ADDIS ABABA UNIVERSITY, COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

DIAGNOSTIC HELMINTHOLOGICAL TECHNIQUES

By: Professor Yacob Hailu

Definition: Veterinary Helminthology is the knowledge and study related to helminthes and their relationship with their hosts. The term helminthes is primarily used to denote parasitic worms.
Helmins (Helminthos) is a Greek word, which stands for worm. Diseases caused by worms are generally known as Verminosis (Helminthosis).
The parasitic helminthes comprise **three (3) Phyla** of Veterinary importance:

Phylum Nemathelminthes

- Class Nematoda (Roundworms)
- Class Acanthocephla (Thorny headed worms)

Phylum Platyhelminthes

Class Trematoda (Flukes)

Class Cestoda (Tapeworms)

Phylum Annelida

- Class Hirudinea (Leeches)

Helminth Morphology

- Trematodes: dorsoventrally flattened but not segmented
- Nematodes: roundworms
- Cestodes: dorsoventrally flattened and segmented

Nematodes belong to the phylum Nemathelminths while Cestodes and trematodes belong to plathyhlminthes

Diagnosis of helminth infections

Helminth parasites are parasitic worms that feed on a living host to gain nourishment and protection, causing poor nutrient absorption, weakness and disease in the host. Helminth parasites include nematode, trematode and cestode. These parasites feed on the tissue or body fluid or competing directly animal food. Diagnosis of Helminth parasites is important to prevent the constraint of these parasitic infections. It is the process of determining the cause of the disease or how to identify a particular parasite species. Veterinary parasitology as a clinical subject endows the future veterinary specialists with theoretical knowledge and correct practical skill of diagnosis, treatment, therapy and prophylaxis of invasive disease of animals. It is frequently impossible to make correct diagnosis based on clinical signs only because they are

similar in majority of helminthiasis that is why helminthiasis are being usually diagnosed by spotting parasitic worm, their fragment, egg and larvae. Diagnosis of infection of parasitic both qualitative and quantitative is largely still depend on relatively inaccurate methods such as fecal worm egg count without which no indication can be obtained of the identities of most of the common worm genera, excepting for those genera with morphologically distinct ova, Although some progresses have been made with computerized identification measuring first stage larvae of protostrongylids and third stage of larvae or length of sheath tail extension can aid identification. Generally, control and treatment of disease can be successful only when preceded by accurate diagnostic techniques. In laboratory and field diagnosis of helminth infection , there are a great variety of techniques, such as direct smear, sedimentation, flotation, McMaster, Baermann technique, molecular technique, postmortem examination, fecal egg count, larvae count and pasture larvae count, Correct diagnostic techniques important to identify parasite species level.

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Why accurate diagnosis is required?

- Proper diagnosis of an infection is important for
    - early detection of parasitism
    - prevention and control
    - evaluation of interventions
    - verification of local disease elimination and resurgence
- The implementation of a diagnostic approach should consider:
    - simplicity and robustness
    - sensitivity and specificity
    - time-consuming or not
    - Inexpensive or expensive: time and resources required per test
    - the techniques needed for large-scale application must be based on cost-effectiveness,

- Many of the techniques used in parasitological works depend on depend on which parasite to be isolated, diagnose, studied etc…
- From which material to separate
- From what to differentiate: we have to know clearly and differentiate properties between the parasite and material from which they are to be separated
- The properties utilized include: particle size, specific gravity, the activity of the live worms and their resistance to digestive enzymes or to corrosive chemicals
- Deep knowledge of parasite biology and morphology

Basic terminologies used in Helminthology

Major terminologies used in Nematode morphology

- Cuticle: outer covering of helminthes parasites. In Nematodes it may modify to form various structures: Papillae, vesicles, alae, leaf crowns etc
- Bursa is an elongated caudal alae supported by bursal rays and present only in male. It consist of two lateral lobes and one dorsal lobe
- Gubernaculum is also a chitinous organ that assist the spiecule during copulation
- Spicule is a chitinous organ of male sex organ used for copulation

Female reproductive organs of nematodes consist of ovary, oviduct, vulva

- ovejector: are structures found just above the vulva and funciton as a store and ejection of eggs during egg laying
- Vulval flap is a structure that covers the vulva. May or may not be present. Different types could also be found Ex. Linguiform, Knobbed, Smooth in Haemonchus spp.
- The digestive system of naematodes may consist of buccal capsule (large or small mouth), Oesophagus (pharynx) and intestine

Shape of oesophagus varies both in its structure and its mode of operation a diversity correlated to their feeding habit

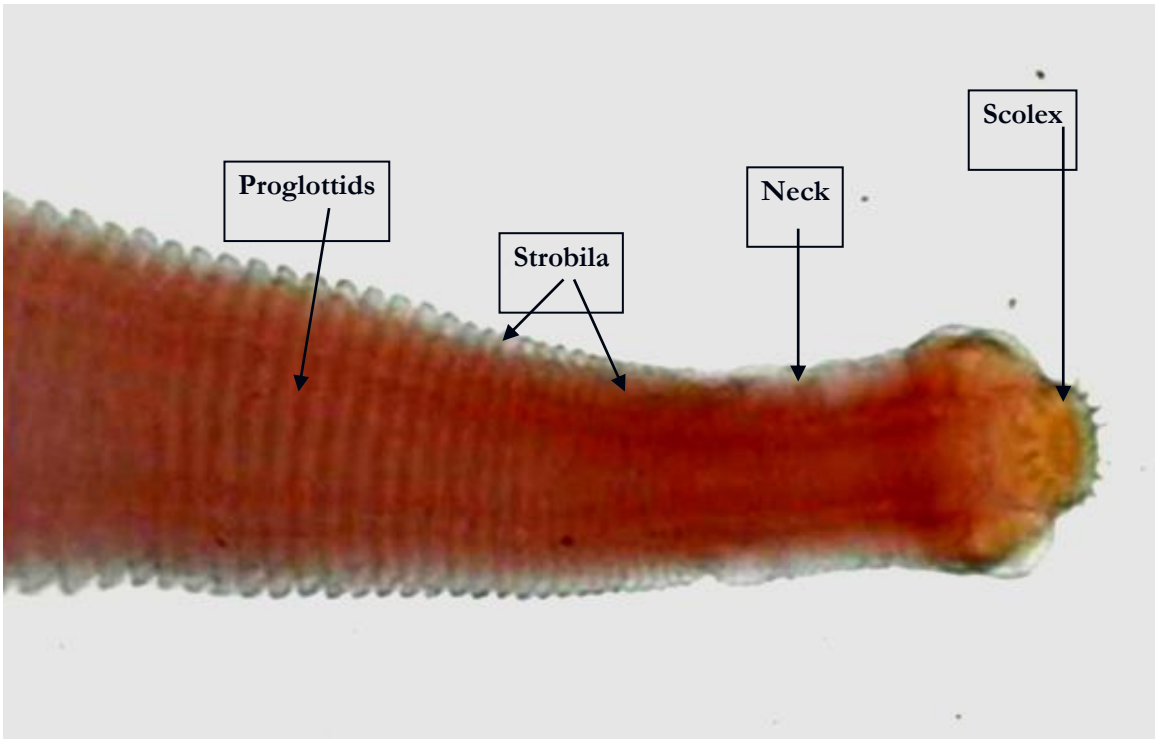
- **Rhabditiform:** with slight anterior and posteripor swelling, Ex. Free living stages, preparasitic stages (L1 and L2), plant nematodes
- Filariform oesophagus; slightly greater in diameter posteriorly than anteriorly. Ex. infective stages & adult stages of strongyles
- Bulb shaped. with large posterior swelling, Ex. Ascaris
- Double bulb shaped. Ex. Oxyuroides

ESSENTIAL MORPHOLOGIES OF CESTODES

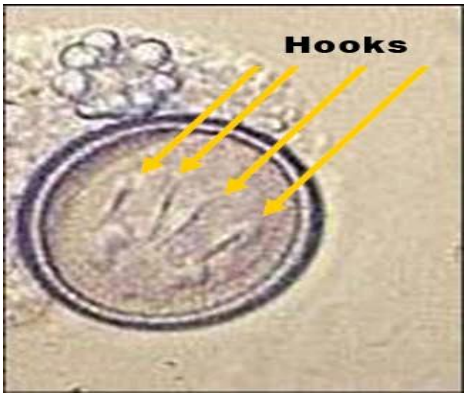
General Characteristic of Cestodes: Cestodes vary from Trematode in having a tape- like segmented body with no alimentary canal. Each segment with one or two pairs of male and female reproductive organs. All cestodes of Vet. Importance are in the order Cyclophyllidea, with two exceptions being in the order Pseudophyllidea. The adult bears head or scolex with

attachment organ (hooks or rostellum), a short unsegmented neck and A chain of segmented body strobila, each segment is known as proglottid. Identification parameters of cestodes include: Type of the host and predilection site, Presence or absence of rostellum, Hooks and their characteristics, Size of proglottids (length and width in cm) and Number of uterine branches

Structure of adult *Taenia saginata*



- The eggs consists of: "striated" shell and several of the larval hooks; approximate size = 40 µm, A thick, dark, radially striated ‘shell’ called the embryosphore, a true shell which is a delicate membrane and is often lost while still in the uterus and 6-hooked embyo, hexacanth or onchosphere.
    - ⇨ Eggs of all species of *Taenia* look like this example



