University of Mosul
Lecture No.: 4
College of Veterinary Medicine
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Unit of Scientific Affairs
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Lecture title: **Coprological examination – Quantitative method.**

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Summary:

Quantitative techniques for separating and concentrating eggs /larvae.

The simplest and most effective method for determining the number of eggs or Oocysts per gram of feces is the Mc-Master technique described below

Principle:

The McMaster counting technique is a quantitative to determine the number of eggs present per gram of feces (e.p.g.). A flotation fluid is used to separate eggs from fecal material in a counting chamber (Mc Master) with two compartments. The technique described below will detect 50 or more e.p.g. of feces.

Advantages and Dis Advantages:

This technique can be used to provide a quantitative estimate of egg output for Nematodes, Cestodes and Coccidia. Its use to quantify levels of infection is limited by the factors governing egg excretion.

Dis Advantages

It will not demonstrate Trematode eggs which have a higher specific gravity density. required a special counting chamber.

Procedure of McMaster technique:

1-Weigh 4g of feces and place into container (1).
2- add 56 ml of flotation fluid.
3- mix the contents thoroughly with a stirring device (fork, tongue blade).
4-filter the fecal suspension through a tea strainer or a double – layer of cheese cloth into container (2)
5-while stirring the filtrate in container (2), take a sub-sample with a Pasteur pipette.
6-fill both sides of the McMaster counting chamber with the sub-sample.
7-allow the counting chamber to stand for 5 minutes (this is important).

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8-Examine the sub-sample of the filtrate under a microscope at 10 Magnification.

9-: count all eggs and Coccidia Oocysts within the engraved area of both chambers.
10-: the number of eggs per gram of feces can be calculated as follows: add the egg counts of the two chambers together.
Multiply the total by 50. This gives the e.p.g. of feces. Example: 12 eggs chamber 2 = (12 + 15) × seen in 50. 50= 1350 e.p.g.).
In the event that the McMaster is negative (no eggs seen), the filtrate in container (2) can be used for the simple flotation method.

4- Preparation of Fecal cultures:

Principle

Many Nematode eggs are alike and species such as Haemonchus, Ostertagia, Trichostrongylus, Cooperia, Bunostomum, and Oesophagostomum, cannot be clearly differentiated from the eggs in fecal samples. For these parasites, differentiation can be achieved by the use of fecal cultures. They provide a suitable environment for the hatching and development of helminthes eggs into the infective stage (L3).

The identification of parasite species present is an important component of initial surveys and of the investigation of clinical disease caused by gastrointestinal Nematodes.

Procedure:

1- : Break up collected feces finely using a stirring device.
2- : feces should be moist and crumbly.
If feces are too dry, add water.
If feces are too wet, add charcoal (or sterile bovine feces) until the correct consistency is obtained.
3- : transfer the mixture to jars or other containers.
4- : leave the culture at room temperature for 14-21 days, by which time all larvae should have reached the infective stage.

5- : if an incubator is available, the culture can be placed at 27 degree and left for 7 to 10 days.
6- : add water to culture regularly (every 1-2 days).
7- : larvae are recovered using the Baermann technique.

5- Isolation and identification of Lung worm larvae harvested from fecal cultures (the Baermann technique

Principle
The Baermann technique is used to isolate lungworm larvae from fecal samples and infective larvae from fecal cultures. It is based on the active migration of larvae from feces suspended in water and their subsequent collection and identification.
Procedure
1- Fit a short piece of tubing which is closed at one end with a clamp or spring clip, to the stem of a funnel of appropriate size.
2- Support the funnel by a stand.

3- weigh or measure about 5-10 g of fecal culture /feces and place it on a piece of double –layer cheesecloth.

4- form the cheesecloth around the feces as a(pouch)

5- close the pouch with a rubber band.

6- fix a supporting stick under the rubber band step -1.fix a supporting stick under the rubber band –step 2.

7- place the pouch containing fecal culture material or feces in the funnel. Trim the surplus cheesecloth off.

8- fill the funnel with Luke warm water, covering the fecal material.

9- leave the apparatus in place for 24 hours, during which time larvae actively move out of feces and ultimately collect by gravitation in the stem of the funnel. Examination for lungworm

10- draw a few ml of fluid from the stem of the funnel into a small petri dish.

11- examine under dissecting microscope for live lungworm larvae (L1). For positive samples a transfer of larvae to a micro slide for identification at 10 × 10 magnification may be required.

It is important to differentiate between *Muellerius capillaries* and other species as the treatment is different.

Examination for infective larvae from fecal culture

12- draw 10-15 ml of fluid from the stem of the funnel into a test tube or other container.

13- leave the tube to stand for 30 minutes. Remove the supernatant with a Pasteur pipette.

14- transfer a small aliquot of the remaining fluid using a Pasteur pipette to a micro slide, add a drop of iodine and cover with a coverslip. Examine under microscope.

15- repeat steps 12 and 13 until 100 larvae have been identified.

16- the count for each species provides an estimate of the composition (%) of the parasite population of the host.

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 uses 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 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.

Parasites of the Gastrointestinal tract:

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Nematodes (Roundworm).
The bovine Trichostrongylus are composed of several genera of nematodes within the Abomasum and small and large intestine of Cattle and other Ruminant. Trichostrongylus type egg are (1-7) type.
These seven-type produce oval. Thin –shelled eggs. They contain four or more cells and are 70 to 120 um long. Some of these ova may be identified to their respective. Identification is usually difficult because mixed infection of bovine Trichostrongyles and identification of species usually can be performed only by Fecal Culture and larvae identification.

Type of Strongyles like egg

Group of (7) type of parasitic type ova of nematodes similar Morphologically. These include 1-7.
1- : *Oesophagostomum columbianum*
2- : *Strongyloides papillosus*
3- : *Bunostomum* spp.
4- : Trichostrongylus axei
5- : Haemonchus contortus
6- : Chabertia ovine
7- : Ostertagia ostertagi

Type of Lung worms

Sheep Lung worm

1. *Dictyocaulus filaria.*
2. *Muellerius capillaries.*
3. *Protostrongylus. Spp.*
4. *Neostrongylus. Spp.*
5. *Cystocaulus nigrescens.*

Cattle Lung worm

1- *Dictyocaulus viviparous*.

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Horse Lung worm

2- *Dictyocaulus arnfieldi*

Dog Lung worm

3- *Filaroides sleri*

References:

Coles, E.H. (1968) Veterinary Clinical Pathology. WB Saunders Company Philadelphia and London,