KASHVET E-LEARNING RESOURCE

A LABORATORY MANUAL OF VETERINARY PARASITOLOGY

Laboratory 1

INTRODUCTION TO THE PARASITOLOGY LABORATORY

Objective:

The purpose of this first laboratory is to introduce you to some of the techniques that a veterinarian uses to detect the eggs, cysts, and larvae of parasites in the feces of animals. The examination of blood for parasites is also described in this handout, although you will not be doing this procedure today. Since most of the diagnostic stages of parasites are microscopic, the proper use of your microscope is very important. Therefore, the last section of this handout covers the proper use of the microscope and gives some suggestions as to correcting some common problems.

A. Use of the microscope for fecal exams:

The following tips will help you adjust to using your microscope for the examination of fecal samples for parasites.

1. The first thing to remember is, unlike a histological section, a wet-mount of parasite eggs is three-dimensional and, therefore, you may find that you must continually adjust the focus to see objects at the bottom or top of the wet-mount.

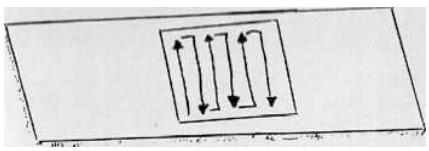
2. Make sure you have the condenser iris diaphragm open so that there is **just enough** light to work with (the higher the aperture, the lower the contrast). When using the 4X and 10X objectives, the diaphragm should be almost closed; open it a little for use with the 40X objective and further for use with the oil lens.

3. The condenser should be moved to almost its top position (you should not be able to see the lamp filament). **Do not use the condenser to adjust the light level, use the diaphragm.**

B. Examining a wet-mount:

When examining a wet-mount for cysts and ova, start in one corner of the coverslip using your 10X objective and cover the slide in overlapping fields (see diagram #1). Use your 40X lens to examine any suspicious objects, and after you have completed the examination, repeat about 1/4 of it using the 40X objective to find the smaller cysts. Note that the addition of a drop of iodine to the sample will stain many eggs and cysts increasing their contrast.

Diagram #1.



C. Fecal examination techniques:

In today's lab you should do the following techniques, making use of the 2 samples of dog feces under the hood. (This feces contains eggs of nematode parasites.) **Record your results (# of eggs per coverslip) on the DATA SHEET (pg 20) and enter your data into the web site [http://cal.vet.upenn.edu/paraav/forms/lab1data.htm] by noon Monday.**

1. Saturated salt flotation - There are numerous devices for doing this type of flotation now in use in local veterinary hospitals. Several manufacturers have donated devices for your use and we will be using them throughout the course in order to allow you to become familiar with each type.

2. **Zinc Sulfate Centrifugal Flotation Technique** - see instructions on Pg. 9.

3. **Ethyl Acetate sedimentation** - see instructions on Pg. 10. **Follow the tip at the bottom of the instructions and do a ZnSO4 float on the sediment. Caution:** Put the ethyl acetate only in the polypropylene test tubes, it will dissolve other types of plastics, including the fecal collection cups!

4. **Direct Smear** - see instructions on Pg. 5.

In a future laboratory you will learn how to count the number of parasite eggs per gram of feces using the McMaster slide, which is in your slide box. The use of the Baermann apparatus for recovering larval nematodes from feces is demonstrated in today's laboratory, you will be using the technique in a future lab.

The methods for examining feces covered in this laboratory are also covered in Foreyt's "Veterinary Parasitology Reference Manual" pp. 1 -10, in Sloss and Kemp's Veterinary Clinical Parasitology" pp. 1 -24.

Collection and Processing of Samples for Parasitology

A. Feces

1. Collection

   a. Ideally, feces should be processed as soon after passage from the animal as possible.

   b. Feces should be collected in airtight containers to prevent desiccation.

   c. If the processing of a fecal specimen must be delayed, it may be:

      I. refrigerated (but not frozen) for several days (not recommended for samples with live larvae that you intend to examine using the Baermann technique).

      II.. fixed, e.g., 10% formalin (5% formalin-saline is better for protozoal cysts). Add fixative to feces at a ratio 3:1 (v:v) and mix well. (Not for Baermann technique.)

   d. If an animal has been treated with antidiarrheal preparations containing bismuth or kaolin, mineral oil, oral contrast material (barium) for radiology (all of these materials float) or antibiotics, then parasites may be difficult or impossible to find. Therefore, repeat fecal exam 5-10 days after treatment withdrawal.

2. Processing

   a. First, examine the feces for blood and other clinical signs, then examine the inside of container for tapeworm segments (which are motile and may move away from the fecal mass).

   b. Many techniques have been devised to increase the likelihood that parasites will be detected in a particular sample of feces. The merits and limitations of representative fecal processing techniques are summarized in the table on the next page. Step-by-step directions for performing the various methods are on the following pages.

3. Repeat fecal exams are suggested in the following situations:

   a. Clinical signs suggest parasitism, but initial fecal exam was negative. Repeat in 2 or 3 days. Repeat for a total of 3 times within 7 to 10 days, if no parasites are seen it is likely the animal is not infected.

   b. Following specific therapy of a parasitic infection, have owner submit a fecal specimen 2 weeks following the last administration of drug. (This is late enough that all eggs and cysts will have been cleared from the gut, but, for most parasites, too early for reinfection to be showing up.)

COMPARISON OF FECAL EXAMINATION TECHNIQUES

Technique	Best Used For:	Problems
Zinc Sulfate Centrifugal Flotation	First choice for standard fecal examinations. Only technique for ***Giardia*** cysts and best technique for *Trichuris* eggs. Will, in most cases, recover nematode larvae.	Trematode, Pseudophyllidean tapeworm and ***Physaloptera*** eggs may not always float. Nematode larvae may be crenated and the Baermann technique may be required for a positive identification. Protozoal trophozoites will usually be too creanted to identify.
Saturated sucrose or saturated salt (sodium chloride or sodium nitrate) flotation	Standard technique used in many veterinary clinics. Will miss most ***Giardia*** cases and many of the mild whipworm cases.	All the problems mentioned above, plus: Nematode larvae and ***Giardia*** cysts may be crenated beyond recognition. Commercial devices allow examination of only a small amount of feces.
Ethyl acetate sedimentation	Best technique for examining samples with a large amount of fat in them.	May take a long time to examine the resulting sediment if not combined with one of the above flotation techniques.
Baermann Technique	Best technique for recovering live nematode larvae for identification.	Takes a minimum of an hour to run and will recover only live nematode larvae. Samples with only a few larvae in them may have to be run overnight.
Direct Smear	Least useful technique. Should be used only on liquid feces to look for protozoal trophozoites. Used as an adjunct to one of the fecal flotation techniques. Also a useful adjunct test when combined with a staining technique.	Examines only a small amount of feces and takes a very long time to examine the sample properly.

Techniques

Direct Smear Fecal Exam

1. Place a **small** amount of feces on a microscope slide.

2. Add a drop of liquid to the feces and mix thoroughly. The type of liquid added depends on what you hope to accomplish with the technique. If you are examining a liquid fecal sample for the presence of protozoan trophozoites (live active protozoa) then use saline (if any extra liquid is needed). If you are looking for helminth eggs and protozoan cysts in a small sample (bird droppings, rectal smear, etc) then either water or iodine may be used.

