Diagnosis of Parasites

3rd stage/2nd Semester/2024-2025

Lec.6 Stool exam techniques for intestinal parasites (Part-III):

2-Intestinal Flagellates:

A flagellate is a cell or organism with one or more whip-like appendages called flagella. Intestinal flagellates include pathogenic *Giardia lamblia* and *Dientamoeba fragilis*, as well as multiple nonpathogenic species. *Giardia*, one of the causative agents of traveler's diarrhea, and *Dientamoeba* are transmitted via infected food or placement of contaminated fingers in the oral cavity. Some of the well non intestinal flagellates will illustrate bellow:

a-*Giardia lamblia:*

Since that *Giardia* trophozoite attached to intestinal mucosa via sucking disc, it is not easy to find the trophozoite in stool specimens. Thus 5-6 slides should be prepared from one specimen to improve presence or absence of giardiasis infection. On the other hands, to make shore that the patient get cure from giardiasis infection, the stool should be re-examined during three successive days after treatment.

Lab. Diagnosis:

1-Direct wet preparation: demonstrate **Giardia trophozoite in diarrheic stool** (watery stool), and **cyst stage in formed and semi formed stool**.

2-Permanent stained specimen is good for cyst and trophozoite **morphology**.

3-Intero-test capsule: useful to obtain duodenal specimens (inspirited materials), which give a high chance to find out *Giardia* trophozoite in a good numbers.

Method:

-Small weighted gelatinous capsule that bound to coiled thread is swallowed by the suspected patient. Free end of the coiled thread is attached to patient cheek.

-When swallowed, the capsule will pass from the stomach to duodenum. Left for two hours.
- Then the thread withdrawn, the capsule put in saline, shaken well, then the washing saline centrifuged.
-After centrifugation, the deposit examined for *Giadia* trophozoite.
-**Note**: entero-test is not recommended because it's caustic.
4-Duodenal biopsy: taken from intestinal mucosa, locking for *Giardia* trophozoite.

5-Serodiagnosis:

-**Antigen detection:** Techniques include ELISA, Immunochromatographic strip and indirect immunoflurescent techniques (IIF) using monoclonal antibody all have been developed to detect *Giardia lamblia* antigen in stool specimen.

Note: The presence of Giardia antigens in feces is an indication to **active giardiasis infection.**

-**Antibody detection**: ELISA &indirect immunoflourecsent techniques are used to detect antibodies against Giardia antigens.

Note: demonstration of **Giardia antibodies is useful for epidemiological and pathophysiological studies.**

-**Specific ELISA kit** was developed to detect giardiasis infection in stool.

-**Immunoflourescent technique** using monoclonal antibodies is **sensitive and specific** for detecting *Giardia lamblia* in stool.

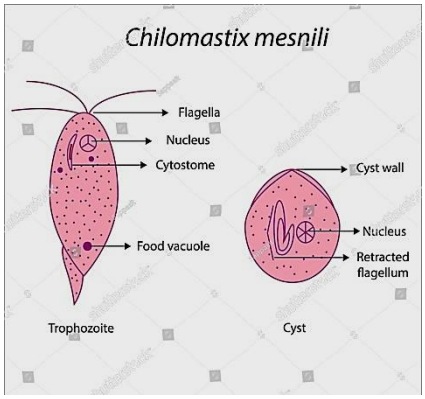
-**Reveres immune-electrophoreses &ELISA** are used together to **visualize and quantify** giardia antigens in stool. Fresh stool preferred to be us because some antigen may lose with time.

6-**PCR**: used to detect DNA of Giardia cysts in stool.

b-*Chilomastic mesnlli:*

Is a large intestine flagellated non-pathogenic organism (cecum and colon) that has cyst and trophozoite stages. The cyst is lemon to pear shape; contain one nucleus and one fiber. Trophozoite contains cytostome.

