الجهاز الهضمي

علم الأحياء الدقيقة

رقم المحاضرة :3

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Common Protozoal infections of the GI tract

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الصورة جيدة لمراجعة انواع ال protozoa من الفصل الماضي لفهم هذه المحاضرة

شكرًا لصاحبها

• I- PROTOZOA:

• Is a phylum of the animal kingdom consisting of unicellular parasites (single cell organisms), divided into 4 classes according to the organ of locomotion (they are divided based on two things: locomotion or if there is sexual production):

• 1- Class sarcodina (also known as Rhizopada): Parasites that move by means of pseudopodia (الأيادي الوهمية) example Entamoeba histolytica (which causes amebiasis الزحار الأميبي).

• 2-Class mastigophora (also known as Flagellate): Parasites that move by means of flagella (السوط) as a part of their morphology, example Giardia lamblia (It causes Giardiasis, also called Beaver Fever in Canada which is an intestinal disease).

• 3- Class ciliates: parasites that move by means of cilia (الشعيرات), for example Balantidium coli (It causes a disease known as Balantidiasis).

The previously mentioned classes reproduce asexually.

• 4- Class Sporozoa: This class of parasites have both sexual and asexual reproductive organs; all these parasites are obligate intracellular so, one of its pathogenesis is cellular destruction, and they have no organ of locomotion (they move via gliding (انزلاق/تزحلق) since they don't have pseudopods nor cilia nor flagella), example Plasmodium parasites causing malaria (causative agent of malaria and they live in RBC's which reflects on their manifestation causing anemia), cryptoporticus & Cyclosporas.

Protozoa of the GI tract

- ***Entamoeba histolytica***
  - Causative agent of amebiasis
- **Giardia Lamblia/ Intestinalis/ Duodenalis**
  - Causative agent of Giardiasis
- **Crypposporidium Parvum and Hominis**
  - Causative agent of cryptosporidiosis

if you remember, in intro we said that protozoa are unicellular microorganisms, we divide them based on two cardinal features: 1- if they possess an organ for locomotion, 2- if they have sexual multiplication in their life cycle.

The first family of protozoa was called Rhizopoda (Amoeba is considered one of them).
Rhizopoda(amoeba): possess a specific organ for locomotion called pseudopodia (أرجل وهمية), and they asexually multiply by binary division.

Amoeba genus is divided into :

1-intestinal amoeba

A- (pathogenic) only Entamoeba Histolytica

B- non-pathogenic commensals like Entamoeba Dispar, Entamoeba Hartmanni, and Entamoeba Moshkovskii.

It is important to know them (non-pathogenic species) to differentiate between them and Entamoeba Histolytica.

2-Amoeba in the buccal cavity like Entamoeba tenax

3-Free living amoeba : Acanthamoeba and Naegleri fowleri

Acanthamoeba which is called " BRAIN EATING AMOEBA" causes primary meningoencephalitis (PAM) and keratitis to people who use eye lenses and Granulomatous Amoebic Encephalitis (GAE).

Naeglerai fowleri causes primary amoebic encephalitis but Entamoeba histolytica causes secondary amoebic encephalitis.

★ All infections of E.histolytica , Giardia Lamblia and Cryposporidium are associated with
1- poor sanitation
2- poor personal hygiene
3- all of them are transmitted by feco-oral route

in the past it was thought that the prevalence rate is very high (third of the people on the earth have Entamoeba histolytica) but the high prevalence was due to confusion between pathogenic and non-pathogenic members.

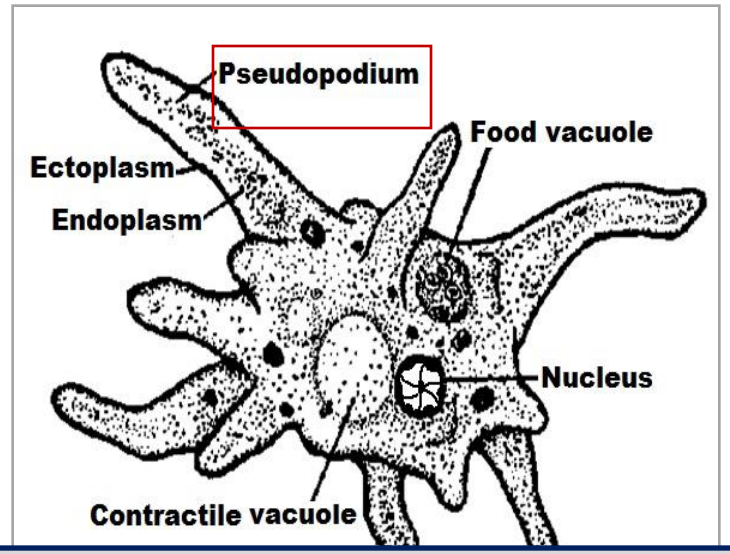
Entamoeba histolytica

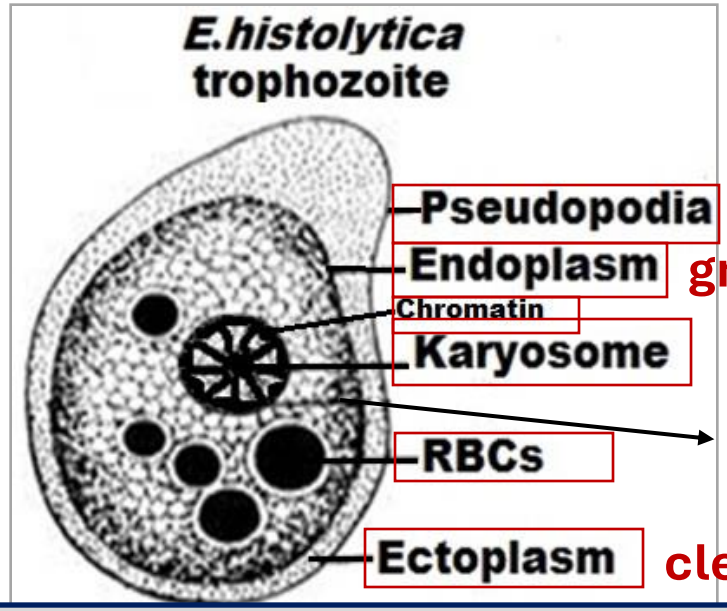
- **Geographical distribution:** Worldwide (found more in tropical and subtropical climates) especially in the temperate zone and more common in areas with poor sanitary conditions and poor personal hygiene (There are always association with these conditions).
- **Habitat:** Large intestine (especially in places that fecal stasis occur at and low peristaltic movement) (caecum, ascending, transverse, descending ,colonic flexures and sigmoidorectal region(places where ulcers occur)).unlike Giardia and Cryposporidium which are in small intestine.
- It is a prototype invasive protozoal infection, invade the mucosa and submucosa of the large intestine
- **D.H (Definitive Host):** Man
- **R.H (Reservoir Host):** Man(the principal reservoir host,mostly asymptomatic(cyst passers), Dogs, pigs, rats and monkeys.
- **Disease:** Amoebiasis or amoebic dysentery. Mainly blood and mucus in patients' stool.

