

Intestinal Protozoa



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Summary

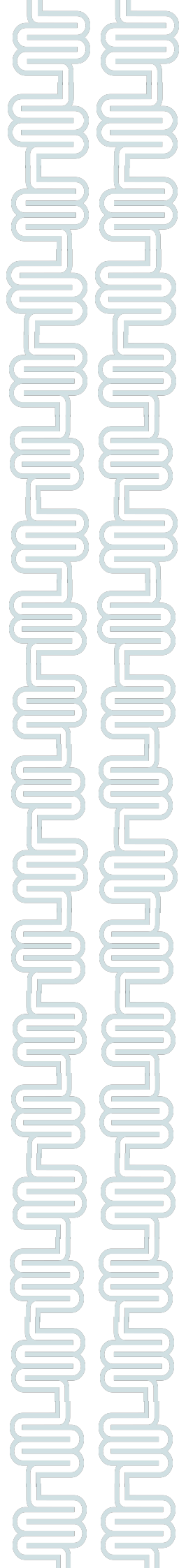
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Objectives:

- Know morphology of cysts and trophozoites of *Giardia lamblia* parasites
- Describe life cycle of *Giardia* parasites
- Describe *Giardia* trophozoites in tissue sections
- Discuss the clinical picture of *Giardia* parasites (Typical and Atypical).
- How to diagnose *Giardia* in the labs
- Know the chemotherapy against *Giardia* parasites.
- Summarize general features of Intestinal *Entamoeba*.
- Know the six types of *Entamoeba*.
- Compare between *E. histolytica* and *E. dispar*.
- Describe Life cycle of *E. histolytica*
- Discuss Pathology of *E. histolytica* (intestinal and extraintestinal).
- Diagnosis and treatment of *Amoebae*
- Life cycle of *Cryptosporidium* and diagnosis



Introduction to Parasitology

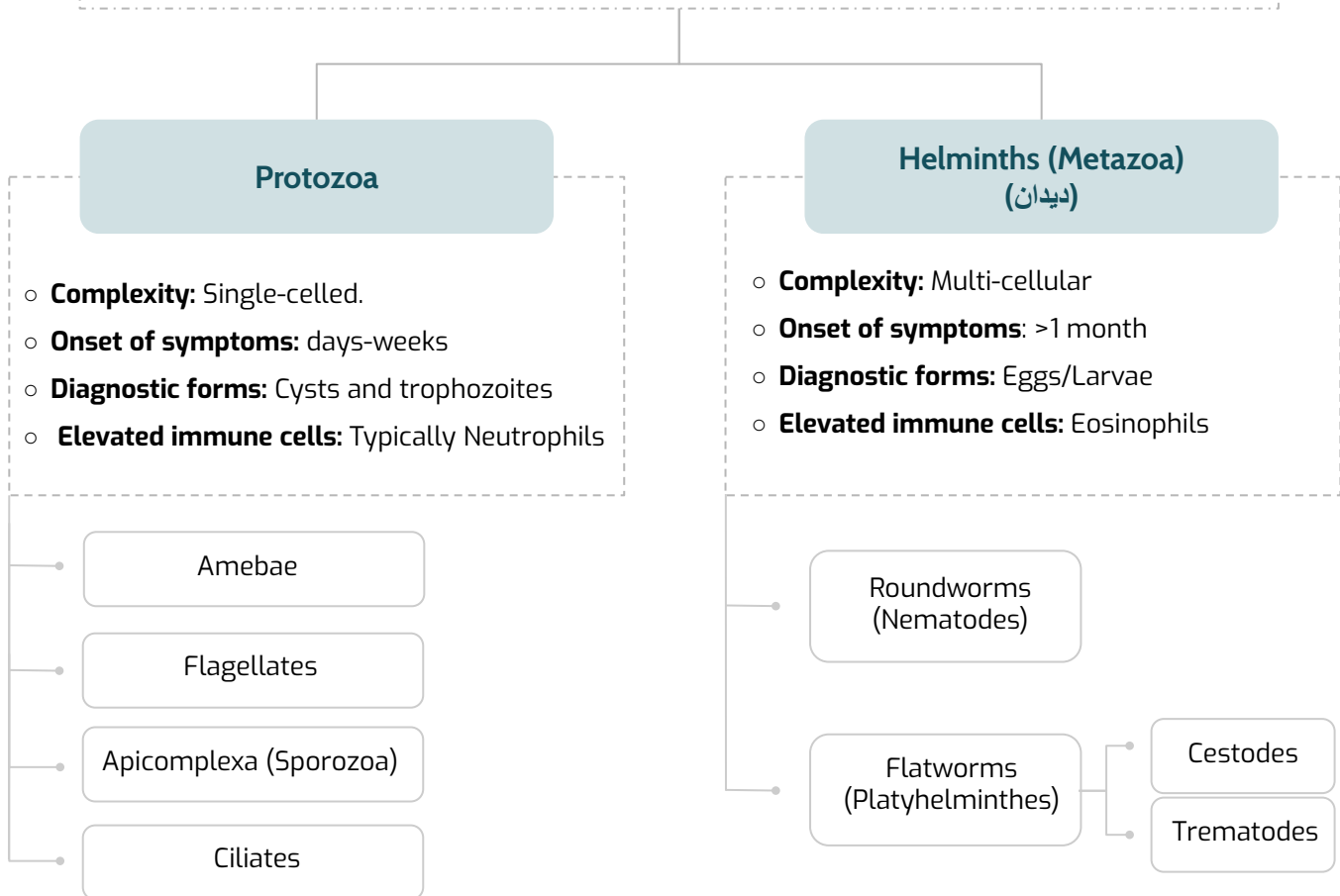
(Extra page for better understanding)

