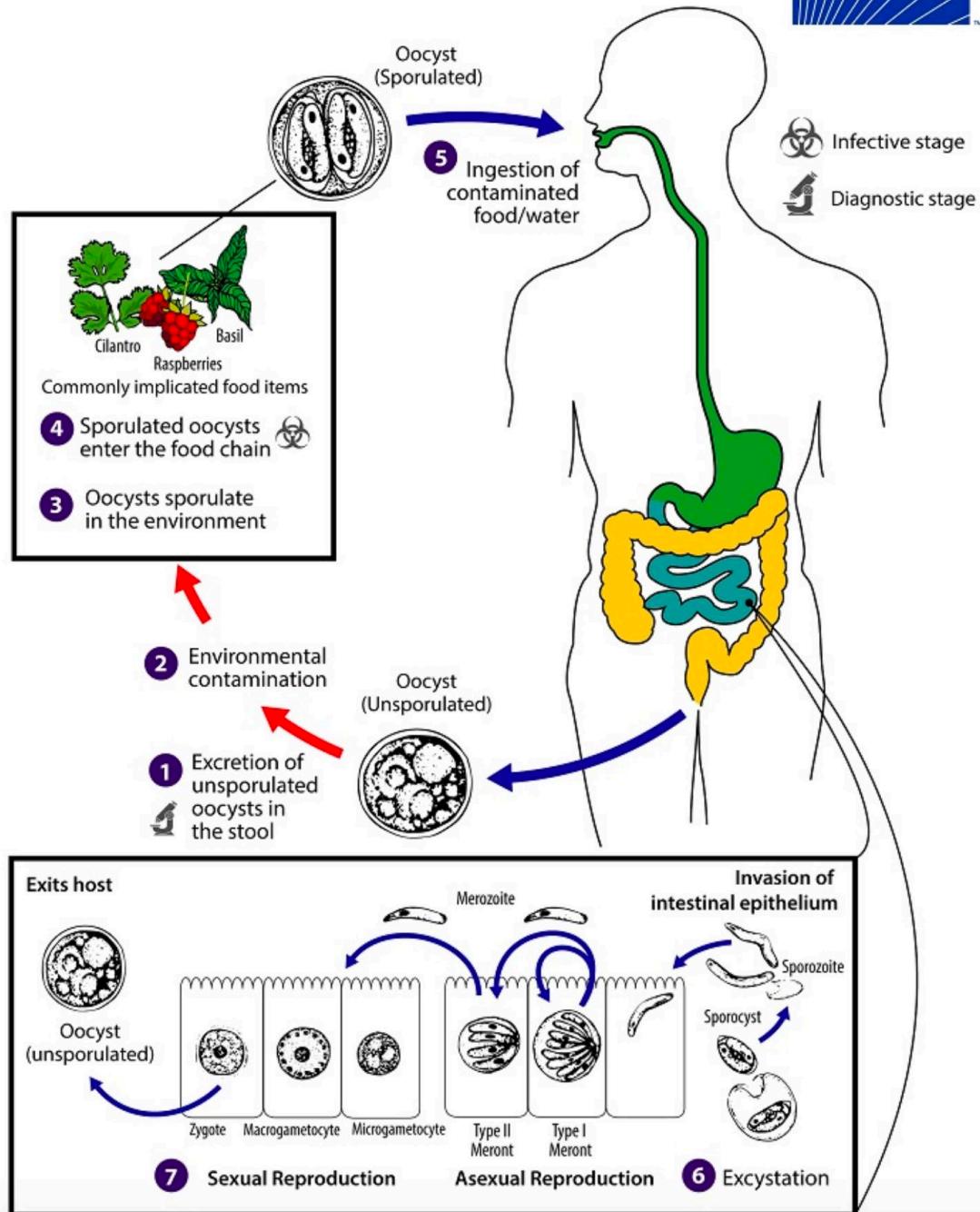
# Cyclospora cayetanesis - cyclosporiasis