We have 2 morphological stages in Entamoeba histolytica and Giardia which are Trophozoite and cyst.
Trophozoite: Active, Motile, Replicating and Feeding form that usually cause pathogenesis.

Morphological characters

1- Trophozoite stage (Vegetative form or tissue form):





Chromatin bodies gives this car wheel appearance to the nucleus, this is one of the things that differentiate between Entamoeba histolytica and other amoeba species(shape of the nucleus).
If ingested RBCs are present, it indicates the presence of Entamoeba histolytica rather than other commensals living in the gastrointestinal tract as part of the microbiome

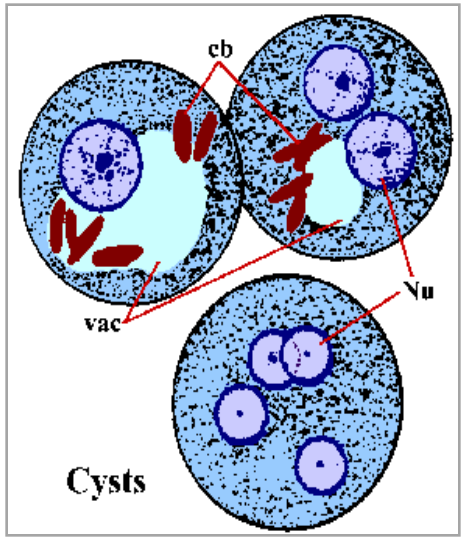
Resistance stage (outside human body) resistance for harsh environmental conditions

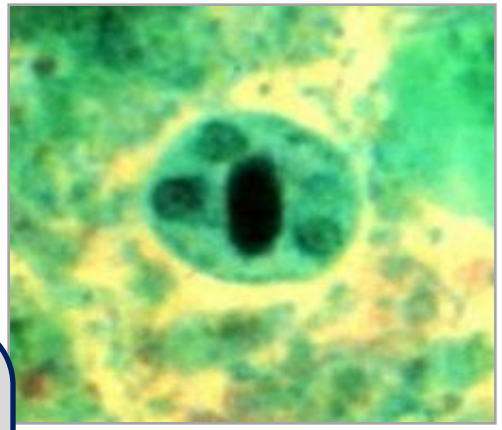
2- Cyst stage (Luminal form):

(a) Immature cyst (Uninucleate cyst and Binucleate cyst):

- **Uninucleate cyst (one nucleus)**
- **Binucleate cyst (2 nucleus)**

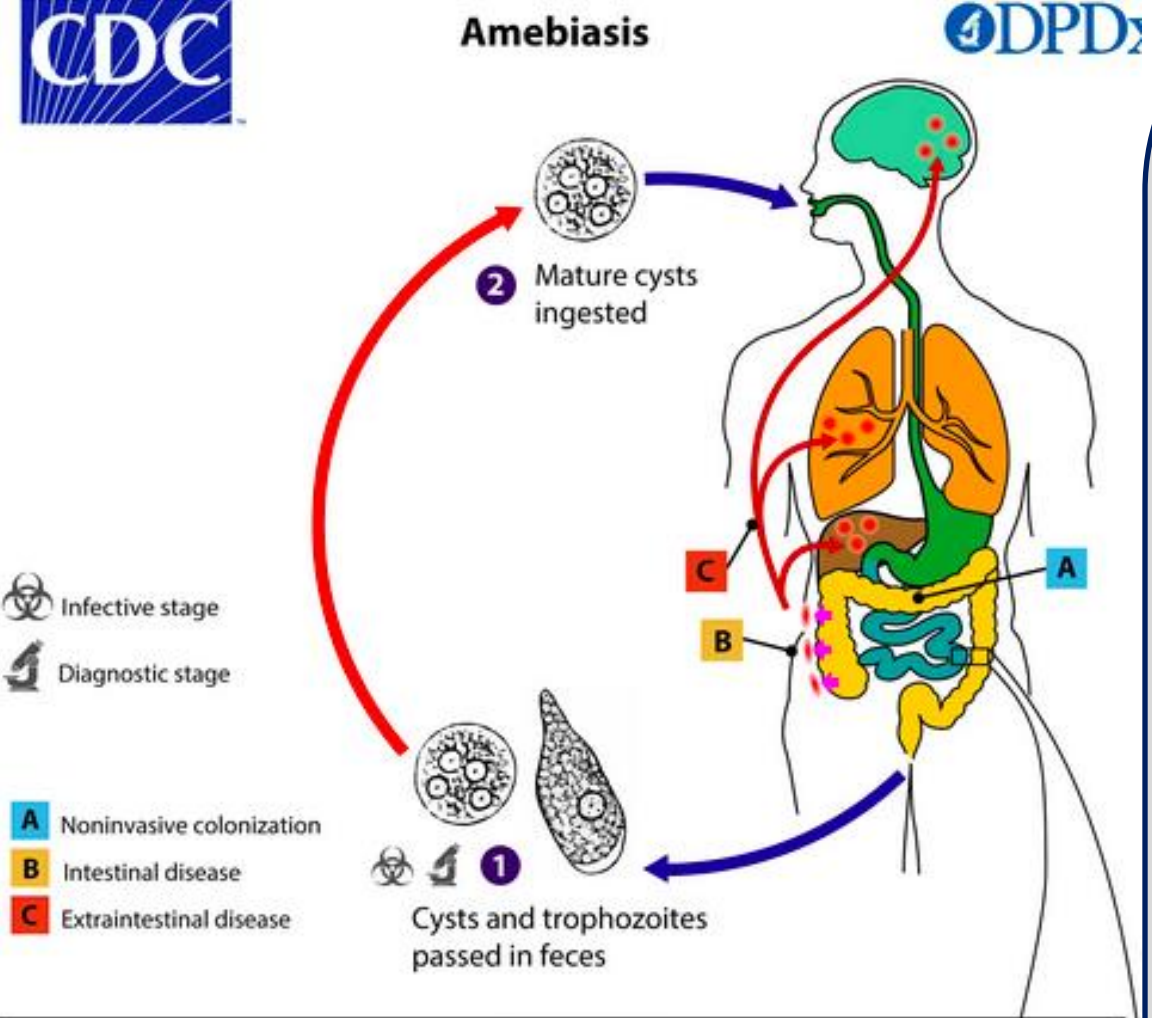
b) Mature cyst (Quadrinucleate cyst)

