Coprological Examination

Macroscopic and microscopic examination of the feces used to identify internal parasites.

Macroscopic Examination

Some of intestinal parasites such as ascarid species, segments of several tapeworms, and

Gastrophilus larvae can be recognized with the unaided eye.

Microscopic Examination

Microscopic examination can be divided into two types of methods: qualitative methods that used for identification of types, and quantitative methods that used for counting of the number of eggs in feces.

I- Qualitative Methods:

1- Direct smear:

The direct smear method is a rapid and easily completed procedure, but eggs and oocysts are not concentrated. It should be used only when:

- Very small samples are available.
- Lack of equipment's.
- Time prevents the use of more accurate techniques.

Procedure of direct smear:

1. Put a small quantity of feces on a slide.
2. Dilute this quantity with water.
3. Thoroughly mix, using an applicator stick, until obtaining homogeneous and sufficiently transparent preparation. The largest particles can be moved aside.
4. A coverglass is applied and the smear is examined under the microscope.

Note:

A negative result with this method is not always reliable, therefore; this method is of little

diagnostic value because it is unlikely that ova will be observed unless the infestation is severe.

2- Flotation:

Fecal flotation method is based on the specific gravity of parasitic material and fecal debris. Specific gravity refers to the weight of an object compared with the weight of an equal volume of water. The specific gravity of most parasite eggs is between 1.100 and 1.200 g/ml, whereas the specific gravity of water is 1.000 g/ml .

To allow for flotation of parasite eggs, oocysts, and other life cycle stages, the flotation solution must have a higher specific gravity than that of the parasitc material. Several salt and sugar solutions work well for flotation. Most have a specific gravity of 1.200 to 1.250. in this range, heavy fecal debris sinks to the bottom of the container, whereas parasitic material rises to the top of the solution.

1- Sugar solution is inexpensive and does not crystallize or distort eggs. Sugar solution may be mad anywhere and sticky the eggs with the coverslip.

2- Zinc sulfate solution is more commonly used in diagnosis laboratories. Zinc sulfate floats protozoal organisms with the least amount of distortion.

3- Sodium chloride solution, this solution corrodes laboratory equipment form crystals and severely distorts parasite eggs. Saturation sodium chloride solution is also a poor flotation medium because the maximum specific gravity obtainable is 1.200, allowing heavier eggs to remain submerged.

Flotation is used to diagnose the nematode eggs, whereas it is not suitable for eggs of trematodes and some cestodes.

a- Examples of intestinal nematodes in cattle and sheep found in stomach (Abomasum) worm (*Ostertagia spp*., *Trichostrongylus axei* ., *Haemonchus spp*.)

b- Intestinal worms (*Cooperia spp*., *Strongyloid papilles*., *Trichostrongylus spp*., *Nematodirus spp*., *Bunostomum spp*., *Oesophagostomum spp*., *Trichuris discolor*., *Toxocara spp*.)

c- Lung worm

In cattle: - *Dictyocaulus viviparous* .

In sheep: - *Dictyocaulus filarial, Protostrongylus rufescens, Cysticaulus ocreutus, Muellerius capillaris* .

Procedure of flotation:

1. In a 100 ml glass beaker, ± 2 g fresh feces and some of concentrated solution are mixed intensity by means of a spatula to obtain a relatively homogenous mixture.
2. Dilute to 90ml with the concentrated solution. Strain the solution through a fine sieve to press out the large particles.
3. Let the solution settle for a few minutes until the air-bubbles have all escaped, then carefully place a coverglass on top of the liquid, or fill a suitable narrow cylinder to the top, and then place a clean coverglass over the top of the cylinder to be in contact with the liquid.
4. After about 30-40 minutes, gently remove the coverglass, and then examined under a low power of the microscope.

Notes:

1. If the eggs are kept to long in such a concentrated solution, they may become deformed and unrecognizable.
2. Saturated sugar solution is the better; because it is sticky, so that the eggs are well attached with the coverglass.

3- Sedimentation:

Fecal sedimentation concentrates parasites eggs, oocysts, and other parasitic material by allowing them to settle to the bottom of a tube of liquid, usually water. A disadvantage of this technique is amount of fecal debris that mixes with the parasitic material which makes microscopic examination somewhat difficult.