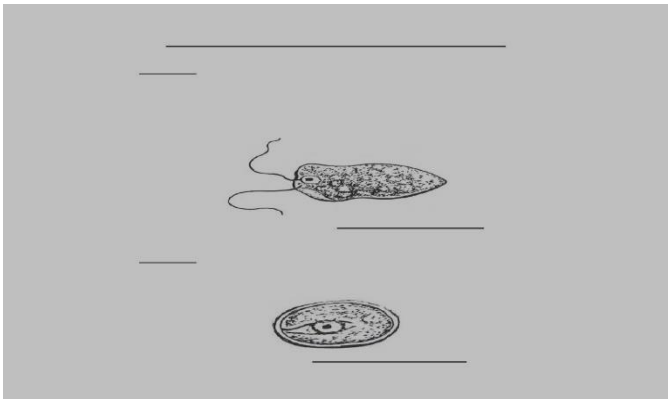
Lab. diagnosis: Permanent stained smear of cyst and trophozoite those are demonstrated in the semi-formed stool.



c-Retortamonus intestinalis:

Is a small flagellated parasite, that have cyst and trophozoite stages. Trophozoite is elongated, dimensions: 5-10X3-4 µm. It has pear shape. The cytoplasm is granulated and vacuolated, it's cleft-like cytoplasm. It is contain one nucleus that is spherical with central karyosome.

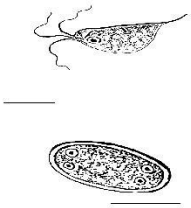
Lab. Diagnosis: direct wet stool preparation to differentiate cyst and trophozoite, Permanent stained method used to identify the species, in which iron hematoxylin stain is used.



e-Enteromonas hominis:

Nonpathogenic intestinal organism, that has cyst and trophozoite stages. Trophozoite: Pear shaped, has no cytostome, contain one nucleus and three anterior flagella and one posterior flagella which are usually not visible.

Lab. Diagnosis: Permanent stained smear, Iron-hematoxilen stain is performed to differentiate between cyst and trophozoite.



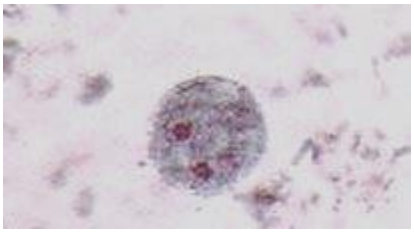
f-Dientamoeba fragilis:

This parasite is classified according to electron microscopy studies, as amoeba-flagellate, rather than amoeba. It is closely related to *Trichomonas* and *Histomonas* species. It has no cyst form.

Morphology: Trophozoite is 20-40 or 60-80 μm in diameter, has one nuclei, nuclear chromatin is fragmented to 3-5 granules, not contain peripheral chromatin on the nuclear membrane. Cytoplasm contain vacuoles, Mode of transmission is not known.

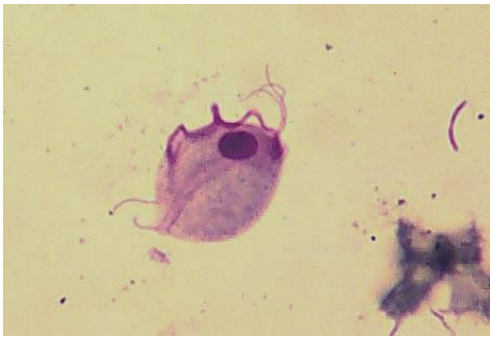
Lab. Diagnosis: wet stool preparation, replicate at least three times.

Permanent stained methods could be useful also.



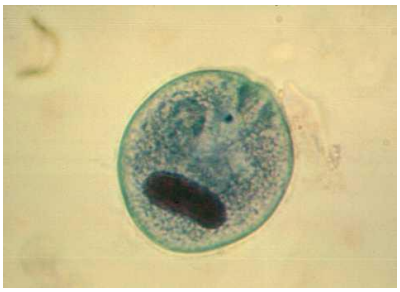
g-Trichumonas hominis:

This flagellated parasite has no cyst stage; there is only trophozoite stage during life cycle. The parasite inhabiting the end part of cecum. Laboratory diagnosis could be performed using direct wet stool preparation. Trophozoite of *T. hominis* could be identified from its distinct axostyle and its movement (twisty movement of flagella and undulating membrane).



3-Intestinal ciliates: (Balantidium coli):

Balantidium coli have trophozoite and cyst stages. Trophozoite has two nuclei, big (the vegetative nuclei) and small (the reproductive nuclei). *Balantidium* trophozoite may be **mistaken with helminthes egg**, especially when it`s cilia are not visible, because it is relatively has **a big size**.



Methods of lab. Diagnosis:

1-Routinly, cysts and trophozoites of *Balantidium coli* could be diagnosed using wet preparation method.

2-**Perminant stained** smear using acidic methyl green stain (specific stain), by which the parasite appear big in size and take a dark stain.