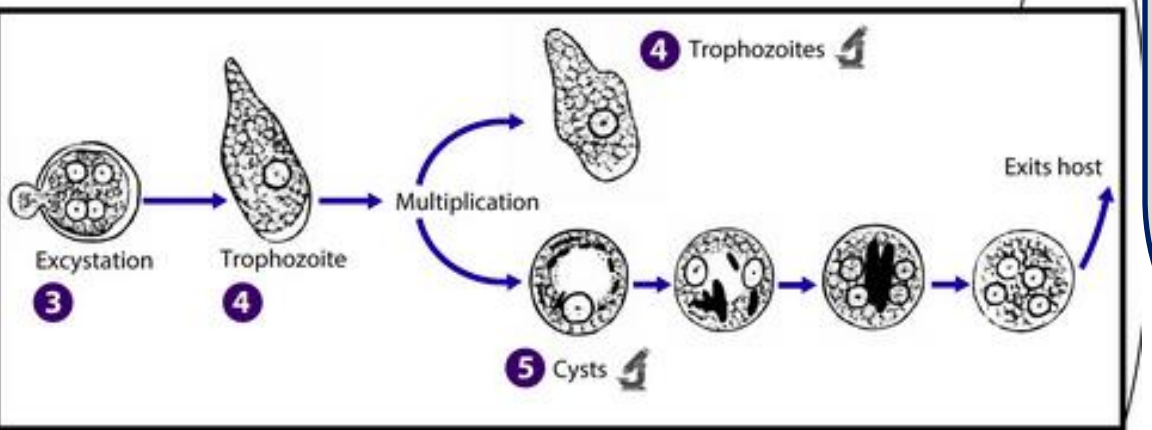




They encyst themselves within a cell wall.

It could be uninucleate, binucleate, or quadrinucleate. Only the quadrinucleate form completes the life cycle of amebiasis.





It is important to know infective stage and diagnostic stage.

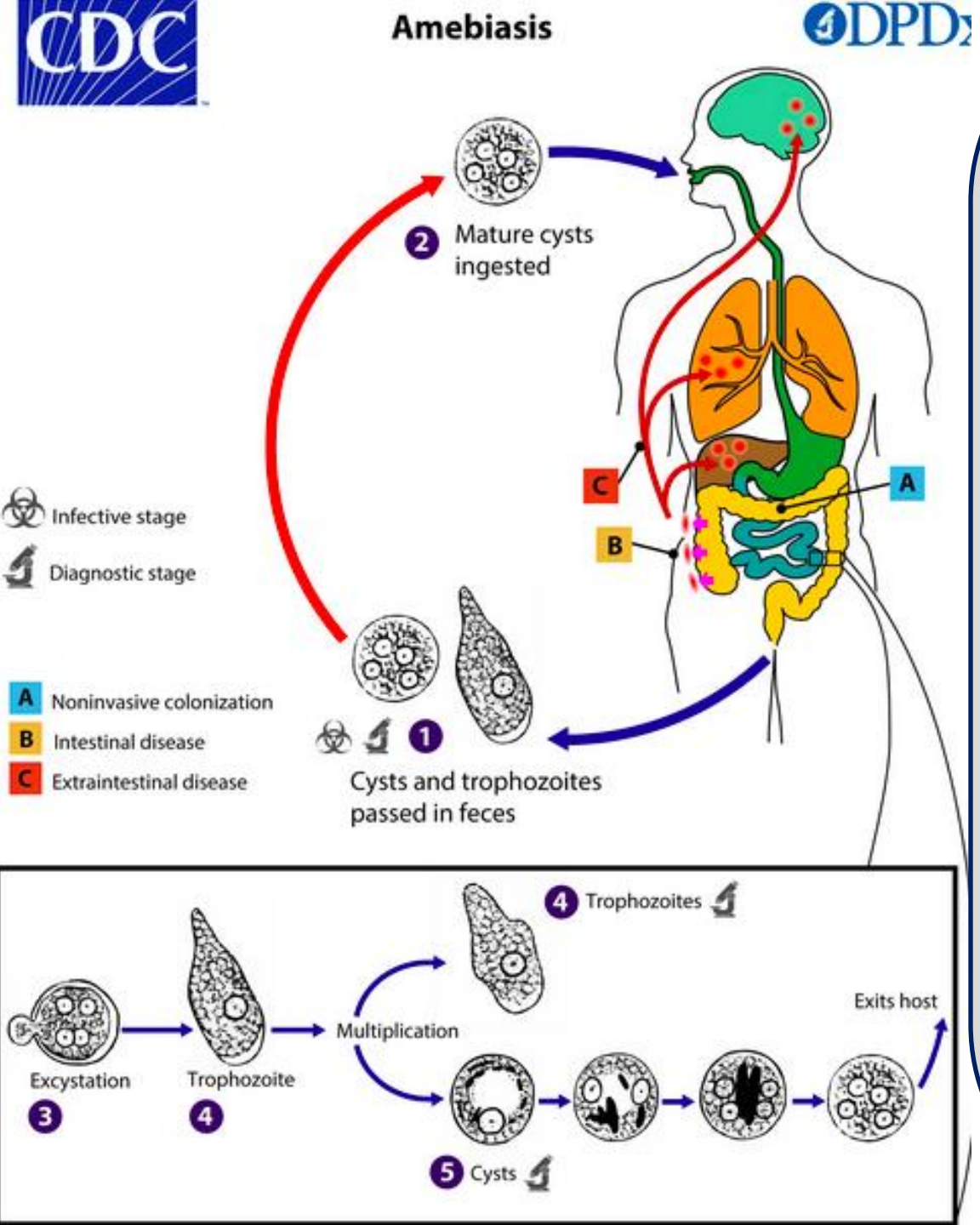
1-Infection starts by ingestion of quadrinucleateed cyst (mature cyst) through contaminated food or water.

2- They pass the stomach and reach the small intestine, a process called excystation happen to these mature quadrinucleate cyst. Each cyst gives 8 trophozoite by binary division.

These 8 trophozoite could :

A-live in the lumen of intestine (in this case we call it (intestinal/lumenal amoebiasis):-

1-They don't invade the mucosa or submucosa of the large intestine (as we said it could be found in places of fecal stasis and low peristaltic movement, colonic flexures)