Essential morphologies of Trematodes

- ✓ Oral and ventral suckers for attachment
- ✓ No body cavity and organs are packed in the parenchyma and have a simple digestive system
- ✓ Excretory system composed of flame cells
- ✓ The adult parasite are with variable hapes: leaf shaped, disc shaped etc
- ✓ Both female and male sex organs in one individual: hermaphrodite
- ✓ Females have a single ovary, males have usually two testis
- ✓ Larval stages vary in shape and are present either in the intermediate host or in the external environment

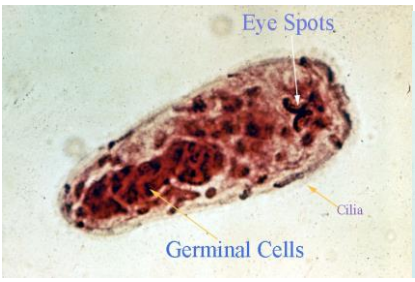
Miracidium-Sporocyst---Redia---Cercaria---Metacercaria (infective stage)

ESSENTIAL LIFE CYCLE OF DIGENETIC TREMATODES

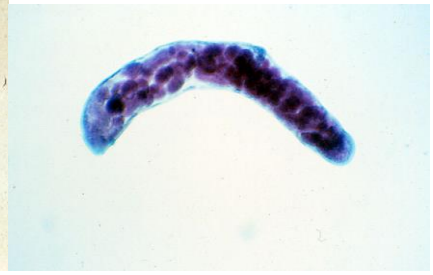
Eggs pass out with faeces (usually) and under suitable environmental conditions of moisture and warmth ($22 – 26^{0}$) hatches **Miracidium** (9 days). The mature fluke egg is operculated (having operculum or lid at one pole only). Five larval stages of development occur in the life cycle: Miracidum Sporocyst, Redia, Cercaria, Metacercaria (5- 6 weeks). Cercariae are young flukes with long tails that emerge actively from the snail in considerable numbers. The actual emergence for cercariae depends on change in temperature and in light stimulus. Cercariae swim on water within an hour or so attaches itself to vegetation (tip of blade), shed (lose) their tails and become encysted. The encysted cercariae are called **Metacercariae**, which is the actual infective stage. Infection of snail with one miracidium can produce over 600 Metacercariae. Once Metacercariae is ingested the outer cyst wall is removed by mastication and digestive secretion (from enzymatic origin) process of the host. From the Metacercariae, juvenile flukes then penetrate the intestine and migrate to the predilection site where it becomes adult after several

weeks. In case of Schistosoma spp there is no Metacercariae stage, it is the **Furcocercaria** that is infective. A single egg produces more than 600 cercariae potentially giving rise to 600 adult parasites. This process is called **Paedogenesis**. Paedogenesis is important in the epidemiology of trematodes. One egg of *Haemonchus* spp gives one L_3 larvae while one egg of fluke gives 600 cercariae.

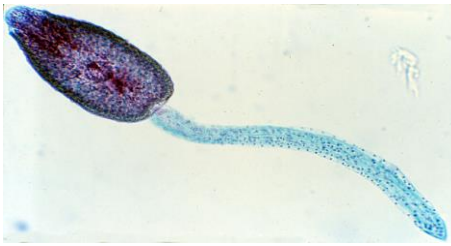
Different Larval stages



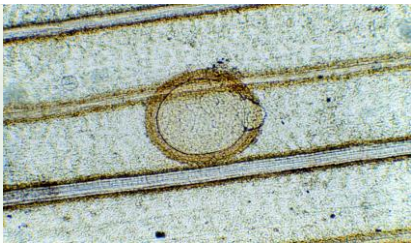
Miracidium



Redia



Cercaria



Metacercaria

Comparison of Fasciola and Paramphistomum eggs

The difference between the eggs of *Fasciola spp* and *Paramphistomum spp*

- Put one or two drops of Methylene blue into faecal suspension, so that the whole suspension will have a blue background:

Fasciola eggs	***Paramphistomum Eggs***
Yellowish brown color	Clear (transparent) color
Relatively smaller in size	Larger in size
Has coarse structure inside the eggs	Has fine structure inside theeggs

Fasciola egg

Paramphistomum egg

DIAGNOSIS OF NEMATODES INFECTIONS

Laboratory diagnostic techniques

- Methods of detecting the parasite itself or the different developmental stages:
  - ✓ Coproscopy: to determine EPG, species of Parasite? reaction of the host
  - ✓ Faecal culture: to identify the different species and genera of strongyles by detecting the infective L3 larvae
  - ✓ Post-mortem examination: to determine the parasite burden, identify the larval stages and to identify the two sexes
- Serological methods: to determine some substances related to the induced pathological disturbances
- Serum level of:
- ↑ Pepsinogen , ↑ Gastrin [Damage to mucosa is related with higher gastrin and pepsinogen release], Normal level: 103.45± 10. 41 pg/ml and 153.61± 13.21 pg/ml
- [Some medical tests report results in picograms per millilitre (**pg**/**mL**). A picogram is one-trillionth of a gram]
  - ✓ ↓ Inorganic phosphates

Field diagnostic methods: Post mortem examination, Pasture larval counts

COLLECTION AND PRESERVATION OF FAECAL SAMPLES

Faecal samples for parasitological examinationshould be collected from the rectum of the animal. Only fresh samples are fit for coproscopy examination for accurate results [in dead animals directly from the intestine]. For massive herd diagnostic purpose can be collected from the pasture and should be selected from the most recently dropped faecal part. Several samples should be collected.

- Collect enough quantities of fecal sample (about 10gm)
- From cattle, sheep, goat, horses and donkeys feces can be collected by inserting two fingers into the rectum: rolling action may stimulate defection
- Dogs and small animals: use spatula or edge of thermometer
- Poultry: from clean floor or cage
- Material for collection: plastic bags, inside of disposable gloves etc
- Screwed containers, plastic or glass are best if the sample is to be transported to another place

Preservation and transportation of Faecal samples

- If the samples to be sent without preservatives, fill the container to its maximum, thus avoiding the presence of air required for development of many parasites
- Samples can be cooled in ice packed for shipment and should reach the lab with in less than one or two days
- Samples to be transported more distance must be treated with 10% formalin

Don't keep fecal samples in a deep freezer

- In parasitological laboratories, faecal samples could be cooled and preserved at 4^0C not less than 6 months
- Faecal samples treated with formaline could stay more
- After taking enough quantityof feaces for coproscopic examination, immediately return the sample in to the refrigerator

GROSS EXAMINATION OF FEACES

- The following points can be noted: consistency of the feces, odour, colour, presence or absence of larvae/adult immature parasites.
    - Most cestodes of veterinary interest pass their proglottids in the feces [hand glass can be used if necessary]
    - Nematodes such as *Oxyuris equi*, larval stages of *Trichonema spp,* etc can be observe in the feces of infected animals.

MICROSCOPIC EXAMINATION OF FEACES

- Qualitative fecal examination: used to know whether an animal is infected or not/presence or absence of infection
        - Direct fecal smear
        - Simple floatation method
        - Sedimentation method
- Quantitative fecal examination: Used to determine the degree of parasitism
    - Based on the number of fecal egg count per gram of feces (EPG)
        - Mcmaster egg counting method
        - Stoll's method

Qualitative fecal examination

- Qualitative fecal examination

- Centrifugation/Sedimentation/technique
- Up to 3gms of faecal sample is mixed/suspended in 30-50 ml of saline
- The mixture is poured into the test tube and centrifugate it at 1500 rpm or 2-3 minutes
- The supernatant is them discarded, agitate the sediment and add equal volume of floatation fluid to the previous level to the test tube
- Certrifugate it at 1500 rpm for 2 minutes for second time
- The surface film is then taken by using wire loop or pipette, put on a glass slide, cover with the cover slip and examine it under the microscope starting with the low power lens.
- The method is used to demonstrate trematode eggs which have higher specific density, hence require floatation fluid with of higher specific gravity

Quantitative fecal examination (Procedure)

- Mc Master Egg Counting Technique :This is a quantitative technique used to count the number of eggs or larvae per gram of faces
- 3gram of faeces dissolved ---------- 42ml saline [1.20]
- The Mc Master chamber will be filled at both sides
- Therefore the number of eggs is multiplied by 100 [If two chambers are examined]
- If two chambers are examined, multiply by 50
- The technique detect the eggs and larvae of most nematodes, cestodes, and coccidia

Importance of Faecal examination

- In the diagnosis of GI parasitosis, it is useful to recover eggs, or larvae or adult parasites from faeces or GIT.
- The demonstration of worms/eggs/ larvae in faeces provides positive evidence that an animal is infected
- Very easy and cheaper method

LIMITATIONS OF FAECAL EXAMINATION

- The failure to demonstrate /eggs/ larvae doesn’t necessarily mean that there is no parasites are present, because:
    - Nodular parasites may not release eggs in to the lumen
- The resistance of the host can depress or entirely suspend ovulation of worms
- No of adult females and fecundity rate

- There is seldom any correlation between the number of eggs or larvae per gram of faces and the total number of adult worms present in cattle. The only exception is primary infection in young grazing animals during their first exposure
- Correlation is stronger in sheep and goats with mixed infections