3-**Biopsy**: examining biopsy and materials scraped from the intestinal ulcer are useful methods when the routine diagnostic methods give a negative result. Both trophozoite and cysts could be observed microscopically in materials biopsied or scraped from *Balantidium* colonic ulcer.

4-Coccidian intestinal protozoa:

Coccidia (Coccidiasina) are a subclass of microscopic, spore-forming, single-celled obligate intracellular parasites belonging to the phylum: apicomplexan, class: Conoidasida. As obligate intracellular parasites, they must live and reproduce within an animal cell. Coccidian parasites infect the intestinal tracts of animals, and are the largest group of apicomplexan protozoa. Infection with these parasites is known as coccidiosis. Coccidia can infect all mammals, some birds, some fish, some reptiles, and some amphibians. Most species of coccidia are species-specific in their host. The following coccidian organisms are examples for human coccidian parasites:

a-Cryptosporidium parvum

Cryptosporidium parasite inhabiting lining epithelial cells of the intestinal villi. This parasite differs from other coccidian protozoa of worm blooded animals that it is not invade deep tissues of the host, but only the superficial epithelial cells of the intestinal villi and confined in cell cytoplasm and extra-cytoplasmic locations.

Each *Cryptosporidium* stage is present inside parasitophorous vacuole within human cell cytoplasm. These parasitophorous vacuoles located inside epithelial cells of the intestinal villi. Oocyst is the diagnostic and infective stage of the parasite.

Methods of lab. Diagnosis:

A-Direct wet stool preparation: this method reveals colorless, spherical oocyst, of 4-5μm diameter and different sized granules (large and small). It is worthy to know that *Cryptosporedium parvum* oocyst **difficult to be recognized by direct wet unstained preparation**.

B-Concentration technique: Sheather`s sugar floatation technique or zinc sulfate floatation are useful methods to demonstrate oocyte in stool specimen, especially if 3 replicate of direct wet stool preparation gave negative results for *C. parvum* .

C-Staining method:

-Modefied Ziehl-Nelson (ZN) staining is the method of choice, by which **oocyst of the parasite appear to have red acid-fast sphear, against a background.**

-Fluorescent staining with **auramine-phenol or acridine orange** is reported to be useful technique for ***C. parvum.***

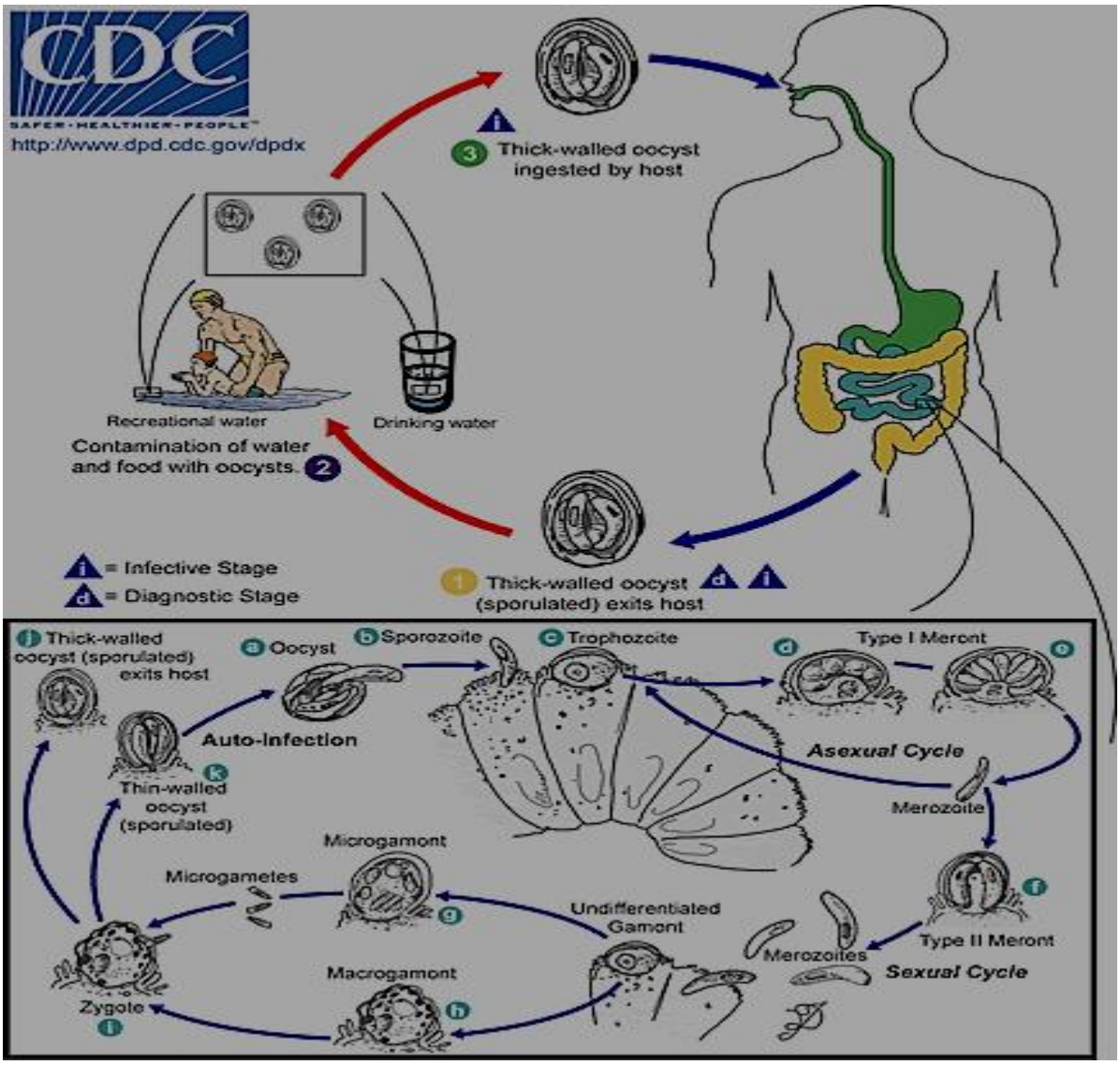
D-**Indirect Immunofluorescence microscopy** using monoclonal antibody is useful method to make **definitive identification of *C. parvum.***