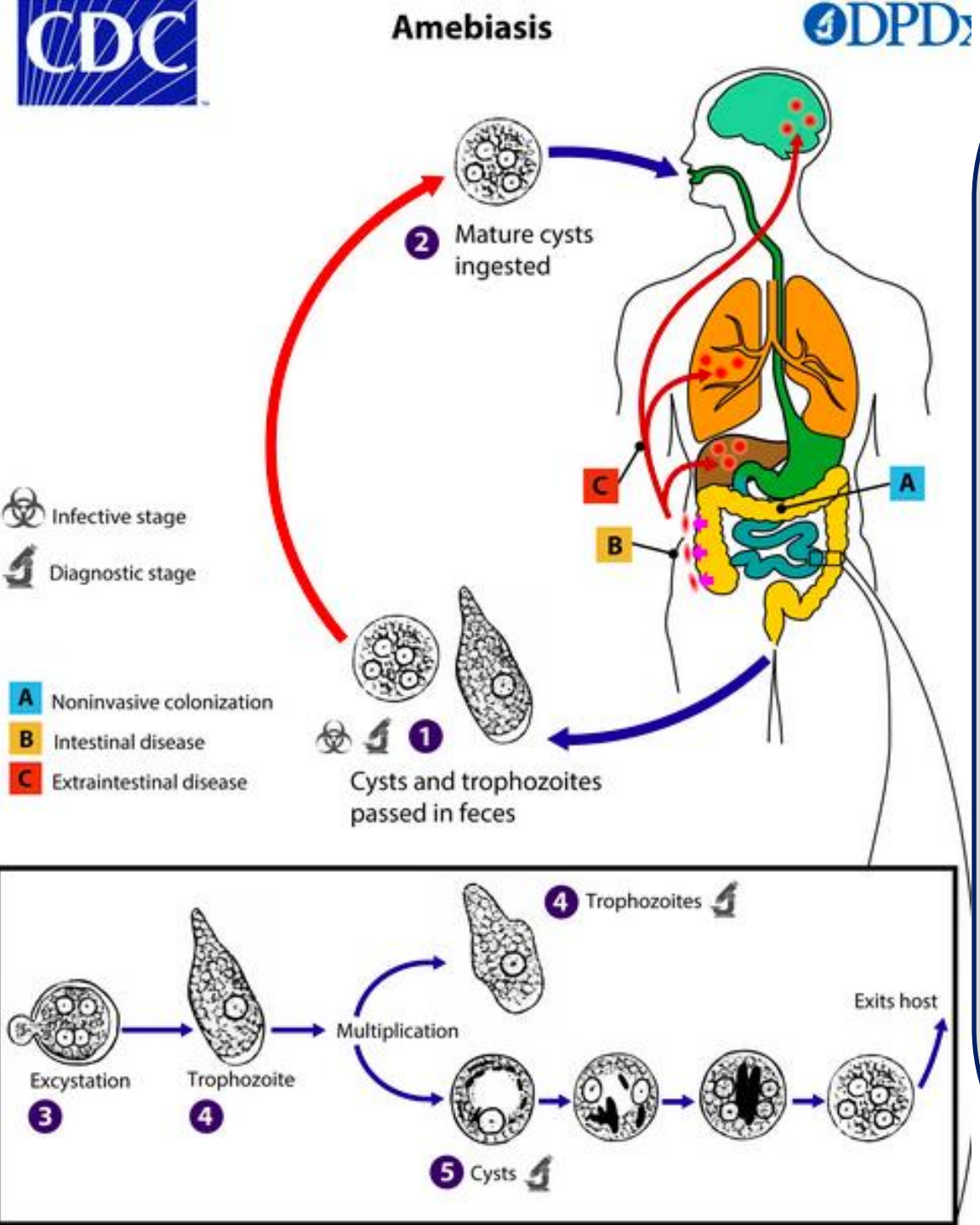
This is the most common scenario in people with Entamoeba histolytica , and we call them asymptomatic carriers or cyst passers. These trophozoite become encysted (encystation process) and get out with stool as cysts.

Diagnosed stage: cyst and trophozoite (when patient has acute amoebic dysentery, it get out with stool without being encysted)

2-In some people, intestinal amoebiasis doesn't only resides in the lumen of the intestine but also begins to bore into the mucosa and submucosa of the large intestine.

These people usually come with acute amoebic dysentery or chronic amoebic dysentery as well as sometimes complications with perforation and penetration for intestinal mucosa and submucosa.

<u>All of this collectively called intestinal amoebiasis</u>



B-In another scenario, after intestinal amoebiasis occurs, invasion and penetration of the intestinal mucosa and submucosa may provide access to the blood circulation , leading to seed in other locations as well as direct extension

(Usually goes to right lobe of the liver, may also cross the diaphragm through either circulation or direct extension from the abscess in the liver and cause abscess in the right lobe of the right lung).

It could also reach the brain through systemic circulation causing secondary amoebic encephalitis or meningoencephalitis.

It could also go to skin, usually if there is stroma above this colonic flexures or perianal region causing Cutaneous amoebiasis (Amoebiasis cutis)

It could also go genital tract.

Mode of infection

Route of transmission: fecal-oral route directly and indirectly.

It could be water born, food born as well as through vectors (fly,cockroaches) they transmit Trophozoite or cyst from feces to food and water.

1- Contaminated water and foods (ex. green vegetables) or drinks or hands with human stool containing mature cyst.

2- Handling food by infected food handlers as cookers and waiters.

3- Flies and cockroaches that carry the cysts from faeces to exposed food.

4- Autoinfection (faeco-oral or hand to mouth infection).

People with poor personal hygiene might develop external autoinfection (finger to mouth if the finger is contaminated with cysts)"external autoinfection" specifically refers to the transmission of pathogens or parasites from an external source (like contaminated hands) to the person's own body.

5- Homosexual transmission.

There are notes on each column in the next slides

Clinical pictures

I) Intestinal amoebiasis

1-Asymptomatic infection

Most common and trophozoites remain in the intestinal lumen feeding on nutrients as a commensal without tissue invasion (Asymptomatic patient known as a healthy carrier and cyst passers)

2-Symptomatic infection

Invasion of mucosa and submucosa

a) Acute amoebic dysentery

Presented with fever, abdominal pain, tenderness, tenesmus and frequent motions of loose stool containing mucus, blood and trophozoites.

b) Chronic infection

-Occurs if acute dysentery is not properly treated.
-With low grade fever, recurrent episodes of diarrhea alternates with constipation.
- Only cysts are found in stool.

3-Complications

- Haemorrhage due to erosion of large blood vessels.
- Intestinal perforation ➲ peritonitis.
- Appendicitis.
- Amoeboma (Amoebic granuloma) around the ulcer ➲ stricture of affected area.

1-Asymptomatic infection

Most common and trophozoites remain in the intestinal lumen feeding on nutrients as a commensal without tissue invasion (Asymptomatic patient known as a healthy carrier and cyst passers)

Majority 60%-80%.

Don't produce bloody diarrhea.

Very important because they are the principal reservoir for continuation of transmission cycle by Entamoeba histolytica.

2-Symptomatic infection

a) Acute amoebic dysentery

Presented with fever, abdominal pain, tenderness, tenesmus and frequent motions of loose stool containing **mucus, blood** (Amoebic dysentery) **and trophozoites.**

They have Tenesmus: rectal straining or rectal spasms (المستقيم إجهاد)

They shed trophozoites because as we said they are getting out of large intestine before encystation occur to them

2-Symptomatic infection

b) Chronic infection

-Occurs if acute dysentery is not properly treated.
-With low grade fever, recurrent episodes of diarrhea alternates with constipation.
- Only cysts are found in stool.

If infection persist, patient might develop chronic diarrhea.

Cysts and trophozoite are diagnostic stage for amoebiasis.

3-Complications

- Haemorrhage due to erosion of large blood vessels.
- Intestinal perforation ➲ peritonitis.
- Appendicitis.
- Amoeboma (Amoebic granuloma) around the ulcer ➲ stricture of affected area.

Invasion of mucosa and submucosa could lead to Haemorrhage.