Factors limiting accuracy of faecal egg count

- Sampling errors: eggs are not evenly distributed through out the faeces
- Quantity of faeces passed will affect the number of eggs per unit weight
- Diurnal fluctuation in faecal egg counts (different samples should be taken at the same day)
- The resistance of the host can depress or entirely suspend ovulation of worms
- Season of the year: higher after short rainy season
- No of adult females and fecundity rate
- Host immunity: cause extension of prepatent period and a lower female fecundity
- Parasite species biotic potential and pathogenicity: *Haemonchus* spp are more prolific and pathogenic with high pasture population of L_3
- Concurrent infections may reduce egg output (Yacob et al., 2002, 2003)
- Immature worms do not produce eggs
- Eggs may not be detected due to low number of them or to low test sensitivity

FAECAL CULTURE AND LARVAL RECOVERY

- Many nematodes egg are alike and nematode species can not be clearly differentiated from the eggs in faecal samples
- Differentiation can be achieved only based on morphological characterization of the infective larvae (L3), which is the only identifiable among larval stages
- The identification of parasite species present is quite important for investigation of clinical diseases caused by GI nematodes

FAECAL CULTURE

Princeples

- Faecal culture enables to identify the different species and genera of trichostrongylid and strongylid
- The infective larvae L3 is the only free larval stage easily identifiable

- Based on the development of eggs to L3 by keeping the faecal sample at 22-27°C, humidity of 85-90% and optimal oxygen for 7 -10 days (incubator) or 14-21 (days at room temperature). After 10 days, it is possible to collect the larvae by using Baermann apparatus
- Disadvantages: yields are never 100% since the proportion of larvae developing may differ between species. Limited to parasitological field studies than routine diagnosis

Procedure

1. Break up collected faeces finely using a stirring device. Faeces should be moisten and crumbly
2. If faeces are too dry, add water and if too wet, add charcoal or sterile bovine faeces until the correct consistency is obtained
3. Transfer the mixture to jars or other containers and leave the culture in incubator or room temperature
4. Add water to cultures regularly every 1-2 days
5. Collect larvae using the Baermann technique

EXAMINATION OF INFECTIVE LARVAE FROM FAECAL CULTURES

1. Take 10-15 ml of fluid from the stem of the funnel into a test tube or other container
2. Leave the test tube to stand for 30 minutes and remove the supernatant with a Pasteur pipette
3. Transfer a small aliquot of the remaining fluid using a Pasteur pipette to a microslide, and add a drop of iodine and cover with a cover slip
4. Examine fewer than 10 x 10 magnifications. The counts for each species provide an estimate of the composition (%) of the parasite population of the host

Post-mortem techniques and differential parasite counts

- Post-mortem parasite counts provide
    - more precise assessment of parasite burdens than parasite egg counts.
- For parasite counts, the intestinal tract from abomasum to rectum is required. The adult and larval nematodes are carefully washed out, counted and identified.
- In addition, a complete postmortem examination of all organs should be done, bearing in mind alternative causes of ill health or death.
- It is important to record all abnormalities and lesions observed.
- A number of parasites will be found in almost every grazing animal, irrespective of the state of its health.
- To assess the significance of parasite infections in field mortalities, it is therefore necessary not only to determine the species present, but also to assess the number of each species.

Procedure

- (a) During the post-mortem examination, ligate the abomasum with string and separate it from omasum and duodenum.

- (b) Place the abomasum in a tray. Open the abomasum along the greater curvature so that its contents fall into the tray: empty the abomasal contents into the total content jar.
- (c) Wash the empty abomasum thoroughly in the tray several times, paying particular attention to cleaning between the folds of the mucous membrane. Add the washings to the total contents jar.
- (d) For cattle, make the total volume of contents and washings in the total contents jar up to 4 liters with water. Occasionally it will be necessary to make the total volume up to 5 liters for cattle. For sheep and goats, make the volume up to 2 or 3 liters.
- (e) Using the large ladle, stir vigorously until all food material, mucus and water are thoroughly mixed.
- (f) Transfer a total of 200 ml of the contents to the wash jar in 5 steps of 40 ml per step, using the ladle container and stirring the mixture continuously.
- g) Fill the wash jar with water. Screw the lid on securely. Invert the jar and shake it till most of the fluid is shaken out. Repeat this process until all faecal culturing matter is removed.
- (h) Add water to make the volume in the wash jar up to 50 ml (for convenience).
- (i) Pour small volumes into petri dishes.
- (j) Add a few drops of iodine solution to the sample in each petri dish. Mix the iodine with the sample and allow to stand for 35 minutes, during which time the worms will stain deeply with iodine.
- (k) Count the number of each species of nematode present in the sample. Repeat the process for each petri dish, and add the species counts for all dishes.

For cattle, multiply the total count for each species by 20 to arrive at the total burden in the animal examined (assuming that an original volume of 4 litres was used). For sheep and goats, multiply the count for each species by 10 or 15 to arrive at the total burden (assuming that an original volume of 2 or 3 litres was used). For *Haemonchus*, small differences in worm burdens may cause significant differences in their pathogenic effect. For this reason, a more accurate assessment of the burden should be obtained by carrying out a total abomasal count of Haemonchus as opposed to the sub-sampling procedure described above.

Key for the identification of the infective larvae (L3) of some GI nematodes of sheep and goats

Total length of larva (μ)	Length, end of larva to end of sheath (μ)	Species, with range of total length (μ)	Other differential features
Short 500-700	Long 85 -115	*Bunostomum* 510-670	Wide body with sudden tapering to long thin tail "Band" constriction on oesophagus
Medium 650-900	Long 20 -40	*Trichstrongylus* 620 -910	Short straight larva, conical tail sheath. Tubercles on tail of larva. Intestinal cells usually prominent
Medium 650-900	Short 30-60	*Ostertgia 790-910*	Long, conical, "finger-like" tail sheath
Medium 650-900	Medium 30 -60	*Haemonchus* 650-750	Tail sheath is usually "kinked" pointed tail of larva
Medium 650-900	Medium 30 -60	*Cooperia* 650 - 750	Oval bodies at anterior end of larva. Tail of larva rounded
Long 900-1200	Long 60 -80	*Chabertia* 710 - 790	Stout body with 24 to 32 rectangular intestinal cells
Long 900 - 1200	Long 60 - 80	*Oesophagostomum* 770 - 920	Usually longer than *Chabertia*
Long 900 - 1200	Extremely long 250-290	*Nematodirus* 922 - 1180	Tail of larva is forked

DIAGNOSIS OF CESTODE INFECTIONS

- **Faecal sample analysis.** For an intestinal tapeworm infection, A laboratory uses microscopic identification techniques to check for eggs or tapeworm segments in the feces. Because the eggs and segments are passed irregularly, the lab may need to collect two to three samples over a period of time to detect the parasite..
- Arecoline test: Treatment of dogs with arecoline hydrobromide
- Immunodiagnosis: sero-epidemiological study
- **Imaging exam:** Certain types of imaging, such as CT or MRI scans, X-rays, or ultrasounds of cysts, may suggest the presence of cysts.
- Necropsy: examination to look for adult cestodes and metacestodes

In human

- Radiographic and ultra sound techniques are also helpful
- Immunodiagnostic tests:
- indirect haemagglutination,
- indirect immuno- florescence,
- latex agglutination and enzyme linked immunosorbant assay (ELISA) are highly sensitive in detecting circulating antibodies in sera from patients with hydatid diseases.
- Immuno electrophoresis and double immunodiffusion tests are also used.

DIAGNOSIS OF TREMATODE INFECTIONS

- History, clinical signs, seasonal occurrence, history, identification of snail habitats can serve as a tentative diagnosis
- For confirmation:
  - ✓ Coprological examination for eggs
  - ✓ Necropsy examination to look for lesions, immature or adult flukes
  - ✓ Hematological tests : Anemia, eosinophils
  - ✓ Biochemical technique to determine plasma enzymes level (Glutamate dehydrogenase [GLDH] due to parenchymal damage and gamma glutamyl transpeptidase [GGT] due to damage to epithelial cells lining the bile duct
  - ✓ Serology : Detection of antibodies against components of flukes
  - ✓ For schistosomes: Eggs are 100-500 µm long, spindle shaped and have lateral or terminal spine; there is no operculum

Veterinary Diagnostic Helminthology VeLT 3101

Part by
Getachew Terefe (DVM, PhD)
50%

Outline and learning outcome

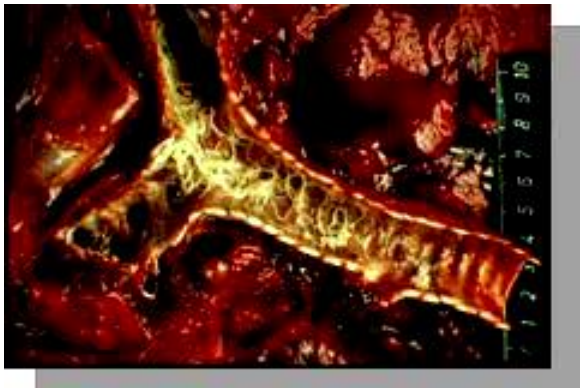
- This portion of the course (50%) consists of the following major sections:
  - Lung worms of domestic animals
  - Helminth parasites of poultry
  - Helminth parasites of equine
  - Helminth parasites of dogs and cats
- At the end of each part please make sure that you are able to:
  - Describe the basic morphological features of each major parasite
  - Explain how samples are collected, preserved and examined for each group of parasites
  - Differentiate parasites within each group by using morphological features of the parasite, its larvae or egg, animal host species affected and the predilection sites of the adult parasites