- Initial discovered during HIV epidemic and confused with Cryptosporidium
- World-wide distribution: Latin America, India, Southeast Asia
- Imported via travel, food (cilantro, ready-to-eat salad)
  - Local outbreaks
  - Approx. 1000 cases per year in US
- Symptoms:
  - Asymptomatic
  - Watery diarrhea +/- mucus, blood, cramping, fever, upper-GI
    - Prolonged disease course with anorexia, wasting is possible
    - More severe with HIV (but not as characteristic as cryptosporidiasis)
- Diagnosis: stools smear
- Treatment: Trimethoprim/ sulfamethoxazole 7-10 days



Cilantro („Koriander“)





1, 2: unsporulated oocyst: non-infective

3, 4, 5: Sporulation („Sporenbildung“)  
after days or weeks 22°C-32°C  
→ infective, enter the food chain

6: Excystation

Sporozoites are freed  
→ invade small intestinal epithelium  
tissue destruction

Asexual cycle

Type I Meront asexual replication

Type II Meront → Sexual cycle

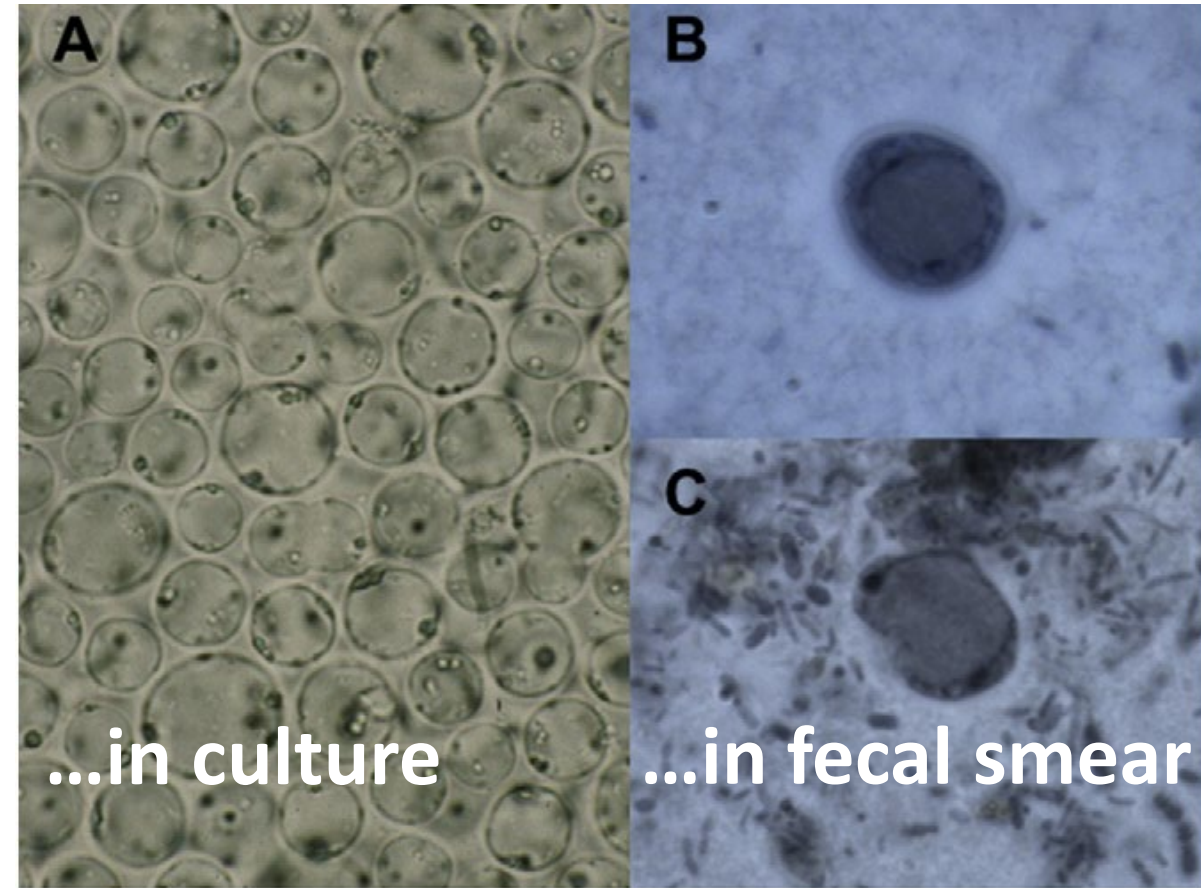
Details unknown

- Microgametes
