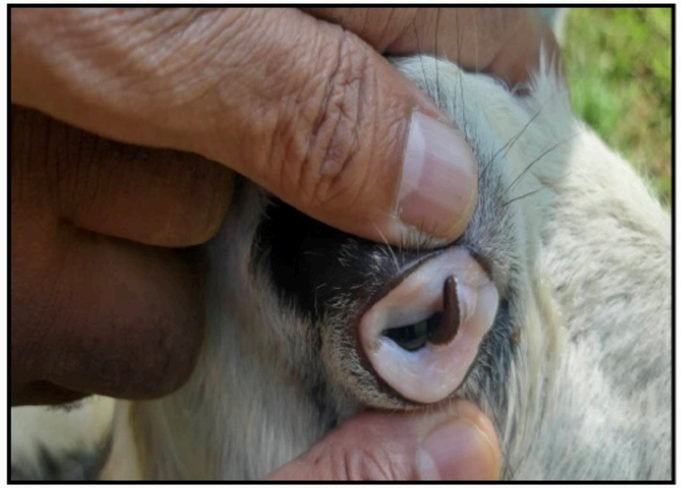
Economic impact and Diagnosis of GI Nematode infections

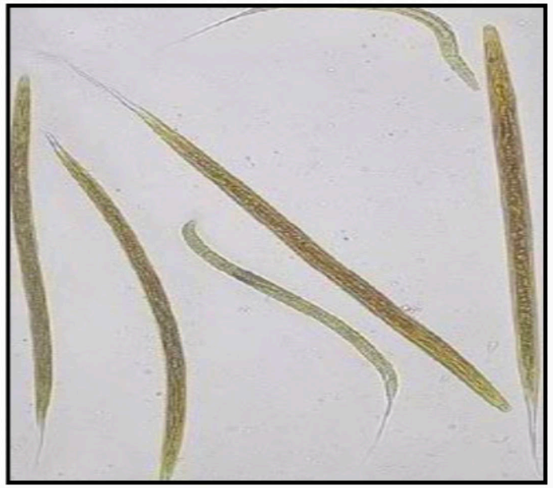
Dr. SENTHILVEL K M.V.Sc., Ph.D.,

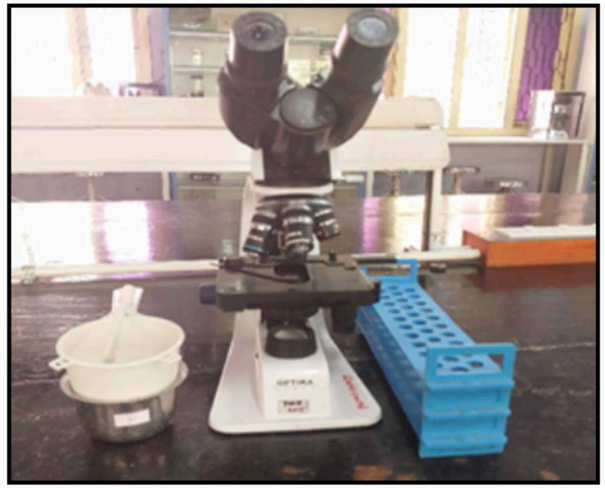
Professor and Head
Department of Veterinary Parasitology,
Veterinary College and Research Institute,
Tamil Nadu Veterinary and Animal Sciences University,
Udumalpet, Tamil Nadu, India - 642 205.
Email Id: senthilvelpara@gmail.com

Economic impact and Diagnosis of GI Nematode infections

- 1. Economic effects of GI parasitism
- 2. Diagnosis of GI Nematode infections
- 3. Faecal culture methods







Economic impact of GI parasitism

GI Parasitism causes

- Reduction in feed intake (10-15%)
- Reduction in weight gain - 20%
- Body weight loss –reduction in market price
- Carcase composition – affect tissue quality and fat deposition

- Devastating effects on young animals - weakness, death
- Affect reproductive performance
- Reduction in wool production
- Cost involved in treatment

Diagnosis of GI nematode infections

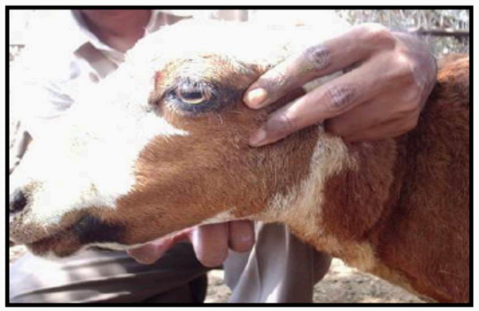
i. Clinical signs

Shared by many diseases and conditions

Presumptive diagnosis by signs, grazing history and seasons

ii. Faecal examination/Feacal egg counts (EPG) / Faecal culture

- Faecal examination - Qualitative
- Lectin binding - Fluorescent peanut agglutination - Haemonchus egg
- Faecal egg counts – Intensity of infection (EPG - Quantitative)
- Faecal culture of strongyle egg – To identify infective L3
- PCR –L1 identification



