Diagnosis of Parasites

3rd stage/2nd Semester/2024-2025

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Stool exam techniques for intestinal parasites (Part-II):

6-Intestinal biopsy:

Biopsy of the small intestinal mucosa may reveal infections with protozoan parasite: ***Giardia***, ***Cryptosporidium*** and ***Microsporidia****, added to* ***Strongyloid*** *larvae.*

7-Methods for estimation worm burden:

You can calculate the **proximate number of worms those inhibiting intestine** if you **estimate the total number of eggs that laid by the female parasitic helminthes during 24horus**.The following method may applied to count helminthes eggs in patient feces:

1-Save entire feces that defecated during 24hrs. Then record its Wight.

2-Wight out 4gm of the collected fecal specimen and place it in a big test tube or calibrated bottle.

3-Add NaOH (10N) solution to the specimen to complete the volume to 60ml.

4-Add few glass beads and shake vigorously to make a uniform suspension.

5-Drew 0.15 ml of the suspension by special pipette, and then put it on glass slide.

6-Count the number of eggs under low power microscopic lens (Not: do not put cover slide).

7- The counted egg number multiplied X 100, the calculated number=Number of eggs/gm. of feces, then multiplies X the Wight of entire feces that collected during 24hrs.

The number of eggs that laid by one female helminthes is varying considerably, it is depending on: **the type of the intestinal worm**, and the **number of female helminthes inhibiting human intestine**.

-**Chinese liver flock** put 2400 egg/24hrs.

-***Ascaris*** female put 200.000 egg/24hrs,
If you record 1000egg of *Ascaris* /gm. of feces, and the entire collected feces from the infected patient was 200gm.
1000egg X 200gm= 200.000egg/24hrs, means that the patient intestine contain one female and male *Ascaris* (One couple).
-***Necator*** female put 2400-8800 egg/24hrs=12-44egg/gm of feces.
If the number of observed eggs of *Necator* helminthes was 2100egg/gm. of feces, it's considered to be **sub clinical** infection.
-***Ancylostoma*** female helminthes put as twice as *Necator* female do.

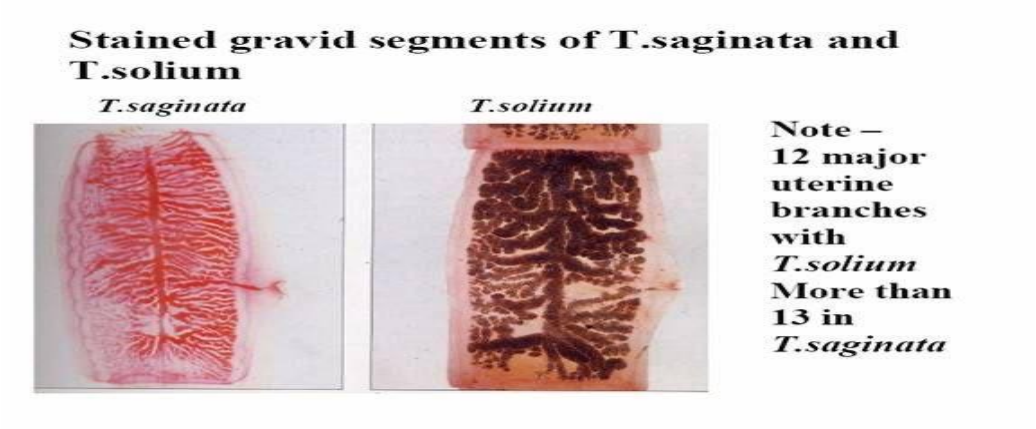
-***Trichuris*** female put about 14.000egg/24hrs.

8-How can you identify tapeworms' genus and species?

The tapeworm segment that found with fecal material should be cleaned with water, and then the segment squeezed between two glass slides to be somewhat flattened, then examined under low power microscopic lens.

Diphyllobothrium segment identified by the presence of a **uterine rosette** in the **middle of the segment**.