Section I: LUNG WORMS

LUNG WORMS

- **Dictyocaulus**
  - Host: Ruminants and equine
  - Site: trachea, bronchi
  - Species:
    - Dictyocaulus viviparus: cattle
      - most pathogenic, cause parasitic bronchitis in cattle (verminous pneumonia)
      - D. filaria: SR
      - D. arnfieldi: equine
  - Morphology
    - Adults: slender thread-like, 8cm
    - Location in the Upper respiratory tract
    - L1 in feces: sluggish while moving, have intestinal cells filled with dark brown food granules
  -

LUNG WORMS: D. viviparus



- Pathogenesis
  - Penetration phase: d1-d7
    - Movement to the lungs, no lesions
    - Prepatent phase:d8-d25
      - Larvae in the alveoli-alveolitis
      - Bronchiolitis, bronchitis w/developing larvae to adult and move upstream
      - Bronchioles blocked by cellular infiltrates-collapse of other groups of alveoli
  - Patent phase: d26-d60
    - Parasitic bronchitis with numerous adult worms in the frothy mucus of the bronchi
    - Broncheal epithelium filled with eosinophils
    - Presence of dark red collapsed areas around infected bronchi
  - Postpatent phase: d61-d90
    - Recovery phase after adult worms are expelled
    - Broncheal and peribroncheal fibrosis

LUNG WORMS: D. viviparus

- Clinical signs
  - Mild: intermittent coughing at exercise
  - Moderate: frequent coughing, at rest, tachypnoea (>60 resp/min)
  - Severe: severe tachypnoea (>80 resp/min), dyspnoea, adopt “air hunger” position:
    - mouth breathing with head and neck outstretched
  - Calves may show clinical signs during prepatent phase: dyspnoea of sudden onset, death in 24-48 hrs
- Diagnosis based on:
  - Clinical signs
  - Demonstration of L1 larvae in the feces using the Vaga technique/ modified Baerman tech
  - Demonstration of adult worms in the trachea and brochi

LUNG WORMS: D. Arnfieldi

- Similar to that of D. viviparus except:
  - Adults commonly in the small bronchi
  - Eggs w/ L1 hatch soon after being passed in the feces
  - PPP. 2-4 months
  - Patent infections are common in donkeys, but only occur in foals and yearlings in horses
  - In adult horses, worms rarely attain maturity
- Pathogenesis:
  - Raised circumscribed areas of over-inflated pulmonary tissue in the caudal lobe
  - On section, at the center of each lesion is a small bronchus w/ lung worms and mucopurulent exudate
- Diagnosis
  - Fecal larvae in donkeys: modified Baerman tech.
  - Eggs are difficult to find in horses

LUNG WORMS: D. filaria

- Similar to that of D. viviparus, except that PPP is 5 wks
- L1 IS similar to that of D. viviparus but has a characteristic cuticular knob at the anterior end
- Differentiated from other SR lungworms by its large size and strait tail

- Diagnosis:
  - History, clinical signs
  - Fecal examination FOR l1 LARVAE

LUNG WORMS: Metastrongylidea

- Introduction
  - Most worms inhabit the lungs or the blood vessels adjacent to the lungs
  - Typical lifecycle: indirect, IH is mollusks
  - Conventionally divided into three according to host:
    - In pig
    - In small ruminants
    - In dogs and cats

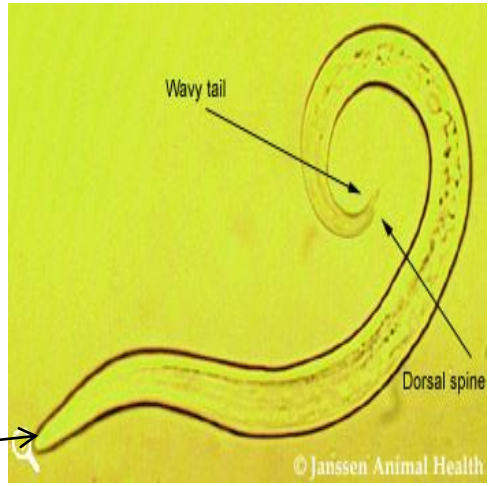
LUNG WORMS: Metastrongylidea

- *Metastrongylus*
  - Host: pigs
  - Species:
    - *Metastrongylus apri/elongatus*
    - *M. salmi and M. pudendotectus*
  - *Morphology*
    - Slender white, 6cm long, have a pair of tri-lobed lips
    - Have long, thin spicules and well developed bursa
    - Vulva near the anus
    - Eggs have rough, thick shells, and larvated when laid

  - Predilection sites
    - Bronchi and bronchioles
  - Prepatent period: 4weeks
  - Pathology: Grayish nodules on the diaphragmatic lobe (postmortem)

LUNG WORMS: Metastrongylidea

- *Muellerius capillaris*
  - In sheep and goats
  - Adult
    - *Well developed bursa, longer comb-like spicule*
    - *Vulva near the anus*
  - Intermediate host: Snails and slugs
  - ovo-viviparous-L1 passes in feces
  - Predilection site: Alveoli and small bronchioles
  - Prepatent period: 6-10 wks
  - Pathology: Small spherical nodular lesions on the lung surfaces (postmortem)
  - Diagnosis: Fecal examination
    - based on L1 morphology
      - Absence of anterior protoplasmic knob

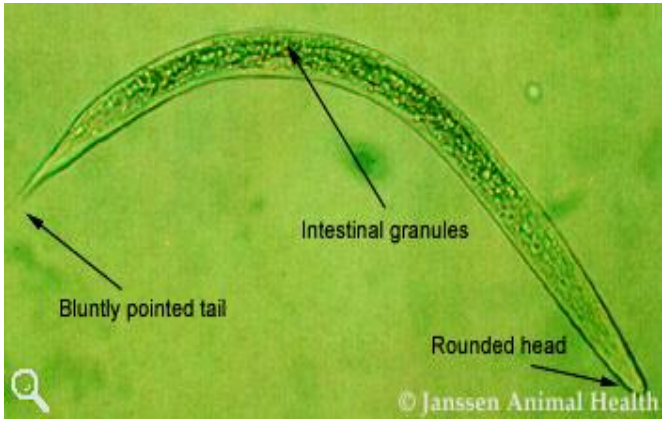


LUNG WORMS: Metastrongylidea

- *Protostrongylus rufescens*
  - In sheep and goats
  - Adult
    - *Well developed bursa, longer comb-like spicule*
    - *Vulva near the anus*
    - Brown hair-like, 1-3cm long
  - Intermediate host: snails
  - Predilection site: Alveoli and small bronchioles
  - ovo-viviparous-L1 passes in feces
  - Prepatent period: 5-6wks
  - Pathology:
    - Occlusion of small bronchus
    - Lesion with conical form with the base on the surface of the lung

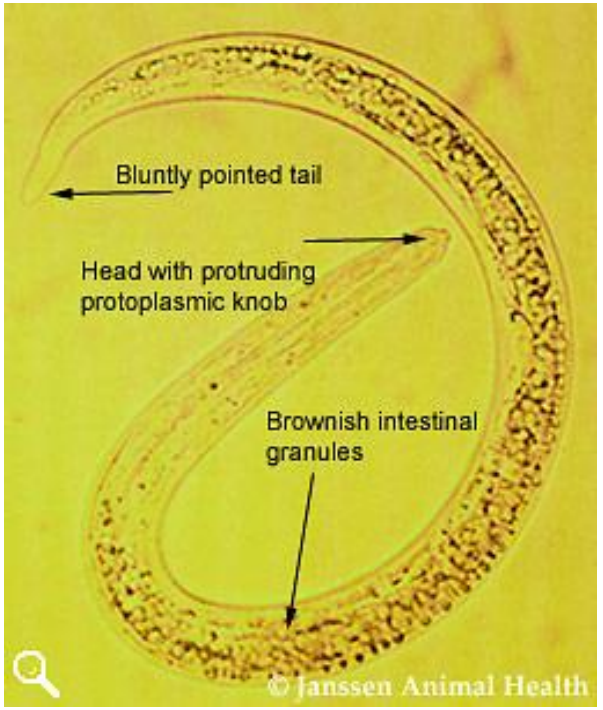
LUNG WORMS

- Morphological differentiation of lungworms on the basis of L1 larvae
  - Free-living nematodes stain deep brown in iodine and can be distinguished by the presence of a double bulbed (rhabditiform) oesophagus.
- D. Viviparous
  - Length 390-450 µm
  - Larva often curved
  - Has dark intestinal granules
  - Sluggish movement
  - Note: Dictyocaulus viviparus does not have a protruding protoplasmic knob on the head unlike Dictyocaulus filarial



