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Received: 14 May, 2018

Accepted: 13 August, 2018

Published: 14 August, 2018

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Research Article

Examination of feces

Fecal examination is indicated in diarrhoeic diseases, constipation, anorexia, jaundice, hepatitis, lungworm infection, anemia, weakness, low milk production and in dysentery. Examination of feces is mainly carried out for the detection of parasites, their ova/larva/oocyst, blood and other foreign material. The fecal examination for diagnosis of parasitic infections is probably the most common laboratory procedure performed in a veterinarian clinic. Sometimes cultural examination of feces is performed for isolation of bacteria or virus in order to establish the etiology of diarrhea/dysentery. The bacterial or viral antigens are also demonstrated in feces using immunological methods like immunofluorescence, enzyme linked immunosorbent assay and dot immunobinding assay. Parasitic worm eggs from the respiratory system may be coughed into the throat and swallowed and appear in the feces. Parasitic forms seen under the microscope have characteristic morphologic features that with a little practice can be diagnostic for a particular parasite. Fecal examination can reveal the presence of parasites in many parts of the animal body. This clinic will concentrate on parasites inhabiting the digestive system. Fecal examination should be done on fresh samples. If fecal samples are used after being in the environment for hours or days accurate reading of parasite indicators cannot be guaranteed. Also, free-living nematodes rapidly invade a fecal sample on the ground and can confuse diagnosis. Several grams of feces should be collected immediately after observing defecation [1].

Collection of fecal sample in domestic animals

When collecting fecal samples, first make certain that the feces is from the animal in question. Secondly, secure a fresh sample that is free from rocks, soil, bedding, and other foreign materials. Place the fecal sample in a plastic vial, glass jar, waxed cup, or plastic bag. If the examination does not follow closely after collection, preserve the sample in a refrigerator [2,3]. In large animals, one should wear gloves and lubricate

the hands with water and collect feces from rectum. If feces is not present in rectum, stimulate the mucosa of rectum with finger, the animal will defecate within few minutes [4].

Collect 5-10 gm feces in a clear, dry glass container. If the feces are to be collected for cultural examination, it should be collected in sterilized glass vials [5]. In small animals like calves, sheep, goat and dogs, the feces is collected by using index finger. In poultry, the cloacal swabs are collected for examination. If the processing of a fecal specimen must be delayed, it may be: Refrigerated (but not frozen) for several days (not recommended for samples with live larvae that you intend to examine using the Baermann technique) and Fixed, 10% formalin (5% formalin-saline is better for protozoal cysts). Add fixative to feces at a ratio 3:1 (v:v) and mix well. Do not fix samples intended for use with the Baermann technique the larvae may destroy by the formalin [6].

The procedure how to take feces:

- * The operator places an obstetrical sleeve on one arm
- * The arm is formed into a cone and the animal's tail held to one side with the opposite gloved hand.
- * Gentle pressure is applied to the anal sphincter until penetration into the rectum is obtained.
- * A fecal aliquot of sufficient size for the intended laboratory procedure is scooped with the sleeved hand and removed from the animal.
- * The fecal sample is placed in a separate container or the obstetrical sleeve is inverted off the arm such that the fecal sample is trapped inside.
- * Small calves, sheep, goats, and swine: restrain manually. Gently pass a gloved, lubricated finger through the anus and massage the rectal wall to stimulate rectal evacuation.
- * If feces are not produced, collect feces with finger.

Techniques of fecal examination in veterinary laboratory

After fecal sample is collected from domestic animals, it would present to laboratory for examination. The methods

for examination of collected fecal sample are direct smear, flotation, and gross examinations.

Direct fecal smear examination

The direct smear method involves mixing a very small amount of feces with a water or saline solution. Place the mixture on a slide, overlay it with a cover glass, and examine the entire smear under a low power microscope. Observe the mixture for worm eggs and larvae and protozoa trophozoites and cysts. The use of a small amount of feces and the presence of fecal debris lower the reliability of this examination method [7,8].

The fecal sample can be examined by qualitative (flotation and sedimentation) or quantitative methods (Mack master method) if the direct smear method interpreted negative result.