- Macrogametes
- Zygotes

→ Oocysts in feces

# Blastocystis species

*«Most important .... is whether Blastocystis causes disease in humans. For every report linking Blastocystis with gastrointestinal or other symptoms there is another that finds no such link. There are a number of factors that have contributed to this apparent lack of progress... We would like to warn the reader at this early stage that we ourselves are convinced only that there are no definitive data yet available to resolve this issue.»*



# *Blastocystis* species (*Blastocystis hominis*)

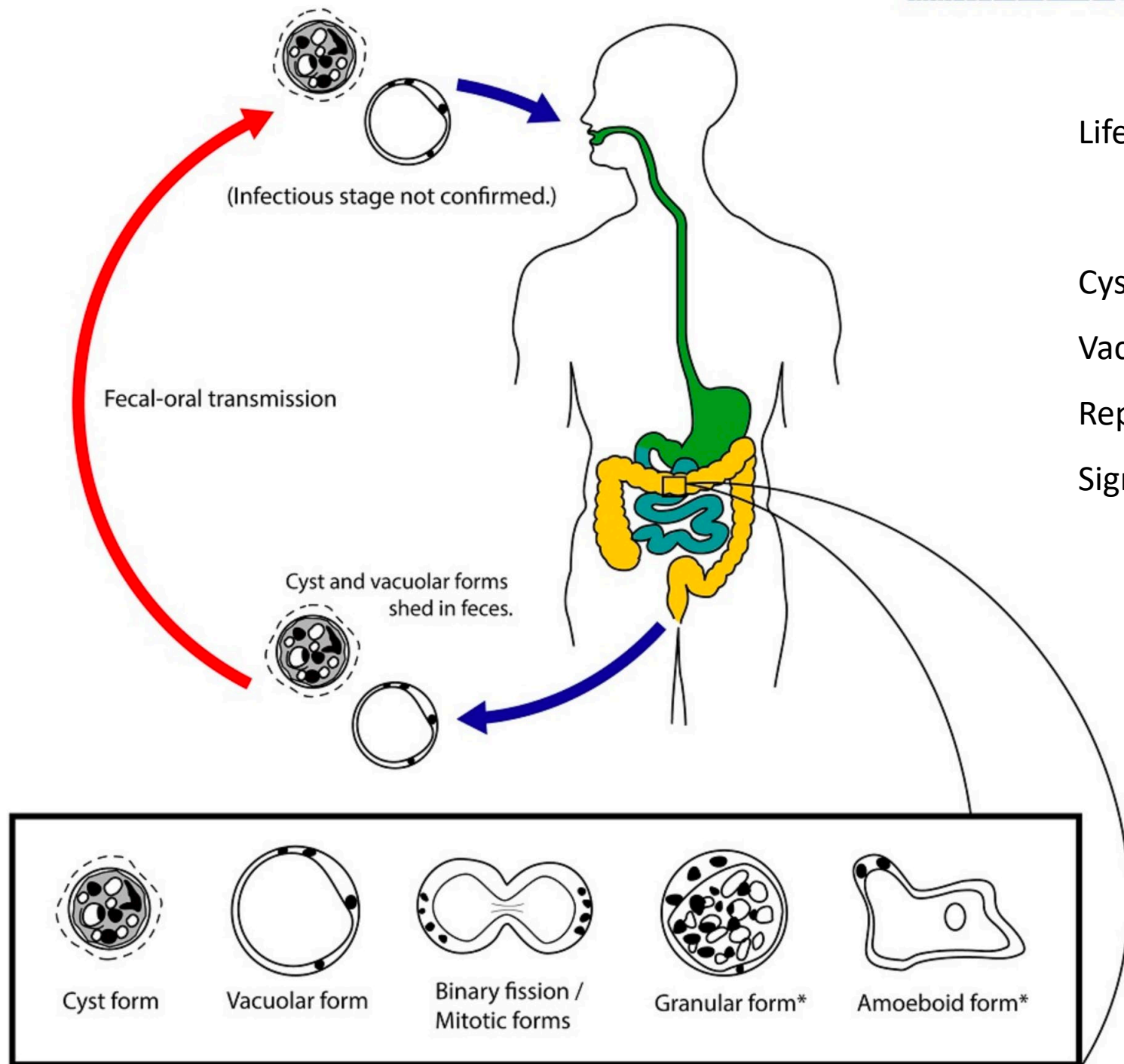
- *Blastocystis* species
  - a number of genetically diverse species with low host specificity
  - 9 Subtypes (ST): ST1, ST2, ST3, ST4 most prevalent
- Mode of transmission unclear
  - Fecal oral? Contaminated water?
  - More frequent upon occupational exposure to animals → zoonotic transmission?
- Prevalence
  - 30-50% in developing countries
  - 5-10% in industrialized countries