Definition

the study of the invertebrate animals and the diseases they cause.

Human parasites' family tree

Parasites are classified as:



Introduction to Parasitology

(Extra page for better understanding)

Important Terminology		
Vectors Are living transmitters (e.g. a fly) of disease and may be:	Mechanical: transport parasite but there is no development of parasite in the vector	
	Biological: some stages of life cycle occur	
Life cycle forms	Infectious: the stage in the life cycle of an endoparasite in which it can initiate infection to its host e.g., cysts in protozoan	
	Diagnostic: e.g., trophozoite in protozoan infections, eggs/worm in helminth infection	
Others	Obligatory: They are always in contact with host and cannot survive without them.	Free living: They can live independently of their host, partially on soil.
	Direct life cycle: When parasite requires only one host to complete its life cycle.	Indirect life cycle: When two or more hosts are required to complete its life cycle
	Definitive host: It is the host in which the sexual reproduction (adult) takes place or most highly developed form exists (usually humans)	Intermediate host: It is the host in which asexual reproduction takes place.
	Reservoir: This is an animal host which serves as the source from which other animals are infected.	Gravid worms: Carrying eggs.
	Embryonated egg (Also called a "Larvated egg"): A nematode egg with a developed larva inside it. Most nematode eggs leave the host in the morula stage and develop in the environment to the embryonated stage (the stage just before hatching). A few nematode eggs are embryonated at the time they leave the host.	Unembryonated egg: Egg without an embryo, due to a lack of fertilization or to zygotic lethality.
		Larva migrans: Means that the larvae (يرقة) living in their abnormal hosts in which they can not grow into adults but can wander everywhere and cause the local and systemic pathological lesions of the hosts.
	Zoonosis: refers to animal's diseases which can be transmitted to humans.	Life cycle: Is the process of a parasite's growth, development and reproduction, which proceeds in one or more different hosts depending on the species of parasites.
	Sporozoite: a motile spore-like stage in the life cycle of some parasitic sporozoans.	Trophozoites: a growing stage in the life cycle of some sporozoan parasites, when they are absorbing nutrients from the host.
	Cysts: a stage in the life cycle of certain parasites, during which they are enveloped in a protective wall, facilitates their survival during unfavorable environmental conditions.	Oocyst (كيسة بيض): a cyst containing a zygote formed by a parasitic protozoan.

Classification of Parasites

Protozoa

Unicellular. A single cell for all functions.