Flotation examination

Examination by the flotation method is more complex and requires more time, but is usually more accurate than the direct smear [8]. The feces is mixed with either saturated sugar solution, saturated salt solution, saturated sodium nitrate solution, 41% magnesium sulfate solution or 33% zinc sulfate solution. The flotation method uses the principle that most fecal particles fall to the bottom of the tube or vial. Parasite eggs and cysts rise to the top of the salt or sugar solution, which is the result of a weight difference between feces, parasite eggs, and cysts within the solution. In pure water, parasite eggs and cysts settle to the bottom rather than float; whereas in the salt or sugar solution, they float due to the higher density of the solution [9].

Instrument and some reagent required:

- Plastic containers or two beakers
- Saturated sugar,
- Saturated salt,
- Saturated sodium nitrate,
- Magnesium sulfate a
- Zinc sulfate solutions.
- Gauze or tea strainer, double layer of cheesecloth
- Measuring cylinder
- Centerfuge
- Strirring rod
- Mortar and pistle
- Test tube
- Test tube rack
- Pipettes

- Balance
- Teaspoon
- Microscope
- Slide and cover slide

The procedure how to process the fecal floatation is listed as following steps [9,10].

The flotation method involves the use of a flotation solution that has a specific gravity, greater than 1.2, such as a salt or sugar solution (Figure 6.1)

- Simple floatation, about one gram of feces is taken and grinded and mixed with 42 ml of saline water
- Then filter it through a fine sieve or muslin cloth or gauze in to test tube or cylinder until it form meniscus (up to top of tube).
- A clean glass slide or cover slide is placed on the mouth of test tube or cylinder. Then left it for 10– 15 minute at room temperature without disturbance, then remove coverslide/ slide and examine under 10 x of microscope.
- In centrifugation floatation, the first step as simple floatation is similar, except this method use centrifugation. Mixed the contents and centrifuge at 1500 rpm for 5 minute, the tube is taken out and placed without disturbance. Transfer the small amount of superficial contents of the tube on a clean and dry glass slide.
- Place the cover slip on a slide and examine it under a microscope and the parasite ova may be observed under microscope as Figure 6.2.



Figure 1: The step of fecal floatation. A. Mix 2 to 5g of feces with approximately 10ml of flotation solution in a disposable cup until the consistency is uniform. B. Strain the mixture. C. Pour the strained mixture into a 15-ml centrifuge tube. D. Fill the tube with flotation solution to form a slight positive meniscus; do not overfill the tube. E. Place a coverslip on top of tube. F. Put the tube in the centrifuge, make sure the centrifuge is balanced, and spin at 1,200 rpm for 5 minutes. G. Remove the tube and let it stand for 10 minutes. H. Lift the coverslip directly upward and place on a glass microscope slide and examine the entire area under the coverslip at 10X magnification.

- The unfiltered material left on sieve or muslin cloth is examined for tapeworm segment.

Sedimentation method

The sedimentation technique is qualitative methods for detection of trematode egg in feces of domestic animals. The majority of trematode eggs are too large and heavy to float reliably in the flotation fluids normally used for nematode eggs. They do however sink rapidly to the bottom of a faecal/water suspension and this is the basis of the faecal sedimentation technique.

Instrument and some reagent required:

- Plastic containers or two beakers
- Gauze or tea strainer, double layer of cheesecloth
- Measuring cylinder
- Stirring rod
- Mortar and pestle
- Test tube
- Centrifuge
- Test tube rack
- Methylene blue
- Pipettes
- Balance
- Teaspoon
- Microscope
- Slide and cover slide

Procedures:

- Weigh or measure 3 g of faeces and grinded it, then transfer it into cylinder [1].