If the uterine rosette is **not observed**, the segment may belong to ***Taenia*** species.
To differentiate between ***Taenia*** **species**, **uterine branches** should be counted.



9-How you can identify the type of small nematodes helminthes?

The helminthes should be treated with clearing agent, like glycerin, as following:

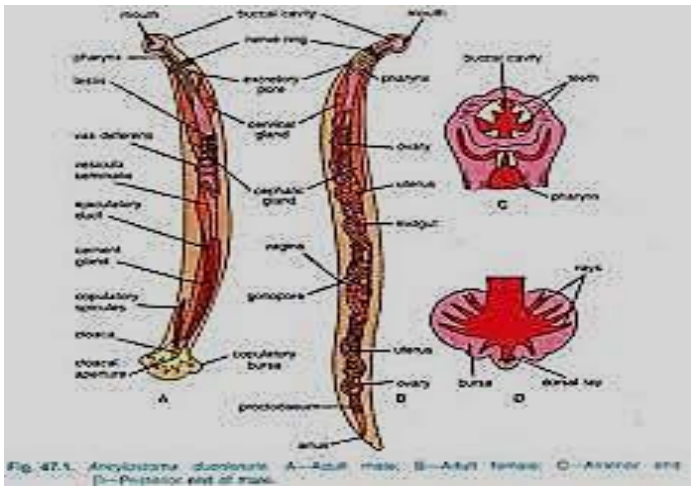
1-Immerse the nematode helminthes in 70% alcohol, heated to 75°C for 3hrs.

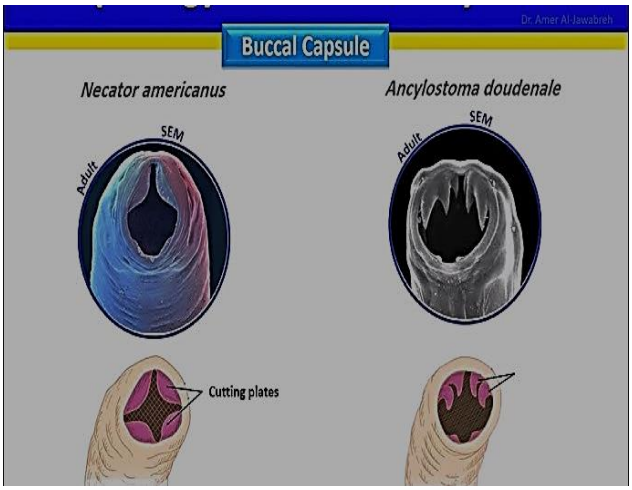
2-If the nematode helminthes was fixed previously in formalin; transfer it to a mixture that contains equal amounts of formalin and 70% alcohol for 3hrs.

3-Transfere the fixed helminthes to new 70% alcohol solution container, leave it for 3hours.

4-The helminthes transferred then to a shallow dish that contain a solution of 10% glycerin and 70% alcohol. Levee uncovered for several days in a dust free location, allow the alcohol to evaporate.

5-Mount the helminthic specimen on a glass slide with glycerin, then covered with cover slide. Then examine microscopically under low power lens, looking for type of mouth parts to identify helminthes type and posteriorly to identify the nematode sex.





Specific Diagnostic methods for intestinal protozoan organisms:

What are the factors that lead to successful diagnosis of intestinal protozoa?

1-Collection of the correct specimens.

2-Number of specimens submitted to the examine.

3-Type of processing methods and the diagnostic technique performed, added to the skill and experience of the examiner that make him able to identify the protozoan parasite depending on movement type and morphological characteristics.

I-Intestinal amoeba: There are about 7types of intestinal amoeba which are:

Entamoeba histolytica, Entamoeba coli, Entamoeba polecki, Endolimax nana, Iodamoeba butschlii and Blastocystes hominis.