E-**Histopathological examination**: Tissue section is made from biopsy of superficial intestinal mucosa (**especially that taken from jejunum**). The tissue section either prepared for light microscopy or electron microscopy examination.

F-**Serodiagnosis**: Not: *Cryptosporedium parvum* **specific antibody** could be detected within **two months of acute infection**.

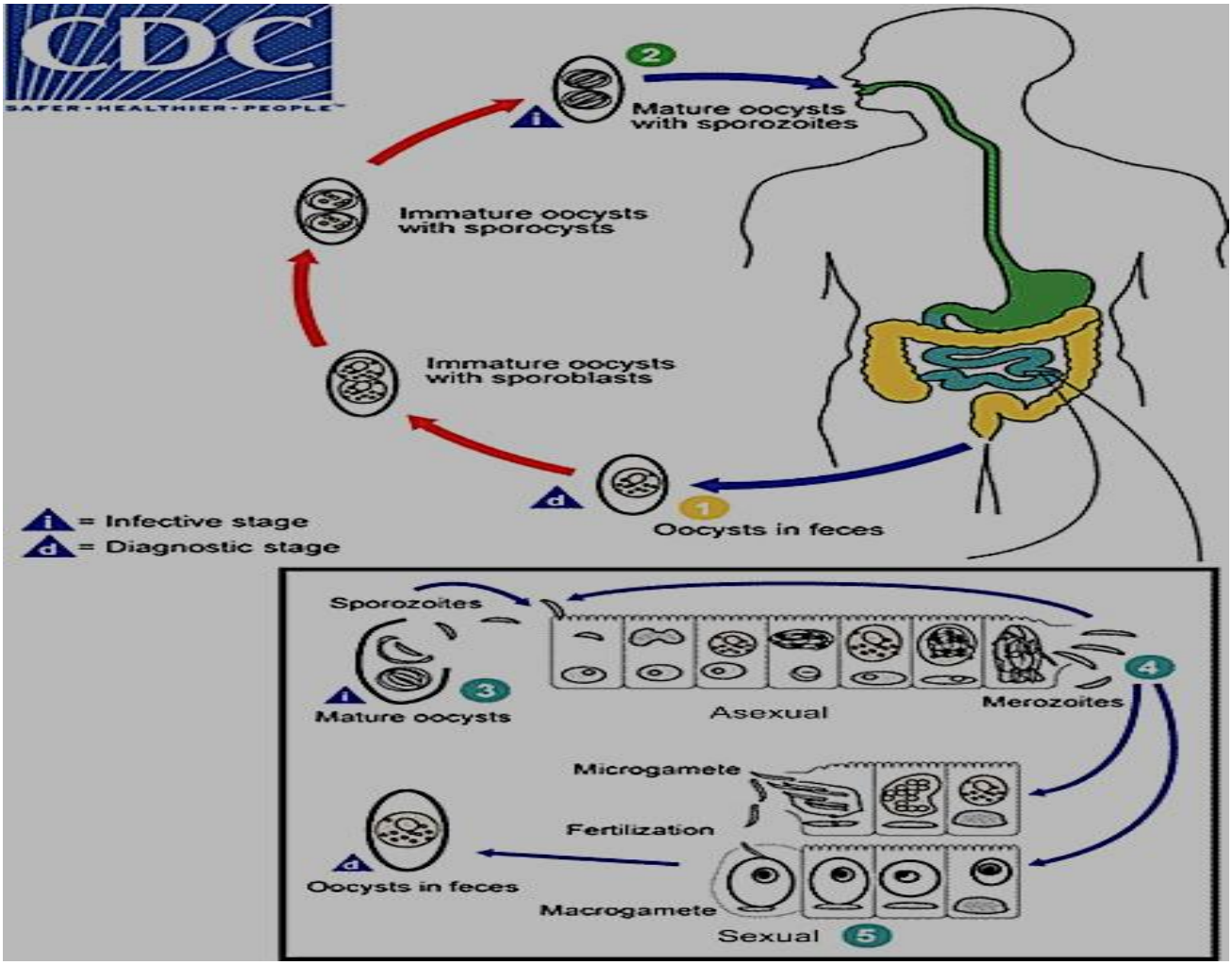
1-**Anti-Oocyst antibody of *C. parvum*** could be detected at least one year after infection. ELISA technique and immunofluorescence are suitable techniques for *C. parvum* antibody detection.