- Amoebae: moves by pseudopodia
- Flagellates: moves by flagella
- Ciliates: moves by cilia
- Apicomplexa (Sporozoa); tissue parasites **Cannot move** e.g. **Malaria**

Helminthes

Multicellular, specialised cells. **With different systems (like us).**

- Roundworms (nematodes):
- elongated, cylindrical, unsegmented
- Flatworms:
- trematodes: leaf like, unsegmented
- cestode: tape-like, segmented

Giardia Lamblia

- Giardia lamblia is a protozoan parasite capable of causing sporadic (**one case in one area**) or epidemic (**many cases in one area**) diarrheal illness.
- Giardiasis is an important cause of waterborne and foodborne disease, daycare center outbreaks, and illness in international travelers.
- Giardiasis is especially common in areas with poor sanitary conditions and limited water-treatment facilities, **Water is a major source of giardiasis transmission.**
- **Giardiasis is transmitted via the fecal-oral route** with the ingestion of cyst (the infective stage)
- Giardia species have two forms, cysts & trophozoites:

cysts^[1]

★ Infectious and diagnostic stage

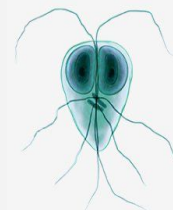
- Resists gastric acidity
- Survives the environment
- **Cysts can stay infectious in the environment for more than 3 months^[2]**
- Excreted in the stool
- infections have an incubation of a week or more before symptoms of acute giardiasis may develop



trophozoites^[3]

★ Replicative and diagnostic stage

- Can't resist gastric acidity
- Can't Survive the environment
- Pear-shaped
- 2 nuclei & adhesive disc
- Multi-flagellated (8)
- Divides by binary fission^[4]



^[1] If you get infected by 2-10 cysts, it is enough to cause disease.

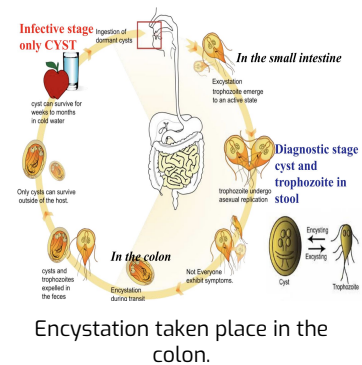
^[2] Because it has a coat that protects it from stomach acidity and external environment

^[3] If you get infected by 1 mil trophozoites, it will not cause disease because it cannot resist stomach acidity.

^[4] It is a local infection on the small intestine it will not go to the blood or attack the mucosal barrier in the small intestine

Life Cycle 

Cyst ingestion (resist acidity) → excystation^[1] occurs in the small intestine → with release of trophozoites (pear-shaped) → trophozoites replicate/multiply by binary fission (it is responsible for causing the disease: diarrhea, vomiting, excessive gas and loss of appetite especially in children) → Trophozoites are then localized in the small intestine, they attach to the mucosal surface of the duodenum and jejunum however, the trophozoite does not invade (It has a local action) the mucosal epithelium → Encystation^[2] takes place in the colon → Excretion in the stool as cyst or trophozoite.



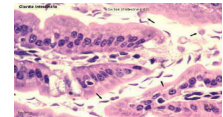
In short: Cyst ingestion → Excystation in small intestine (Cyst becomes Trophozoite) → Trophozoite replicate & causes local disease in duodenum → Encystation in colon (Trophozoite becomes Cyst) → Excretion in the stool as cyst or trophozoite.^[3]

Clinical manifestations

- It is mainly an asymptomatic infection that occurs in both children and adults.
- Asymptomatic cyst & trophozoites shedding can last six months or more.
- If symptoms occur, they include diarrhea, malaise, abdominal cramps, flatulence, weight loss & vomiting.
- The parasite mostly asymptomatic or can produce a wide range of gastrointestinal symptoms especially in children
- Symptomatic infections either:
 - Typical : Incubation period 1-2 weeks followed by diarrhea, vomiting & flatulence for about 6 weeks
 - Atypical : Severe diarrhea , Malabsorption especially in **children** and cholecystitis

Complications
Usually in children

- In a small number of patients.
- persistent infection is associated with development of malabsorption (poor absorption of proteins and vitamins) and weight loss
- Chronic giardiasis may affect growth and development in children.



