University of Mosul
Lecture No.:
College of Veterinary Medicine
Date:
Unit of Scientific Affairs
Website:

Lecture title: Helminthology

Lecturer Affiliation: Department of Microbiology , College of Veterinary Medicine, University of Mosul, Mosul, Iraq. **Enas saddi@uomosul.edu.iq**

Summary: *Helminthology*

Laboratory diagnosis of parasitism

Samples collection and examination

Diagnostic stages of most parasites can be detected in :

1. Feces : used to diagnose parasite eggs, larvae, oocysts, cysts, Trophozoites,cestode segments adults.
2. Blood : used to diagnose blood parasites: *Babesia, Theileria, Trypanosoma , Dirofilaria immitis*.
3. Sputum : used to diagnose lung parasite eggs, larvae for example *Dictyocaulus* species (eggs) is cattle and sheep.
4. Urine : used to diagnose eggs in urinary system for example :*Dioctophyma renale* (giant kidney worm), *capillaria* species in dogs and cats.
5. Skin : used to diagnose external parasites such as : Mange *(Sarcoptes, Psorptes)*.
6. Autopsy : from dead animals .
7. Biopsy from live animals.

used for studying the pathological changes and diagnosing some parasites such as : *Eimeria, Toxoplasma, Theileria*.

* Collection and submission of samples

1. Fecal Samples :

- Fecal exams should be conducted for fresh fecal material.

- ❖ In large animals : feces should be collected directly from the rectum by using disposable plastic glove.
- ❖ In small animals feces should be collected immediately after defecation.
- ❖ Feces should be placed in a sealed glass or plastic container, clearly marked (Label) with the :

1. Time and date of collection.
2. Species of animal, sex, age.
3. History of clinical disease.
4. Owner's name , and any other information relevant to the case.

- ❖ If collected feces can not be examined within a few hours, the sample should be stored at $4C^0$ until examined.
- ❖ Feces should not be frozen, because freezing can distort parasite eggs and trophozoites .
- ❖ If feces inspected for the presence of protozoan Trophozoites (e.g. *Giardia, Trichomonad*) should be examined immediately after collection
- ❖ When the material is to be sent to another laboratory it should be packaged in cold packs, helminthes eggs may also be preserved in equal volum of 10% formalin or 70% isopropyle alcohol

Examination of the fecal samples :

There are several procedures commonly used to examine feces for internal parasites :

1. Gross examination of feces :

- Consistency : The condition of the feces that is : soft, watery (diarrheic) or very hard soild , this description will vary with the animal species , for example , cattle feces are normally softer than those of horses or sheep .
- Color : Unusual fecal colors should always be reported.
- Mucous : Mucous on the surface of fresh feces may be associated with intestinal parasitism or some other metabolic diseases .
- Blood :blood may indicate severe parasitemia.
- Age of feces : If the feces appear old and dry , this should be noted in aged sample, parasite eggs have embryonated or larvated , oocyst may have sporulated or pseudo parasites may be present .
- Gross parasites : Tapeworm segments , round worms , and larval arthopods (bots) may be present .

Microscopic examination of feces :

1. Direct smear :

procedure of direct smear :

1. Small amount of feces placed directly on the microscope slide ,by a stick
2. Dilute this quantity with water or normal saline .
3. Mixed by using applicator stick.
4. A cover slip is applied and the smear is examined under the microscope.

The advantages of this method are : short of time and minimal equipment needed and coster.

Disadvantages : negative result with this method is not always reliable and the animal may be incorrectly assumed to be free of parasite. This method also leaves a lot of fecal debris on the slide.

2. Concentration methods for fecal examination :

A. Qualitative methods : these methods used for determination the types of infection

1. Fecal flotation : this procedure based on differences in specific gravity of parasite eggs and larvae and that of fecal debris.

Specific gravity refers to the weight of an object (for example : parasite eggs) compared with the weight of an equal volume of pure water. Most parasite eggs have a specific gravity between 1.1 and 1.2, whereas tap water is only slightly higher than 1, therefore, parasite eggs are too heavy to float in tap water, to make the eggs float, a liquid with a higher specific gravity than the eggs must be used, such liquid are called flotation solution consist of concentrated sugar or salts solution added to water to increase its specific gravity.

Flotation solution usually have specific gravity between 1.2 and 1.25.

Flotation method is used to diagnose the nematode eggs, oocyst and cysts.