Patient come with characteristic bloody diarrhea with only RBCs and mucus while in dysentery caused by bacilli species which usually contain RBCs, WBCs and mucus.

Complete penetration of mucosa and submucosa end up by perforation, so these patients have peritonitis.

Granuloma formation occurs during the healing process for ulcers caused by Entamoeba histolytica, which can progress into mass-like structures resembling amoeboma.

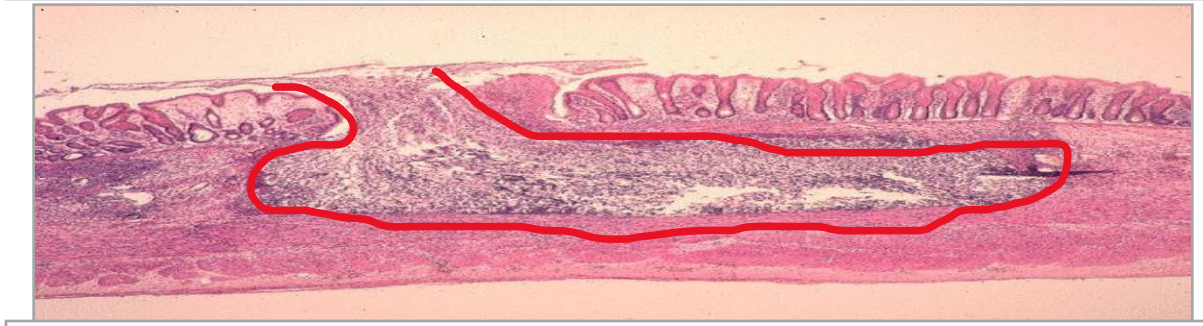
Amoeboma: One of the differential diagnosis for masses or malignancy that present in large intestine.

Additionally, the ulcer may be located in the right iliac fossa, causing pain in that area similar to the presentation of appendicitis.

With heavy infection and lowering of host immunity

The trophozoites of *E. histolytica* invade the mucosa and submucosa of the large intestine by secreting lytic enzymes ➲ amoebic ulcers

The ulcer is flask- shaped or inverted flask shaped with deeply undermined edges containing cytolyzed cells, mucus and trophozoites.



transverse section

The most common sites of amoebic ulcers are caecum, colonic flexures and sigmoidorectal regions due to decrease peristalsis & slow colonic flow at these sites that help invasion.

II) Extra-intestinal amoebiasis

Due to invasion of the blood vessels by the trophozoites in the intestinal ulcer ➲ reach the blood ➲ to spread to different organs as:

Liver

The most common site

-Amoebic liver abscess or diffuse amoebic hepatitis. especially in young age people 20-40

-Affect commonly right lobe either due to spread via portal vein or extension from perforating ulcer in right colonic flexure.

-CP (Clinical presentation): include fever, hepatomegaly and pain in right hypochondrium.

Lung

•Lung abscess ➲ pneumonitis with chest pain, cough, fever.

•Amoebic lung abscess usually occur in the lower part of the right lung due to direct spread from the liver lesions through the diaphragm or very rarely trophozoites may reach the lung via blood.

Abscess treatment: Incision and Drainage (I&D).

When we drainage the abscess, their content have characteristic appearance called Anchovy paste appearance
Anchovy: texture like toothpaste.

Patient might start with Extra-intestinal amoebiasis without passes through Intestinal amoebiasis (without showing symptoms related to GI tract), almost the case of 50% of the cases of Extra-intestinal amoebiasis.

It might reach the brain through systemic circulation causing secondary amoebic encephalitis.
We have to differentiate between this Entamoeba histolytica and Naegleria fowleri that cause primary amoebic encephalitis and Acanthamoeba that cause Granulomatous amoebic encephalitis.

→ **Brain** → Brain abscess ➲ encephalitis (fatal).

→ **Skin** → **Cutaneous amoebiasis (Amoebiasis cutis) due to either extension of acute amoebic colitis to the perianal region or through rupture on the abdominal wall from hepatic, colonic or appendicular lesions.**

It could also go genital tract.

It could also extend to the skin, especially If the patient undergoes abdominal surgery in colonic flexure regions, Cutaneous amoebiasis (Amoebiasis cutis) can occur at this places.
Perianal region is the most common affected areas of Cutaneous amoebiasis.

Laboratory diagnosis

I)Intestinal amoebiasis

CFT: complement fixation test
IHAT : indirect hemagglutination assay
IFAT : indirect fluorescent antibody test

a) Direct

•**Macroscopic: Offensive loose stool mixed with mucus and blood.**
The presence of fecal leukocytes is amoebic dysentery not bacillary dysentery

•**Microscopic:**
1-Stool examination: Reveals either trophozoites (in loose stool) or cysts (in formed stool) by direct smear, iodine stained & culture.

- **2-Sigmoidoscopy:To see the ulcer or the trophozoites in aspirate or biopsy of the ulcer. Because of Entamoeba histolytica**

3-X-ray after barium enema: to see the ulcer, deformities or stricture.

b) Indirect

-Serological tests: CFT, IHAT, IFAT, ELISA and GDPT (gel-diffusion precipitin test).
Detect either Antigen or Antibody
✍**N.B. These serological tests are positive only in invasive intestinal amoebiasis but negative in asymptomatic carriers.**(luminal amoebiasis)
Both are NEGATIVE in case of inactive amoebiasis or luminal amoebiasis or asymptomatic carrier or cyst passive.
Only POSITIVE in case of invasive acute amoebic dysentery

II)Extra- intestinal amoebiasis

According to the organ affected

a) Direct

1- X- ray:

In liver ➲ space occupying lesion.

In lung ➲pleuritis with elevation of the diaphragm

2- Ultrasonography, CT scan& MIR: For liver abscess.

3- Aspiration of abscess content: For liver abscess to detect trophozoites.

b) Indirect

1- Serological tests: As intestinal amoebiasis. They are positive and can persist for years.

The most 2 famous tests are for (parasite lactase dehydrogenase(PLDH) + GAL/GAL lectin antigen)