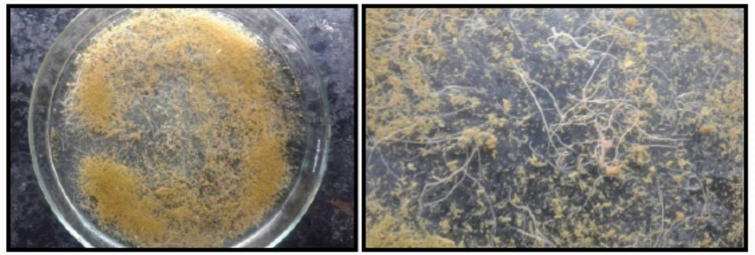
Diagnosis of GI nematode infections

iii. Post mortem examination

- Status of GIN in the rest of herd/flock
- Worm counts can be made
- Significance of number of worms present varies to worm and host species (*Haemonchus* sp -100 numbers & *Ostertagia* sp.- 5000-10000 to produce clinical signs)

iv. Biochemical parameters (supportive)

- Estimation of PCV – Heamonchosis (Decreased PCV level, 33 to 22 %)
- Plasma pepsinogen levels (tyrosin levels >3 IU)- Ostertagiosis



Faecal Examination Methods

A small laboratory unit can be installed at reasonable cost and effort

Materials required

- Compound microscope (objectives-10,40,100X; eye piece 10X)
- Dissecting microscope and hand lens
- Slides, cover slips and labels
- Pasteur pipettes, beakers and petridishes
- Spatulas, tea strainer and measuring cylinders
- Centrifuge and centrifuge tubes
- Plastic bags and plastic specimen containers
- Dissection needles and forceps
- Flotation solutions and measuring cylinders

Points to Remember in faecal collection

- Samples should be fresh, collect directly from the animal's rectum.
- If not, collect faeces immediately after defecation from clean ground
- Flock samples are often pooled
- 5-10 grams of faeces is needed (8-12 pellets) from 10-15 animals in a flock
- Don't mix samples from different groups or pastures
- Examine fresh sample at lab
- Old samples will give a false negative result as the eggs may hatch and not visible

Fresh pellets

MICROSCOPIC EXAMINATION

Eggs discharged by helminths find their way out along with faeces

Different methods of examination are,

I. Direct smear examination

II. Concentration methods

1. Sedimentation Technique
2. Flotation Technique

DIRECT SMEAR EXAMINATION

- Place pin's head size faecal sample on slide along with 3-4 drops of water/io-dine
- Mix and examined under the low power of microscope
- Method is easy and useful in heavy infections

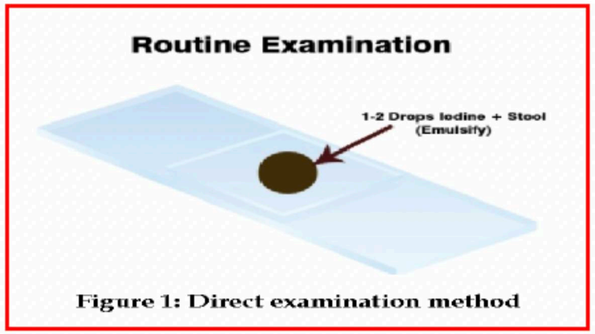


Figure 1: Direct examination method

Faecal Examination By Concentration Methods

Concentration techniques principle works on specific gravity

concentration By Sedimentation

Use solutions of lower specific gravity than the parasitic eggs, thus concentrating the latter in the sediment

1. Direct Centrifugal sedimentation technique
2. Formal-Ether sedimentation technique

concentration By FLOTATION

This method use an emulsifying fluid of a greater specific gravity, allow to float the ova and can be improved by centrifugation.