Giardia trophozoites in tissue section seen by duodenal aspirate. So, we do biopsy in severe cases.

Diagnosis

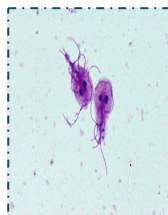
- Antigen detection assays: a number of immunoassays using antibodies against cysts or trophozoites antigens have been developed for stool analysis.
- Examination of duodenal contents for trophozoites.
- Stool examination: Microscopy for cysts & trophozoites.
- **Note that: infective stage: Cyst only / Diagnostic stage: both Cyst and Trophozoite**

Treatment

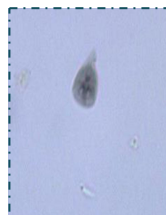
- Chemotherapy
- Drug of choice is **Metronidazole**.



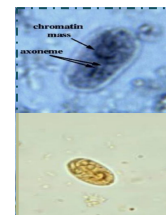
Giardia trophozoites (electron microscopy)



Giardia trophozoites (Trichrome stain)



Giardia trophozoites (Light Microscopy)



Giardia Cyst (Light Microscopy)

^[1] Removal of the protective coat of cyst and emergence/development of trophozoite.

^[2] Trophozoite differentiates into cyst stage to move to the next host.

^[3] Both of them are passed in the stool but trophozoite will not survive in the environment

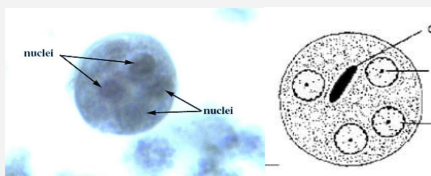
- Amebiasis occurs worldwide; the prevalence is increased in developing (tropical) countries because of poor socioeconomic conditions and sanitation levels.
- It is a **waterborne infection**, there is pathogenic and non pathogenic strains, but we can't distinguish between them by microscopic examination.
 - 500 million people worldwide are infected.
 - 100,000 deaths per year.
- Intestinal Amoebae
There are a number of intestinal commensal amoebae , the only pathogenic species is E. histolytica
- There are 6 species of Entamoeba : Just focus in E.Histolytica the other species are not important
 - E.Histolytica^[1] amoebae are pathogenic & invasive.
 - E.dispar is non-pathogenic, non-invasive form.
 - E.coli
 - E.gingivalis
 - E.hartmanni
 - E.polecki
 - histolytica and E.dispar can't be distinguished by microscopic observation.
- The parasite exists in two forms:

Stained	Entamoeba coli	Entamoeba histolytica	Entamoeba dispar	Entamoeba gingivalis	Entamoeba hartmanni	Entamoeba polecki
Cystium inclusions	With haematoxylin, often black-grey	None	None	None	None	None
Major characteristics	Shin-lake vesicle (pyrogen in diet area)	None	None	None	None	None
Membrane	Thick	Thin	Thick	Very delicate	Delicate	Delicate
Characteristics membrane	Oxide	None	Sometimes granular	None	None	None
Karyosome	Coarse, generally eccentric	Large, regular	Large lateral	Central granules	Small central	Small central
Flagellation	May be chromatin patches	No chromatin	No chromatin	Delicate filaments	None	None
Pathogenicity	Harmless commensal	Harmless commensal	Harmless commensal	Dependent	Invasive	Harmless commensal (Non-invasive)

cysts

★ Infective and diagnostic stage

- Resist to the harsh conditions of the environment. .
- No more than 4 nuclei
- Chromatin bar
- Peripheral chromatin of the nuclei



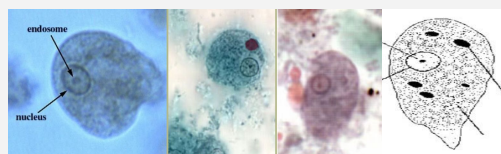
- The infective stage is the cyst but we can detect both in the stool cyst and trophozoites diagnostic stage

trophozoites

★ Vegetative and diagnostic stage

- causes invasive disease.
- fragile structure.
- must encyst to survive in the environment

Consumes RBCs.