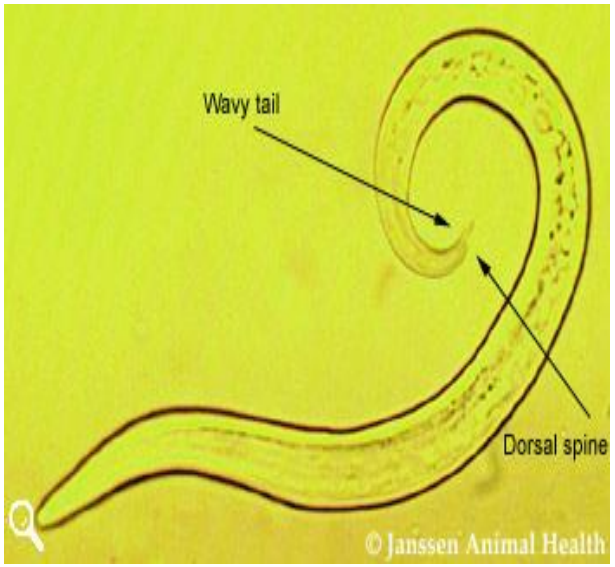
LUNG WORMS

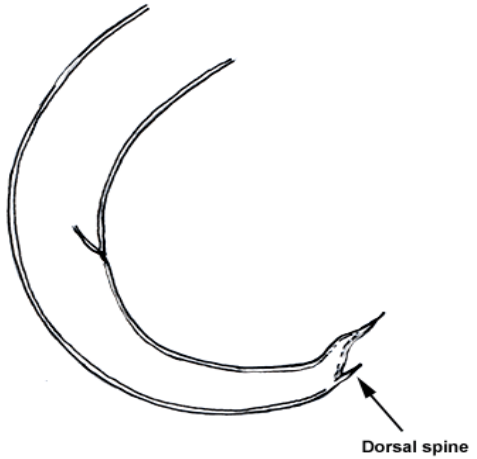
- D. filarial
  - Length 550–585 µm
  - Larvae often curved
  - Sluggish movement
  - head with protruding protoplasmic knob
  - bluntly pointed tail
  - brownish intestinal granules



LUNG WORMS

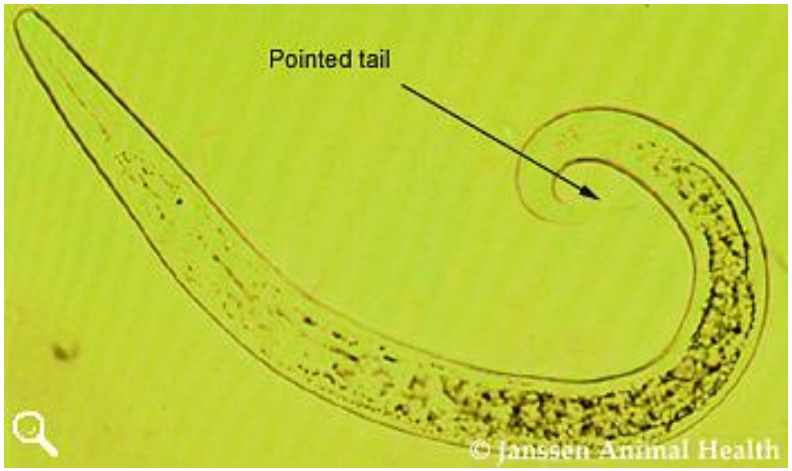
- Mullerius capilaris
  - Length 300–320 µm
  - Posterior end of body often curled
  - has wavy tail with dorsal spine





LUNG WORMS

- Protostrongylus
  - Length 320–400 µm
  - Posterior half of body may be curled
  - Pointed tail with no spine



Section II: Helminth parasites of poultry

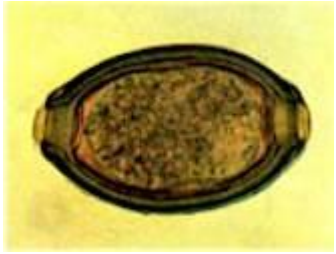
Nematodes

- Capillaria (Poultry, game birds: Crop, S.Intestine)
- Ascaridia (Poultry, game birds: SI)
- Heterakis (poultry: cecum)
- Syngamus (chicken and others: respiratory tract)

Nematodes-Capillaria

- Capillaria is a genus of parasitic roundworms that infects chickens, and other birds.
- They belong to the group of hairworms or threadworms,
- Adult Capillaria worms are very thin worms (hairworms), up to 8 cm long (depending on the species) and of a whitish color.

- C. contorta and C. annulata (Crop and esophagus)
- C. obsignata, C. caudinflata, C. anatis (SI)
- Capillaria eggs are ovoid, about 30x70 micrometers with a thick shell, two polar plugs, and contain a single cell (i.e. they are not embryonated when shed).

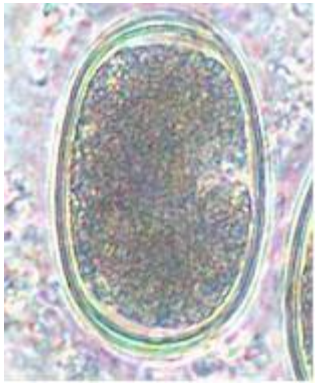


Nematodes- Ascaridia

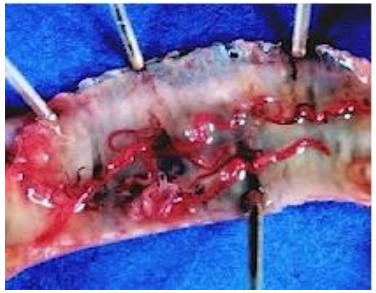
- Adult Ascaridia worms are largest roundworms found in domestic birds.
- They are up to 12 cm long and of a whitish color, rather transparent.
- Ascaridia eggs are ovoid, about 50x80 micrometers, with a thick and smooth shell.
- Ascaridia is a genus of parasitic roundworms belonging to the ascarids that infects small intestine of chickens
- Ascaridia galli infects mainly chicken, but also turkey, geese, guinea fowls, etc.
  - Diagnosis
    - Post mortem: large whitish worms in the SI
    - Antemortem: eggs less common

Heterakis

- Heterakis is a genus of parasitic roundworms belonging to the pinworms that infects chickens and other birds
- They are also called cecal worms
- Adult Heterakis worms are rather small roundworms, not longer than 15 mm, very thin and of a whitish color.
- Heterakis eggs are ovoid, about 45x75 micrometers, with a thick and smooth shell, and contain a single cell that fills also the egg poles. T
- hey are very similar to those of Ascaridia galli



Syngamus



- Syngamus trachea, also called gapeworm, red worm or fork worm, is a parasitic roundworm that infects the respiratory system of poultry (trachea and bronchi)
- Syngamus trachea is a medium-sized worm.
- Males are up to 6 mm, females up to 20 mm long.
- Characteristic for these worms is that the adults live in permanent copulation.
- Diagnosis
  - Postmortem: nodular tracheitis with hemorrhages, Y-shaped pairs of worms collected,
  - Antemortem: the signs are those of asphyxia, double capped egg in feces

Cestodes of poultry

- Davainea proglottina, also called the minute tapeworm or the small chicken tapeworm, is a parasitic worm that infects the small intestine of chicken and other domestic and wild birds
- It is usually not longer than 4 mm with only 4 to 7 segments (proglottids).
- The head (scolex) has numerous hammer-shaped hooks and suckers armed with spines,
- The segments are whitish to translucent. Only the last one is gravid, i.e. filled with eggs.
- Sheds one proglottis/day

Cestodes of poultry

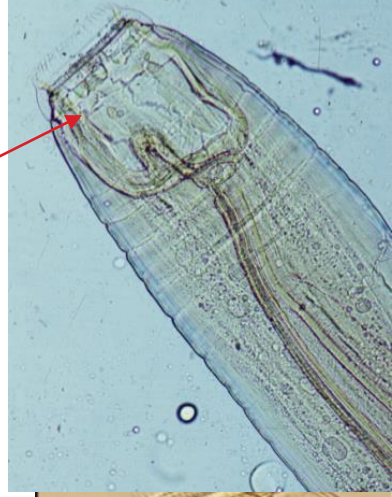
- Raillietina is a genus of parasitic tapeworms that infects chicken and other birds
- Adults are whitish, medium-sized tapeworms 5 to 30 cm long and 1 to 4 mm wide, depending on the species.
- The head (scolex) is armed with numerous hooks, spines and 4 suckers.
- The segments (proglottids) are wider than long.
- The eggs measure ~75x95 micrometers.
- Examination of faeces:
  - Wash faeces on a fine sieve and look for segments
  - Release eggs by teasing segments on a slide

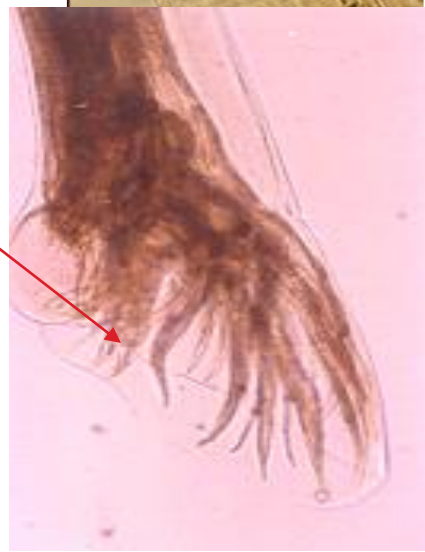
Section III: HELMINTH PARASITES OF EQUINES

Site	Genus/species	Description
Stomach	*Draschia megastoma*	▪ Found in large fibrous nodules in the stomach wall ▪ Nodule contain grayish-yellow purulent material ▪ Adults have funnel-shaped buccal cavity
	Habronema microstoma	▪ Larger than Draschia ▪ Cylindrical buccal cavity ▪ Less significant pathological reaction
	H. muscae	▪ Left spicule is 5x as long as the right one
S. Intestine	*Parascaris equirum*	▪
L. intestine	*Oxyuris equi*	▪ Large white pinworm ▪ Oesophageal bulb and tail tapers to a point in females ▪ L4 may be found attached to mucosa
	Large strongyles Strongylus Tridontophorus Oesophagodontus	
	Small strongyles Cyathostomum	Little/no pathological significance
Eye	Thelazia lacrimalis	On the surface of the eyeball, under the eyelids and in the lacrimal glands