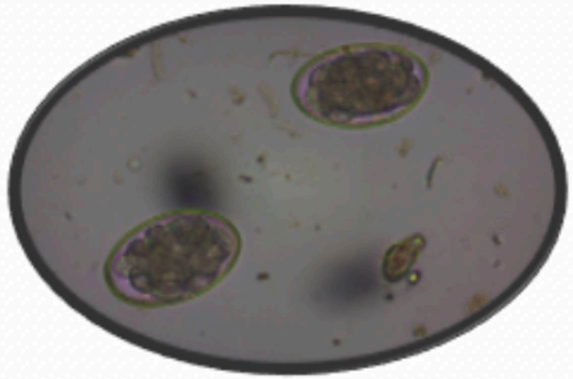
Common flotation fluids

- Saturated solution of common salt (NaCl) - Sp.Gr. 1.18-1.19
- Sheather's sugar/sucrose solution - Sp. Gr. 1.25
- Zinc sulphate solution (32.5 %) – Sp.Gr. 1.18

Sedimentation Techniques

A. Centrifugal sedimentation technique

- Small quantity (2 g) of the faeces
- Add water about 3/4th of the centrifuge tube and mix
- Strained through a sieve to remove all debris
- Transfer into a centrifuge tube & centrifuge (2,000 r.p.m for 2 minutes)
- All the eggs get packed at the bottom of the tube along with the sediment
- The supernatant fluid is poured off
- A drop of the sediment is examined under low power (10 x) of microscope



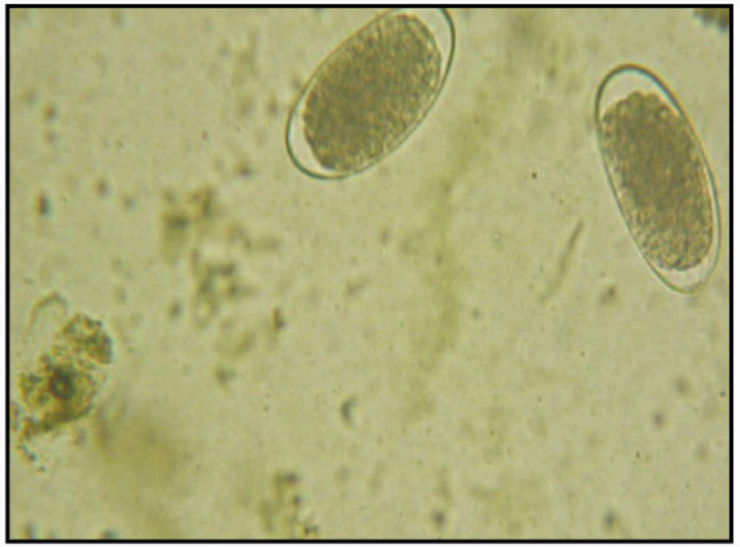
Sedimentation Techniques

- **Advantages**

  Most reliable method and identify the eggs of all types of helminths.

- **Disadvantages**

  Presence of too many faecal materials and fibers may hinder the visualisation of eggs

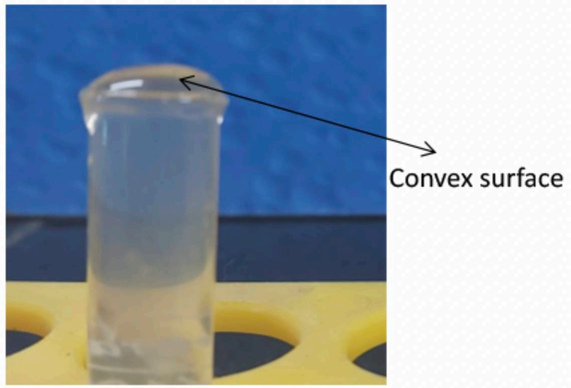


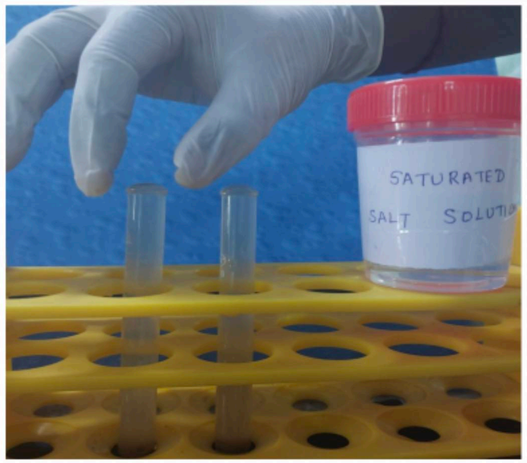
Further Reading-
https://www.rvc.ac.uk/review/parasitology/FaecalSedimentation/Purpose.htm