3. Cover with a cover slip. Move the cover slip around until it lays flat. You should be able to read through the smear (light from the microscope must be able to pass through the sample in order for you to examine it).

4. Examine the slide using the 10X objective, and then go over it with the 40X objective.

Because this technique examines only a very small amount of feces, it should only be used in the following circumstances:

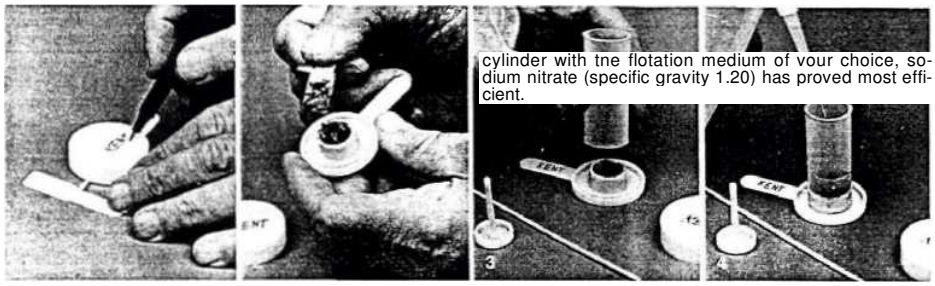
a. Liquid feces where protozoan trophozoites may be present.

b. Fecal samples where the amount of feces obtained is too small to handle with any other technique.

c. As an adjunct to a flotation technique where you are looking for eggs that do not float. (In this case you probably would be better off running an ethyl acetate sedimentation and examining the resultant pellet using the direct smear method.)

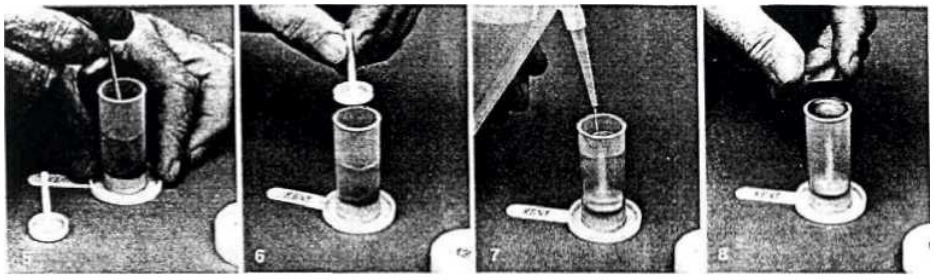
Note: Circumstances "b" and "c" occur frequently when dealing with small fish, birds, amphibians and reptiles and thus the direct smear has some utility in dealing with fecal samples from these animals.

Follow this simple procedure to set up the OvATECTOR[r] system in less than 45 seconds.



1> Using the special Indelible marking Den provided, Identify the fetal collection Container and dispense to Client, 21 Client separates spatula from container and fills center cylinder with tne flotation medium of vour choice, sodium nitrate (specific gravity 1.20) has proved most efficient.

When fecal container Is returned, the cylinder, with lip end up. Is placed over trie center receptacle and snapped into position forming the flotation system *A)* Half fill tne receptacle with fecal matter (holds 2 gram sample), SI

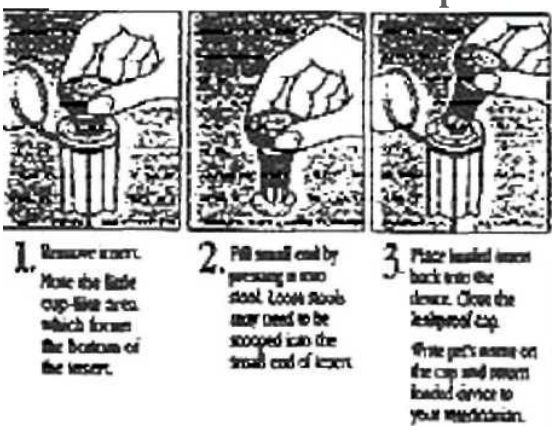


Si Mix fecal specimen and solution thoroughly with applicator stick provided, fit Push strainer gently down Into cviinder until handle is below top lip. 71 Add more flotation medium until convex meniscus is formed at the top of cylinder. 8> Float a 22mm cover slip on the meniscus. Allow to stand for at least 15 minutes for ova to float through strainer and adhere to cover slip. Lift cover slip with a smooth motion and place on microscope slide. Examine under low power and 100X for ova 9) When procedure is completed, pour off liquid and discard all components of the ovaTector.[m]

The OvaTector Kits used in this lab were a gift from Henry Schein, Inc., Melville, NY.

The OVASSAY *PLUS* Kits used in today's lab were a gift from Synbiotics Corp., San Diego, CA.

How to Collect a stool sample



1. Remove insert. Note the little cup-like area which forms the bottom of the insert.
2. Fill small end by pressing it into stool. Loose stools may need to be scooped into the small end of insert.
3. Place loaded insert back into the device. Close the dispenser cap.

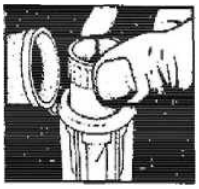
Write pet's name on the cap and return loaded device to your veterinarian.

OVASSWPtus

Ana^zing the Fecal Sample

Instructions

How to collect a stool sample with your Fecalyzcr*



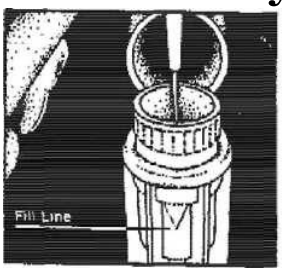
EVSCO PHARMACEUTICALS

1. Lift cap and remove insert.

2. 1*11!* small end of green insert into sipol sample. (If stool sample is ioose, scoop imo small end of *ptta* insert.)

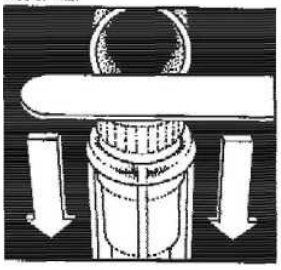
3 Replace green insen and close cap. Reeum to your veterinarian,

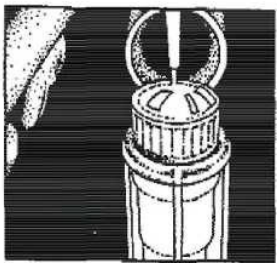
Laboratory Instructions



1. Lift op hur (to IKH returns g!Wn insert. Fill green vial with FECASOL* Ftamion Mtdmlothciipofsheinweatod U i S side of vial.

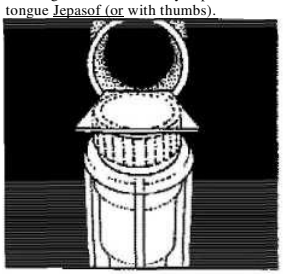
2. Rotaa jr*** insert «al bKk and fonh 10 separate ova frara ftai sample Mix

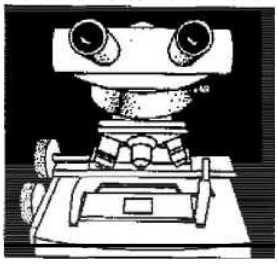




3. Scat grrcn inssfl vi^i fimly in pla<c wilh tongue Jepasof (or with thumbs).

4. F.!! twldrr ™mpl«ely to 6)rm a meniscas widi rfditkmal FECASOL Ffcmtion Mcdhira.





5. Float *iZmm* cover slip on marticos for [5-20 minutes,

6. Transfer cover slip to siide tor IHICID-u^k esamiiasion it I0QX nugnifjcaiion.

7. Close cap ftiuJ dispose of FECALYZER* ;o prevent cross-coMaminaiion

The Fecalyzer units used in this lab were donated by EVSCO Pharmaceuticals, Buena, NJ (a division of Vétoquinol).