ORDER STRONGYLIDA

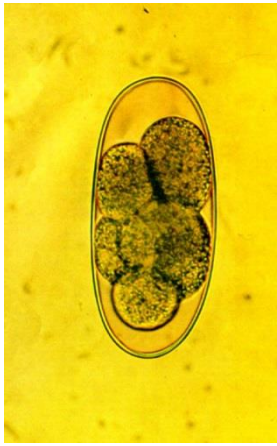
- Characteristics
  - Well-developed buccal capsule, mostly armed w/ teeth
  - Males have caudal copulatory bursa supported by muscular processes, called rays
  - The disposition and configuration of these rays are used in classification and identification
  - The strongylid uterus has two horns, equipped w/ strong muscular ovijector





Su/F Strongyloidea, Family: Stronglidae (1)

- s/F. Strongylinae: Large strongyles
  - Parasites of the large intestine of equines
  - More pathogenic than small strongyles
  - Identification based on microscopic appearance of buccal region
  - Egg identification between small and large strongyles is difficult (only by culture)
  - Eggs: 70–90 µm x 40–50 µm , 8–16 cell morula
    - Strongylus spp are migratory blood-sucking parasites, and are the most destructive parasites of equine
    - Draw a plug of mucosa deep to the submucosa/muscularis
    - Their larvae undergo migrations that inflict more damage
  - *Triodontophorus* spp, *Oesophagodontus* spp, *Craterostomum* spp: nonmigratory



Su/F Strongyloidea, Family: Stronglidae (1)

- **Large Strongyles:**
  - **Strongylus vulgaris**
  - **S. equinus**
  - **S. edentatus**
- **Morphology:**
- Large Strongyles are long, fat worms with biting mouths.
- Since they are full of their equine victims' blood, they usually are reddish-brown in color.

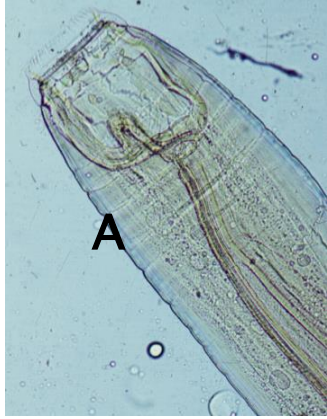
S.F Strongyloidea, Family: Stronglidae (1)

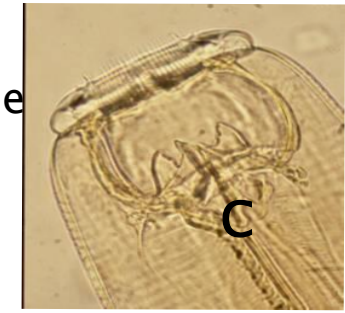
- sb/F. Strongylinae: Large strongyles:
  - Parasites of the large intestine of equines
  - More pathogenic than small strongyles
  - Adult morphology:
    - Robust, dark-red
    - Well developed buccal capsule and male bursa
    - Species Identification based on size and the presence and shape of the teeth

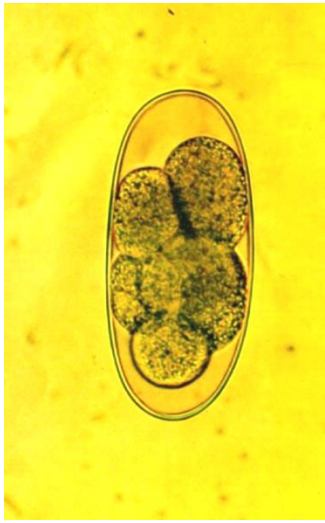
    A) S. vulgaris: 1.5-2.5cm, 2 ear-shaped rounded teeth

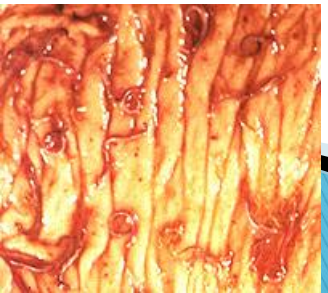
    B) S. edentatus: 2.5-4.5cm, no teeth

    C) S. equinus: 2.5-5cm, 3 conical teeth
  - Egg identification between small and large strongyles is difficult (only by culture)
  - Eggs: 70-90 µm x 40-50 µm , 8-16 cell morula
  - pathology
    - Nodule development, immune reaction
    - Arteritis, thrombosis, embolism of cranial mesenteric artery, colic, lameness (S. vulgaris)

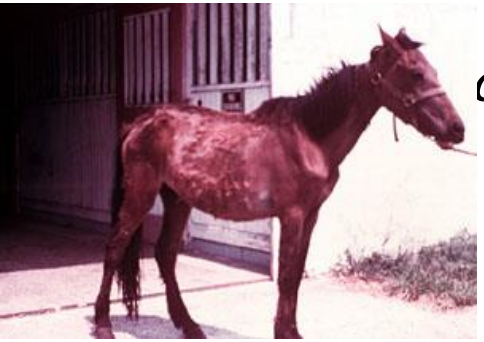






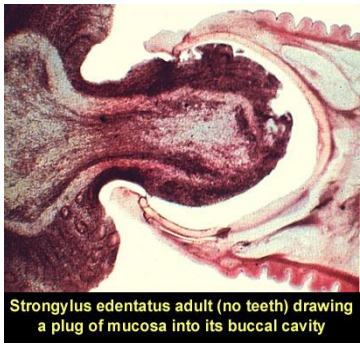


S.F Strongyloidea, Family: Stronglidae (1)



ongylus spp: migratory

- Strongylus spp are blood-sucking parasites, the most destructive
- Draw a plug of mucosa deep to the submucosa/muscularis
- Their larvae undergo migrations that inflict more damage



Strongylus edentatus adult (no teeth) drawing a plug of mucosa into its buccal cavity

- *Other large strongyles:* nonmigratory
  - *Triodontophorus* spp,
  - *Oesophagodontus* spp,
  - *Craterostomum* spp

Robust, reddish, 1-2.5cm long

Head end of Triodontophorus showing its large buccal cavity with teeth at the base and fringed by a prominent leaf crown around the opening

Su/F Strongyloidea, Family: Stronglidae (2)

- s/F. Cyathostominae: small strongyles
  - Parasites of the large intestine of equines
  - Have smaller buccal cavities than strongylus species
  - Species identification by comparing dorsal and lateral aspects of the buccal region of fresh/cleared specimen
  - *Major genera are Cyathostomum and Trichonema* sp
  - Have little clinical significance
  - Eggs difficult to differentiate from others : culturing is necessary for identification

Su/F Strongyloidea, Family: Stronglidae (2)

- s/F. Cyathostominae: Cyathostomes/Trichonema
  - small strongyles: < 1.5 cm
  - Parasites of the large intestine of equines
  - smaller buccal cavities than strongylins
  - Species identification by comparing dorsal and lateral aspects of the buccal region of fresh/cleared specimen
    - *Cyathostomum*
    - *Trichonema* sp
    - *Cylicocyclus* sp
    - *Cylicodontophorus sp* etc...
  - Have little clinical significance
  - The major damage is by larval stages
    - L3 passing to mucosal glands and then to lamina propria
    - Surrounded by fibroblastic reaction (nodule)
    - Molt to L4, emerge into the lumen and molt to L5
    - Prepatent period: 3 months
  - Eggs difficult to differentiate from others (by culture)

Su/F Ascaridoidea: *Parascaris equirum*

- Mainly in Small intestine of young foals
- Large, robust nematodes, up to 50 cm
- Eggs in feces
  - Round/oval, pigmented, thick shell w/ granular surface
  - 90-100 µm in Ø, recovery by floatation technique
- Diagnosis
  - Characteristic eggs in feces
  - Adult parasite in feces or intestine

Su/F Habronematoidea: Habronema and Draschia

- Adults
  - Parasites of equine stomach
  - Draschia megastoma is about 13 mm long
  - Habronema species are 22-25 mm w/ cylinderical buccal cavity
  - The former is associated with nodules , the later not
  - Diagnosis
    - Egg by floatation: elongate, thin-walled, 40-50 µm x 10-12 µm
    - Adults from the stomach

Order: Oxyurida

- Oxyuris equi: pinworm of horse
  - Esophagus is a spherical bulb immediately anterior to its junction with the intestine
  - Have long tapering tail (pinworm)
  - Adults in LI, often protrude from the anus
  - attach sticky eggs to the exterior of the anus –causing pruritis ani
  - Eggs are smooth, thin-shelled, operculate, may be larvated
  - Diagnosis
    - Eggs from the perineum
    - Adult worms in feces/rectum
    - Pruritis ani (itching at the anal region)

Order: Oxyurida

- Oxyuris equi: pinworm of horse
  - Esophagus is a sherical bulb immediately anterior to its junction with the intestine
  - Have long tapering tail (pinworm)
  - Tiny males with caudal alae & single spicule
  - Anterior vulva in females
  - Adults in LI, often protrude from the anus
  - attach sticky eggs to the exterior of the anus –pruritis ani
  - Eggs are smooth, thin-shelled, operculate, may be larvated
  - Life cycle
    - Eggs attached to skin: develop to infective stage in 4-5 days, detach and drop and adhere to feeding materials, walls etc
    - L4 feeds on the mucosa
    - Prepatent period: 4-5 months
  - Pathophysiology
    - Adults and L4 in the LI
    - Eggs on the skin of the perineum
  - Diagnosis
    - Eggs from the perineum
    - Adult worms in feces/rectum
    - Pruritis ani