^[1] E.Histolytica = large intestine. Don't forget that Giardia lamblia = small intestine.

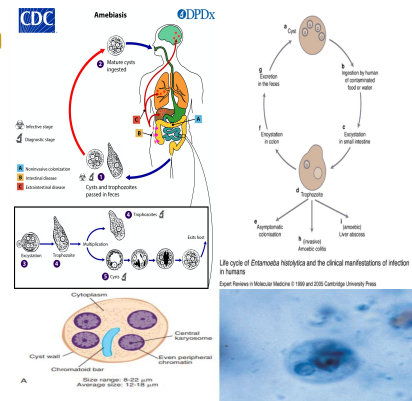
Entamoeba Histolytica

Life Cycle ^[1]

Important: Entamoeba histolytica is **invasive** (unlike giardia)

- The cysts pass through the stomach → to the small intestine, where they excyst to form trophozoites → The **trophozoites can invade and penetrate the mucous barrier of the colon (large intestine)** → causing **tissue destruction (ulcer)**, colitis and increased intestinal secretion and can thereby ultimately lead to **bloody diarrhea**.
- The infective dose can be as little as 1 cyst (highly virulent organism).
- The incubation period can be from few days to few weeks depending on the infective dose. **IF the TROPHOZOITE is ingested it disintegrates in the stomach without producing infection.**
- Cysts can survive for weeks at appropriate temperature and humidity
- Excystation** occurs in the lower region of the **small intestine** with production of 8 small amoebae which enter the large intestine and may :
 - invade the tissue,
 - live in the lumen of *large intestine* without invasion .
 - encyst (become cysts and pass in the stool), in the carrier.

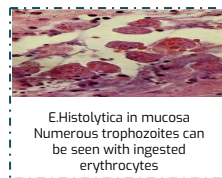
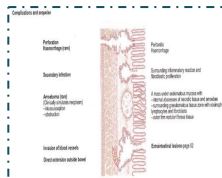
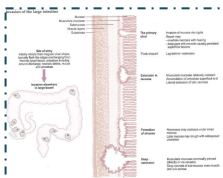
Only the Cysts can survive in the outside environment for weeks at appropriate temperature and humidity after excreted from stool of infected patients or carrier.



Pathology

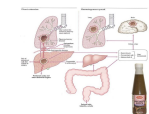
Intestinal amoebiasis & complications

- Remarkable and unique ability to produce enzymes that lyse host tissue.
- Trophozoite has the ability to hydrolyze host tissues with their active enzymes present on the surface membrane of the trophozoite (causing ulcer and tissue perforation), also trophozoite has the ability to ingest blood cells.
- Lesions are found mainly in the colon** and they may heal or cause serious complications:
 - Perforation of the colon** → **★ flask shaped ulcer in large intestine** ,this may lead to peritonitis that can lead to death
 - Ameboma** : Granulomatous mass obstructing the bowel (**tumor from surrounding cells that will lead to bowel obstruction**)
 - Blood invasion; **Amoebic liver abscess**, lung, brain.
 - Direct extension
- As a complication ,severe intestinal hemorrhage or rarely perforation may occur ,lesions are found in cecum ,appendix or colon.
- The presenting symptom is diarrhea which is accompanied by blood ,mucus and sometimes tenesmus. ^[3]
- They may heal. If perforation of the colon occurs, this may lead to peritonitis that can lead to death.



Dr: MCQ Extra-Intestinal amoebiasis

A 30-year-old male experienced diarrhea for two weeks with fever of 39° C, nausea, vomiting, malaise and right upper abdominal pain. Physical examination revealed hepatomegaly 6 cm below the right costal margin. CT scan showed a single hypodense mass in the right lobe of 7.8 x 5.2 cm, round, with well defined borders. Serology was positive for Entamoeba histolytica at 1/512. Amoebic liver abscess was diagnosed.