Saturated salt solution flotation technique

Procedure

- Faecal sediment made from centrifugal sedimentation technique
- Add few ml of floatation solutions and emulsify it.
- Fill up to one third of a small flotation tube with a thick emulsion and fill the tube
- Add one or two drops more of the solution till a convex surface is formed
- Allow it to stand for 10-15 minutes by which all the eggs have floated up
- A drop from the topmost layer is then examined under 10X for egg

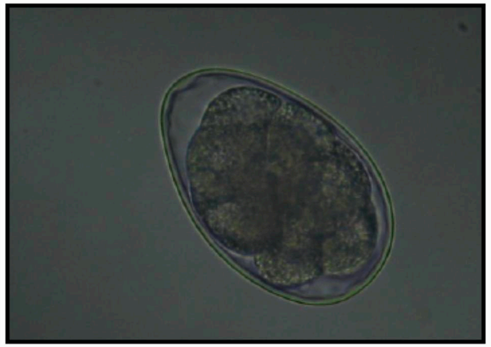




Sheather's sugar centrifugal flotation technique

- Modified Wisconsin Sugar Faecal Worm Egg Flotation Method
- https://midamericaagresearch.net/swine_parasites_guide.php

Flotation methods



Advantages

- Light infections are detected by this technique. Only eggs are clearly visible without the
- hindrance of fiber materials

Disadvantages

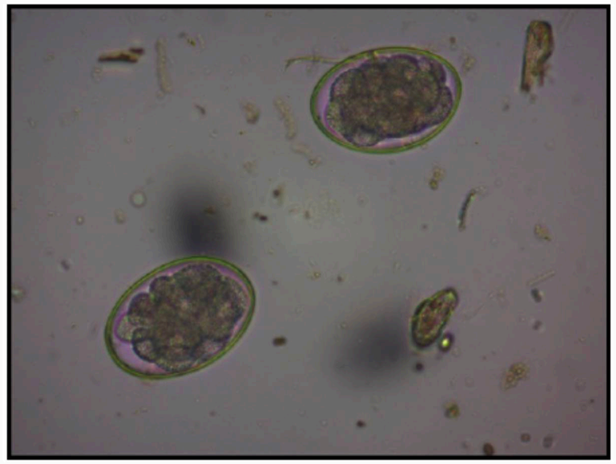
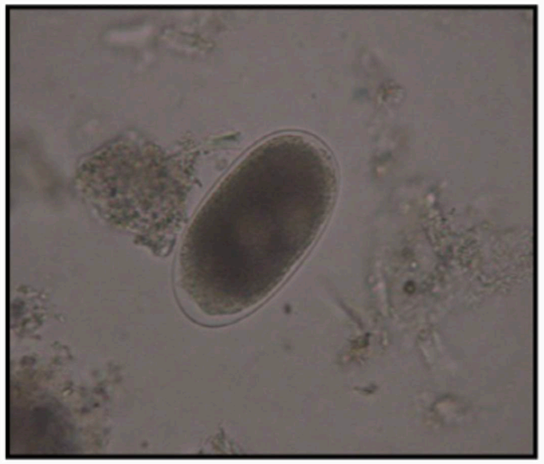
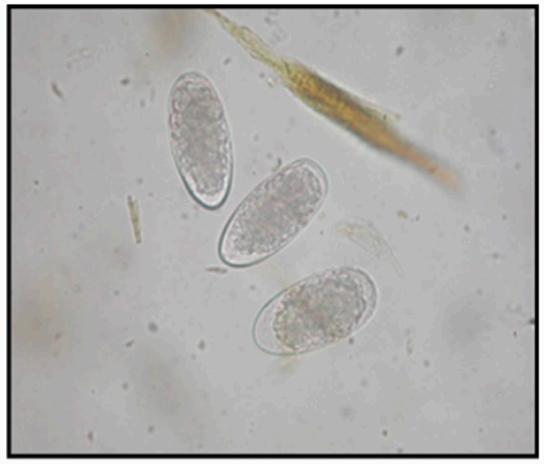
- Useful in the examination of nematode infection only, since eggs of trematodes and most of the cestodes can not be floated up.
- Eggs may distort if kept in a floatation solution for long time

Further reading

- https://www.rvc.ac.uk/review/parasitology/Flotation/General.htm
- http://ecoursesonline.iasri.res.in/mod/page/view.php?id=59989

Interpretation of microscopic examination of faecal sample

→ No egg in the whole sample	Negative (-)
→ Very stray eggs in the whole sample	Mild infection (+)
→ Few eggs in the whole sample	Moderate infection (++)
→ Few eggs in each microscopic field	Medium infection (+++)
→ Many eggs in each microscopic field	Heavy infection (++++)