2-***C.parvum* antigens** could be detected in stool specimen performing ELISA technique and using monoclonal antibody.


CDC
SAFER·HEALTHIER·PEOPLE™
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
j Thick-walled oocyst (sporulated) exits host
a Oocyst
b Sporozoite
c Trophozoite
d Type I Meront
e
Auto-Infection
k Thin-walled oocyst (sporulated)
Asexual Cycle
Merozoite
Microgamont
Microgametes
g
Undifferentiated Gamont
f
Type II Meront
Merozoites
Sexual Cycle
Macrogamont
h
Zygote
i

b-Isospora belli:

It is developed in intestinal mucosa of duodenum and proximal jejunum (small intestine) of man. This parasite discovered in human biopsy. It is lead both Schizogony and sporogony life in the lining epithelial cells of human intestine.



Methods of lab. Diagnosis:

a-Stool diagnosis: Which may provide two types of Isosporiasis infection evidence those observed microscopically in the examined stool sample:

A- Indirect evidence, which includes:

1-High fat content in the fecal material

2-Presence of fatty acid crystals with fecal materials

3-Presence of Charcot-Lyden* crystals in stool.

***Charcot-Lyden crystals:** are hexagonal bipyramidal structures localized in the primary granules of the cytoplasm of eosinophils and basophils. Their presence, along with eosinophilic infiltrate, is an indirect evidence of parasitic infestation particularly with *Isospora, Toxocara, Capilliriasis, Ascariasis, or Fasciola.*

B-Direct stool features evidence:

It is may be difficult to demonstrate the presence of *Isospora* oocysts in direct wet preparation of stool specimen**, thus the following techniques may apply:**

I-Concentration techniques of stool specimen.

II-Permanent stained method: two types of stool stain could be used:

-Auramine-rhodamine &Giemsa stains.

-Modified Ziehl-Neelsen (zn) stain (Kinyoun acid fast stain), by which: Oocyst appear pink in color, with diameter >25µm.

b-Duodenal aspiration: Oocyst may observed in material aspirated from duodenum of the infected patient**.** This method is **useful especially after repeated negative stool exam.**

c-Intestinal biopsy: Oocyst may demonstrated in biopsy taken **from upper GIT wall** of patient with **isosporiasis** during endoscopy exam**.**

d-Blood exam: Eosinophilia observed in patient who has **isospriasis** infection, **but not observed** in other intestinal protozoan infections.