1-Entamoeba histolytica

*** Methods for detecting intestinal amebiasis infections**:

a- Direct wet preparation: Useful to identify the **movement of trophozoite** stage, with its **food vacuoles that may contain RBC**. And the **shape of the amoeba pseudopodia**. **Direct iodine stain** is useful also to identify **protozoan cysts**.

b-**Concentration techniques**: Useful to identify the **protozoan cysts** even it was found in **small numbers** in the stool specimens.

c-**Permanent stained smear**: In which **trichrom stain** or **Iron - hematoxilin stain** used. It is useful to demonstrate intracellular **characteristic features of the Trophozoite and cyst stages** of the protozoan parasite.

d-**Culturing stool specimen** using specific culture media is useful to detect **chronic and a symptomatic** infections.

e-**Intestinal biopsy** should be submitted to histopathological examination.

f-**Molecular methods**: PCR and real time PCR in which DNA specific sequence amplified, then could be identified by DNA prob. It is useful technique to identify amoeba species and strain. Radioimmunoassay also is rapid and specific method for detecting enatmoebiasis infections.

g-**Serodiagnosis of Intestinal amoebiasis infections**: serodiagnosis of intestinal amoebiasis infection give positive results just in case of **invasive amoebiasis**. There are several methods of serodiagnostic methods for detecting intestinal amoebaiasis:

1-Amoebic antibody detection: Amoebic antibodies appears in serum only in **late stage of the intestinal amoebiasis**. Test for *Entamoeba* **antibodies** in serum is useful mainly in diagnosis of **extraintestinal amoebiasis**.

Serodiagnostic methods in which you can detect *Entamoeba* **antibodies** are include**:**

-Indirect hemagglutination assay (IHA): In which serum antibody titer of 1.256 considered to be significantly positive.

-Indirect fluorcent assay (IFA): In which serum antibody titer of 1.200 considered to be significantly positive.

-Enzyme linked immunosorbent assay (ELISA)

-Counter-count immunoelectrophoreses (CIEP)

2-Amoebic antigen detection: in serum detected only in **patients with active infection** and **disappear after clinical cure.**

Antigens like: lipophosphoglycan (LPG), amebic lectin, serine rich Entamoeba histolytica protein (SREHP) are all may be detected using monoclonal antibodies by **ELISA technique.**

3-Stool antigen detection: detection of copro antigen of *Entamoeba histolytica* in stool by micro well ELISA is more sensitive than direct stool examination and stool culture. Commercially available ELISA tests like Tech lab *Entamoeba histolytica* are more easily performed with increasing frequency.

***Methods for detecting extraintestinal amoebiasis (systemic amoebiasis):**

1-Hepatic amoebiasis:

a-**Diagnostic aspiration**: including examination of aspirated materials (pus) from hepatic amoebic abscess, in which *Entamoeba histolytica* **trophozoite** demonstrated under microscope.

b- **Liver biopsy**: Biopsied specimen taken from liver abscess, which then prepared histopathologically and examined under microscope locking for *E. histolytica* **trophozoite.**

c-**Blood examination**: when **leukocyte** count **15.000-30.000 cell/ml of blood**, and **70-75% of the counted WBC were polymorphous nuclear cells**, it is **positively significant** for parasitic infections.

d-**Serological test**: Systemic amoebiasis could be detected using several serosiagnostic methods like: IHA, IFA, ELISA, Agglutination test.

e-**Moleculer methods**, like: DNA prob and PCR, its sensitivity is about 87%.

