MICROBIOLOGY

بسم الله الرحمن الرحيم

Final | Lecture 4

Protozoal Infections of the GIT

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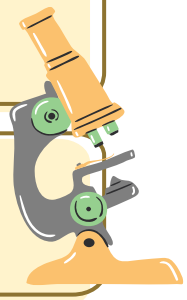
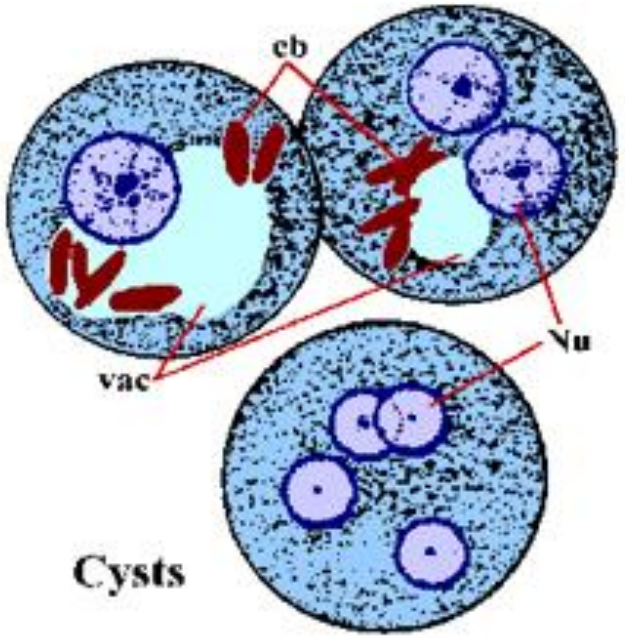


﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

اللهم استعملنا ولا تستبدلنا

Click on the infective stage of *E. histolytica*
(this is from last semester)



Common Protozoal Infections of the GI Tract

Done By: Nader Alaridah MD, PhD

Important notes from general microbiology

- Parasitic kingdom include three phyla, protozoa, helminths and arthropods.

This lecture *Next lecture*

- Protozoa (our scope in this lecture) is a phylum consisting of **unicellular** parasites that are divided into 4 classes according to their locomotion and the presence sexual reproduction in their life cycle.
- Protozoa classes are:
  - ✓ **Rhizopoda**, move by **pseudopodia (crawling motion);** replicate **asexually**.
  - ✓ **Flagellates**, move by **flagella;** replicate **asexually**.
  - ✓ **Ciliates**, move by **cilia;** replicate **asexually**.
  - ✓ **Sporozoa**, move by **gliding** as they don't possess an organ of locomotion. They are the only class that replicate **sexually (they can also reproduce asexually),** and they are **obligate intracellular pathogens**.

Protozoa of the GI tract

❖ *Entamoeba histolytica*

- ✓ The causative agent of **amoebiasis**.
- ✓ It belongs to **Rhizopoda class**; they multiply **asexually** by binary division.

❖ Giardia Lamblia

- ✓ The causative agent of **giardiasis.**
- ✓ It belongs to **flagellates class**; they multiply **asexually** by binary division.

❖ Cryptosporidium

- ✓ The causative agent of **cryptosporidiosis.**
- ✓ It belongs to **Sporozoa class and coccidia subclass**, they multiply **sexually**.

All the diseases that are caused by these protozoa share the fecal-oral route of disease transmission by ingestion of the infective stage.

Entamoeba histolytica

❖ **Geographical distribution: Worldwide especially in the temperate zone and more common in areas with poor sanitary conditions.**

✓ Common **protozoal diseases** show **more prevalence** in **developing** countries, as lower standards of sanitation and hygiene there contribute to more spread of infections.

❖ **Habitat: Large intestine (caecum, colonic flexures and sigmoidorectal region).**

✓ **They prefer** to stay at these sites that show **low peristaltic movements** and **fecal stasis**, **ulcers** caused by amoeba are **commonly found there**.

❖ **Definitive host (DH): Man**

❖ **Reservoir host (RH): Man, Dogs, pigs, rats and monkeys.**

✓ **Man** is also the **main reservoir host,** as **90%** of infected people are **asymptomatic carriers** (also called **cyst passers**). These patients don't develop symptoms, as *Entamoeba histolytica* colonize their large intestine lumen without invading the mucosa and the submucosa, carriers with poor personal hygiene working as a food handlers could cause outbreaks of amoebiasis.

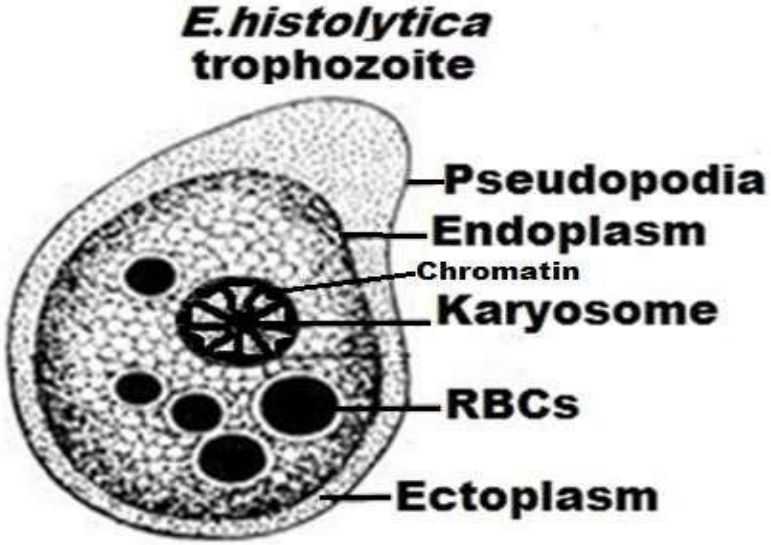
❖ **Disease: Amoebiasis or amoebic dysentery ⇔ الزحار الأميبي**

✓ Compared with bacterial infections, protozoal infections are insidious and progress slowly, while bacterial infections are rapid and acute. Protozoa are not known for their toxic-related pathogenesis unlike the toxicity associated with bacterial endotoxins.

