Diagnosis of Parasites

3rd stage/2nd Semester/2024-2025

Lec.3 Stool exam techniques for intestinal parasites (Part I):

The available methods of stool exam to detect parasitic diseases are either applicable or sometimes have limited prepossess. Thus, the chosen technique should be suitable to the type of parasite we locking for in stool specimen. Initially, you should have knowledge about the features and content of normal fecal specimen before detecting the abnormalities in stool samples.

What are the main features of normal stool specimens?

Stool or feces is the eliminated digestive waste products from the body. The content of jejunum and terminal ileum are liquid. And about 400gm in weight/24hrs. As they pass through caecum and colon, most of the water is absorbed converting the content to a soft but formed mass, about 150-200gms in weight.

Physical examination of fecal material for parasites:

1-Fecal material should be examined for macroscopic parasites by necked eyes. Pinworm may found on the surface of the specimen, Tapeworm proglotted (segments) may found in the interior of the specimen, therefore, the specimen should be broken with applicator stick to make check for helminthes. Eggs of helminthes could be observed microscopically either in formed or liquid stool.

2-If bright red blood observed on the surface of formed stool specimen, it is almost a sign of bleeding hemorrhoid. If mucus found with blood on loose or liquid fecal materials, it is highly suggested amoebic ulceration in large intestine. (Note: *Entamoeba histolytica* is the causative protozoa of human amoebic ulcer).

3- Trophozoites (Trophic form) of the intestinal protozoan parasites could be observed Microscopically in liquid or soft stool, and never be found in fully formed stool. Cysts of intestinal parasites usually observed in formed stool. Both trophozoites and cysts could be observed after administration of anti-parasitic agents.

Stool examination techniques:

Combination of two or more techniques is required to confirm an infection with intestinal parasite, because using single technique is not satisfactory to improve the identity of certain parasitic diseases. The following lab. Techniques are used to detect intestinal parasites infections:

1-Direct wet film method:

This method is used to detect trophozoite forms of amoebas and flagellated parasites, by tracking their movement and so helminthes larvae. Each of these organisms has its distinct motility method. Simultaneously, eggs of helminthes and protozoan cysts could be observed using direct wet preparation.

The intestinal content that taken during large intestine endoscopy, could also tested for presence of intestinal protozoan parasites using direct wet preparation method. The drawn intestinal specimen when contain blood and mucous it may indicate infection with amoebic ulcer.

Logal iodine is usually used to stain wet stool preparation, by which you can detect eggs and cysts of intestinal parasites but not trophozoites or helminthes larvae.

2-Concentration methods:

Concentration of stool specimen is performed to demonstrate Cysts and eggs when they present in small number. These techniques should be routine step of the parasitology examination. Many concentration methods were applied to detect eggs of helminthes and cysts of protozoan parasites. Concentrated methods are mainly divided to **two classes**:

a- Sedimentation techniques: Principle: Cysts and eggs which are heavier than the suspending liquid, become concentrated in bottom of tube.

b-Flotation methods: Principle: using a heavy liquid (that have high specific gravity), thus eggs and cysts those have specific gravity lower than that of the flotation fluid will lift up on the surface.

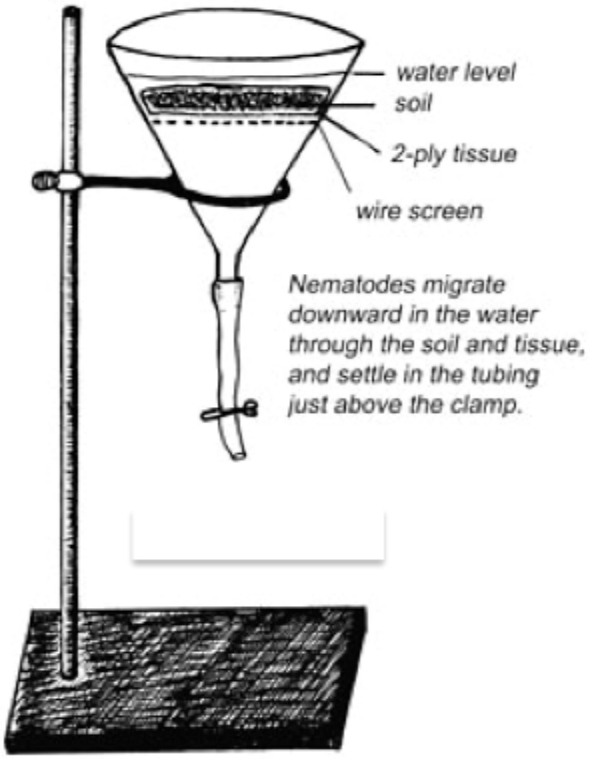
3-Methods for detecting *Strongyloid stercuralis* larvae:

a-Baermann Apparatus for S*trongyloid* larvae:

Strongyloid larvae do not always concentrate well with either or concentrated techniques. The Baermann technique yields a good concentration of the living larvae of *Strongyloid stercoralis.* It should be used when there is a high index of suspension and routine stool examination is negative.