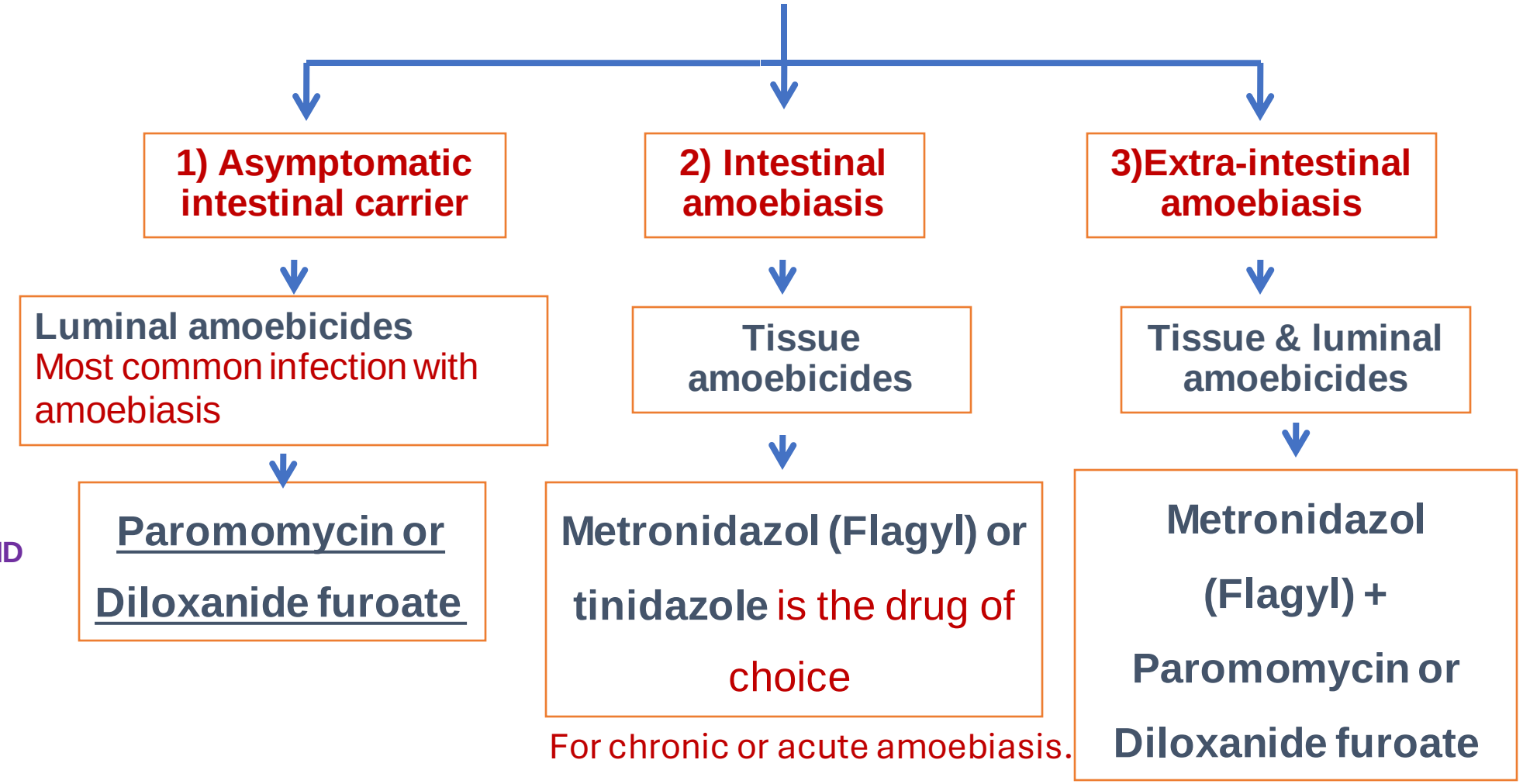
Positive in extra intestinal amoebiasis.

2- Molecular by PCR. For serum or stool to find out agents of Entamoeba histolytica

3- Blood examination: Leucocytosis.

4- Liver function tests: Increased in amoebic liver abscess.

Treatment


1) Asymptomatic intestinal carrier
2) Intestinal amoebiasis
3)Extra-intestinal amoebiasis
Luminal amoebicides
Most common infection with amoebiasis
Tissue amoebicides
Tissue & luminal amoebicides
DON'T RESOPOND to Flagyl
Paromomycin or
Diloxanide furoate
Metronidazol (Flagyl) or tinidazole is the drug of choice
For chronic or acute amoebiasis.
Metronidazol (Flagyl) + Paromomycin or Diloxanide furoate

- **Prevention:**

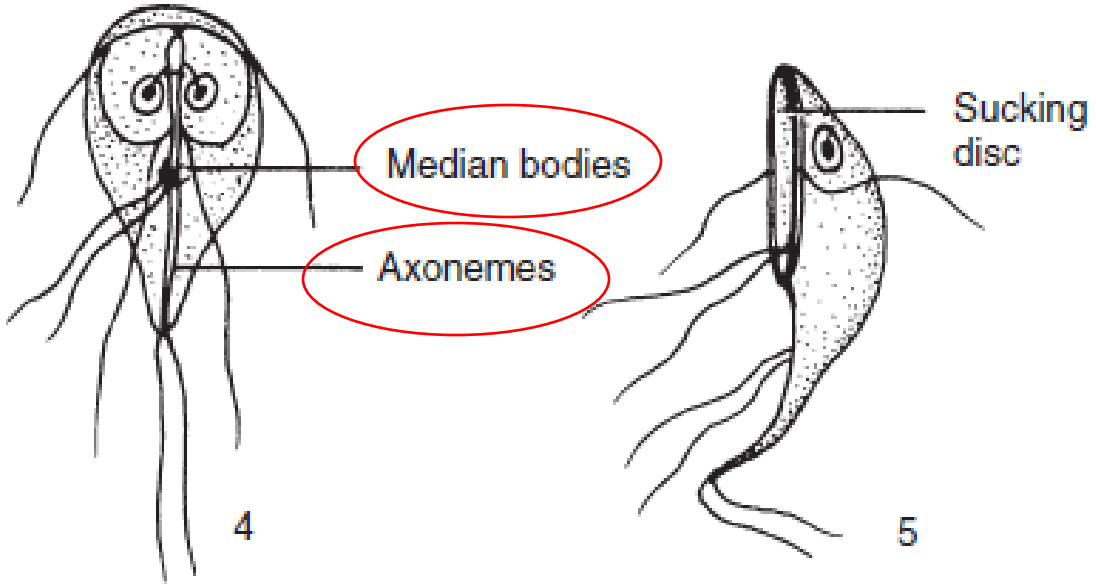
- Amoebic infection is prevented by eradicating fecal contamination of food and water
- Water is a prime source of infection and therefore the most contaminated foods are vegetables such as lettuce
- Amoebic cysts are not killed with low doses of chlorine or iodine, **so the best way to sterilize water is boiling as well as mechanical filtration.**
- Bringing water to a boil ensures the absence of amoeba

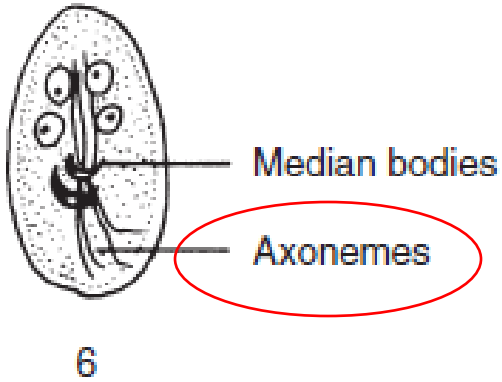
Giardia duodenalis

known nowadays as " Giardia intestinalis"

- Common cause of intestinal infection worldwide
- **Causes a disease known as " giardiasis" and "beaver fever" in Canada.**
- Flagellated
- Both the trophozoite and the cyst are included in the life cycle.(both are the diagnostic stage and the infective stage is only cysts)
- found most commonly in the crypts in the duodenum.
- Trophozoites are attached to the epithelium of the host villi by means of the **ventral disk.** **So no bloody diarrhea**
- Cyst formation takes place as the organisms move down through the jejunum after exposure to biliary secretions. **(infective stage).**
- **Usually associated with epidemics ,pandemics and outbreaks in refugee camps or at day care centers between children.**
- **Associated with poor sanitation and hygiene.**
- **In E.histolytica we said that there is invasion in mucosa and submucosa , here there is only attachment on brush boarder of enterocytes so there is no bloody diarrhea , in symptomatic infection (minority) it is characterized by profuse diarrhea which is foul smelling and floating watery greasy stools**

it has 2 Morphological stages : Trophozoites and cyst





1-Trophozoite

Looking like it's wearing glasses and having whiskers!