Procedure of flotation :

1. put about 2 gm. of the fecal sample in 100 ml glass beaker.
2. Add 15-30 ml of flotation solution
3. Mix the feces solution with solution .
4. Strain the solution through a fine sieve (tea strainer) to remove the layer objects .
5. Pour the mixture in to (10 ml) test tube and fill the tube to the top.
6. Place a glass cover slip gently on the top of the fluid and allow the cover slip to remain for 10 to 20 minutes.
7. Remove the cover slip carefully and immediately place it on the microscope slide.
8. examine the area of the slide under the cover slip with the microscope.

2. Sedimentation :

This method is suitable for trematodes eggs and some cestodes and nematodes whose eggs do not float readily in common flotation solutions .

Procedure of sedimentation :

1. Place 3-6 gmof the fecal sample in 100 ml glass beaker.
2. Add 30-40 ml of tap water or normal saline
3. Mix the water with the feces.
4. Strain the solution through a fine sieve
5. Pour the strained mixture to the centrifuge tube and centrifuged for 1-2 minutes on 1500 rpm, if a centrifuge is unavailable, allow the mixture to sit undisturbed for 20-30 minutes.
6. Pour off the liquid in the top of the tube without disturbing the sediment at the bottom.
7. Using the pastour pipette, transfer a small amount of the top layer of the sediment to a microscope slide.
8. Apply a cover slip to the drop and examine the slide microscopically.

3. Baermann method :

- This method used for detection the lung worm larvae and cultural method for specific identification of the third larval stage of the Strongyles and Trichostrongyles.
- Baermann apparatus consist of :

a. A funnel clamped to a metal stand

b. A short piece of tubing with a clamp is attached to the end of the funnel(Fig 3).

Procedure of Baermann technique :

1. Apply 5-20 gm of fresh feces or any suspected soil to a gauze and placed it in the funnel.
2. The sample is covered by the warm water.
3. Let the apparatus at room temperature for 8-24 hours.
4. Release the clip and collect the first 3-4 drops of water on a microscope slide and examine the slide, or collect 10 ml into a centrifuge tube, spin in the centriguge for several minutes and examine the sediment.

4. Fecal culture :

is used in diagnostic parasitology to differentiate parasites whose eggs and cysts can not be distinguished by examination of fresh fecal sample. For example eggs of some nematodes like Strongylus species in horses. The feces allowed to incubate at room temperature for several days until the eggs hatched and the larvae developed to infective third stage (L3).

Procedures of fecal culture :

1. Place 20-30 gm of fresh feces in a jar and moisten slightly with the tap water, until it become soupy.
2. Place the jar on a shelf, away from direct sun light and for 7 days at room temperature if the culture is dried add few drops of water . .
3. After incubation, concentrate the larvae by means of the Baermann technique and examine .

Quantitative fecal examination :

Quantitative procedure indicate the number of eggs or cyst present in each gram of feces (severity of infection). Several procedures are used to estimate the numbers of parasite eggs per gram of feces, including :

1. Stoll´s technique.
2. Mcmaster technique.

Stoll technique:

1. Place 5 gm of fresh feces sample in 100 ml graduated measuring cylinder.
2. Add 0.1 N (4%) solution of NaOH (sodium hydroxide) in water up to 75 ml .
3. Shaking the liquid with glass beads .
4. By a graduated pipette, apply 0.15 ml suspension immediately to a microscopic slide and cover the liquid with a cover slip(22x45) and examine the slide.

It is advisable to check four, preparations the average number of eggs multiplied by 100, equals the number of eggs per gram feces (EPG,). Larvae (LPG)

$$Y \times \frac{75}{5} \times \frac{1}{0.15} = Y \times 100$$ (y = Number of eggs)

McMaster technique:

Used the counting slide (McMaster slide) procedure of Mc- Master method (Fig 4)

1. Two gm of fresh feces are dissolved in 60 ml saturated solution (flotation solution) such as sodium chloride.
2. Strain the mixture through a fine sieve.
3. Using a Pasteur pipette, fill one compartment of the counting cell at once
4. Repeat the same operation to fill the second counting chamber.
5. After a few minutes, the eggs float up to the surface of the concentration solution and stick to the cover glass.

2 gm 60 ml

1 gm. 60 ml/2

each champartment contains 0.15 ml liquid.

$$\frac{60}{2} \times \frac{1}{0.15} = 200$$

EPG = Y × 200 in which Y (number of eggs).