ZINC SULFATE CENTRIFUGAL FLOTATION METHOD

1. Fill a 15 ml centrifuge tube with ZnSO4 solution (1.18 specific gravity)[1] and pour into a glass dish or plastic specimen cup.

2. Using a tongue depressor, push the feces (2 to 3 grams, a piece the size of a grape) through the strainer into the ZnSO4 solution in the dish. **Note:** the more feces you use, the more likely you will be able to find eggs which are present in low numbers.

3. Using a funnel, pour the ZnSO4-fecal mixture back into the centrifuge tube.

4. Centrifuge for 2 min at high speed (1500 - 2000 rpm).

5. Using a headed-rod or loop, remove a sample from the surface of the solution and place on a microscope slide. (You may have to take **several samples** with the rod or loop to get enough material to examine, you want the equivalent of a large drop on the slide.) Add a drop of iodine[2] (to stain the cysts and ova) and a coverslip. Examine at 10X.

NOTE: To increase the sensitivity of this technique either use more feces or do the following: After removing the tube from the centrifuge, fill the tube with ZnSO4 to just over the top of the tube, place a coverslip over the top of the tube and wait 5 to 10 min. Place a drop of iodine on a slide and place the coverslip onto the drop of iodine and examine at 10X.

Note: If the sample contains a large amount of fat or other material that floats in water, you may want to wash the sample before doing the flotation. To do this, start at step 1 but use water instead of ZnSO4. When you centrifuge the water-fecal mixture, the eggs, being heavier than water, will sink but the fat and other material will float. After centrifugation pour off the supernatant, add the ZnSO4 solution and mix well. Centrifuge as in step 4 and examine as in step 5.

1. **ZnSO4 solution** (1.18 sp. Gr.) is made by adding 386 grams of ZnSO4 to 1 liter of water. The mixture should be checked with a hydrometer and adjusted to 1.18. The ZnSO4 solution should be stored tightly capped to prevent evaporation (and the resulting change in the specific gravity of the solution).

2. **Iodine solution:** 10 gms Potassium Iodide (KI) added to 1 liter of distilled H_2O. Shake to dissolve. Add 10 gms of Iodine (I_2) to the above solution.
Allow to stand over-night with stirring, at this time you may still have Iodine crystals at the bottom, this is OK, just leave them there. This solution will stain (and kill) most parasite eggs and cysts (coccidial oocysts are an exception, they do not take in the iodine).

ETHYL ACETATE SEDIMENTATION METHOD

1. Pass a grape-sized piece of feces through a sieve into about 9 ml of water and pour into a 15 ml centrifuge tube. **CAUTION:** Test materials before placing Ethyl Acetate into them. This solvent will dissolve many types of plastic!! The white plastic centrifuge tubes used in the lab are OK, but clear plastic tubes and the disposable polystyrene cups will dissolve.

2. Add about 3 ml of ethyl acetate, plug the tube with a rubber stopper and shake the tube vigorously.

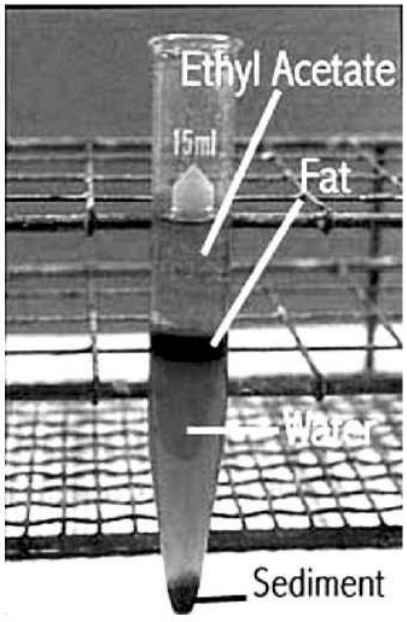
3. Remove the rubber stopper and centrifuge the tube (1500-2500 rpm) for 1 to 2 minutes.

4. Using a stick, "ring" the plug of fat at the water - ethyl acetate interface (the plug adheres to the side of the tube and must be detached before the liquid contents of the tube can be poured off).

5. Pour off the supernatant, being careful to leave the pellet at the bottom of the tube intact. (Flush the ethyl acetate down the sink with plenty of water.)

6. Transfer some of the sediment from the bottom of the tube to a slide and examine. The sediment can be transferred in several ways: 1) If some liquid remains, the pellet can be resuspended and a drop transferred with a pipette. 2) Add a drop of iodine to the pellet to resuspend it and then transfer with a pipette. 3) Use a stick to remove some of the pellet and smear it on a slide as you would when making a direct smear.

NOTE: For this technique to be as sensitive as a flotation method, you must examine the entire pellet!



When removed from centrifuge, your tube will have clearly defined layers:

A. An ethyl acetate l
B. A plug of dissolv
C. A layer of water.
D. A pellet of sedim

Because formalin fixed eggs and cysts may not float (they may now have a specific gravity of greater than 1.2) this technique is preferred for formalin fixed samples.

TIP: If you did this technique just to remove fat, you can resuspend the pellet in flotation solution, centrifuge, and remove the material from the top of the float to examine for eggs (see $ZnSO_4$ technique on Pg. 9).

BAERMANNIZATION

In 1917, while working in Java, the Dutch physician Dr. Baermann developed a simple method for isolating nematodes from soil. Today veterinarians use his method for the extraction of live larval stages of nematode parasites from the feces.

Technique:

1) Place a sieve in a custard dish or other similar container.
2) Spread about 10 grams of feces* on a piece of tissue paper and place it into the sieve.
3) Place warm* * water in the custard dish until it just covers the feces, taking care not to disrupt the feces.
4) Allow to sit for about one hour.
5) Lift off sieve.
6) Pour liquid into a 50 ml centrifuge tube.
7) Let sit for 20 minutes.
8) 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. (Be careful not to resuspend the sediment before you take a sample from it.)

* Use **fresh** feces - refrigeration may kill *Strongyloides* stercoralis larvae.

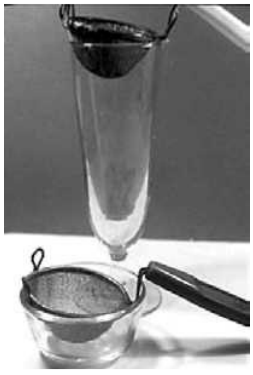
** This technique makes use of two characteristics of parasitic larval nematode behavior:
1) The warmer it is, the more active the larva (up to a point, you don't want to cook them!; 37 to 40° C is as warm as you want to get), and, in addition, some larvae are thermotaxtic and will move towards the warmer water under the filter paper.
2) 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.

f 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.

Baermann apparatus

Modified Baermanns



STOLL EGG COUNTING TECHNIQUE

A method for determining the number of nematode eggs per gram of feces in order to estimate the worm burden in an animal. The advantage of this technique is that it requires no specialized equipment, the disadvantage is the counting takes a long time because of the amount of extra (non-egg) material on the slides.