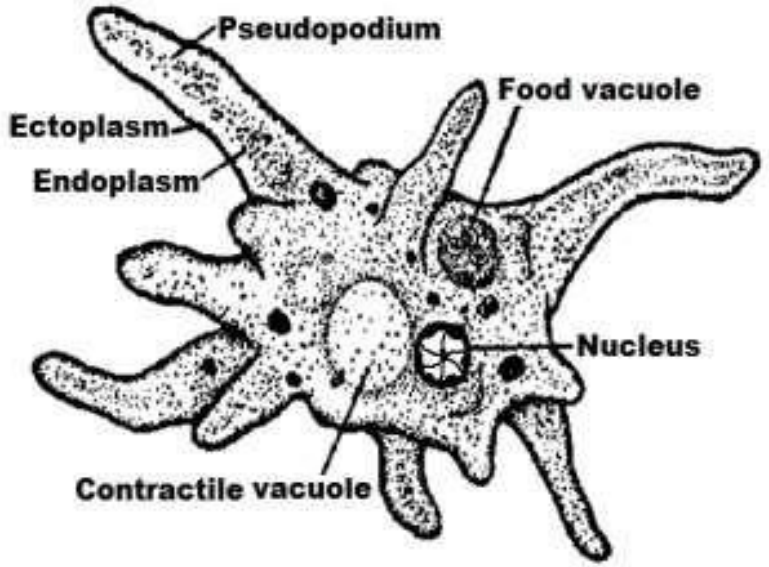
Morphological characters

1.Trophozoite stage (vegetative form or tissue form):

- ✓ It is the active, motile, and feeding form.
- ✓ ***Entamoeba histolytica*** has an ectoplasm and a granular endoplasm. They both contribute to the formation of pseudopods for crawling. The endoplasm contains a central nucleus that has a central nucleolus (or karyosome) surrounded by a ring of fine chromatin; this nucleus morphology is called **cartwheel appearance**.



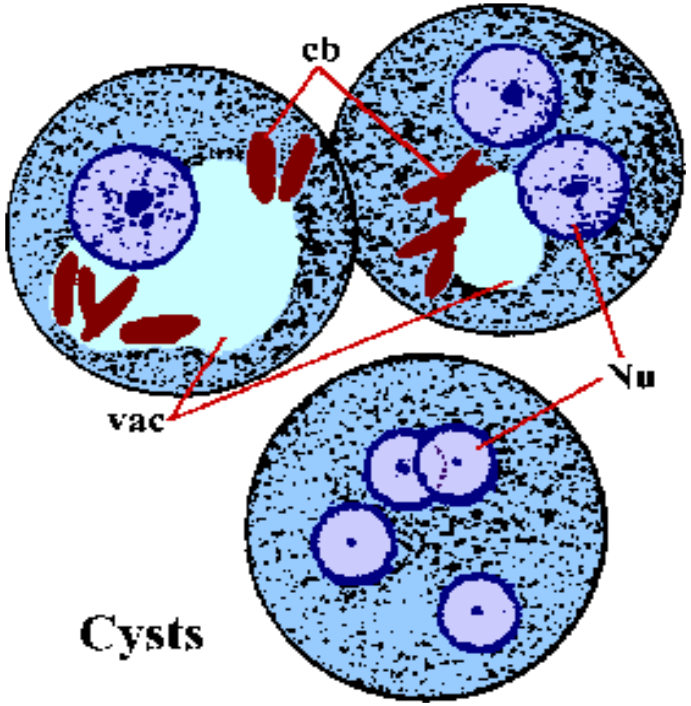
- ✓ The endoplasm also shows **glycogen granules** and **engulfed RBCs**; the presence of RBCs inside the endoplasm is a **pathognomonic** sign of ***Entamoeba histolytica*** trophozoites, as this distinguishes them from other **commensal species** that are part of the microbiome of the gut such as ***Entamoeba coli, Entamoeba dispar and Entamoeba moshkovskii***, which lack the ability to phagocytose RBCs.



- ✓ They are normally found rounded as in the first picture; the second picture draws pseudopodia as an illustration of their motility.

2. Cyst stage (Luminal form):

- ✓ The **Cyst stage** represents the quiescent phase of the protozoal life cycle. During this stage, protozoa encyst themselves to survive the harsh conditions. These cysts may be either:



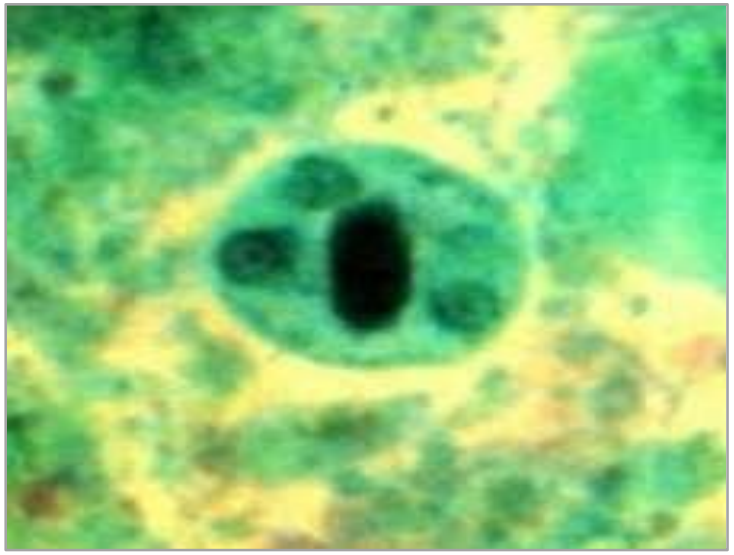
A) Immature cyst:

- ✓ **Immature cysts** are either **uninucleate** or **binucleated**, whereas **mature** cysts are **quadrinucleated**, containing four nuclei per cyst.
- ✓ Exposure to immature cysts does not result in infection, as they lack the ability to establish a new infection in the host.