Large and small strongyles

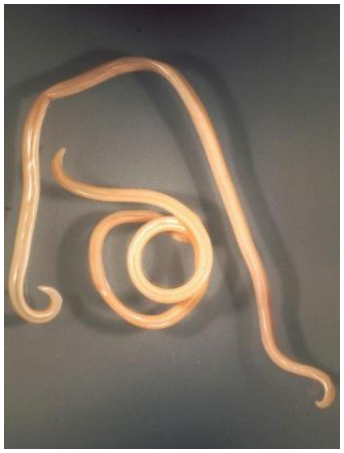
- diagnosis
  - Clinical signs:unthriftness, anaemia, diarrhoea, lamness, colic etc
  - Eggs: oval, thin-shelled strongyle type
    - Consider Low fecundity
    - Consider Long PPP
- Treatment
  - Common anthelmintics

Section IV: HELMINTH PARASITES OF DOGS AND CATS

NEMATODES

Toxocara species

- Causes “visceral larval migrant” in man- severe eye damage
- Up to 10cm long
- Male has small finger-like projection on its tail
- The anterior end has two wing-like projections (alae)
- The head has three prominent lips armed with small teeth
- Egg is dark brown, subglobular, with thick pitted shell
- Egg containing L2 is infective after 4 wks of development
- Transmission:
  - ingestion : L2 in egg
  - transmamary : L2 during the first 3 wks of lactation
  - Transplacental: L2 3wks prior to parturition



- Produce up to 20,000 egg/female/day
- No need to use floatation since the eggs are many and readily detected in direct smears

NEMATODES

- Toxocara canis in dogs
  - Male tail has small finger-like process
- T. cati in cats
  - Cervical alae seems an arrow head, male tail similar to T. canis
- Toxoascaris leonina dog and cat
  - Different from other ascaroids of dog and cat only by the absence of the above morphological descriptions

NEMATODES

- Lifecycle
  - **T. canis:**
    - Ingestion (direct)
      - In less than 3 months old dogs
        - ingestion of egg w/L2-hatching-liver-lung-L3-trachea-intestine-L4,L5-adult
      - > 3 months less frequent hepatic tracheal migration, no at 6 months
        - L2 travel to tissues like liver, lungs, brain, heart etc-
    - Prenatal infection (horizontal)
      - Larvae mobilized from tissues 3 wks prior to parturition-lungs of foetus-L3 before birth cycle completed as above after birth
    - L3 in milk (vertical)
      - During the 1st 3 wks of lactation
      - No migration in the pup
    - Paratenic hosts: rodents, birds
      - Ingest infective egg, L2 in tissues-eaten by dogs-development in the Si
    - PPP: ingestion of egg/paratenic host: 4-5 wks
    - Prenatal infection: 3 wks

NEMATODES

- **T. cati**
  - Similar to T. canis except that prenatal infection does not occur
  - PPP from egg infection: 8 wks
- Toxoascaris leonina
  - Ingestion of egg w/L2 or L2 in the tissues of mice
  - Development entirely in the wall and lumen of the SI
  - No migratory phase
  - PPP: 11 wks

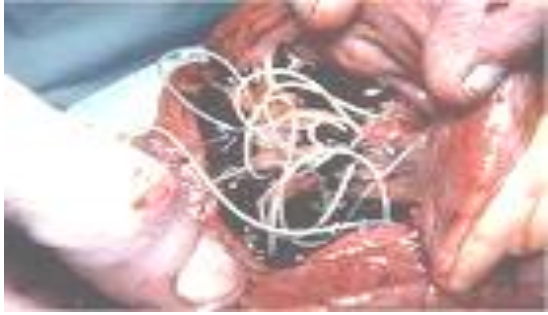
NEMATODES

- Ascarids
  - Diagnosis
    - Eggs
    - Floatation: Zinc/magnisium sulphate > NaCl
    - Direct: as large No of eggs are produced
    - Adult worms
    - Postmortem (birds)
    - Remember that the prepatent period is long

NEMATODES

- Spirocerca lupi
  - Adults in granulomatus nodules of the oesophagus
  - IH: copropgagous beetles
  - Migrating larvae produce lesions in the aorta
  - Distribution: tropical, subtropical
  - Morphology:
    - Pink worms, coiled in the granuloma
    - Thin-shelled elongated egg contains L1
  - Pathology and clinical signs
  - Scarring of wall of aorta, stenosis, rupture
    - Granulomas containing adult worms-dysphagia, vomiting, due to obstruction and inflammation
    - Eggs in feces/vomit-if fistula of egranuloma
    - Endoscopy/radiography

NEMATODES



Dirofilaria immitis: Heart worms of dog/cat/man

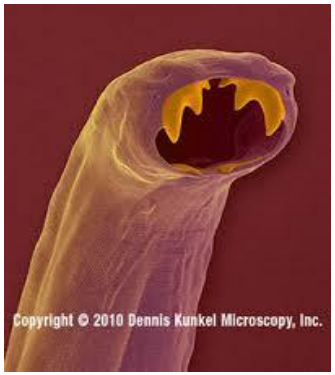
- IH: mosquitoes
- Site: adults in the R.V., pulmonary A. and anterior VC
- Identification:
  - slender worms, 20-30cm, male tail has loose spiral shape, microfilariae 300-330μm with tapered anterior and blunt posterior ends
- Lifecycle:
  - Female release microfilariae into blood -ingested by female mosquitoes-L3-mosquitoes feed on blood-L3 into the host-migration to subcutaneous/subserosal tissue-2 molts-young-adults pass to heart via venous system
  - Minimum PPP. 6 months, adult worms survive several years

- Diagnosis:
  - Signs of CV dysfunction, microfilariae in blood

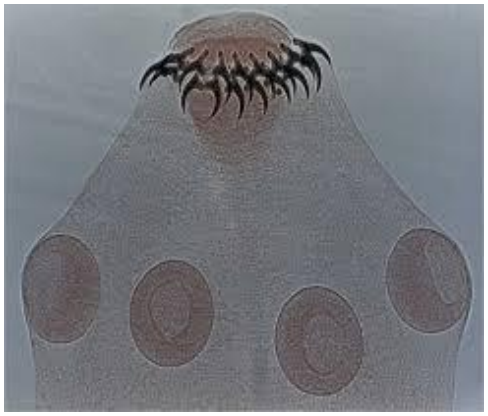
NEMATODES

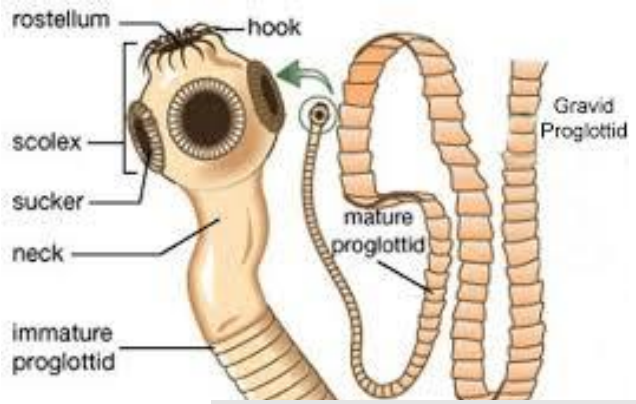
Ancylostoma species (hook worms)

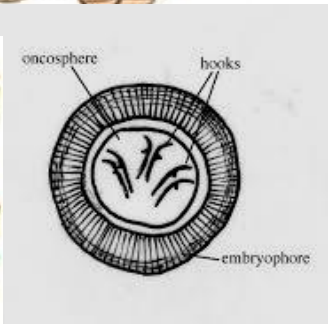
- *Characteristic hook posture of anterior end*
- *1-2cm long*
- *Has 3 pairs of teeth in the buccal cavity*
- *Infection is by skin penetration or by ingestion of L3*
- *Transmammary infection is possible until 3wks after whelping*
- *Diagnosis: floatation tech.*



CESTODES

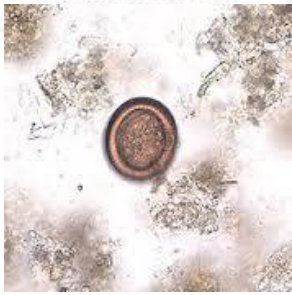






- *Taenia multiceps*
- *Echinococcus species*
- *Dipylidium caninum*
- *Other cestodes*
  - *Taenia hydatigena*
  - *T. ovis*
- ***Basic structure***
  - Scolex: head
  - Strobila: chain of segments
  - Proglottid: segment
  - Scolex has 4 suckers , rostellum
  - Rostellum may be armed (has hooks) or not
- Each egg contains an already developed larva (*oncosphere* or *hexacanth*) with 3 pairs of hooks and is surrounded by a thick, striated shell.
- It is not possible to visually distinguish the eggs of the different *Taenia* species under the microscope, and they are also very similar to the eggs of Echinococcus granulosus and Echinococcus multilocularis

CESTODES



Taenia multiceps (adult), coenurus cerebralis(cyst)

- Adult: up to 100 cm long
- The definitive hosts: dogs and other canids
- Eggs and gravid proglottids are shed in feces into the environment, where they are ingested by an intermediate host
- Many animals may serve as intermediate hosts, including rodents, rabbits, horses, cattle, sheep, and goats

CESTODES

T. multiceps:

• Coenurus (metacestode or larval stage) takes about 8 months to mature in the CNS