1. Weigh out 3 grams of feces.
2. Measure out 42 ml of water and place it into a dish. Using a tongue depressor, push the 3 grams of feces through a sieve into the water. Lift the sieve and hold over the dish. Push out any remaining water from the feces.
3. While stirring the water-feces mixture, take 0.15 ml of the suspension and spread over 2 slides. Cover each slide with a long coverslip (or 2 regular size coverslips).
4. Examine both slides for worm eggs, **the total number of eggs counted X 100 represents the number of eggs per gram of feces.**

5. The mathematics: 0.15 ml is 1/300 of 45 ml (42 ml water and 3 gm feces) so the number of eggs in 0.15 ml X 100 is equal to 1/3 of the total number of eggs in the original 3 grams and thus equal to eggs per gram **(EPG).**

McMASTER EGG COUNTING TECHNIQUE

Another method for determining the number of nematode eggs per gram of feces in order to estimate the worm burden in an animal. The advantage of this method is it is quick as the eggs are floated free of debris before counting, the disadvantages are you must use a special counting chamber and it has a detection limit of 100 EPG (unless multiple counts are done on the same sample).

1. Weigh out 2 grams of feces.

2. Pass the feces through a sieve into a dish containing 60 ml of $ZnSO_4$ or saturated salt solution. Lift the sieve and hold over the dish. Push out any remaining solution from the feces.

3. While mixing vigorously (you may want to put the solution into a flask to prevent spillage) take a sample of the mixture with a pipette and transfer it to one of the chambers of the McMaster slide. Repeat the procedure and fill the other chamber.

4. Wait 30 sec then count the total number of eggs under both of the etched areas on the slide. Use your 10X objective (first check to see that this objective can be swung into place without hitting the slide, if it hits the slide, count with the 4X lens). Focus first on the etched lines of the grid, then go down a tiny bit, the eggs will be floating just below the top of the chamber. **Multiply the total number of eggs in the 2 chambers by 100, this is the eggs per gram (EPG).**

5. **The mathematics:** The volume under the etched area of each chamber is 0.15 ml (the etched area is 1 cm X 1 cm and the chamber is 0.15 cm deep) so the volume examined is 0.3 ml. This is 1/200 of 60 ml. Since you started with 2 gms of feces and then multiplied by 100, the final result is **eggs per gram** of feces.

Modified Wisconsin Sugar Flotation Method

This method of determining the EPG is probably the most commonly used method. (First used by the University of Wisconsin's Parasitology Laboratory, it is a modification of the Stoll technique.) It is the most accurate as it counts all the eggs in 3 grams of feces and because it is a flotation method it has little debris to interfere with the count. However, if the EPG is high, there may be too many eggs to count.

1. Fill a 15 ml test tube with 10 ml of Sheather's* solution.

2. Weigh 3 grams of feces and place into a cup.

3. Pour the Sheather's* solution from the test tube into the cup and mix well.

4. Place a funnel into the test tube, place a strainer into the funnel and pour the fecal-sugar solution mixture through the strainer into the test tube. Using a tongue depressor, squeeze the liquid out of the feces that is left in the strainer.

5. Centrifuge the tube for 2 to 4 minutes.

6. Fill the tube to just over the top and place a cover slip onto the meniscus.

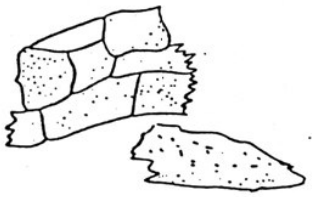
7. Let sit for about 5 minutes, then remove the cover slip and place on a slide.

8. Examine the entire cover slip and count the number of eggs that you find.

9. The number of eggs counted is the number per 3 grams of feces, so divide by 3 to find the EPG.

* Sheather's Solution: Add 454 gm (1 lb) of table sugar to 355 ml of very hot water. Stir until dissolved and allow to cool. This solution will grow mold if left out, so keep refrigerated and use quickly. Some people add 6 ml of formaldehyde to the solution to preserve it.

Objects Sometimes Mistaken for Helminth Eggs and Protozoan Cysts



plant cells

plant hair

seed fibers

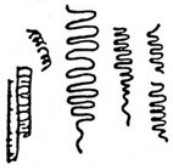
starch granules

protein particles

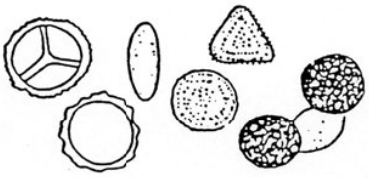
vegetable cells

fat droplets -. oil droplets

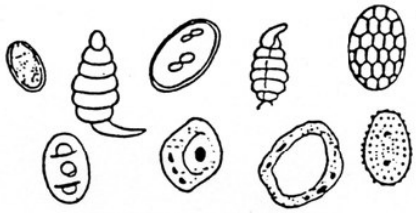
air bubbles scratches on slide



plant spring cells



pollen grains



fungus and yeast spores

Blood

1. Collected for two basic procedures:

   a. Concentration - to detect microfilaria (i.e., *Dirofilaria* and *Dipetalonema)*
   b. Smears - to detect protozoal and rickettsial infections (e.g., *Trypanosoma, Babesia, Anaplasma).* Smears must be fixed and stained to reveal organisms.

2. If blood is not to be processed immediately upon removal from the patient, an anticoagulant must be added to the sample. Among those commonly used:
   a. Heparin - effect lasts only for a matter of hours
   b. EDTA - effect lasts several days

3. **Procedure for making Blood smears (thin films):**

   a. Clean slide by wiping with alcohol. Handle slides by edges only. (Any grease on the slide will cause the dried blood to flake off during staining).
   b. Place a very **small** drop of blood near the end of a slide.
   c. Place the end of another slide (the "spreader") on the sample slide so that the edge of the spreader is just ahead of the drop of blood.
   d. Holding the spreader at an angle of about 30° (relative to the sample slide), draw it back until its edge just touches the drop of blood. The blood will then run along the entire edge of the spreader slide
   e. Push the spreader briskly in one fluid motion completely across the sample slide. Note that the blood is being **dragged behind** the spreader, not pushed in front of it.
   f. If the correct amount of blood was applied, the smear should end before the end of the slide, and the smear should end in a "feathered edge," a region where the blood cells are well separated.
   g. Air dry.
   h. Fixation and staining - various methods can be used. Normally a commercial staining kit is utilized following the manufacturer's instructions.

4. **Procedure for concentration of blood (Knott's Test):**
   see Laboratory #5.