B) Mature cyst (quadrinucleate cyst).

- ✓ The mature cyst is the only form that can continue the cycle of infection. This **quadrinucleate cyst** must undergo **two successive mitotic divisions** to produce **eight trophozoites.**

- ✓ The **nucleus** shows a **cigar-shaped** morphology; it has a **central karyosome** with **chromatoid bars** (or chromatoid bodies).

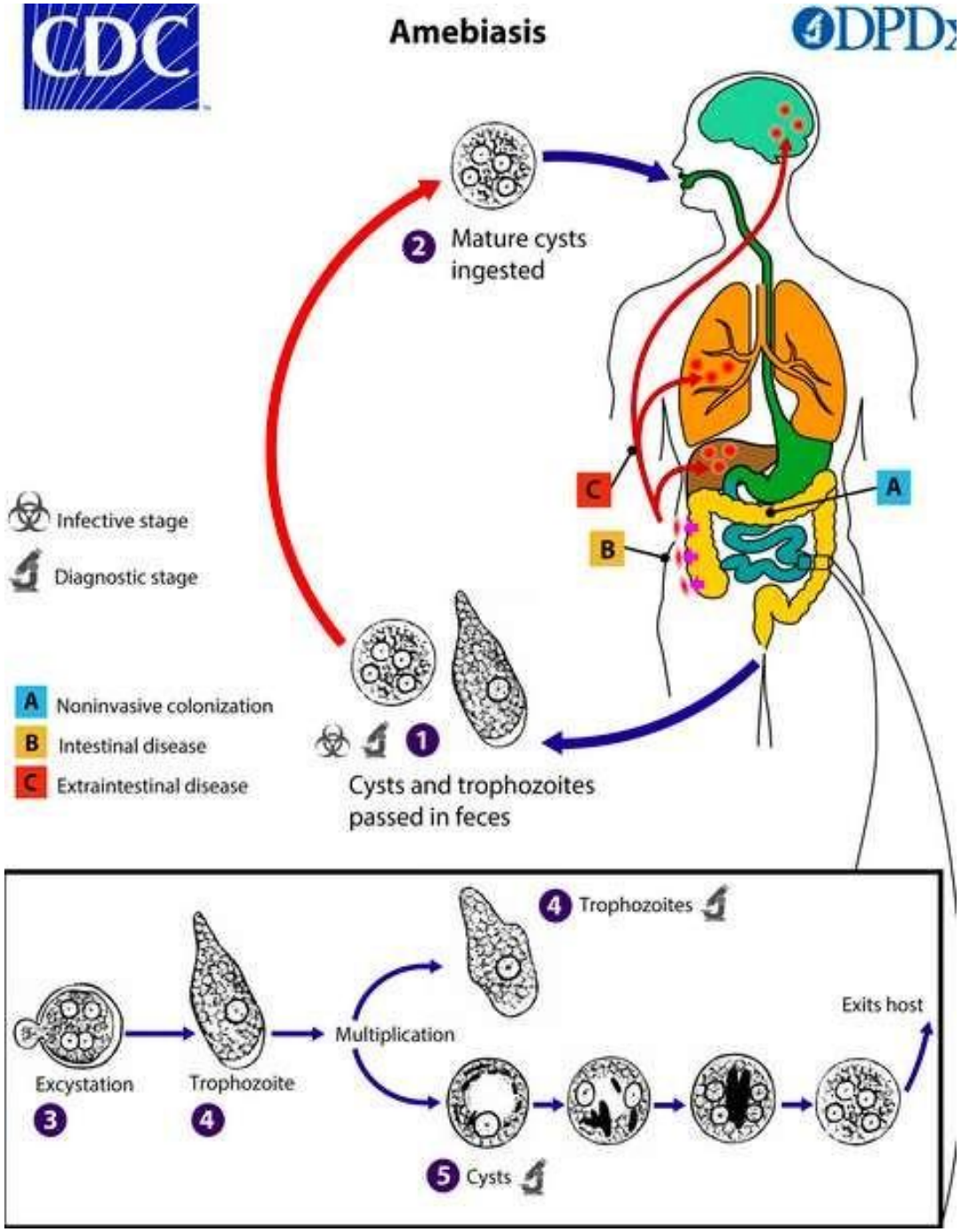


Life cycle of *Entamoeba histolytica*

- ✓ The **INFECTIVE stage** of ***Entamoeba histolytica*** is the mature quadrinucleate cyst. After ingesting these cysts by contaminated food or water, they travel through the GI tract surviving the acidity of the stomach until they reach the small intestine, where each cyst excysts in the small intestine, releasing **eight trophozoites.**

- ✓ After excystation, the **trophozoites** may remain **confined to the lumen** of the large intestine and re-encyst, a condition known of **asymptomatic carriers** or **cyst passers** (this is the case in 90% of infected people).
- ✓ **The DIAGNOSTIC stage** could be the **mature** or the **immature cyst** as well as the trophozoites (as patients with acute amoebic dysentery have frequent bowel movements; these trophozoites didn't have the time to encyst themselves).

- ✓ **Trophozoites** may **invade** the mucosa and submucosa of the intestinal tract, causing **acute** or **chronic amoebic dysentery**. In severe cases, complete penetration of all the layers of the large intestine and the blood vessels there will lead to perforation and haemorrhage, this can progress to extra-intestinal amoebiasis by direct or hematogenous spread, typically starting with the **right lobe** of the **liver,** as it is close to the right colonic flexure**,** and potentially spreading to the **lungs**, **brain**, or the **skin area above the large intestine.**



Modes of infection

1. Contaminated water and food (e.g. green vegetables) or drinks or hands with human stool containing mature cysts.
2. Handling food by infected food handlers as cookers and waiters.
3. Flies and cockroaches that carry the cysts from feces to exposed food.
4. Autoinfection (faeco-oral or hand to mouth infection).