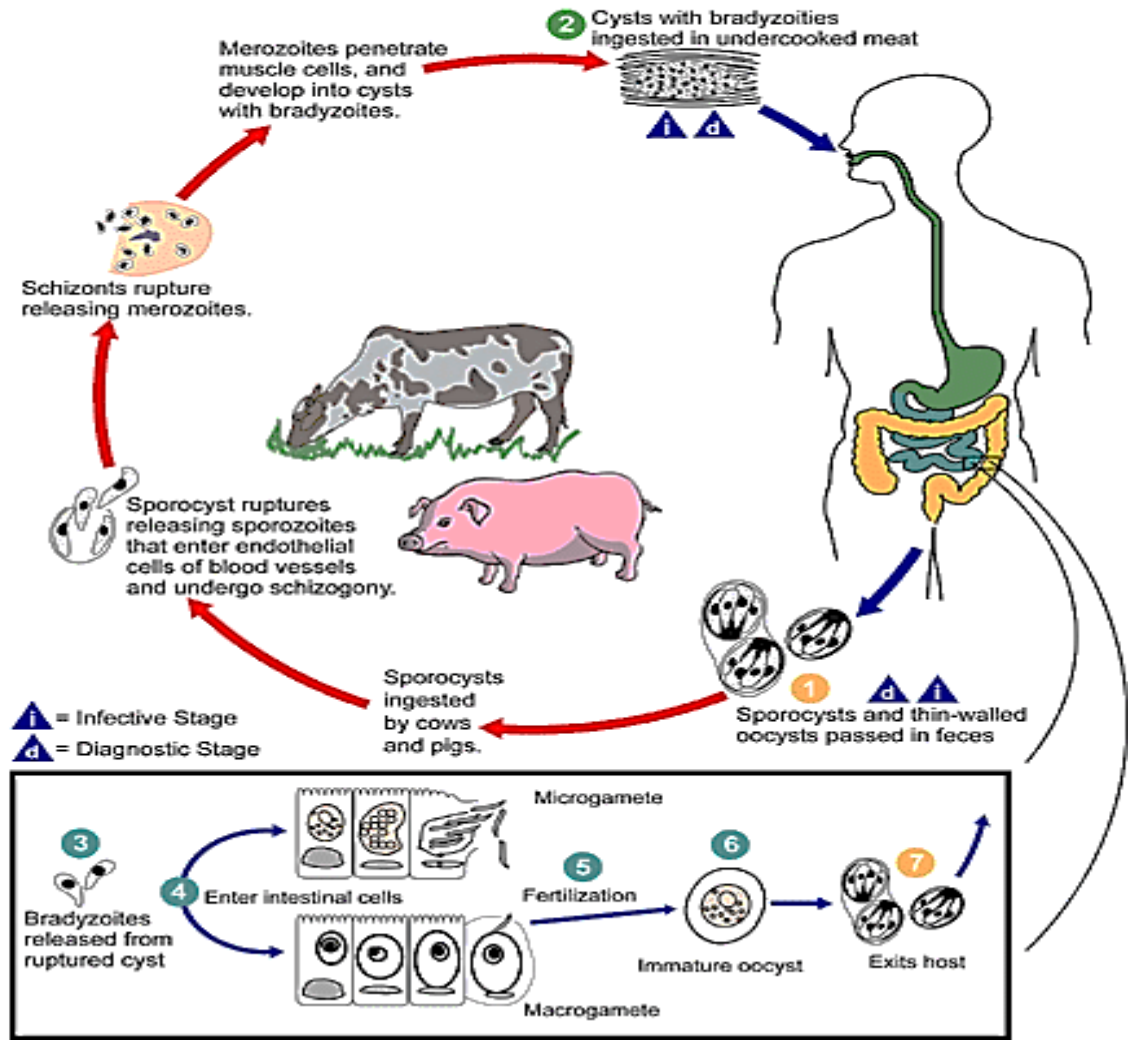
c-Sarcocystis spp.