PROPER USE **OF THE MICROSCOPE**

A. Illumination

1. Condenser should always be racked up as high as it will go.

2. BRIGHTNESS is controlled by (not all microscopes have all features):
   a. varying voltage to lamp by adjusting rheostat
   b. neutral-density filter over illuminator
   c. adjusting illuminator iris diaphragm

3. CONTRAST is controlled by:
   a. adjusting condenser iris diaphragm

4. Centering condenser -
   a. For microscope <u>with</u> iris diaphragm on illuminator:
      i. focus on a specimen with 10X objective ii. close diaphragm almost completely iii. focus spot of light by slightly lowering condenser iv. if necessary, adjust centering screws (two knurled rods), which protrude from condenser assembly, until the spot of light is centered in the field of view v. open the illuminator diaphragm until the light just in the field
   b. For microscopes with no iris diaphragm on illuminator:
      i. focus on a specimen with 10X objective ii. remove an eyepiece (pull straight out) iii. look down tube and close condenser iris diaphragm until a small spot of light is seen surrounded by black iv. center bright spot as above

B. Focusing

1. Use coarse adjustment first, then fine adjustment (the fine adjustment may have a limited range of travel in some instruments)

2. Oil immersion objective:
   a. If objectives are parfocal, focus at lower power, put drop of oil on slide, swing oil immersion objective into position, and adjust focus carefully with fine adjustment
   b. If objectives are not parfocal:
      i. view objective <u>from the side</u> ii. place drop of oil on specimen iii. lower oil imm. objective with coarse adjustment until its tip just touches the slide. Note the direction the focusing knob was turning! iv. looking into the microscope, turn the coarse focusing knob in the opposite direction slowly until the specimen comes into focus. Adjust, if necessary, with the fine-focus knob.

3. To make obj ective parfocal:
   a. adjusting screw on each objective (expensive models)
   b. if no adjusting screws on your instrument, use shims between each objective

and turret (available from microscope supplier ~ cheap)

4. If you find it impossible to focus on a specimen:
   a. coverslip too thick (usually only a problem with oil immersion)
   b. slide is upside down
   c. oculars not matched (binocular microscopes)

C. Tips for eyeglass wearers:
   1. install rubber guards over eyepieces to prevent scratching, or

   2. trade in your eyepieces for "high eyepoint" ones ("exit pupil" further away from end of eyepiece)

D. Cleaning
   1. Locating dust specks (assuming that slide is clean):
      a. if specks disappear when condenser is moved, then dust is on illuminator bulb or filter
      b. if specks disappear while focusing, then dust is on condenser
      c. if neither of the above manipulations works, then
         i. rotate eyepiece(s) - specks will rotate as well if dust is on them ii. rotate objective - ditto above

   2. Removing dirt and film from lens surfaces:
      a. try using a special brush or air jet first (blower/brushes available at photo stores)
      b. wipe, using lens paper, after breathing on surface
      c. if necessary, use some alcohol - xylene is the last resort! (solvents may attack lens mounting cements)
      d. immersion oil should always be removed from objective soon after use by wiping with lens tissue - no solvents should be necessary

E. Measuring objects under the microscope
   1. The purchase of an ocular micrometer is highly recommended for parasitology work. It is relatively cheap and easy to install. It must be calibrated before it can be used; this procedure is simple and is described in a separate information sheet.

F. All students should have the following microscope accessories:
   1. lens tissue
   2. immersion oil
   3. spare illuminator bulb
   4. lens brush

CALIBRATION OF THE OCULAR SCALE

Calibrating a ocular scale in a microscope is simply a matter of converting an arbitrary measure (ocular micrometer units) to a standard unit of measure (microns).

Follow the directions below in calibrating your microscope's ocular scale.

Place a stage micrometer on the microscope stage and focus on the scale using reduced illumination. Notice that the scale has large divisions which are 0.1 mm or 100 microns in length. At one end of the scale, two of the 0.1 mm divisions are each divided into 10 smaller divisions each measuring 0.01 mm (10 microns).

1. Superimpose the ocular scale over the micrometer scale so that the zero point of each scale will coincide.

2. Count the total number of divisions from the 0 of the ocular to one of the numbers near the end of the ocular where it exactly coincides with one of the lines on the stage. Record both numbers.

3. Divide the stage measurements in microns by the ocular units to obtain the number of microns/ocular unit.

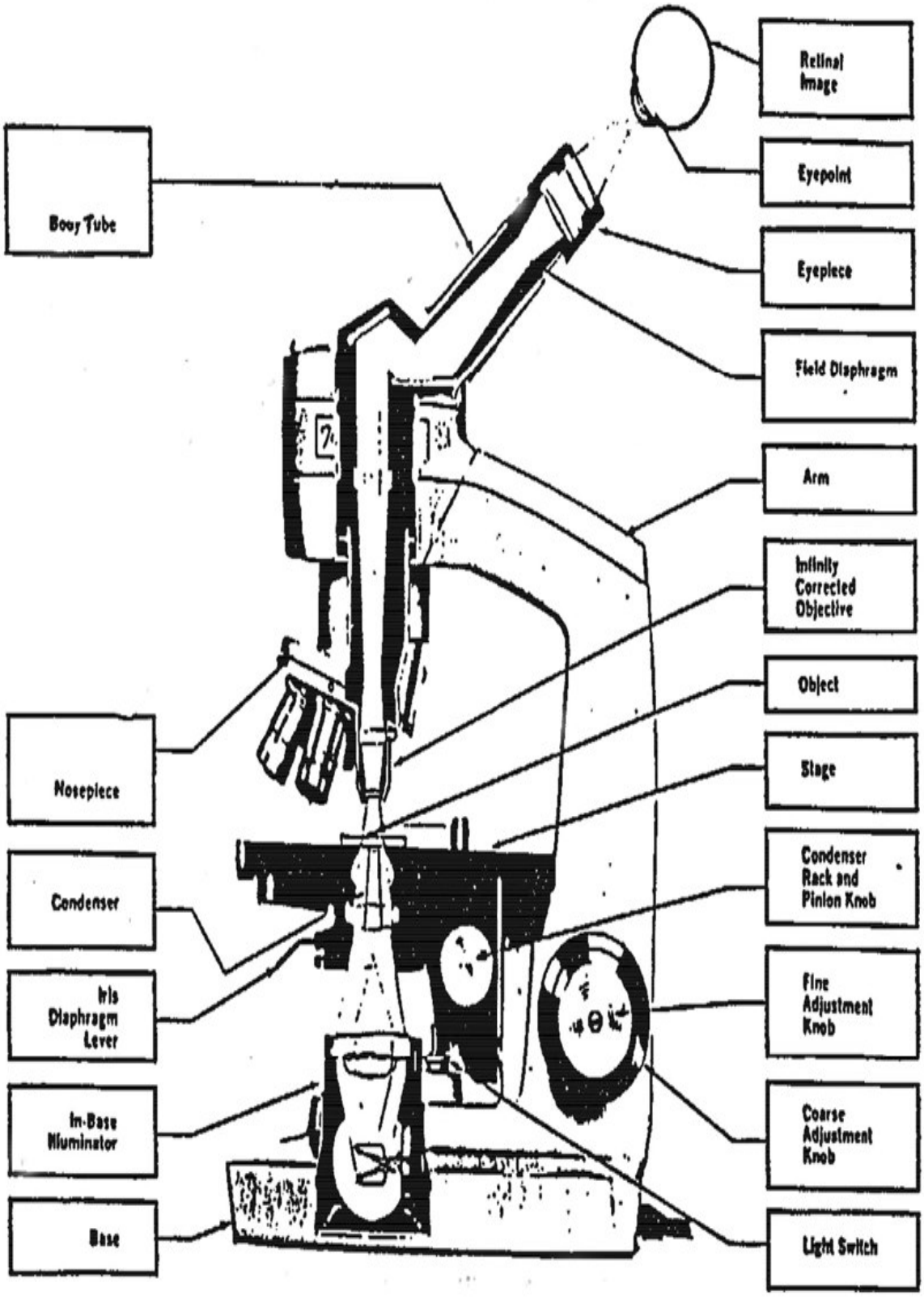
4. Repeat the measurement twice to ensure that you have made no errors.