- ✓ Occurs when a person contaminates their own hands or surroundings with feces containing infective cysts and then ingests them through hand-to-mouth contact. In this case, it is external autoinfection, which is different from **internal autoinfection** that will be discussed with ***cryptosporidium*** infections.

5. Homosexual transmission.

- ✓ Can also occur during **anal-oral sexual practices**, especially in men who have sex with men (MSM).

CLINICAL PICTURES OF INTESTINAL AMOEBIASIS

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

2. **Symptomatic infection**

   a) Acute amoebic dysentery
   Presented with fever, abdominal pain, tenderness, tenesmus and frequent motions of loose stool containing **mucus, blood, cysts and trophozoites**.

   b) Chronic infection

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

3 - Complications

- **Hemorrhage** due to erosion of large blood vessels.
- **Intestinal perforation ➪ peritonitis.**
- **Appendicitis.**

✓ If located near the ileocecal junction.

- **Amoeboma (Amoebic granuloma)** around the ulcer ➪ stricture of affected area.

✓ It should be distinguished from intestinal tumors.

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

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.

EXTRA-INTESTINAL AMOEBIASIS

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

A) Liver

- ✓ Most common site
- ✓ Amoebic liver abscess or diffuse amoebic hepatitis.
- ✓ The contents of the abscess is described as "anchovy paste" colored golden-brown.
- ✓ Affect commonly right lobe either due to spread via portal vein or extension from perforating ulcer in right colonic flexure or hematogenously.
- ✓ Clinical picture:
  - ✓ Fever
  - ✓ Hepatomegaly
  - ✓ Right hypochondriac pain
  - ✓ Jaundice

B) Lungs

- ✓ Lung abscess ➔ pneumonitis
  - ✓ 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.

C) Brain

- ✓ Brain abscess

➔ meningoencephalitis (fatal).

D) 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.

E) Urogenital Amebiasis

LABORATORY DIAGNOSIS
I) Intestinal Amoebiasis

A) Direct

Macroscopic:

- ✓ Offensive loose stool mixed with mucus and blood.

Microscopic:

- ✓ **Stool examination:** Reveals either **trophozoites (in loose stool) or cysts (in formed stool)** by direct smear, iodine stained & culture.
- ✓ RBCs and WBCs can also be seen by microscopy.
- ✓ **Sigmoidoscopy:** To see the ulcer or the trophozoites in aspirate or biopsy of the ulcer.
- ✓ **X-ray after barium enema:** to see the ulcer, deformities or stricture.

B) Indirect

Serological tests:

- ✓ **CFT** (Complement Fixation Test)
- ✓ **IHAT** (Indirect Hemagglutination Test)
- ✓ **IFAT** (Indirect Fluorescent Antibody Test)
- ✓ **ELISA** (Enzyme-Linked Immunosorbent Assay)
  - ✓ **Such as ELISA for PLDH2 (parasite lactate dehydrogenase 2), which is only present in *E. histolytica* (differentiates it from other commensal species).**
- ✓ **GDPT** (gel-diffusion precipitin test)

N.B. These serological tests are positive only in invasive intestinal amoebiasis (dysentery) but negative in asymptomatic carriers (most common).

LABORATORY DIAGNOSIS
II) Extra-Intestinal Amoebiasis

Depends on the organ involved

A) Direct

✓ **X-ray:**

In liver → space occupying lesion.

In lung → pleuritis with elevation of the diaphragm.

✓ **Ultrasonography, CT scan & MRI:**

For liver abscess.

✓ **Aspiration of abscess content:**

For liver abscess to detect trophozoites.

Hepatomegaly can also be detected

The doctor did not focus on the difference between the techniques above; he only emphasized on that space-occupying lesions can be detected.

B) Indirect

✓ **Serological tests:**

As intestinal amoebiasis.

They are positive and can persist for years.