This procedure is used to detect heavy eggs that would not float in flotation solution or eggs that would become distorted by the flotation solution. Trematode eggs are often considered too heavy for flotation and are often found with fecal sedimentation.

This method is suitable for trematode eggs (Such as *fasciola, paraphistomum, schistosoma, Dicrocoelium dendriticum*) and some of cestodes (such as *Monezia expansa* , *Monezia benedeni*) and nematodes whose eggs do not float readily in common flotation solution.

Procedure of sedimentation:

1. In a 100 ml glass beaker, ± 10 g fresh feces are intensively mixed with water by means of spatula.
2. The suspension is strained through a fine sieve. Then transfer strained mixture to a centrifuge tube.
3. Centrifuge for 1-2 minutes at 1500-2000 rpm to obtain the sediment.
4. The supernatant is decanted, while a portion of the sediment transfer to a microscope slide and then examined with a low power.

4- Baermann techniques:

This technique used for detection of lung worm larvae, and cultural method for specific

identification of the third stage larvae of the strongyles and trichostrongyles.

Baermann apparatus consist of:

1. Glass funnel 20-25 cm in diameter.
2. Rubber tube about 10 cm in long.
3. Clip, for pinching of the tube. The apparatus is supported in a retort ring on a stand

Procedure of Baermann technique:

1. Apply ± 20 g of fresh feces to a gauze.
2. Fill the funnel with tap water, so that the feces are completely immersed.
3. Let the whole settle at room temperature for 24 hours. The larvae will come out of the soaked feces and fall through the meshes into the funnel neck where they are concentrated at the bottom.
4. Release the clip and collect the first 3-4 drops on a microscopic slide, or collect 5-10 ml of the liquid, which may be drained off into a centrifuge tube where the larvae will be in the sediment.
5. Examine the nematode larvae under low power without coverglass. The larvae will be swimming actively.

Note:

In heavy infestation, larvae can be drawn off in a drop of water after an hour, but when few larvae are present, it may be necessary to leave the Baermann set up overnight.

Shapes of larvae of lung worms

1- *Dictyocaulus filarial*: The head contain protoplasmic knob and bluntly pointed tail.

2- *Dictyocaulus viviparous*: The head round in shape and bluntly pointed tail.

3- *Protostrongylus rafescens*: The head round in shape and tail wavy and pointed .

4- *Muellerius capillaris*: Round head and the tail is end up in dorsal spine and wavy tail .

5- *Cysticaulus ocreutus* : Round head and wavy tail .

5- Feces culture:

It is used for detection the presence of helminth ova and ensuring their incubation, and the development of larvae to the infective third stage.

Procedure of feces culture:

1. The feces broken up and placed in a glass jar
2. Glass jar is closed and kept at a temperature of about 26°c for a suitable time, usually 7 days.
3. After incubation, concentrate the larvae by means of the Baermann technique.

6- Adhesive-tape method:

This method used in horses for the determination of *Oxyuris equi*; because their eggs stick to the anal region and usually are not found in the feces. The method use a transparent adhesive tape 2.5 cm wide and ± 15 cm long.

1. Clean the area surrounding the anus day before the sample is to be taken.
2. Stick the tape onto the right thumb, and firmly press the adhesive tape to the anal skin folds.
3. The adhesive tape is stuck on to a microscope slide.
4. To examine the preparation, a drop of water is placed under the tape.
5. The strip is then again firmly stuck and the preparation is examined under the microscope.

II- Quantitative Methods:

To obtain more accurate information with regard to the severity of an infection, egg counting methods have been devised. There are two methods can be used: Stoll's dilution method and McMaster method.