5. Carry out the same procedure for each of the objectives on your microscope. It is not necessary to use oil with the oil immersion objective in this instance.

6. Prepare a chart converting ocular units (at the left) to microns (at the right) for each of the microscope objectives.

OPTICAL AND MECHANICAL FEATURES OF

THE MICROSCOPE



LAB1 DATASHEET

This sheet is for your records and should remain in your lab manual. Enter your data into the web site [http://cal.vet.upenn.edu/paraav/forms/lab1data.htm] by Monday.

1] Count the number of nematode eggs that you find under the coverslip for each procedure.
2] Estimate the time it took to do the procedure (from when the feces was obtained until the egg count was recorded).

PROCEDURES FOR SAMPLE "A"

	Egg Counts	Time needed to do procedure
Saturated Salt (Fecalyzer)	_____	_____
ZnSO4 Centrifugal flotation		
Ethyl Acetate		
Direct Smear		

PROCEDURES FOR SAMPLE "B"

ZnSO4 Centrifugal flotation

Ethyl Acetate

LABORATORY 2

STRONGYLES

Objectives:

The strongyles of livestock all have similar eggs ("strongyle- type"), most of which hatch and develop to the infective third-stage on pasture. However, the life-cycles differ to some degree and the different species can cause different diseases. While the newer anthelmintics kill a broad range of strongyles, control measures may vary for each worm. Therefore, it is important to differentiate these worms based on their morphology and the location from which they were recovered upon necropsy.

Introduction:

The strongyles are bursate worms; that is, the males all have a copulatory bursa at their posterior end which is wrapped around the female during mating. All these worms have "strongyle-type" eggs which have a thin shell, and an 8-16 cell morula visible inside (as passed in the feces).

Sheep and Cattle

1. Estimate the number of strongyle type eggs per gram of sheep feces using the McMaster Egg counting method. Fecal samples for use with this technique are provided in tubs marked #1. Information on this technique can be found in Lab #1 handout. **Note: You can make one flask of the diluted feces and everyone at the bench can use it to do a McMaster count.**

2. Examine the worms found in the dishes marked **"abomasal nematodes:** A, **B, C".**

The following nematodes might be found in a sample of the abomasum's contents (differentiate based mainly on size, but remember, male and female worms of the same species may be of slightly different sizes):

A. *Haemonchus contortus*

The largest of the nematodes found in the abomasum, they are 2 to 3 cm in length. The adult female will have her white ovaries wrapped around her intestine, which, when full of blood gives the appearance of a "barber pole," hence the common name "Barber pole worm." The male worm will have an asymmetrical dorsal ray (i.e. the dorsal ray arises from one side of the mid-line). (See pg 19 of the text: Urquhart, et al.), **however, you are not responsible for identifying this feature on the males, it is enough to know that the worm is a male and because of its size it probably is *Haemonchus contortus.***

Take a worm from this dish (A) and examine it under your microscope by using the technique ("rolling nematodes") found in the appendix. (See Figure 1).

B. *Ostertagia* sp.

Of the 3 nematodes found in the abomasum of sheep, this species is intermediate in size (about 1 cm long).

C. *Trichostrongylus axei*

The smallest of the abomasal nematodes, less than 7mm long (hard to see with the naked eye). (See pg. 23 of Urquhart et al. and Figure 1.)

3. The following worms may be found in the sheep's small intestine (these worms are shown in the **demonstrations [DEMO]):**

A. Other *Trichostrongylus* spp. - similar to *T. axei*

B. Cooperia sp. - small worm (4-6 mm). The worm may be tightly coiled, giving the appearance of a watch-spring ("watch-spring" worm) the cuticle of the anterior end is slightly swollen (cephalic vesicle) and striated. (See pg. 25 of Urquhart et al.).

C. *Nematodirus* sp. - a long worm (about 1 to 2 cm). The spicules of the male extend past the bursa. The egg is twice as large as any other strongyle - type egg (pg. 75, Foreyt).

Swine

4. *Oesophagostomum* - (DEMO) and *Stephanurus* (eggs in bottle #132).

Oesophagostomum sp. - ("nodule worm") causes the formation of nodules in the intestine. **DEMO.** Since the acute disease is associated with the larvae, eggs are not usually present in the feces at this time.

Stephanurus sp. - Large (4-5cm), stout worm, found around and in the kidney of pigs **(DEMO).** Fresh specimens are pinkish in color. The size of the worm and site (kidney) are enough to identify this worm. Eggs are found in the urine, however, the disease's main pathological effects occur during the prepatent phase.

5. Material contained in trays distributed throughout the classroom, was removed from the large intestine of an equine at post-mortem. **Notice the difference in size between the large and small strongyles.** The large strongyles can be differentiated by size and the number of "teeth" in the buccal capsule.

The following worms may be found: (Note: the color of preserved specimens differs from that of fresh, and even varies depending on the initial state of the worm and how it was preserved. Therefore, do not use color as an identifying characteristic. Also you can't see the teeth in these bile-stained, formalin fixed specimens, therefore use the size to separate *S. vulgaris* from the other two large strongyles.)

A. *Strongylus vulgaris* - the smallest (1.5-2.5cm) of the 3 species of *Strongylus* sp. found in the horse. All of the adult *Strongylus* spp. have a large buccal capsule, but differ in the number of teeth in the capsule. *S. vulgaris* has two, ear-shaped teeth. (see pg 41 of Urquhart et al. and **DEMO)**

B. *S. edentatus* (2.5 to 4.5 cm) No teeth in the buccal capsule (see pg. 42 of Urquhart et al. and **DEMO).**

C. *S. equinus* (2.5 to 5.0 cm) 3 cone-shaped teeth in the buccal capsule (see pg 42 of Urquhart et al. and **DEMO).**

Note: both *S. vulgaris* and *S. equinus* have a pair of teeth situated on both sides of the mid-line at the bottom of the buccal capsule. When viewed directly from the side these two teeth may overlap and appear as one tooth.

D. *Cyathostoma* sp. - one of the many "small" strongyles (<1.5 cm)., the buccal capsule is shallow and contains no teeth. **(DEMO)**

6. Examine the stained sections provided which show the buccal capsule of a *Strongylus* sp. attached to the intestinal wall. (Student Slide Box #30).

Checklist of Objectives for Lab 2

1. Use of the McMaster counting chamber for determining EPG.

2. Identify sheep **abomasal parasites:** *Haemonchus*, *Ostertagia* and *Trichostrongylus* by size.

3. Identify sheep **small intestinal nematodes:** *Trichostrongylus* by size, *Cooperia* by its "watch-spring" coiling, and *Nematodirus* by size and by its very large egg.