✓ **Molecular by PCR.**

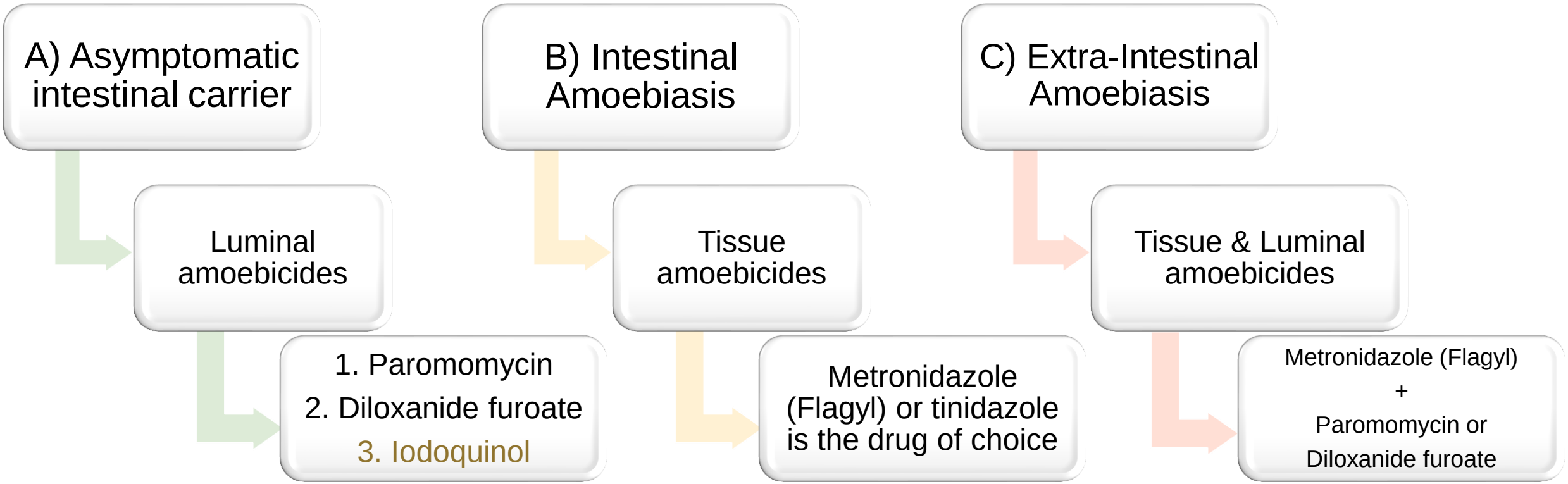
✓ **Blood examination:**

Leucocytosis.

✓ **Liver function tests:**

Increased in amoebic liver abscess.

Treatment



Flagyl cannot be used for this group because it doesn't reach cysticidal levels in the lumen. In other words, it cannot kill the cysts in the lumen of the gut of those cyst-passers.

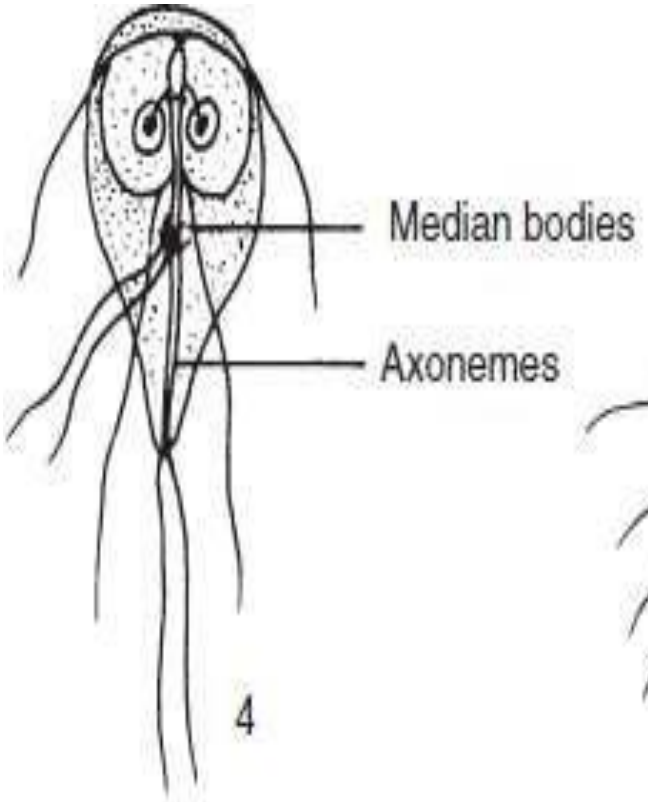
Prevention

- ✓ 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.
- ✓ *E. histolytica* cysts can be viable in the environment for at least 3 weeks.
- ✓ Amoebic cysts are not killed with low doses of chlorine or iodine, and if the concentration is increased to a cysticidal level, it will not be safe for human use.
- ✓ Bringing water to a boil ensures the absence of amoeba (also works with *Giardia* and *Cryptosporidia*); filtration also can be used.

Giardia duodenalis & Giardia lamblia

Pathogenesis is in the small intestine (duodenum and jejunum), unlike *E. histolytica* (large intestine).

- ✓ Common cause of intestinal infection worldwide.
- ✓ Giardiasis in Canada is also known as beaver fever (misleading name).
- ✓ Flagellated and reproduce by binary fission.
- ✓ Both the trophozoite and the cyst are included in the life cycle.
- ✓ 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.
- ✓ Cyst formation takes place as the organisms move down through the jejunum after exposure to biliary secretions.
- ✓ It doesn't invade (no bloody diarrhea), but instead it can cause steatorrhea, yet most cases are either asymptomatic or mild.
- ✓ Part of the differential diagnoses of both infantile (EPEC) and traveler's (ETEC) diarrheae.



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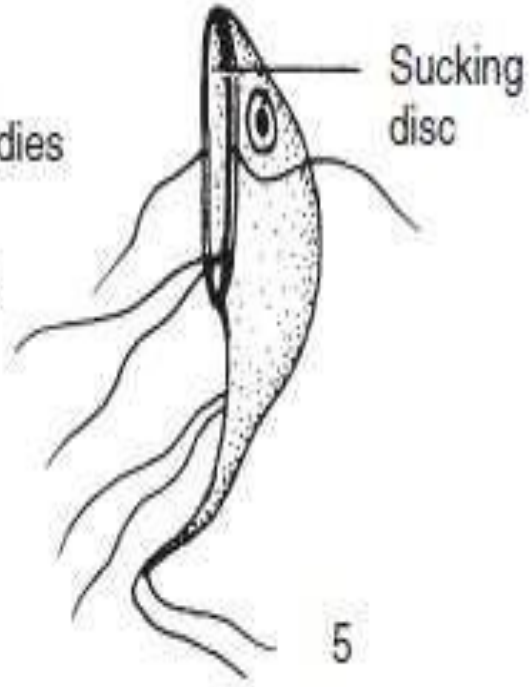

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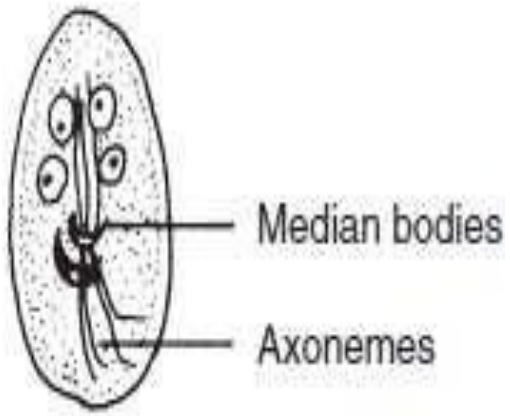

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The quadrinucleated cyst is the infective stage just like *E. histolytica*.