It has:

- **2 nuclei**
- **Parabasal bodies (ventral disc)uses them in attachment on brush boarder of enterocytes of small intestine**
- **4 pairs of flagella.**

2-cyst (oval)

It has :

- **4 nulei (quadrinucleated cyst)**
- **axonemes(source of flagella)**

Epidemiology

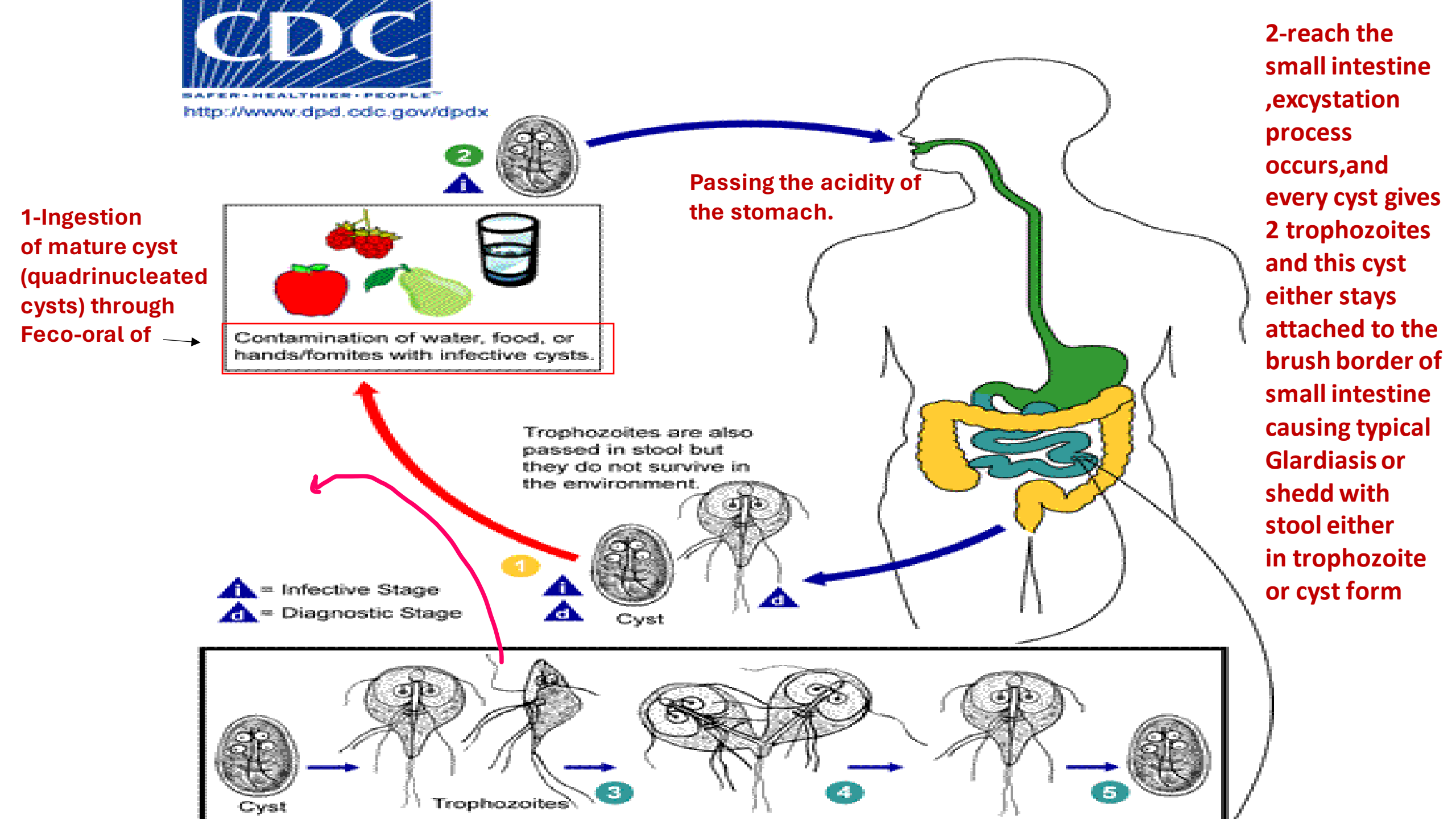
- Transmission of *G. lamblia* occurs by ingestion of viable cysts by fecal oral route. Highly person to person transmission unlike E.histolytica.
- high incidence of giardiasis occurs in patients with immunodeficiency syndromes and immunoglobulin A deficiency.
- The incubation period ranges from approximately 1-2 weeks and infectious dose is 10.(low dose of infection to establish the disease)
- **One of the main causes of diarrhea and mental retardation in children.**

clinically

majority

- Asymptomatic Infection (treatment not recommended)
- Symptomatic:

- Diarrhea usually watery: profuse watery diarrhea that later becomes greasy foul smelling and may float (steatorrhea)
- Abdominal cramps, bloating, malaise, weight loss,
- Malabsorption **because of taking up the surface area of brush borders so the reabsorption decreases.(espically of fat, disaccharides and protein)** and weight loss.(may cause chronic diarrhea-->mental retardation)
- Vomiting and tenesmus are not common


http://www.dpd.cdc.gov/dpdx
2
i
1-Ingestion of mature cyst (quadrinucleated cysts) through Feco-oral of
Contamination of water, food, or hands/fomites with infective cysts.
Passing the acidity of the stomach.
2-reach the small intestine ,excystation process occurs,and every cyst gives 2 trophozoites and this cyst either stays attached to the brush border of small intestine causing typical Glardiasis or shedd with stool either in trophozoite or cyst form
Trophozoites are also passed in stool but they do not survive in the environment.
1
i = Infective Stage
d = Diagnostic Stage
Cyst
Cyst
Trophozoites
3
4
5

Lab Diagnosis

- **Routine Methods:** **(microscopy)**
  - Stool analysis: cysts and sometimes trophozoites
- **Antigen Detection:**

  - Sensitive and specific in detecting *G. lamblia* in fecal specimens.
- **Another method is Entero test (string test) which contains a gelatin capsule attached to a string**