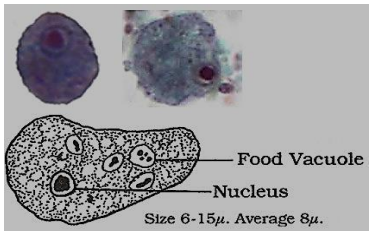
Methods for detecting non-pathogenic intestinal amoebas:

1-*Entamoeba coli*

Distinguished by the **cyst which is large and contain 8 nucleus when mature**. It could be demonstrated by wet preparation stool smear, but **permanent stained smear is the method of choice.**

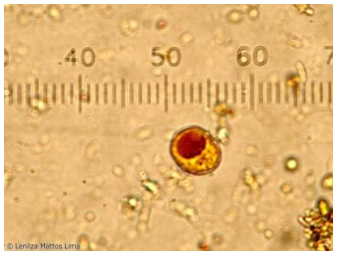
2-*Endolimax nana*

Permanent stained smear is the method of choice to diagnose the organism, although it is non-pathogenic intestinal amoeba. It is important to differentiate between pathogenic and non-pathogenic amoeba.



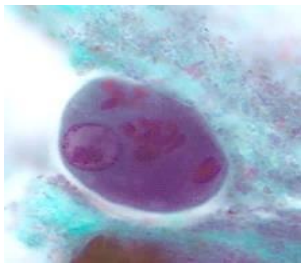
3-Iodamoeba butshlii

The **best method** for diagnosis is the **permanent stained smear**, although the **large glycogen vacuole could be demonstrated using wet iodine stained** direct preparation film.



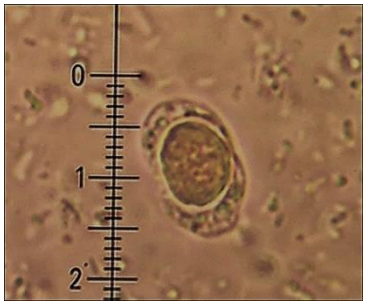
4-Entamoeba poleki:

Permanent stained film is the method of choice added to direct wet preparation. Its characteristic features are: **Originally found in pigs and monkey intestines**, added to **man intestine**. 10-12µm in diameter, **trophozoite has sluggish movement**, small nuclei with **small karyosome fine chromatin granules**. Food vacuole contains the ingested bacteria. **Cyst** has single nuclei and contain inclusion body.



5-Blastocystis hominis:

Permanent stained smear is the method of choice but it could be demonstrated using direct wet stool preparation. This amoeba has **yeast-like shape**, contain central large **oil-vacuole** and **clear peripheral cytoplasmic layer with many nuclei**. It is reproducing by **binary fission and sporulation**. Movement by pseudopodia extension and retraction.



C**ultivation of *Entamoeba histolytica*:**

Boek and Drobohlav`s Lock-egg-serum medium (LES) is successfully used to culture *E. Histolytica.* This medium is not axenic, and antibody is not added.

Content of Boek and Drobohlav`s Lock`s solution; NaCL 9.0gm; CaCL2 0.2gm; KCl 0.4gm; NaCO3 2.5gm; DW. 1Litter.

This solution should be autoclaved before storage.

LES medium is prepared by:

1-Wash 4 eggs, brush with alcohol to sterilize and break into a sterile flask containing glass beads.

2-Add 20 ml Lock`s solution, shake until homogenized.

3-Dispense in test tube sufficient quantity to produce a 2.5 -3.5 cm slant in bottom of tube.

4-Slant plugged tubes and place in inspissation at 70°C until slant are solidified. If an inspissation is not available, a substitute may be devised by leaving the door of the autoclave partially open.

5-When slant have solidified, autoclaved at 15 pound pressure for 20 minutes. Discarding any badly broken slant.

6- Cover 1cm with mixture of 8 parts sterile Lock`s solution to 1 part in activated sterile human blood serum.

Sterility of mixture of Luck`s solution and serum should be insured by filtration, sterilization followed by incubation at 37°C for 24hrs or longer before use.

A loopful of sterile rice starch or powder is added to each tube before inoculation.

Inoculation with portion of stool the size of a small pea, break up well in medium, and incubated at 37°C.

Examine after 2-3 and 4 days. Inoculation by removing a small amount of sediment with pipeate. The sediment is transferred to a slide, covered with cover glass slide and examined under low power.

Primarily, isolation may yield few organisms, whereas subsequent transfer shows a considerable increase in parasite number.