- Pour 40–50 ml of tap water into cylinder.
- Mix faeces and water thoroughly.
- Filter the suspension through a tea strainer or double-layer of cheese cloth into cylinder [2].
- Pour the filtered material into a test tube. And allow to sediment for 5 minutes
- Remove the supernatant with a pipette very carefully.
- Re-suspend the sediment in 5ml of water.
- Allow to sediment for 5 minutes.
- Discard the supernatant carefully.
- The eggs of liver flukes are heavy and do not float on water and settle in sediment.
- Stain the sediment by adding one drop of methylene blue
- The dyes stain the faecal particles a deep blue or green leaving the trematode eggs unstained. The egg of trematode is revealed in figure
- Transfer a small drop of the stained sediment to a microscope slide using a pipette.
- Cover droplet with a coverslip and examine under a microscope at 10 x 10 magnifications.

McMaster egg counting technique

McMaster is the quantitative methods for determining the number of nematode eggs per gram of feces in order to estimate the worm burden in an animal. Advantage of this technique is quick as the eggs are floated free of debris before counting.

Equipment required:

- * Beakers or plastic containers
- * Balance
- * Tea strainer or cheesecloth
- * Measuring cylinder
- * Stirring device (fork)
- * Pasteur pipettes and rubber teats
- * Flotation fluid
- * McMaster counting chamber (Figure 3)
- * Microscope

Procedure of McMaster techniques [8,13-15]:

- * Weigh out 2 gm of feces and transfer in to container 1
- * Add 60 ml of saturated salt solution into container 1

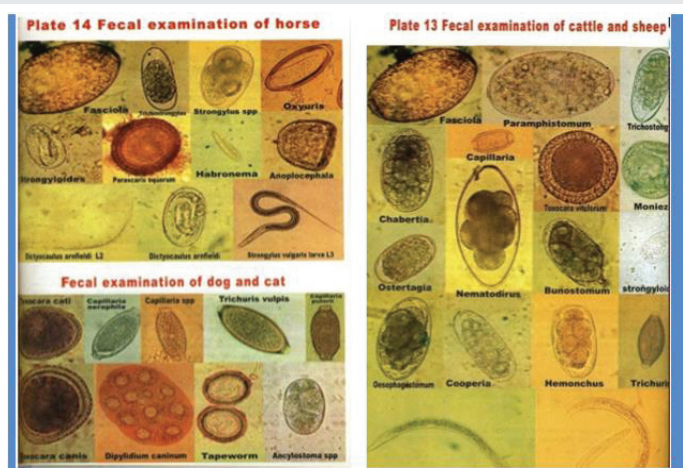


Figure 2: The larvae eggs and larve of different species of helminthes in domestic animals.



Figure 3: Macmaster two chamber and equation for egg per gram of faeces.

- * Mix feces with saturated salt solution by stirring device
- * Filter fecal from container 1 into container 2 or cylinder by gauze or sieve
- * Take a sub- sample with a Pasteur pipette from container 2
- * Fill both side of the McMaster counting chamber with the sub sample
- * Allow the counting chamber to stand for 3-5 minutes
- * Examine the sub sample of filtrate under a microscope at 10x 10 magnification
- * Count all eggs and coccidian oocytes within the engraved area of both chambers
- * Focus first on the etched lines of the grid, then go down a tiny bit, the egg will be floating just below the top of the chamber.
- * The calculation of egg from chambers is: Multiply the total number of eggs in the 2 chambers by 100= eggs per gram (EPG) or multiply the total by 50. This gives the EPG (egg per gram of feces) of faeces. (Example: 50 eggs seen in chamber 1 and 100 eggs seen in chamber 2 = $(50 + 100) \times 50 = 25,000$ epg.)

Baermannapparatus technique

In 1917, while working in Java, the Dutch physician Dr. Baermann developed a simple method for isolating nematodes from soil [16]. Nowadays, the veterinarians and nurses are using this method for the extraction of live larval stages of nematode parasites from feces. The Baermann technique is used to isolate lungworm larvae from faecal samples and infective larvae from faecal cultures. It is based on the active migration of larvae from faeces suspended in water and their subsequent collection and identification [17].