McMaster egg-counting technique:

1. Weigh 2 g of feces accurately.
2. Soaked it in a 60 ml of saturated solution, then strain the solution through a fine sieve.
3. Fill the compartment of the counting cell in the McMaster slide.
4. After a few minutes the eggs float up to the surface of McMaster slide and they are all in the focus against the upper slide Count the eggs at least in two compartment, according to the following calculation:

$$\frac{\textit{Total number of eggs}}{\textit{Number of chembers}} \times 200 = (\ ) \text{ epg}$$

Examination of Feces for Protozoa

All of previously described qualitative fecal examination procedures may be used to detect protozoan oocysts . However, some protozoans pass out of the host in trophozoites form, which are one-cell motile organisms that lack the rigid wall of a cyst, making flotation without distortion or death of the trophozoites impossible. Therefore; the direct smear, using saline and a stain, is the preferred procedure for examination of a fecal sample for protozoal organisms, by recognizing their movement. Stains also may be used to recognize certain structural characteristics. Lugol's iodine and new methylene blue are the common stains used with direct smear. The acid-fast staining procedure is used to identify cryptosporidium species in the feces.

Acid-fast staining procedure:

1. Make a thin smear of feces using a glass pipette and allow to air dry.
2. Fix the smear by heating on a hot plate for 2-3 minutes or by passing the slide through a Bunsen burner flame, specimen side up.
3. Flood the slide with carbol fuchsin stain and heat the slide until the stain steams.
4. Remove the slide from the heat and let it sit for 5 minutes. Rinse with tap water.
5. Decolorize by adding acid alcohol for 10-20 seconds, then rinse with tap water. Repeat this step until the film on the slide appears light pink.
6. Add new methylene blue stain for 20-30 seconds. Then rinse with tap water.
7. Blot dry with a paper towel and examine by the oil-immersion objective.
8. Oocysts will appear as bright fluorescent red objects, whereas bacteria and yeasts stain blue.

Causes of failure of faecal examination in the diagnosis of parasitic infection which include:

1- Infection with immature stage of parasite .

2- Infection with male parasites .

3- Infection with adult worm which are located in enclosed space such as brain .

4- Infection with larval stage when mammalian is an intermediate host such as *cysticerccus bovis* , visceral larval migration .

5- Infection with old and damaged worm such as after use antihelminthic drug .

Factors affecting egg count

The number of helminthes ova pass per gram of feces depend on such factors :-

1- Consistency of the feces.

2- Egg laying capacity of species of the worms.

3- Total production of feces.

4- Immunity of the host.

5- Number of female worms present.

6- Age of the parasites.

7- Diet of the animals.

8- Season of the year.

Blood Examination

The blood of domestic animals infected with various pathogenic and nonpathogenic parasites. These blood-borne parasites include numerous species of protozoa and several species of larval nematodes. The diagnosis can be made based on

identification of the parasite in a proper prepared blood films with an oil immersion objective.

Blood Protozoa

Most of the protozoan parasites that invade the blood destroy erythrocytes. Blood should be examined for erythrocytes of abnormal size, shape, and poor staining reaction. The cell and plasma should be searched for evidence of parasites. A routine blood smear can be examined and is appropriate for the diagnosis. A Giemsa stain is most effective, but Wright's stain can also be used. In case of chronic infection, few circulating parasites may be present and diagnosis becomes more difficult. Immunologic tests are also now available for some hemoprotozoal infections.

1- Direct examination

Dicret examination of the blood for microfilariae the simplest procedure to perform. Trypanosomes also can diagnosis by direct examination of the blood.

The following nematodes include those filariids whose microfilariae may be seen during microscopic examination of the blood of domestic animals:

1. *Setaria equina*, the peritonial worm of horses.
2. *Setaria cervi*, the peritonial worm of cattle.
3. *Elaeophora schneideri*, the arterial worm of sheep.
4. *Dirofilaria immitis*, the heartworm of dogs and occasionally cats.
5. *Dipetalonema reconditum*, the subcutaneous worm nonpathogenic filariid of dogs.

<u>Direct examination of the blood</u>

1- Put 1-2 drops of fresh blood on clean slide .

2- Put a coverslip on the blood drops .

3- Examine the smear under a microscope .

2- Hematocrit (Buffy coat) method:

This method is a concentrated technique, used for diagnosis microfilariae. The procedure as follows :

1- Draw fresh whole blood into a microhematocrit tubes, as for a routine PCV test .

2- Centrifuge for 3 minutes .

3- Read the PCV, if desired, then find the buffy coat layer between the red blood cells and plasma .

4- Using a file, scratch the tube at the level of the buffy coat. Carefully snap the tube and save the part of the tube containing the buffy coat and plasma .