Spreading:

- Direct extension: To liver (progress to abscesses) → Subdiaphragmatic abscess → To lung (as pleuro-pulmonary abscess).
- Haematogenous spread: Through liver (progress to abscesses) → To ectopic sites (Brain and Lung).

^[1] **Cyst ingestion** → **Excystation in small intestine (Cyst becomes Trophozoite)** → **Trophozoite replicate & causes tissue destruction and invasion of colon (Large intestine)** → **Excystation in colon (Trophozoite becomes Cyst)** → **Excretion in the stool as cyst or trophozoite.**

^[2] Whole colon is infected and inflamed with severe pain

^[3] Severely painful sensation of inability or difficulty in bowel emptying (even if the bowel contents have already been evacuated)

Entamoeba Histolytica

Clinical outcomes of infection	<ul style="list-style-type: none"> • Symptomatic : intestinal (Amoebic dysentery), extraintestinal (perforation & abscess) • Asymptomatic : carrier (We should take care of people working in the kitchen)
Clinical manifestations	<p>Majority are asymptomatic but some may have:</p> <ul style="list-style-type: none"> • mild diarrhea • Severe amebic dysentery ^[2] (abdominal pain, bloody diarrhea, mucus in stools) • Weight loss occurs in about half of patients, fever.^[3] • Fulminant amebic colitis <p>Fulminant colitis with bowel necrosis leading to perforation and peritonitis has been observed in approximately 0.5 percent of cases; associated mortality rate is more than 40 percent</p>
Mood of transmission	<ul style="list-style-type: none"> • Fecal-oral route, Water & Food. • Flies can act as vector • Can be sexually transmitted person - to - person contacts (homosexual) ^[1] • Not a zoonosis, the infective dose can be as little as one cyst, the incubation period can be from few days to few weeks depending on the infective dose. (Highly virulent)
Diagnosis	<p>Intestinal :</p> <ul style="list-style-type: none"> • Stool Examination (Microscopy) mostly used <ol style="list-style-type: none"> 1. Wet mount (cysts and trophozoites) 2. Concentration methods (only cysts) • Molecular testing Detection of parasitic DNA or RNA in feces via probes, can also be used to diagnose amoebic infection & to differentiate between the different strains. • Serology antigen detection (Mainly for invasive infections) <ol style="list-style-type: none"> 1. IHA (indirect haemagglutination assay) 2. ELISA • Colonoscopy with biopsy and histological examination (in severe cases) <p>Extra-intestinal: (liver/lung/brain abscess)</p> <ol style="list-style-type: none"> 1. Serology: IHA, ELISA. 2. Surgical aspirate (needle aspiration not done as a diagnostic procedure due to risk of extension): to look for Trophozoite.^[4] 3. Sigmoidoscopy and/or colonoscopy and taking biopsy: Trophozoite.
Treatment	<ul style="list-style-type: none"> • Intestinal: <ol style="list-style-type: none"> 1. Asymptomatic (cysts only): diloxanide furoate (Furamide). 2. Symptomatic (cysts and trophozoites): Metronidazole (same as Giardia) • Extra-intestinal: Metronidazole.

^[1] Because it's transmission is through feces

^[2] Differential diagnosis: Shigella (both cause blood and mucus in stool), and EHEC (Enterohemorrhagic E.coli)

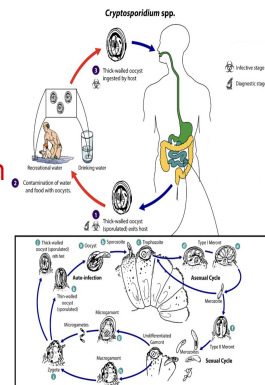
^[3] Because it facilitates secondary bacterial infection (due to invasion of colon which is full of bacteria)

^[4] We find trophozoite only (cyst can only be found in the intestines)

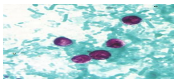
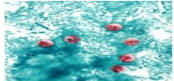
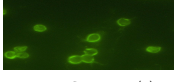


Overview and Life Cycle

An intracellular protozoan parasite that is associated with:

- **Self-limited** diarrhea in normal immunocompetent hosts (normal).
- **Severe debilitating diarrhea with weight loss and malabsorption in (AIDs) patients** and those undergoing immunosuppressive therapy. infection may not be self-limiting, leading to dehydration^[1] and, in severe cases, death



Team438: Before the parasite was not known, because it didn't cause diseases, but with the spreading of HIV especially when it reaches the end stage (AIDS) it caused infection through the cyst entering the small intestine

<h3>Mood of transmission</h3>	<ul style="list-style-type: none"> • Infection is caused by ingestion of sporulated oocysts (infective stage) transmitted by the fecal-oral route • Spread from an infected person or animal by fecal-oral route • From a contaminated environment, such as food or water source contamination
<h3>Diagnosis</h3>	<ul style="list-style-type: none"> • Cryptosporidium species cannot be cultivated in vitro. (so diagnosis of cryptosporidiosis is generally based upon microscopy and Ag detection in stools). • Stool Examination: The diagnosis of cryptosporidiosis is made by finding oocysts in: <ul style="list-style-type: none"> ○ fecal smears when using modified Acid –fast stain(ZN)^[3] ○ by Antigen detection by using ELISA,IF. • Duodenal aspirates, Bile secretions & biopsies from affected gastrointestinal tissue: <ul style="list-style-type: none"> ○ Polymerase Chain Reaction(PCR) ○ Enzyme immunoassays: (ELSA) & IF. <div style="display: flex; justify-content: space-around; align-items: flex-start;">  <p>Cryptosporidium oocyst in feces by stain modified acid-fast stain ZN</p>  <p>Cryptosporidium, safranin Ziehl-Neelsen</p>  <p>Immunofluorescence (IF)</p>  <p>Crypto-Giardia FAI</p>  <p>Crypto-Giardia: Ag detection test in stools</p> </div>
<h3>Treatment & Prevention</h3> <p><small>Dr.Ibrahim: We will not focus on the treatment</small></p>	<ul style="list-style-type: none"> • The most effective way to prevent the spread of C. parvum is to avoid contact with contaminated feces. Avoiding this contact, especially with young children, Hygiene is the most effective way to combat this difficult-to-prevent parasite, • Infection is generally self-limiting in immunocompetent patients. • In immunocompromised patients, such as those with AIDS or those undergoing immunosuppressive therapy, infection might not be self-limiting, leading to dehydration and in severe cases death. • In AIDS patients: Paromomycin^[2] .. Dr.mona: not important.

^[1] like cholera, it causes severe watery diarrhea and loss of electrolytes.

^[2] antiparasitic treatment

^[3] Same as TB

Dr. Mona

- The life cycle of *Giardia Lamblia* is composed of 2 stages: (1) the trophozoite, which cause the disease. (2) The cyst, which is passed into the environment.
- When someone drinks water filled with trophozoites only, he will not get the infection. (because when trophozoite is ingested, it will be eradicated/killed directly by stomach acidity). However, one can easily be infected when drinking water with cysts because it can survive acidity.
- **Trophozoite of *Giardia Lamblia* is not an invasive parasite. It has a local action only** (it does NOT pass to the blood or cross small intestine barrier), so it only causes diarrhea, vomiting, excessive gas and loss of appetite.
- In stool examination: both cyst and trophozoite can be seen.
 - **Infective stage of giardia? Cyst**
 - **Diagnostic stage of giardia? Both**
- To cause an infection, *Giardia Lamblia* enters the body via fecal-oral route as cyst. Then, it reaches small intestine and convert into trophozoite by **excystation**. It replicates there and reside in **duodenum** to cause the disease (non invasive). When it reaches the colon, it converts back to cyst (to prolong its life cycle) by **encystation**. Finally, the cysts will pass in stool to infect someone else.
- There are many types of Amoebae, but the most important in causing the disease is *Entamoeba Histolytica* (other types might be seen, but they are not pathogenic -they do not cause disease-).
- *Entamoeba Histolytica* is more serious than Giardia because it is invasive. (invade mucosa of intestine).
- **Disease caused by *Entamoeba Histolytica* is very severe and painful due to invasion & tissue destruction.** (it starts as mild diarrhea to severe amebic dysentery with blood & mucus in stool).
- To cause an infection, *Entamoeba Histolytica* enters the body via fecal-oral route as cyst. Then, it reaches small intestine and convert into trophozoite by **excystation**. It replicates and **invade the large intestine and colon to cause tissue destruction & disease**. Then, some will converts back to cyst by **encystation**. Finally, the cysts will pass in stool to infect someone else.
- Potentially, *Entamoeba Histolytica* can lead to: (1) perforation (**flask shaped ulcer**), (2) formation of granulomatous mass/**ameboma** (tumor → obstruction of bowel), and it can cause (3) blood invasion (**mainly liver abscess**).
- *Entamoeba Histolytica* is a **highly virulent organism** (one cyst is enough to cause infection).
- When a patient complains of bloody stool with mucus, we suspect *Entamoeba Histolytica* or *Shigella*. (we check microscopy to look for cyst/trophozoite. If microscopy was negative, we culture for *Shigella*).
- *Cryptosporidium Parvum* is considered to be normal flora (commensal) in healthy subjects. However, nowadays, it is extremely important especially in cases of AIDs patients (it is opportunistic).
- **What is the difference between HIV infected patient and AIDs?**
 - HIV infected patient: early stages.
 - AIDs patient: end stage of HIV infection. (the stage where *Cryptosporidium Parvum* causes the disease)