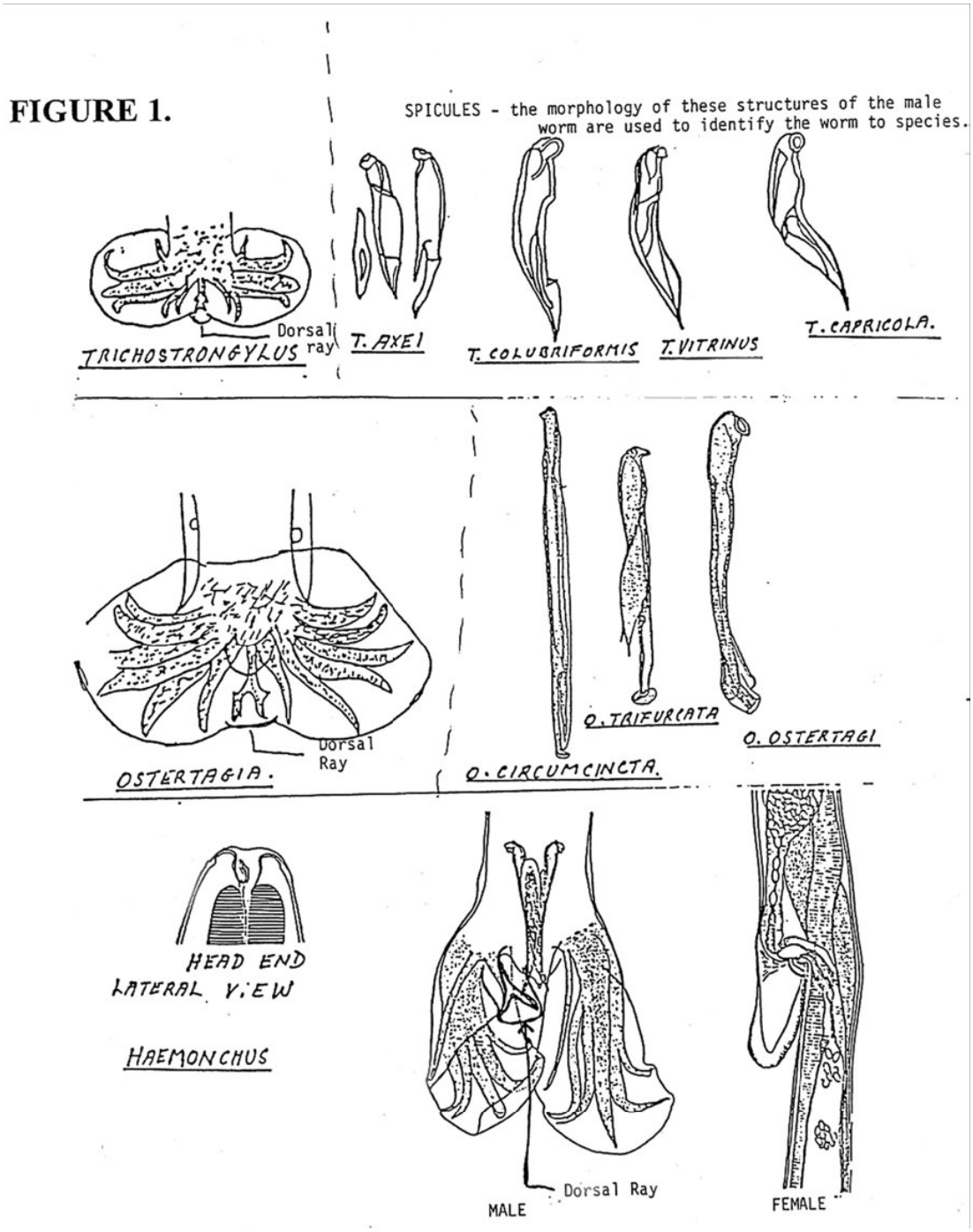
4. Identify *Oesophagostomum* and *Stephanurus* from the swine **(by location).**

5. Identify the strongyles of horses: the species of *Strongylus* by the number of teeth in the buccal capsule of a cleared specimen, and *Cyathostoma* and *S. vulgaris* by size.

6. Be able to identify a "strongyle-type" egg.

7. Answer the Review Question at the end of the demonstrations.

FIGURE 1.


SPICULES - the morphology of these structures of the male worm are used to identify the worm to species.
Dorsal ray
TRICHOSTRONGYLUS
T. AXEI
T. COLUBRIFORMIS
T. VITRINUS
T. CAPRICOLA.
Dorsal Ray
OSTERTAGIA.
O. CIRCUMCINCTA.
O. TRIFURCATA
O. OSTERTAGI
HEAD END
LATERAL VIEW
HAEMONCHUS
Dorsal Ray
MALE
FEMALE

Appendix for Laboratory #2 ROLLING NEMATODES

This technique is used by parasitologists to examine the morphology of small nematodes in order to identify them as to species.

Place a worm on a slide with a few drops of water and a coverslip. Place the slide on your microscope under low power and roll the worm by moving the coverslip around. If the worm is a male try to get it in such a position that the bursa is spread out so the dorsal ray is visible. If the specimen is a female, roll it until the vulva is visible.

SIGNIFICANCE OF EGG COUNTS

(These are only approximate and should be considered in association with the clinical signs.)

Parasitic gastritis in lambs	2000 - 6000 EPG
Parasitic gastritis in cattle	300 - 600 EPG
Strongylosis in equines	1500 - 2500 EPG
Fascioliasis in sheep	300 - 600 EPG
Fascioliasis in cattle	100 - 200 EPG

EGG LAYING CAPACITY OF SOME NEMATODES

Haemonchus contortus	5000 -10000 eggs per day
Ostertagia and *Trichostrongylus* spp.	500 - 2000 eggs per day
Nematodirus filicollis	50 - 250 eggs per day

SEVERITY OF INFECTION

Fatal effects seldom seen with less than:

Haemonchus contortus	1,000 worms
Ostertagia circumcincta	8,000 worms
Trichostrongylus spp.	10,000 worms
Chabertia ovina	100 worms

Laboratory #3 Hookworms,

Lungworms, and *Strongyloides*

Objectives: Both lungworm and *Strongyloides stercoralis* infections are diagnosed by finding the larvae (L1s), rather than eggs, in fresh feces. In dogs, hookworm larvae may also be present in **old,** non-refrigerated feces ($24 hours old), and, therefore, you should be able to distinguish these 3 kinds of larvae.

The adults of these nematodes, when found at necropsy, can be distinguished on the basis of size, shape, and their location within the host. The several species of hookworms, some of which are of public health importance, can be distinguished from each other by the structure of their mouth cavities.

The objectives of today's laboratory are to learn the diagnostic stages of hookworms, lungworms and *Strongyloides stercoralis* from dogs, as well as to learn to identify the adults (based on size and anatomical site from which they were recovered). Also, learn to identify the adult hookworms of the different species by the number of teeth or the presence of cutting plates in their oral cavities (see Figs. 1-4).

Finally, learn to recognize the larvae of *Aelurostrongylus* of cats and the eggs of the other *Strongyloides* spp. that parasitize a great diversity of hosts, both wild and domestic. (Diag. 1)

Wet Preparations - make a wet preparation from each bottle, containing the diagnostic stages, by adding one drop of the bottle suspension and one drop of Lugol's iodine (optional, but highly recommended) on a slide. Cover with a cover glass.

HOOKWORMS

Ancylostoma spp.

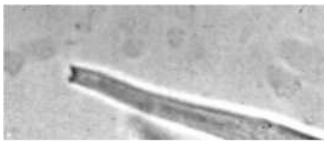
A. Do a fecal float to recover *Ancylostoma caninum* ova (60 x 40 :m, pg. 21, Foreyt) from dog feces.