Important features of the trophozoite:

- ✓ Pair of nuclei.
- ✓ Ventral (sucking) discs → used for **attachment**.
- ✓ Axoneme (axostyle) → causes the **falling leaf** pathognomonic motility under microscopy.
- ✓ Heart-shaped.
- ✓ 4 pairs of flagellae.

Under the microscope, the trophozoite's paired nuclei resemble eyes, contributing to its 'face-like' appearance.

Epidemiology

- ✓ Transmission of *G. lamblia* occurs by ingestion of viable cysts by fecal oral route.
- ✓ It is more associated with contaminated **water** sources.
- ✓ Commonly involved in clustering of cases, such as in daycare centers, mental institutions, or in family members.
- ✓ High incidence of giardiasis occurs in patients with immunodeficiency syndromes, as well as cannabis smokers or people suffering from malnutrition or overcrowdedness.
- ✓ The incubation period ranges from approximately 1-2 weeks and infectious dose is 10.

Clinically

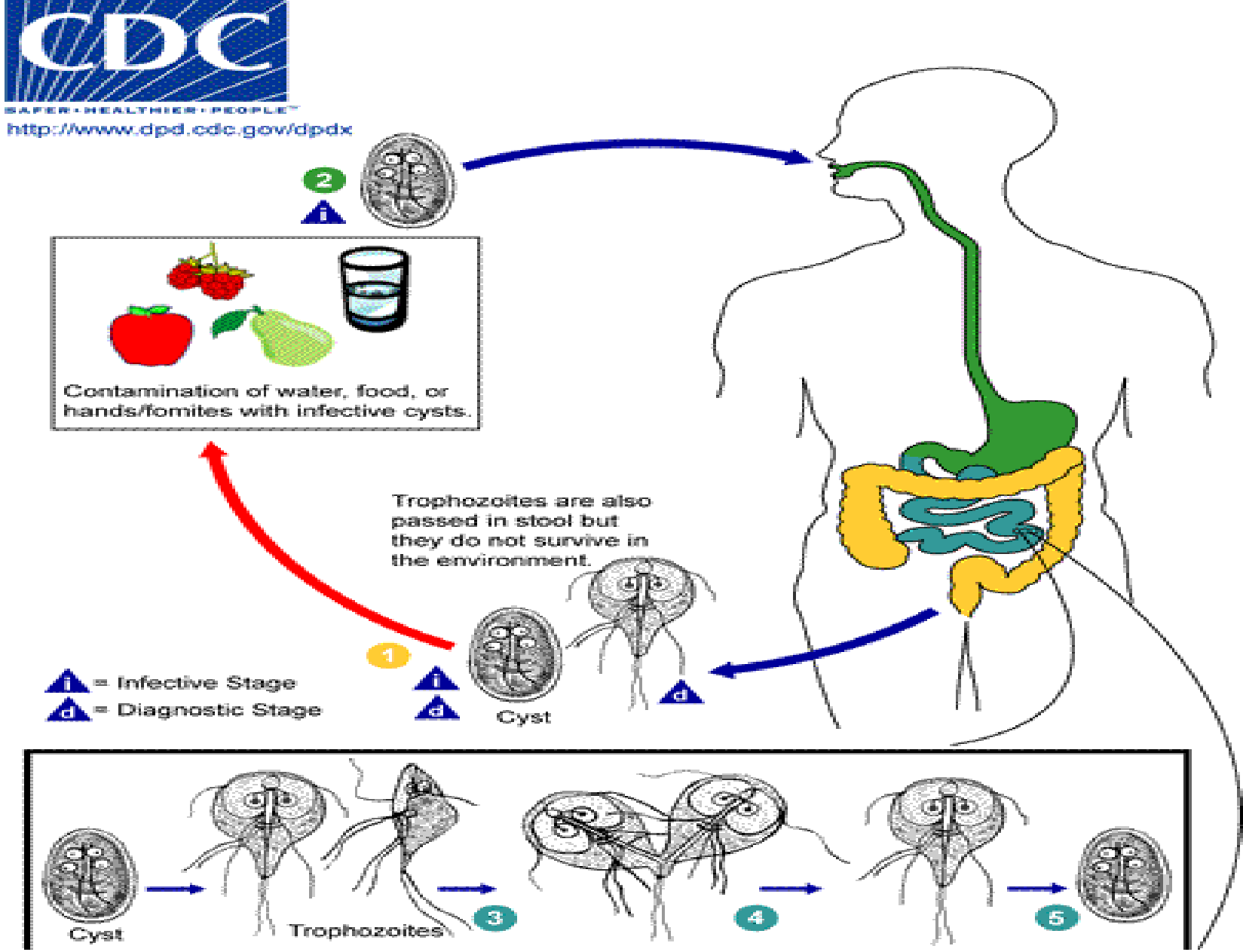
Pathogenesis is by attachment to duodenum or jejunum by their ventral discs, causing mild irritation, causing malabsorption of fat and proteins and some vitamins, such as vit A, vit B12 & folic acid.

- 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 and weight loss.
- Vomiting and tenesmus are not common.

Life Cycle:

- ✓ Ingestion of quadrinucleate cyst (infective Stage).
- ✓ Excystation is in the small intestine, forming **2 trophozoites** per cyst.
- ✓ It encysts when it reaches the large intestine.
- ✓ The diagnostic stage can be both cysts and trophozoites depending on the severity of the diarrhea, just like amoebic dysentery.



Lab Diagnosis

- **Routine Methods:**
  - Stool analysis: cysts and sometimes trophozoites.