Procedure:

1-A glass funnel with a diameter of 10cm or greater is set up in a ring stand, with a short piece of rubber tubing attached to its stem and a pinchcock closing the tubing.
2-A wire circle or sieve, of slightly smaller diameter than the top of the funnel, is covered with two layer of gauze.
3-The funnel is filled with lukewarm water to a level just covering the gauze, and a specimen of stool is placed on the gauze, partially in contact with the water.
4-The apparatus is left to stand at room temperature for 8-12 hrs. Then a few drops of fluid are drawn off through the tubing into glass dish.
5-Examine for larvae under low microscopic power.



b-Filter paper strip procedure for *Srongyloid stercuralis* and T*richostrongyloid* larvae:

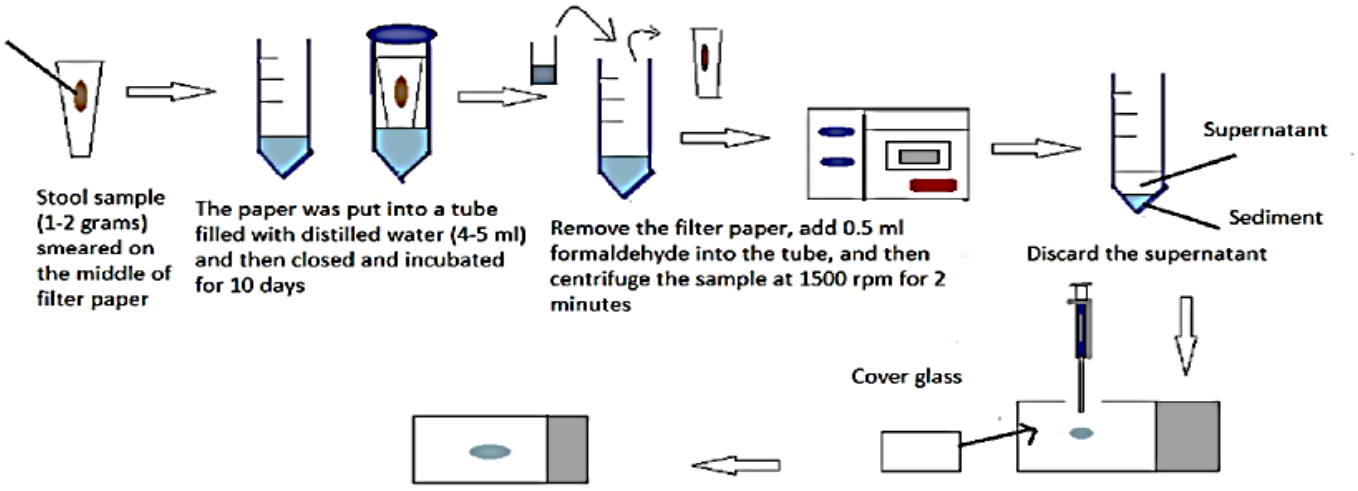
This method is well adapted for failed or survey use, were quick results are not necessary. The method described by Harada and Mori (1955).

Procedure:

1-Place 0.5-1gm of fecal materials on filter paper (20×30cm), The stripted Filter paper then inserted in 15ml test tube that already contain 3-4ml of D.W.

2-The tube placed at upright position or slightly slanted, so that the filter paper is kept moist by capillary flow. Water may add if needed, to maintain the original fluid level.

3-Small amount of the fluid is withdrawn from the bottom of the test tube after 10 days, and then examined for helminthes larvae.



c- Agar plate method for *Strongyloid stercoralis* larvae finding:

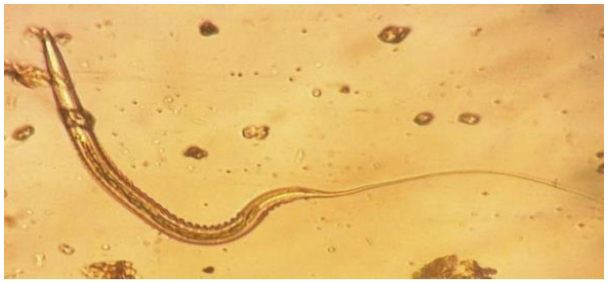
More sensitive than either Bearman or filter strip method, an agar plate method such as described by Koga et al (1991).