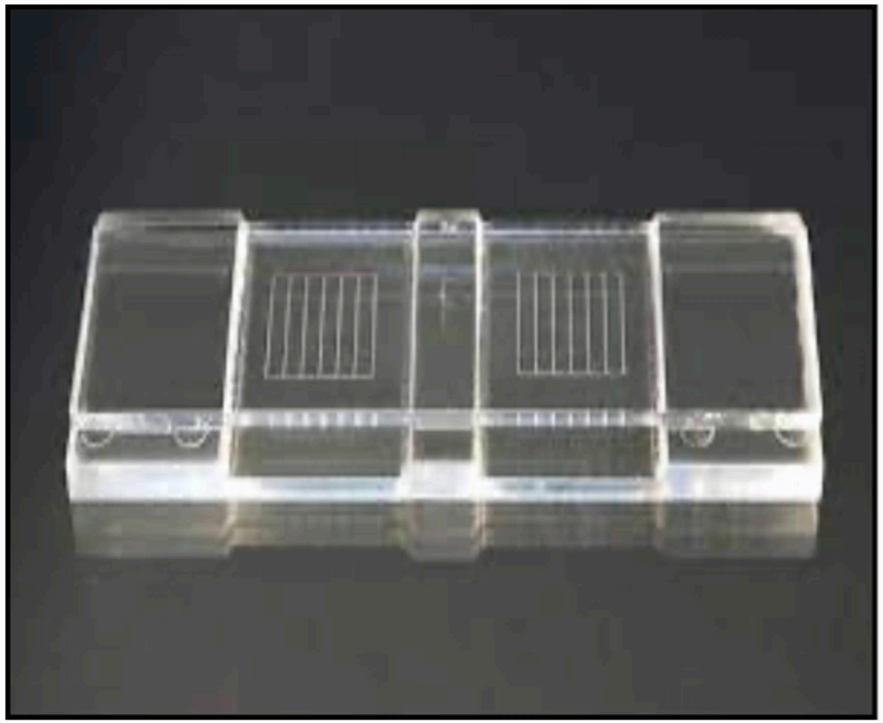




Faecal egg counts (EPG)

- Gives an indication of the worm burden and the intensity of infection
- Used to determine the level of dewormer efficiency
- To identify susceptible and resistant animals
- Used to indicate potential parasite contamination

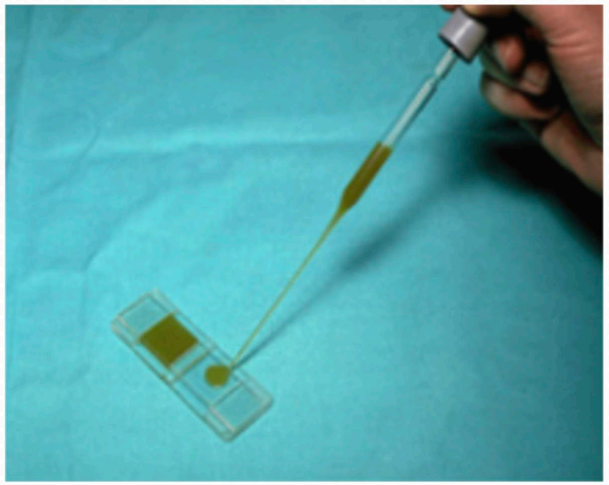
Gorden–Whitloc method - Mc-Master method

- A counting chamber called Mc-Master slide is used.



How to do egg count?

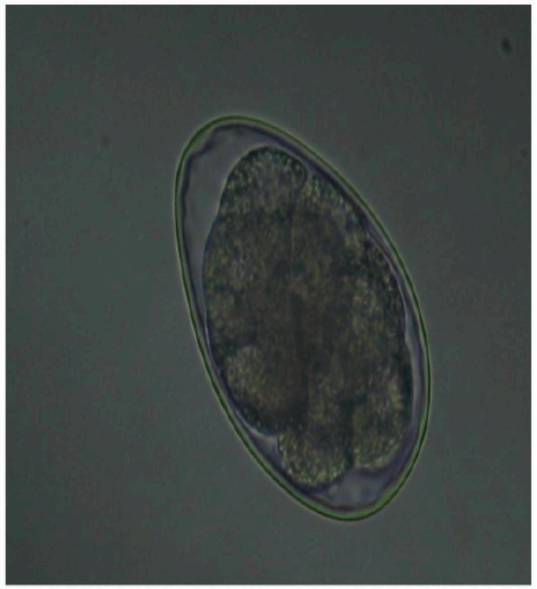
- Weigh out 2 grams of faeces.
- Mix faeces with 60 ml of saturated NaCl until the mixture is homogeneous and filter it
- Fill chamber with little quantity of emulsion, thereby it can hold 0.15 ml of emulsion.
- Repeat the procedure of mixing and drawing off a sample and fill the other chamber.
- Slide will be kept undisturbed, so that all the eggs would have floated up.
- Count all the eggs present in each chamber
- Total number of eggs in the 2 chambers multiplied by 100 is the eggs per gram of faeces (EPG).