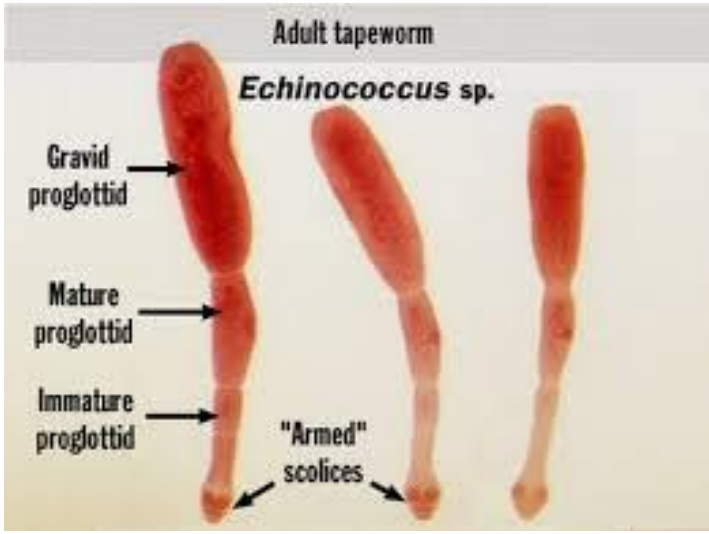
•**Coenurus cerebralis causes** coenurosis in sheep/goat/man

•Mature proglottids are longer than they are wide

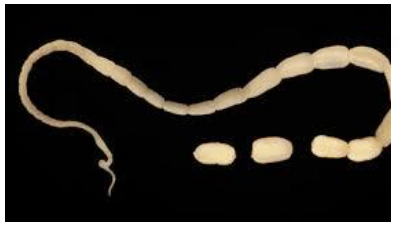
•Each gravid proglottide has 9–26 lateral branches and a lateral genital pore

CESTODES

- **Echinococcus granulosus**
  - 6.0mm long
  - Has scolex and three or four segments
  - Egg is radially striated and has six-hooked oncosphere
  - Prepatent period in dogs is 40-50 days
  - Diagnosis in dogs:
    - segments are 2-3mm with ovoid shape and single genital pore
    - Use of purgative: **arecoline** hydrochloride to expel adults
    - Egg by floatation, but difficult to distinguish from others

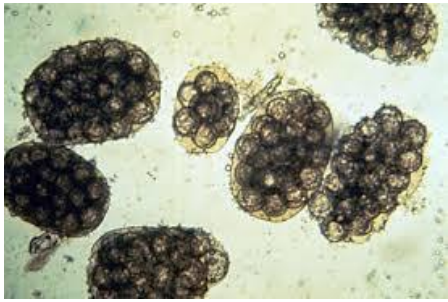


CESTODES



Dipylidium caninum

- Commonest tapeworm of dogs
- Has armed rostellum with several rows of hooks
- IH: fleas
- Measures about 50cm
- Proglottid (segment):
  - is elongate like a large rice grain
  - Has 2 sets of genital organs with a pore opening on each margin
- Diagnosis:
  - Presence of segments on the coat around the perineum. If fresh, look for the typical elongate shape and double genital organs
  - If the segment is dry, break it up with a mounted needles in water, look of the egg packets under the microscope
  - NB. In other Taenia species , numerous individual eggs /onchospheres are seen



RESTRAINING DOGS

- A stray dog may be fearful, hungry, injured, or diseased—all of which could put your health and safety at risk (and his as well) if you do not know the humane and proper way to catch him
- Remain as still as possible. Stray dogs tend to be very fearful. Any sudden movements you make, or even just moving toward him normally, could look threatening to him. In turn, his 'fight or flight' response will be activated, causing him to turn and run away from you. Staying still will allow him to see that you are not a threat to him
- Do not hold your hand out for him to sniff it. He may bite your hand out of fear
- Even if the dog is displaying aggressive behavior, remember that he is likely more afraid of you than you are of him

RESTRAINING DOGS

- Do not run away. If the stray dog is demonstrating erratic or aggressive behavior and you become fearful, fight the temptation to run away
- If you start to run, chances are good that he will chase you
- If you want to distance yourself from him, walk away slowly and do not turn your back on him
- Entice the stray dog with food. Food is a great way to encourage a stray dog to come closely enough to you to catch him.
- Pretend like you are picking the food off the ground and eating it yourself.
- The stray dog will likely become curious at your actions and begin walking toward you
- To appear less threatening, sit on the ground while you are enticing him with food

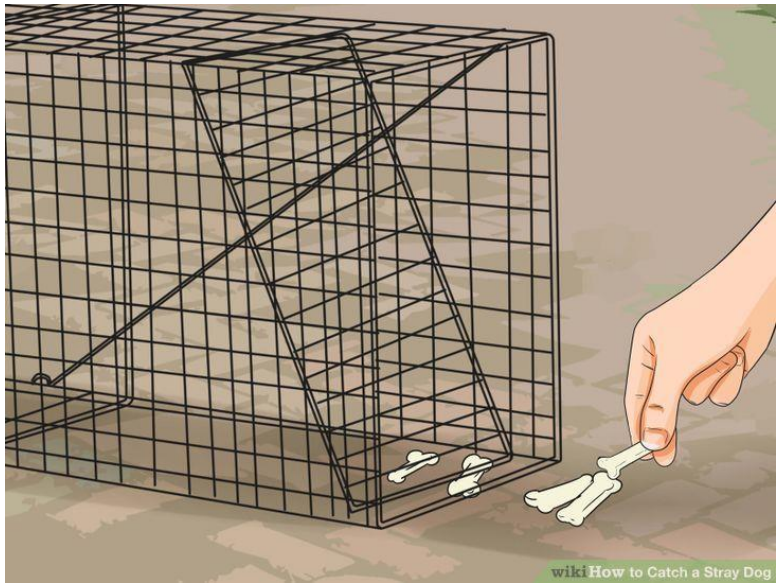
RESTRAINING DOGS

- **Use a dog catch pole**. A dog catch pole is a humane way to catch a stray dog. However, you should use it only if your own safety will not be at risk when using it
- Before trying to catch a stray dog in this way, set up a **crate** nearby in which you can place the dog after catching him
- Make the crate comfortable by setting blankets inside of it, along with some food and water
- If you can entice the stray dog with food, allow him to come closely enough to you where you can quickly hook the pole around his neck
- Move him quickly to the crate when you have hooked him. Release the pole when he is safely in the crate

- Never call a stray dog. Don't look at it, don't pat your leg, and don't walk towards the dog

RESTRAINING DOGS

- **Use of humane trap.** Traps come in a variety of shapes, sizes, and configurations
- Place the trap in a good location. The trap should be in an area where the stray dog frequents
- Ideally, this area should be where the stray dog would be comfortable approaching without feeling threatened
- Set up the trap. It is important for the trap to be inviting to the stray dog. If he does not feel comfortable around it, he may approach it but not walk in it.
- For example, cover the trap with blankets—this will help him feel safer when he steps inside of it
- Place tasty food in the trap
- Set the trap out when the stray dog is most likely to start roaming: dusk, dawn, or midnight



RESTRAINING DOGS

- After catching the dog, you can use **dog muzzles** during close examination, sampling or giving treatments

TAKING FECAL SAMPLES

- **Materials**
  - Plastic bag or
  - Sampling bottles
  - Gloves
  - Spatula/fecal loop/tongue bl
  - 10% formalin as necessary
- **Voluntary : from the ground**
  - Use freshly dropped feces
  - Take from the upper surface or inner part but avoid the part in contact with the ground
  - Collect the specimen using spatula/fecal loop/tongue blade in a clean dry screw-capped top container

TAKING FECAL SAMPLES

Involuntary: from the rectum

- Restrain the animal properly
- Get assistance from the owner or others
- Insert sampling **curette or your gloved finger** into the rectum gently
- Stimulate or remove as much feces as possible
- Examine also the perennial area for any attached parasites or fecal material that may contain cestode eggs or segments

- The stool specimen must be enough for satisfactory examination using the technique of choice
- Collect the specimen in a clean dry screw-capped top container

PRESERVING FECAL SAMPLES

- Most viable parasites are susceptible to desiccation or temperature variation.
- If time lapse between collection and observation is considerable, it may be necessary to add some form of preservative to feces specimen to retain morphology
- Formed samples can be kept in a refrigerator at 4 C° for few days
- Any whole worms or segments passed should be placed in a separate container
- If you are sampling feces for immediate examination for eggs or for culturing, take it without preservation or in an ice box
- If the sample is from distant location and/or culturing is not required, the feces can preferably be preserved in 4% formalin (for eggs and larvae) and transported with or without cold chain to the lab
  - 2/3 of the volume should be covered by formalin
- The container with the specimen should be clearly labeled with the following:
  - Animal ID and information
  - Date and place of collection

PRESERVING FECAL SAMPLES

Cestodes

- Wash in water to remove the mucus. Large tapeworms such as Taenia can be washed for several hours to relax the musculature, and can then be fixed in 10% formol saline between two glass slides to give flatter specimens.

Nematodes

- Adult are washed in saline to remove mucus. Worms up to about 7 cm in length are fixed in hot(60-70˚C) 70% alcohol, which straightens out living worms, except those which have natural curvatures at the head or the tail. Alternatively, they can be fixed in hot 5% formalin.
- Large worms such Ascaris groups can be fixed and preserved in cold 5% formalin