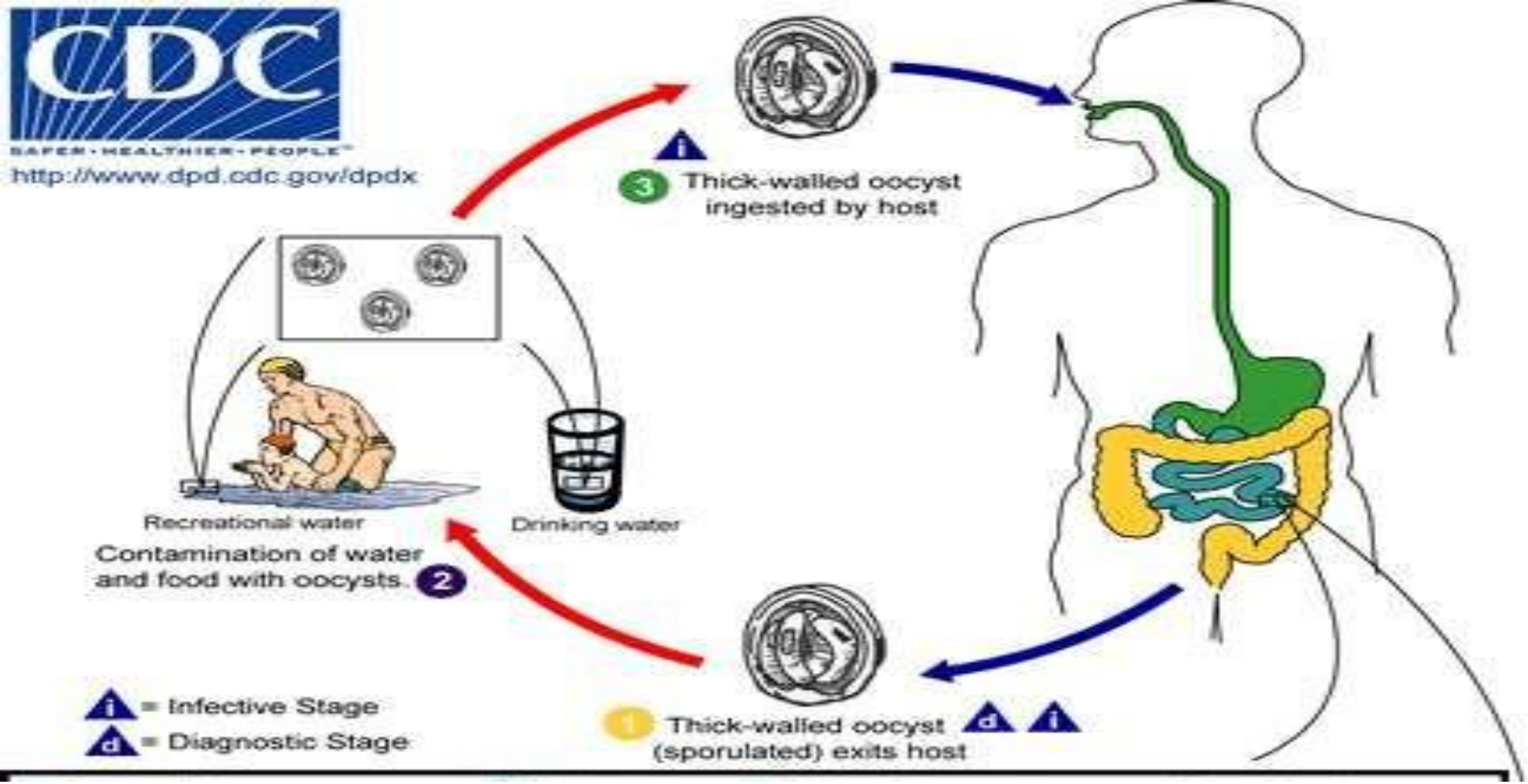
- **Antigen Detection:**
  - ✓ Immunochromatographic enzyme immunoassay for GSP65 – giardia-specific protein 65 (kilo-Dalton).
  - ✓ Sensitive and specific in detecting *G. lamblia* in fecal specimens.

- ✓ The string test (enterotest) can be used. The patient swallows a gelatinous capsule connected to a string that is attached in their mouth, and after the capsule dissolves after 4–5 hours, the string is retracted, and trophozoites can be detected if present.
- ✓ Serology can be used to screening and epidemiology.

Treatment:
Metronidazole or tinidazole.

Cryptosporidium spp.