What is the significance of faecal egg counts ..

- Can also help in making deworming decision but NOT as main crieteria
- EPG are not mathematically correlated to worm numbers/clinical disease
- Infection is clinical significance, If EPG in lambs is

1500+	in *Haemonchus contortus*
500-2000	in *Trichostongylus spp.*
500-1000	in *Oesophagostomum spp.*
500+	in *Teladorsagia spp.*



Further reading-
https://www.rvc.ac.uk/review/parasitology/eggcount/Principle.htm#
https://www.youtube.com/watch?v=rkSGe-L4Sec

Faecal larval culture

Purpose:

- To diagnose GI nematode infections
- To identify the third stage larvae of GI strongyle nematodes present in faeces which are recovered using faecal culture methods

Faecal culture methods

Principle:

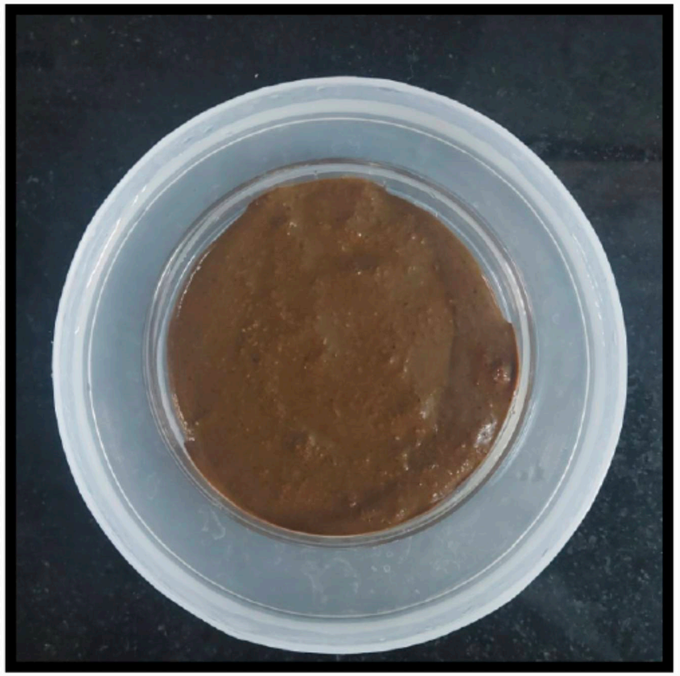
- To provide suitable environment/ conditions for the hatching of eggs and larval development of the infective third stage larvae (L3)
- Recovered L3 can be identified to the genus level

Methods:

- Petri dish method
- Bottle jar method
- Baermann's technique

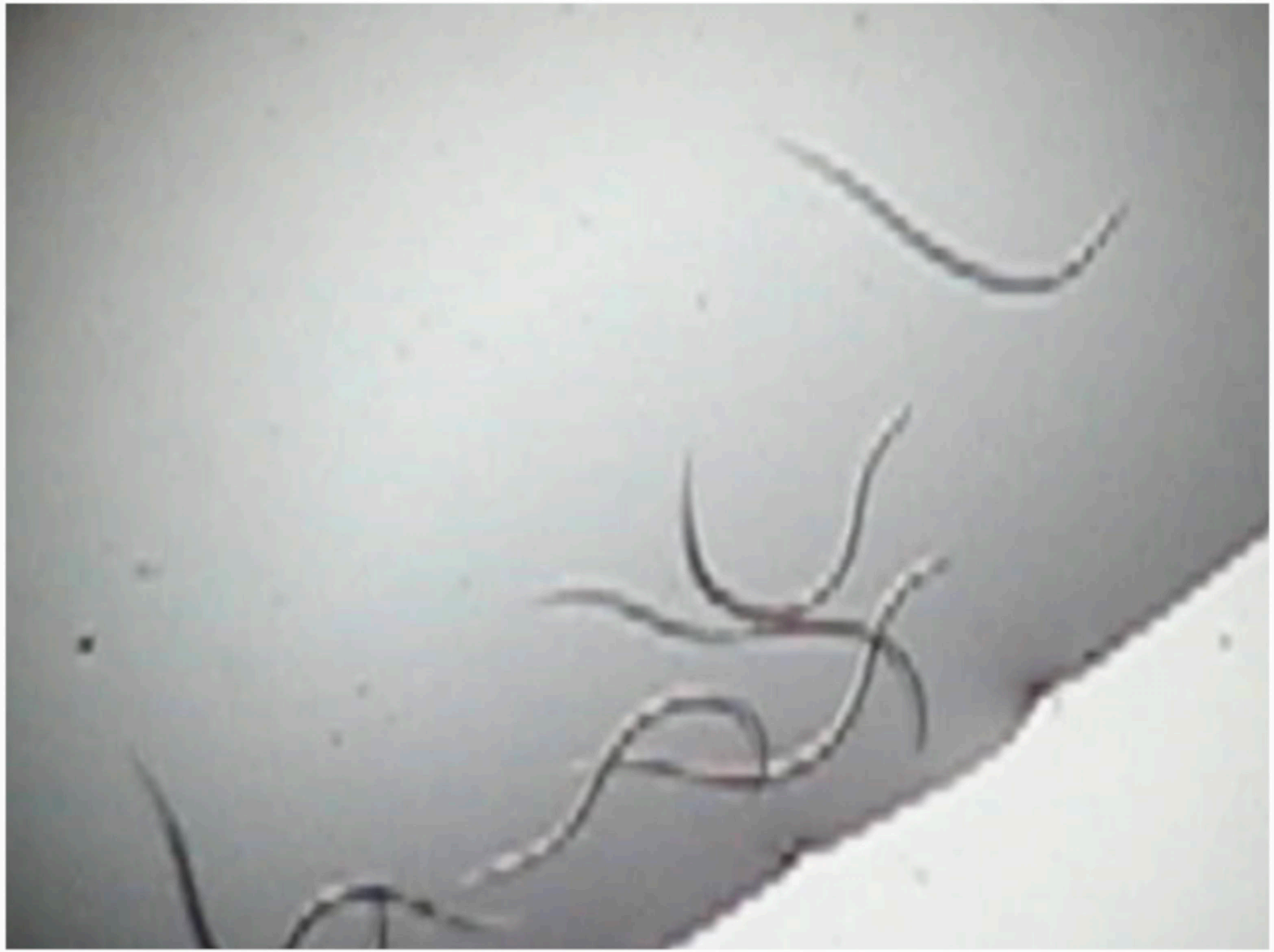
I. Petridish method

- Take a little quantum of fresh faeces in a small petridish
- Add little quantum of water to that faeces and make a uniform mixture
- Place it in a another petridish which contains water for 5-7 days
- Eggs in the faeces would have hatched, reached the L3 and migrated to the other petri dish which contain water.
- Examination of drops of water from the larger dish will reveal L_3



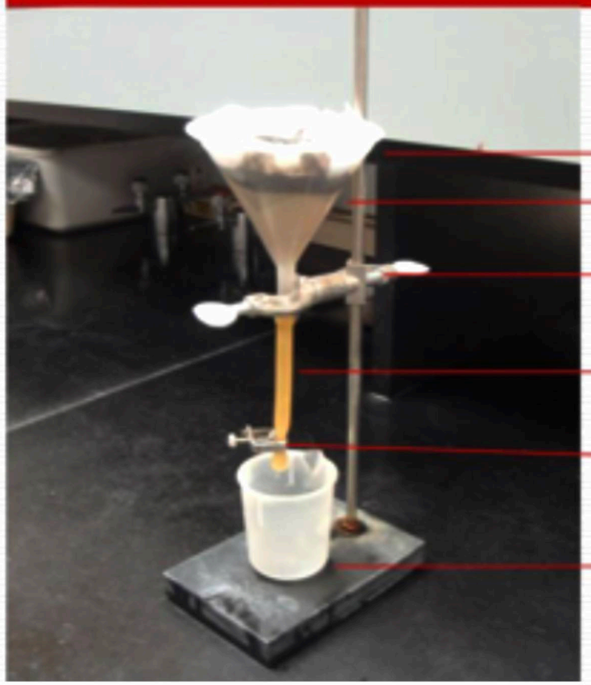
II. Bottle jar method

- Take 20-30g of fresh faeces in a glass jar
- Add little quantum of water to faeces and make a uniform mixture
- Cover it with muslin cloth and keep it in a dark place of the laboratory for 5-7 days
- Enough moisture should be present so that droplets of condensed water can be seen on the sides of jar
- Within 5-7 days ,eggs hatched, reached the infective larvae (L3), and migrated to the walls of jar
- Examination of drops of water from side wall of jar will reveal the infective larvae (L3)