There are three species of the genus Sarcocystis that can parasitize human:

1-***Sarcocystis hominis*** (transmitted through cattles)

2-***Sarcocystis suihominis*** (transmitted through pigs)

3-***Sarcosistis linemanni***



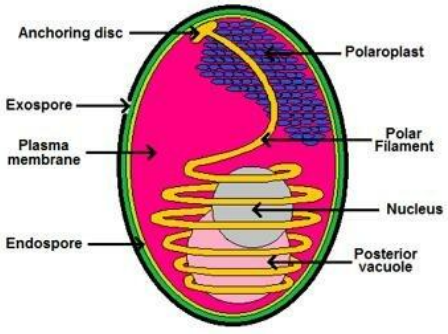
Methods of lab. Diagnosis:

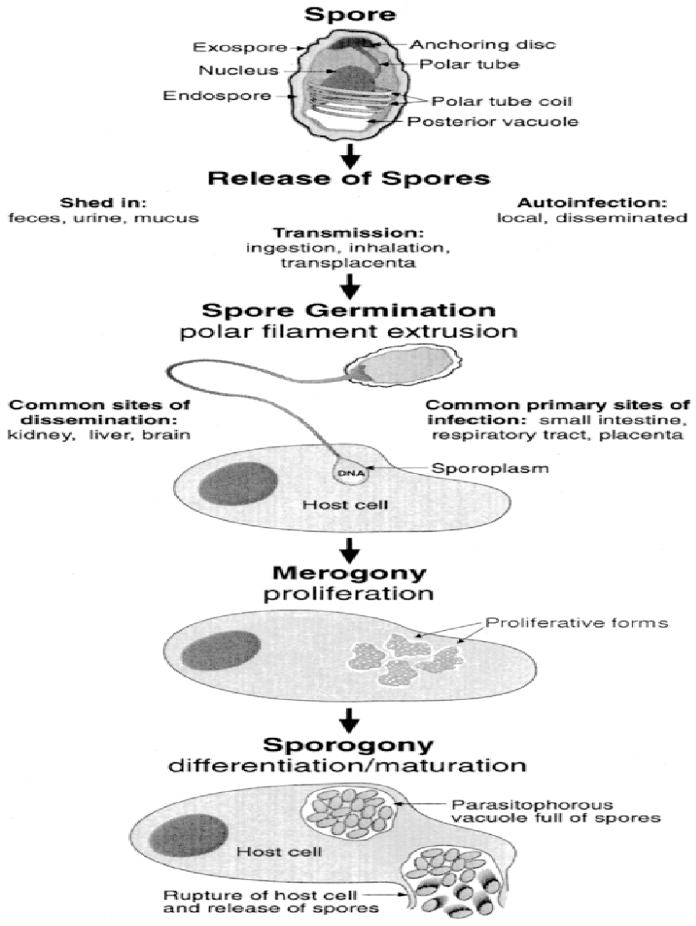
1-Stool examination: Sporocysts and sometimes oocystes could be demonstrated in human fecal materials. **Note: Differentiation between Sarcocystis species is not possible under light microscope.**

2-Musculer Sarcocystis: biopsy or autopsy taken from skeletal muscles or cardiac muscles of the infected patient is useful to identify **tissue cyst**.

5-Microsporidia:

Are uniloculer obligate intracellular parasite. The parasite reproduces by forming spores (sporogony). Spores are the infective stage of Microsporidia parasite, that is resistant and the only stage that can exist out of host's cell. It is oval to cylindrical in shape, 2-4 µm in diameter. Each spore surrounded by double layer: **1-the outer layer (exospore**) is protienacous and electron dence. 2-**the inner layer (endospore)** is chitins and electron lucent. Microsporidia spore contain also polar filament (or polar tubule). Polar tubule is considered as extrusion mechanism that helps in injecting spore content in to the host cell. The spore is Gram positive and acid fast.





Methods of lab. Diagnosis:

1-Microscopically:

Diagnosis of microsporidia is made by detecting the spore in: **stool, urine, cerebrospinal fluid (CSF), or small intestinal biopsy specimen**.

-Microsporidia spore is stained with Graims`s stain and periodic acid-stiff (PAS). Note: Microsporidia spores are **poorly stained** with hematoxylin and eosin stain.

-Although intracellular spores can be visualized under light microscope, **electron microscope is the golden stander.**

-Differentiation between genera and species of microsporidia is **based on the morphology of pore under electron microscope**.

2-Serodiagnosis:

Direct fluorescent method using monoclonal antibody is used to detect microsporidia infections in clinical samples.

3-**Moleculer methods**: PCR (polymerase chain reaction) technique is used to amplify and detect microsporidia DNA.

4-**Cell culture:** Microsporidia spores could be cultured **intracellulary** in **monkey and rabbit kidney cells added to human fetal liung fibroblast.**

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