5- Gently tap the tube onto a slide, ejecting the buffy coat layer with a small amount of plasma. Save the rest of the plasma for a total protein determination, if desired .

6- Add a drop of saline and a drop of stain to the buffy coat. Apply a coverslip and examine for microfilariae .

3- Modified Knott's technique :

This method is a concentration technique. Used for microfilariae exanimation. It is a simple technique that allows differentiation of microfilariae through staining them while lysing the red blood cells to make the microfilariae more visible. The procedure as follows :

1- Collect a sample of venous blood, do not allow it to clot, and use it promptly .

2- Mix 1 ml of blood with 9 ml of 2% formalin in a centrifuge tube .

3- Centrifuge the tube at 1300-1500 rpm for 5 minutes .

4- Discard the supernatant fluid, leaving the sediment at the bottom of the tube .

5- Add 2-3 drops of 0.1% methylene blue to the sediment. Using a pipette, mix the sediment with the stain .

6- Place a drop of this mixture onto a glass slide . Apply a coverslip and examine for microfilariae using the 10X objective .

Filter technique:

Filter techniques are a common method used in veterinary practices for detection of microfilariae in the blood. Used test kit contain complete with filter, lysing solution, stain, and directions for use.

1- Most kits require 1 ml of whole blood to test for heartworm.
2- The blood is mixed with nine parts of lysing solution and passed through a filter.
3- The filter is rinsed, removed, and placed on a slide.
4- A drop of stain is added, a cover slip is applied, and the filter is microscopically examined for microfilariae.

Mites

The common genus of the mites include: *Sarcoptes, Pseroptes, Chorioptes, Notoedres, Knemidocoptes,Otodectes, Trixacarus, Demodex* and *Cheyletiella*. Most common mite infestations are diagnosed by skin scraping as follows:

1. The area of skin to be scraped is coated with mineral oil.
2. The site selected for scraping should be at the periphery of a lesion or the predilection site of the suspected parasite.
3. The blade should be scraped back and forth over the skin until capillary bleeding is occurring.
4. The debris collected on the scalpel blade is then placed on a microscope slide, coverslipped and examined (10X).

Digestion concentrated technique

Agents that needed 10% KOH and saturated sucrose solution. These technique is most useful in mites, lice that cause epidermal hyperplasia and for concentrating parasite from animals.

1- Mixed scraping material with 3-4 ml of 10% KOH .

2- Heat gently in a beaker covered by funnel the concentration should be retaining to the digestion solution.

3- When hair has dissolved remove mixture from heat and allow to cool.

4- Centrifuge and discard the supernatant.

5- Resuspend the sediment in water and recentrifugation .

6- Discard the supernatant and examine the sediment. If no parasite found resuspended sediment in the saturated sucrose solution and centrifuge.

7- Remove the parasite from the top of solution with wire loop for examination.

Ticks

Ticks are usually larger than mites, ranging in length from 3-4 mm, and may reach 12 mm or more in case of engorged females. There are two groups of ticks are parasitic:

1. Argasidae (soft ticks) that have a leathery integument.

- *Otobius megnini* (their larval and nymphal stages feed on horses, cattle, goats and dogs.
- *Argas persicus* (fowl ticks).

2. Ixodidae (hard ticks) that have a hard dorsal shield called the scutum.

- *Boophilus spp.*
- *Rhipicephalus spp.*
- *Dermacentor spp.*
- *Amblyomma spp.*
- *Ixodes spp.*

Ticks should be submitted for identification in 70% alcohol.

Identification of adult ticks:

Identification of adult ticks to the level of genus is not difficult in veterinary practice. One of the most useful magnification for identifying the genus of a hard tick is the shape of the basis capitulum and mouthparts, the decoration of the scutum, or ornamentation, is also helpful.

- Use a magnifying glass or other source of low magnification will be helpful.
- For close observation (e.g. mouthparts), standard compound microscope can be used.

Identification of immature ticks:

Identification of immature tick stages is much more difficult.

- Larvae can be recognized by the presence of only 6 legs.

- Nymphs can be recognized by the presence of 8 legs, and absence of a genital pore.