III. Baermann's method

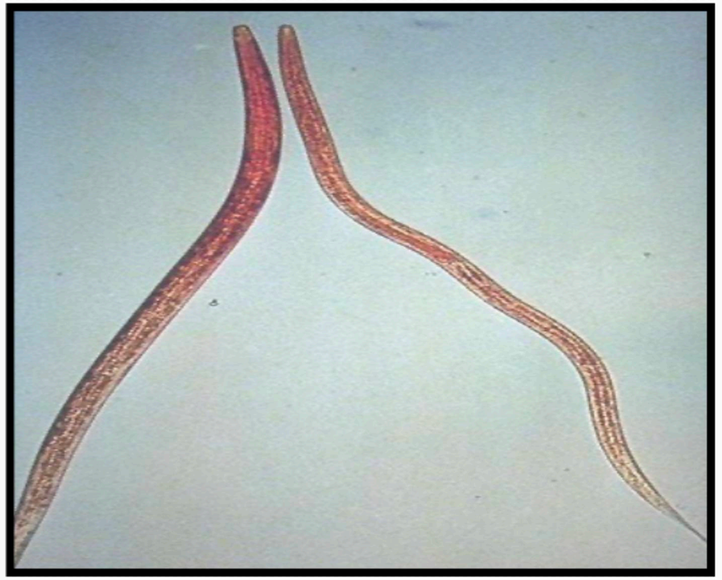
Baermann Funnel set up



- Funnel
- Soil sample over wire mesh
- Clamp to hold funnel
- Rubber tubing
- Clamp
- Beaker

Larval identification

- Once larvae are washed from the jar, collected and concentrated
- A small drop of Lugol's iodine is added to straighten, kill and stain the larvae to study diagnostic features such as
- length of the tail sheath, number and shape of the gut cells, and shape of the head region.
- One hundred larvae are counted and typed under a microscope.

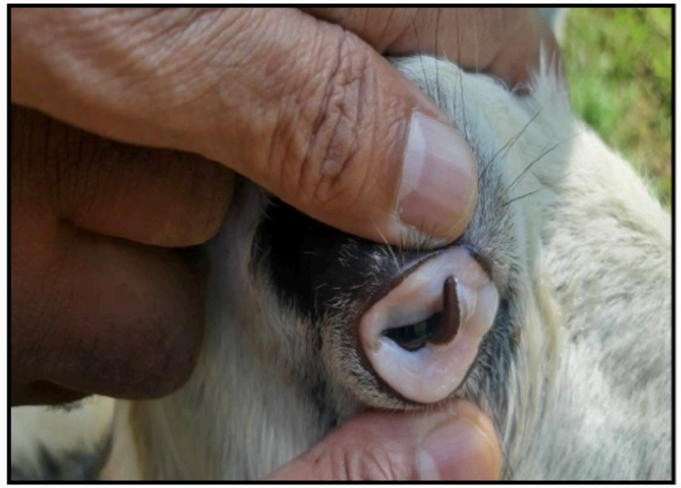


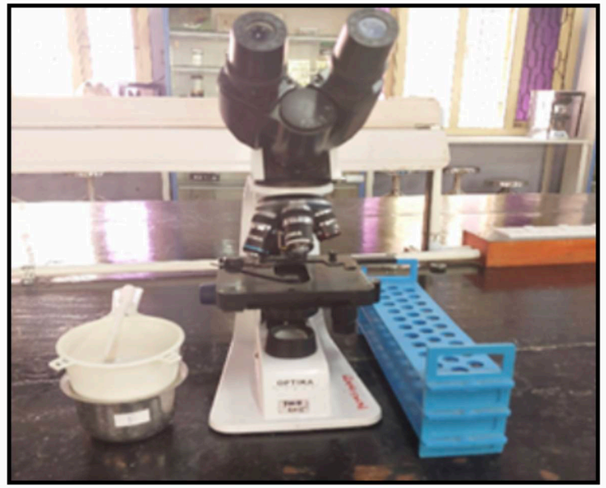
Haemonchus contortus - L_3

Note the kinked tail end

Economic impact and Diagnosis of GI Nematode infections

- 1. Economic effects of GI parasitism
- 2. Diagnosis of GI Nematode infections
- 3. Faecal culture methods





Thank you