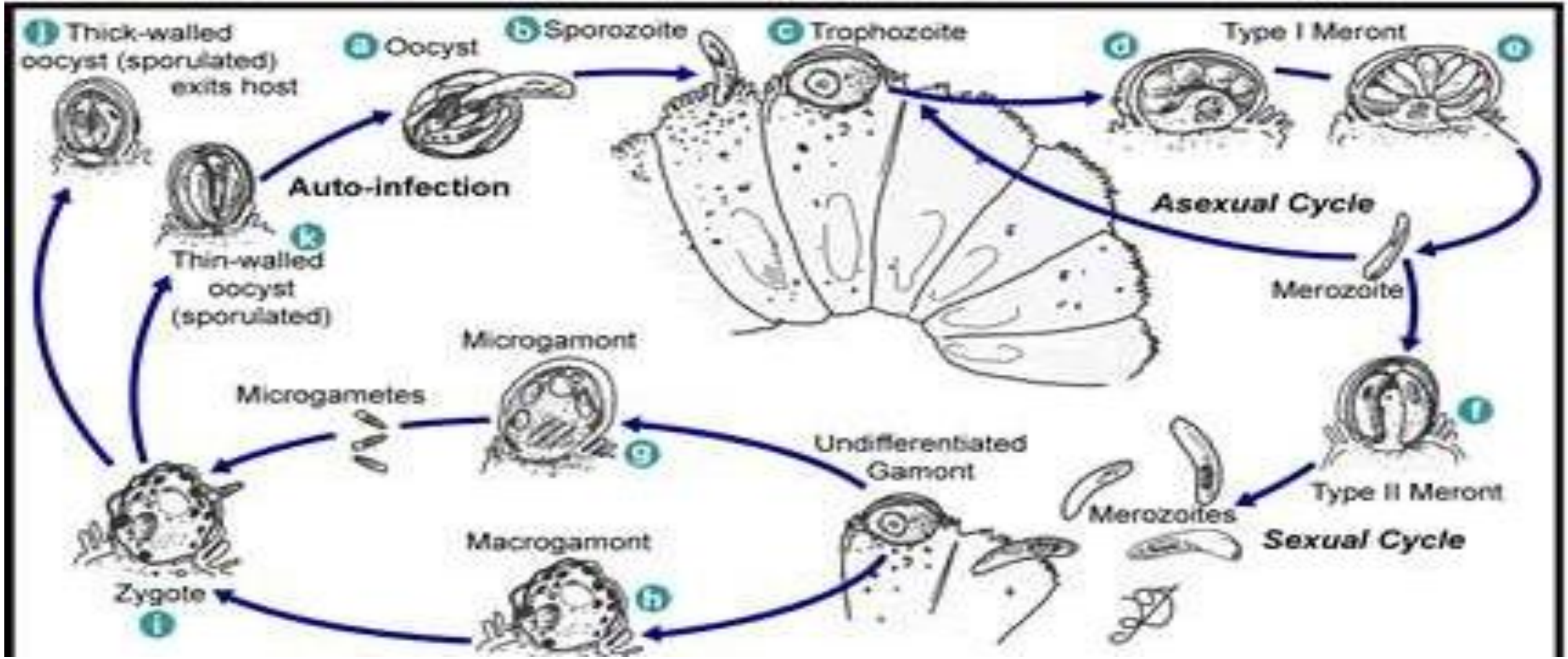
- Intracellular enteric parasites that infect epithelial cells of the stomach, intestine, and biliary ducts.
- *C. parvum* (mammals, including humans) and *C. hominis* (primarily humans).
- Infections begin with ingestion of viable oocysts, each oocyst releases four sporozoites, which invade the epithelial cells (crypts) of the small intestine and develop into merozoites then oocyst.
- Prevalence of fecal oocyst 3-10%



In addition to the transmission discussed in amoebiasis and giardiasis, cryptosporidiosis in known to contaminate recreational water sources.

In the **small intestines**, oocysts, inside which there are sporocyst, which in turn enclose sporoblasts, finally form merozoites (type I and type II meronts).

See next slide for **type I vs. type II.**



Internal autoinfection is well-known in Cryptosporidia. It results from **sexual** reproduction of **type II meronts**, forming **thin-walled oocysts**. On the other hand, **type I meronts asexually** form **thick-walled oocysts** which is the infective stage that can withstand **environmental conditions**.

Clinically:

➢ Copious Diarrhea in immunocompromised:

These patients may have 3-17 liters of stool per day

➢ Abdominal pain and vomiting

✓ **Cryptosporidiosis** is typically asymptomatic or with mild transient diarrhea in immunocompetent individuals, however in immunocompromised individuals, it can cause severe debilitating intractable diarrhea.

Diagnosis:

Oocyst in stool using modified acid-fast stain (also called Kinyoun technique).

- It is used for nocardia and the coccidia subfamily: cryptosporidium, cyclospora & isospora.
- The gold standard in the US is the direct fluorescence antibody test from a stool sample.

Treatment:

✓ Nitazoxanide and other antiparasitic agents can be tried for immunocompromised patients, but the efficacy is not well proven.

➢ Usually, self limited with Oral or intravenous rehydration.

➢ Nitazoxanide is used for immunocompetent individuals. HAART is vital for HIV patients.

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	15	on the space-occupying lesions can be detected.	on that space-occupying lesions can be detected.
	16	Metronidazole (Flagyl) is the drug of choice	Metronidazole (Flagyl) or tinidazole is the drug of choice
V1 → V2			

Additional Resources:

رسالة من الفريق العلمي:

Sheet 2 of last semester

وَقَضَيْنَآ إِلَىٰ بَنِىٓ إِسْرَٰٓءِيلَ فِى ٱلْكِتَٰبِ لَتُفْسِدُنَّ فِى ٱلْأَرْضِ
مَرَّتَيْنِ وَلَتَعْلُنَّ عُلُوًّا كَبِيرًا ﴿٤﴾ فَإِذَا جَآءَ وَعْدُ أُولَىٰهُمَا بَعَثْنَا
عَلَيْكُمْ عِبَادًا لَّنَآ أُو۟لِى بَأْسٍ شَدِيدٍ فَجَاسُوا۟ خِلَٰلَ ٱلدِّيَارِ ۚ
وَكَانَ وَعْدًا مَّفْعُولًا ﴿٥﴾ ثُمَّ رَدَدْنَا لَكُمُ ٱلْكَرَّةَ عَلَيْهِمْ
وَأَمْدَدْنَٰكُم بِأَمْوَٰلٍ وَبَنِينَ وَجَعَلْنَٰكُمْ أَكْثَرَ نَفِيرًا ﴿٦﴾
إِنْ أَحْسَنتُمْ أَحْسَنتُمْ لِأَنفُسِكُمْ ۖ وَإِنْ أَسَأْتُمْ فَلَهَا ۚ فَإِذَا جَآءَ
وَعْدُ ٱلْءَاخِرَةِ لِيَسُۥٓـُٔوا۟ وُجُوهَكُمْ وَلِيَدْخُلُوا۟ ٱلْمَسْجِدَ
كَمَا دَخَلُوهُ أَوَّلَ مَرَّةٍ وَلِيُتَبِّرُوا۟ مَا عَلَوْا۟ تَتْبِيرًا ﴿٧﴾