# Pathogen? No pathogen? – key question.

## Inconclusive

- Observational studies, case reports, different diagnostic methods, heterogeneity of *Blastocystis* spp., prolonged shedding
- Co-pathogenicity (controversial)
  - Marker of dysbiosis
  - Presence of *Blastocystis* spp indicates presence of other pathogen?
  - *Blastocystis* is easier detected in liquid stool?
- Symptoms:
  - watery diarrhea, nausea, anorexia cramps, bloating, fatigue
  - IBS patients have a 2.3-fold higher risk for *Blastocystis* infection





Life cycle is incompletely understood...

Cyst form (3-5  $\mu\text{m}$ )

Vacuolar form (5-40  $\mu\text{m}$ ), most prevalent in stool

Replication via binary fission

Significance of granular and amoeboid forms unclear

# Diagnosis + Treatment

- Diagnosis: stool smear (frequently in colon biopsies)
- Asymptomatic: no treatment
- Symptomatic
  - 1) Look for other pathogen
  - 2) Check for persistent symptoms (frequently self-limiting)
  - 3) Consider therapeutic trial with observation
    - Metronidazol 750 mg 3x per day for 5-10 days
      - Effective in 1 RCT (88% vs. 14% placebo), ineffective in others
    - Tinidazol 2g once
    - Paromomycin 500 mg
      - Effective in 1 RCT (77% paromomycin, 38% metronidazole, 22% placebo)
    - Trimethoprim/ sulfamethoxazole

Protozoan Infections of the GI Tract					
Disease	Pathogen	Signs and Symptoms	Transmission	Diagnostic Tests	Antimicrobial Drugs
Amoebiasis (amoebic dysentery)	<i>Entamoeba histolytica</i>	From mild diarrhea to severe dysentery and colitis; may cause abscess on the liver	Fecal-oral route; ingestion of cysts from fecally contaminated water, food, or hands	Stool O&P exam, enzyme immunoassay	Metronidazole, tinidazole, diloxanide furoate, iodoquinol, paromomycin
Cryptosporidiosis	<i>Cryptosporidium parvum</i> , <i>Cryptosporidium hominis</i>	Watery diarrhea, nausea, vomiting, cramps, fever, dehydration, and weight loss	Contact with feces of infected mice, birds, farm animals; ingestion of contaminated food or water; exposure to contaminated water while swimming or bathing	Stool O&P exam, enzyme immunoassay, PCR	Nitazoxanide, azithromycin, and paromomycin
Cyclosporiasis	<i>Cyclospora cayetanensis</i>	Explosive diarrhea, fever, nausea, vomiting, cramps, loss of appetite, fatigue, bloating	Ingestion of contaminated food or water	Stool O&P exam using ultraviolet fluorescence microscopy	Trimethoprim-sulfmethoxazole
Giardiasis	<i>Giardia lamblia</i>	Diarrhea, nausea, stomach cramps, gas, greasy stool, dehydration if severe; sometimes malabsorption syndrome	Contact with infected individual or contaminated fomites; ingestion of contaminated food or water	Stool O&P exam; ELISA, direct fluorescence antibody assays	Metronidazole, tinidazole