Procedure:

1-Agar medium is prepared as following: 1.5 % agar+ 0.5 %meat extract+1%pepton+0.5% NaCl dissolved in D.w. then autoclaved and dispensed in sterilized dishes, plate are the dried at room temp. for 4-5 days to eliminate excess moisture. Finally, stored in sealed plastic bags.

2-Cultivation of the eggs: approximately 2g of fresh or refrigerated stool specimen is placed at the center of the plate. The plate then sealed with adhesive tape to prevent escape of larvae. Incubation applied at room temp. (26-33°C) for 48hrs.

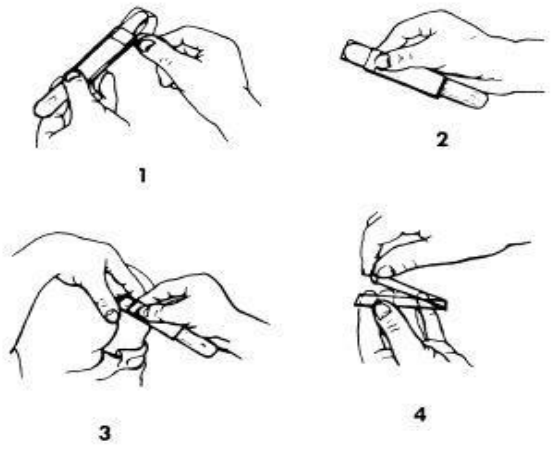
3-Plates are then examined microscopically looking for larvae or larvae tracks. When the result is negative try reexamine under low magnification power using green filter. The rhabdetiform larvae of *Stronigyloid* are said to exhibit whip like movement as they progress, while hookworm larvae glide like snake.

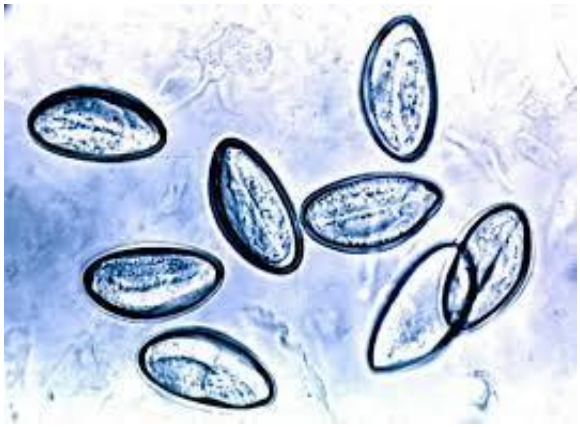


4-**Celluphane tape swab for detecting *Enterobius vermicularis* and *Taenia* eggs:**

Pinworm eggs, which are generally deposited at night, will be found scattered around the perianal region. The tape should be used in the morning before the patient washed or defecates. A number of commercial pinworm detection kits are now available, but if cellophane are used. The Procedure is:

1-Fold together sticky surface of a piece of cellophane tape 1×8cm, for both 1 cm at each end.
2-Stretch tape, sticky side out, over butt end of a test tube or wooden tongue blade, holding non sticky end firmly with thumbs and forefinger.
3-Apply tape to anal area, rocking back; froth to cover as such of mucosa and mucocutanous area as possible.
4-Remove tape and apply to microscope slide, sticky side down. Press firmly into position.
5-Examine for egg under lower power microscope.





5-Duodenal inspiration:

Duodenal content is useful to identify intestinal parasites like: *Strongyloid stercuralis*, eggs of the helminthes *Cloronorchis, Opiasorchis and Fasciola and Protozoan* intestinal parasites like*: Giardia, Isospora, Cryptosporidium.*

Specimen collection: specimen may collect from duodenal content using enteric capsule or string test (Intero-test). Number of gelatin capsule containing 90cm line for children , or 140 cm for adults, composed of 20cm silicon rubber covered thread and a 70cm or 120cm soft nylon yarn, is swallowed by the patient, while the thread , which is protrude from a hold in the capsule is held firmly. To the end of the nylon yarn is attached a 1 g Wight. Which eventually help carry the string in duodenum. The free end of the line taped to the parient neck or cheek. After 4 hrs., may pull up. The bile stained mucous adhering to its distal end is examined microscopically.