Dr. Ibrahim

- In *Giardia Lamblia* the cyst is round shaped while it is pear shaped in trophozoite.
- Unlike trophozoite, cyst has some properties that enables it to survive acidity and external environment.
- Intestinal Amoebae move by pseudopodia.
- Differentiation between entamoeba species by microscope is difficult, and it needs more experience and advanced investigations. It is NOT important for us to know the species (**just focus on the *Entamoeba Histolytica* which is the pathologic one**).
- In *Entamoeba Histolytica*, one cyst is capable of causing the infection (highly virulent)
- *Cryptosporidium* infection is an opportunistic infection, and it became evident with AIDs patients. (its life cycle is not well established).

Quiz

MCQ

Q1: duodenal aspirate is a good specimen for diagnosis of:

- A- giardiasis
- B- teniasis
- C- amoebic dysentery
- D- cysticercosis

Q2: Liver abscess is known complication of:

- A- Giardia Lamblia
- B- fasciola hepatica
- C- schistosoma mansoni
- D- Entamoeba histolytica

Q3: Which of the following is sexually transmitted ?

- A- sporulated oocysts.
- B- Giardia Lamblia.
- C- Entamoeba histolytica
- D- Cryptosporidium Parvum

Q4: Which of the following protozoa is self-limiting?

- A- Giardia Lamblia
- B- Entamoeba Histolytica
- C- Cryptosporidium parvum
- D- leishmaniasis

Q5: What is the best stain used for cryptosporidium diagnosis?

- A- H&E stain
- B- acid-Fast stain(Ziehl-neelsen)
- C- silver stain

Q6: An AIDS patient came to the ER complaining of diarrhea and abdominal pain. Examination confirm the presence of parasite, which of the following is the more likely pathogen? And what is the drug of choice ?

- A- Cryptosporidium Parvum, Metronidazole.
- B- Cryptosporidium Parvum, Paromomycin.
- C- Entamoeba histolytica, Paromomycin.
- D- Entamoeba histolytica, Metronidazole.

Answers: Q1:A | Q2:D | Q3:C | Q4:C | Q5:B | Q6:B

SAQ

Case: A 3 year-old child came to the clinic complaining of diarrhea, malaise, abdominal cramps, flatulence, weight loss & vomiting for the past 2 weeks. The doctor noticed that the patient's growth and development is affected. Stool examination microscopy showed cysts & trophozoites, and examination of duodenal contents showed trophozoites. He attends the daycare regularly and lives in a poor village.

Q1: What is the most likely diagnosis?

A: Giardiasis infection

Q2: What is the infective stage of this disease?

A: Cysts

Q3: What is a major source of giardiasis transmission?

A: Water and food

Q4: What is the treatment?

A: Metronidazole

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