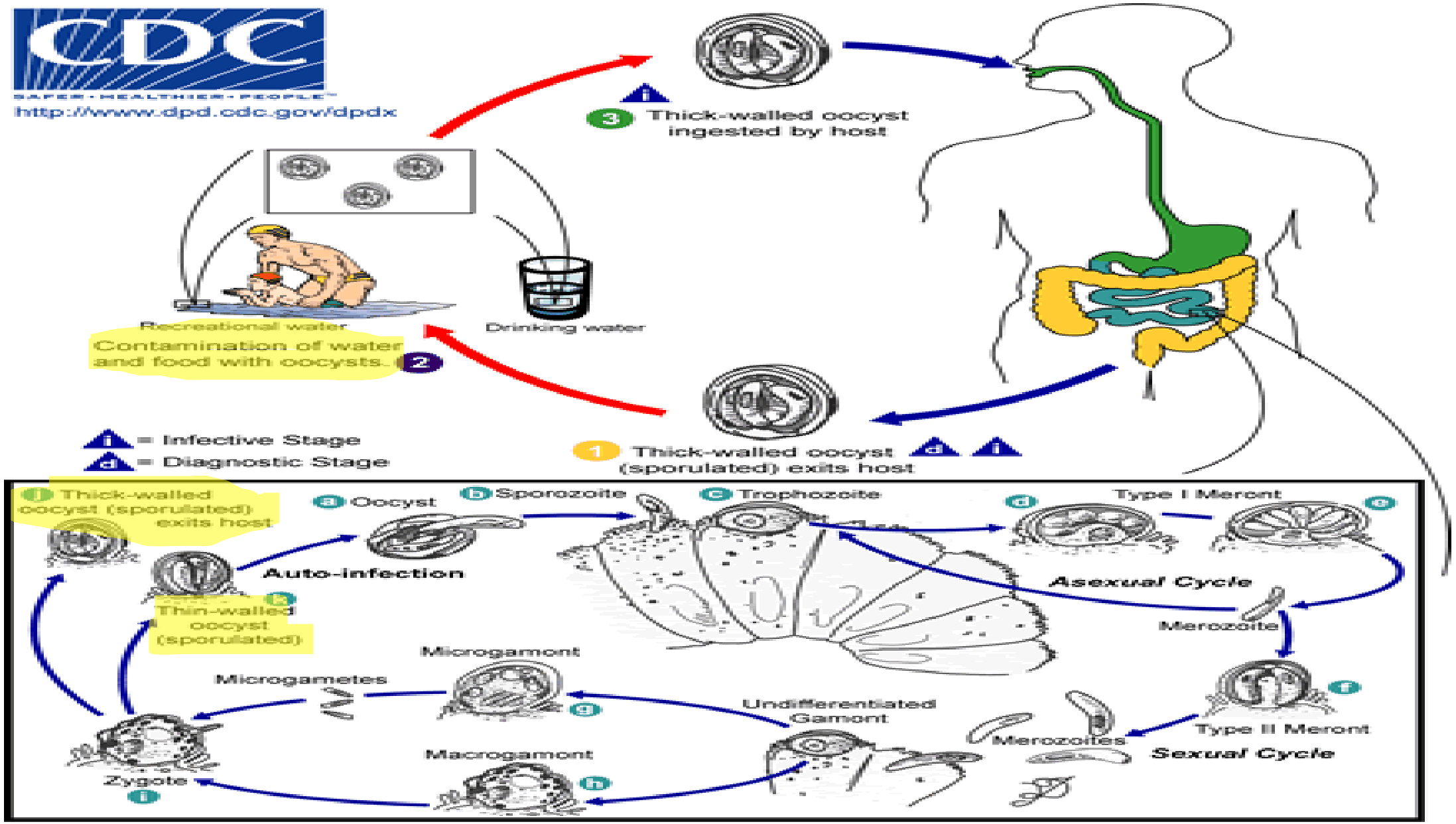
  The patient swallow it and after several hours the doctor pulls it up by the string and test the duodenal content of the capsule.

Treatment: Metronidazole or tinidazole

Cryptosporidium spp.

Remember, the fourth category of protozoa is sporozoa which includes the causative agent of malaria and intestinal sporozoa (Coccidia) which includes Cryptosporidium (includes two species: C.hominis which infects humans and C.parvum infects humans and animals.

- Intracellular enteric parasites that infect epithelial cells of the stomach, intestine, and biliary ducts.
- *C. parvum* (mammals, including humans) and *C. hominis* (primarily humans).
- infections begin with ingestion of viable oocysts, each oocyst releases four sporozoites, which invade the epithelial cells and develop into merozoites then oocyst.
- Prevalence of fecal oocyst 3-10%
- **They can switch between sexual and asexual production.**
- **They are intracellular extra-cytoplasmic**


http://www.dpd.cdc.gov/dpdx
3 Thick-walled oocyst ingested by host
Recreational water
Drinking water
Contamination of water and food with oocysts. 2
i = Infective Stage
d = Diagnostic Stage
1 Thick-walled oocyst (sporulated) exits host
Thick-walled oocyst (sporulated) exits host
a Oocyst
b Sporozoite
c Trophozoite
d Type I Meront
e
Auto-infection
Thin-walled oocyst (sporulated)
Asexual Cycle
Merozoite
Microgamont
Microgametes
g
Undifferentiated Gamont
f
Type II Meront
Merozoites
Sexual Cycle
Macrogamont
h
Zygote
i

The diagnostic stage and infective stage are thick wall oocysts.

Oocysts was given this name due to sexual multiplication in their life cycle.

الي بتطلع برا الجسم --------> Thick wall oocysts
جوا الجسم الي بتضل-------> Thin wall oocysts (continue the life cycle of cryptosporidiosis without the need to leave the host

Life cycle starts by
1-ingestion of contaminated food and water (mainly water pools when you swim if you swallow water, it may contain cryptosporidiosis.
2-after it reaches the small intestine ,sexual and asexual multiplication occurs.

Sexual multiplication:(Gametogony), give type 2 meront which are converted to macro and microgametocytes , they will form the zygote and this zygote will form the thick wall oocysts (that will go out with the stool and continue the transmission outside the host).

Asexual multiplication:(schizogony) , the daughter cells are called merozoites(meront) they go asexual multiplication and give type 1 meront which will convert to thin wall oocyst (continue life cycle without the need to leave the host).

- **Clinically:**

  Majority of patient are asymptomatic

➢ Copious Diarrhea watery diarrhea (cholera like diarrhea): These patients may have 3-17 liters of stool per day Especially in immunocompromised individuals (HIV patients)

➢ Abdominal pain and vomiting

- **Diagnosis:** oocyst in stool using modified acid fast stain as MTB
- **Treatment:**

➢ Usually self limited with Oral or intravenous rehydration.

➢ Nitazoxanide is used for immunocompromised individuals e.g HIV patients.

Immunocompetent usually result in self limited mild gastroenteritis

V2 : string not sting

SLIDE 31

The End