Berman technique makes use of two characteristics of parasitic larval nematode behavior like; the warmer it is, the more active the larva (37 to 40 °C is as warm as you want to get), and, in addition, some larvae are thermotaxtic and will move towards the warmer water under the filter paper. And the other characteristic is like most parasitic larval nematodes are poor swimmers. Therefore, the following events take place when the sieve is placed in the water: The larvae will be moving around in a random fashion and within any given time interval

some of them will migrate through the tissue and fall into the water. Because they can't swim they sink to the bottom and over time a number accumulate there. The more active the larvae are (i.e. the warmer the water) the greater the number of larvae that accumulate at the bottom in a given time interval. The longer you wait, the more larvae will fall to the bottom of the dish, but with time, the fecal sample breaks down and begins to pass through the tissue leading to an accumulation of sediment along with the larvae [8,18].

Equipment required:

- * Funnel
- * Funnel stand
- * Rubber or plastic tubing
- * Rubber bands
- * Clamp or spring clip
- * Cheesecloth
- * Simple thin stick
- * Strainer
- * Microscope
- * Test tube
- * Pasteur pipette
- * Small petridish

The procedure ofbaermann apparatus technique [18] (Figure 4):

- * First of all, construct the funnel with its standing; fit a short piece of tubing which is closed at one end with a clamp or string clip to the stem of a funnel
- * Support the funnel by a stand
- * Weigh the fecal culture/ faeces about 5- 10 gm and place on a piece of double layer cheesecloth
- * Form the cheesecloth around the faeces as a pouch
- * Close the pouch with a rubber band
- * Fix a supporting stick under the rubber band
- * Place the pouch containing faecal culture material or faeces in the funnel and trim the surplus cheesecloth off
- * Fill the funnel with lukewarm water and covering the faecal material
- * Leave the apparatus in place for 24 hours, during time larvae actively move out of faeces and ultimately collect by gravitation in the stem of the funnel.
- * Draw 10-15 ml of fluid from the stem of the funnel into a test tube or petri dish.



Figure 4: The procedure of baermann apparatus technique for diagnosis of larvae of nematode like lung worm. A. Place a sieve in a custard dish or other similar container, B & C. Spread about 10grams of feces on a piece of tissue paper and place it into the sieve, D&E. Place warm water in the custard dish until it just covers the feces, taking care not to disrupt the feces. Allow to sit for at least one hour, f. Lift off sieve, G. I and K. using a pasteur pipet, remove a drop of the sediment at the bottom of the tube and place it on a microscope slide for examination.

- * Leave the tube to stand for 30 minutes. Remove the supernatant with a Pasteur pipette.
- * Transfer a small aliquot of the remaining fluid using a Pasteur pipette to a microslide, add a drop of iodine and cover with a coverslip. For positive samples a transfer of larvae to a microslide for identification at 10 x 10 magnifications may be required. It is important to differentiate between *Muellerius capillaris*, *Dictyocaulus* spp and other species as the treatment is different.

Examination of feces for blood

Take 2-3 ml of saturated solution of benzidine in glacial acid in to small test tube, and then add 2-3 ml of hydrogen peroxide. The feces 2 gm is mixed with distilled water and fecal suspension is poured in the benzidine solution drop by drop. If blood is present in faeces, it would generate blue colour.

Preparation of fecal culture

Many nematode eggs are alike and species such as *Haemonchus*, *Mecistocirrus*, *Ostertagia*, *Trichstrongylus*, *Cooperia*, *Bunostomum*, and *Oesophagostomum* cannot be clearly differentiated from the eggs in faecal samples. For these parasites, differentiation can be achieved by the use of faecal cultures. They provide a suitable environment for the hatching and development of helminth eggs into the infective stage (L_3).

Equipment required for fecal culture:

- Fork
- Spoon depressor

- Spatula
- Water
- Jars
- Containers
- Charcoal (It should be added with equal amount with feces and used to make moist feces to become damp.)

Procedure of fecal culture:

- Break up collected faeces finely using a mortar and pestle or stirring device
- Faeces should be moist and crumbly
- If faeces are too dry, add water
- If faeces are too wet, add charcoal until the correct consistence is obtained
- Transfer the mixture to jar or other containers
- Leave the culture at room temperature for 14-21 days, at this time all larvae should have reached the infected stage
- If an incubator is available at laboratory, the culture should be placed at 27 °C and left for 7 -10 days.
- Add water to culture regularly, every 1-2 days
- Larvae are identified using baermann technique

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