B. Canine feces (24 hr.-old), hatched *Ancylostoma* eggs. Find rhabditiform larvae (L1) and note the shape of the characteristic esophagus (rhabditiform), prominent mouth tube, inconspicuous genital rudiment and simple conical tail. These are the diagnostic features.

A charcoal-feces mixture is under the hood. Each group of students should take a teaspoon-sized sample and run the modified Baermann technique (see lab 1) to recover larvae.

C. *Ancylostoma caninum* third-stage (infective) larvae (L3), compare and note the difference between the mouth and esophagus of *Ancylostoma* and *Strongyloides* infective (L3) larvae. (Center Bench) *A. caninum* L3 will have a sheath (the cuticle of the L2 which has been retained for protection from the environment), while *Strongyloides* will not. The esophagus of the hookworm is bulbed and runs only about 25% of the length of the worm, while *S. stercoralis* will have a long (about 40 to 50% the length of the worm) straight esophagus. Also, *S. stercoralis* L3 will have a notch in the tail, while *A. caninum* has a straight tail. **Note:** It is sometimes useful to culture *Strongyloides* larvae to the L3 stage for diagnostic purposes. Because multiplication occurs during the heterogonic cycle (free-living cycle) a sample with too few larvae (L1) for diagnosis initially may be found to be positive for *S.*

stercoralis larvae after amplification in culture. Also, you should recognize these third-stage larvae because such larvae could be present in dogs with hyperinfective strongyloidiasis (they may be seen in tracheal washes) and may be present in "old" stools. If the dog whose stool you cultured also had hookworms you would have to tell these L3 from those of *Strongyloides.*



Tail of *S. stercoralis* L3. Note the notch in the tail.

D. *A. caninum,* Adult female (student slide box #29): Note the size of this worm, its large mouth capsule, and its teeth. In dorsoventral view 3 pairs of teeth (6 total) will be visible. (See Figure 1. If your specimen is mounted in lateral view, only 1 - 3 of the teeth will be visible at any one depth of focus, depending on the microscope objective used.)

A. caninum

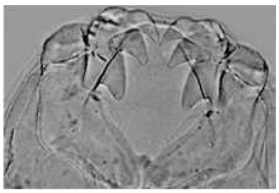


Fig. 2
A. *braziliense*

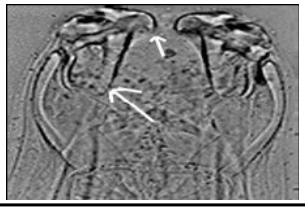


Fig. 3.
Uncinaria

stenocephala

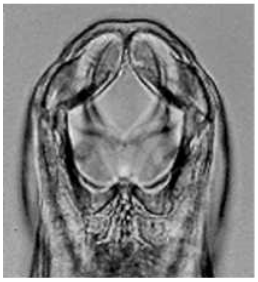


Fig. 4
Bunostomum sp.

E. Other Hookworms - Demonstrations of Adults.

A. braziliense - dogs and cats. 1 large and 1 small tooth per side. This worm is the main causative agent of cutaneous larval migrans in humans. (Fig. 2)

Uncinaria stenocephala - wild and domestic canines. Common in Europe and Canada its range extends into the northern U.S. Note the **cutting plates** instead of teeth. (Fig. 3)

Bunostomum sp. - the sheep/cattle hookworm. This large hookworm also has cutting plates in the mouth capsule of the adult worm. (Fig. 4)

LUNGWORMS

Dogs

1. *Oslerus (Filaroides) osleri* - larvae are found in fresh feces.

Bottle #113- first-stage (L1) larvae (pg. 25, Foreyt), esophagus is longer than the distinctly bulbed rhabditiform esophagus (i.e. less distinctly bulbed) of the first-stage *Strongyloides* larvae. Strongyliform esophagus, no mouth tube, and irregular, digitiform or "kinked" tail. There also are larvae in this sample with very long tails, these are equine larval "contaminants", ignore these. (However, these are occasionally naturally present in the feces of dogs examined in suburban/rural practices, in which they may cause confusion for the veterinary technician.)

Cats

1. *Aelurostrongylus* sp. - bottle #111 - larvae from tracheal aspirate. Make a wet preparation and examine. Note the S-shaped (kinked) tail. (Pg. 49, Foreyt) Swallowed larvae will, of course, appear in the feces.

Sheep

1. *Muellerius capillaris* - in the lungs of sheep.
   DEMO: Section of lung.

Cattle

1. *Dictyocaulus viviparous*

Note: This lung worm is, taxonomically speaking, a trichostrongyle, not a metastrongyle. It is presented here because diagnostically it has larvae in the feces and adults in the lung and thus the diagnosis is similar to that of the metastrongyles.

A. adults obtained from lungs of cattle - Demo.

B. larvae from feces - bottle #10. (Pg. 81, Foreyt)

Pigs

1. *Metastrongylus apri* - Demo.

Sheep

1. *Strongyloides papillosus* - Bottle #49, ova. These eggs are smaller than strongyle eggs and typically contain a larva when freshly passed in feces. Size = 40-60 :m X 20-25 :m. (See pg. 75 in Foreyt)

Horse

1. *Strongyloides westeri* - eggs almost identical to *S. papillosus* (Bottle # 49).

Pigs

1. *Strongyloides ransomi* - eggs almost identical to *S. papillosus* (Bottle # 49).

Dogs

1. Strongyloides stercoralis - 1st stage larvae are found in fresh feces. (Larvae, rather than eggs, pass in feces. In other species of *Strongyloides* a larvated egg is passed.) Additionally, a few precociously developed infective larvae (L3) may be present in fresh feces of *S. stercoralis* infected animals (also see "C" under *Ancylostoma).*

Living L1s and L3s were obtained, by using a Baermann apparatus and fresh feces or 7-day-old fecal-charcoal cultures, respectively. **(Remember:** L3s, but not L1s, are infectious for humans!!).

A. Bottle on center bench - *Strongyloides* first-stage larvae from canine feces. Size is between 280: and 310: in length, rhabditiform esophagus, no mouth tube, simple conical tail, and **large** genital rudiment. (Pg. 21 in Foreyt)

B. Bottle on center bench - *Strongyloides* third-stage (infective) larvae, size is 525: to 600: in length, long filariform esophagus, notched tail tip. Compare to the ensheathed L3 of *Ancylostoma caninum* (and similar bursate nematode parasites).

C. Demo - *Strongyloides stercoralis* adult females from a post-mortem of a heavily infected dog. Note the small size and **all** are females. The esophagus is nearly cylindrical and at least one fourth as long as the body. Parasitic females of Strongyloides are parthenogenetic. Therefore, males are **not** found in the small intestine along with the adult female worms.

Differential diagnosis of canine nematodiases based on L1 larvae, the stage that typically appears in the feces in *Strongyloides* and *Oslerus (Filaroides)* infections:

Distinguish between the larvae of the following species. These are larvae that might occur in a 24-hour-old canine fecal sample: a) *Strongyloides stercoralis* (L1) b) *Ancylostoma caninum* (L1) c) *Oslerus (Filaroides) osleri* (L1). See the diagram below for details. The following "key" may help you identify the L1s:

A. *Ancylostoma* spp. are found as eggs in fresh feces; if the feces are fresh and only larvae are found, eliminate hookworms from consideration. If only eggs are found eliminate *Filaroides* and *Strongyloides.*

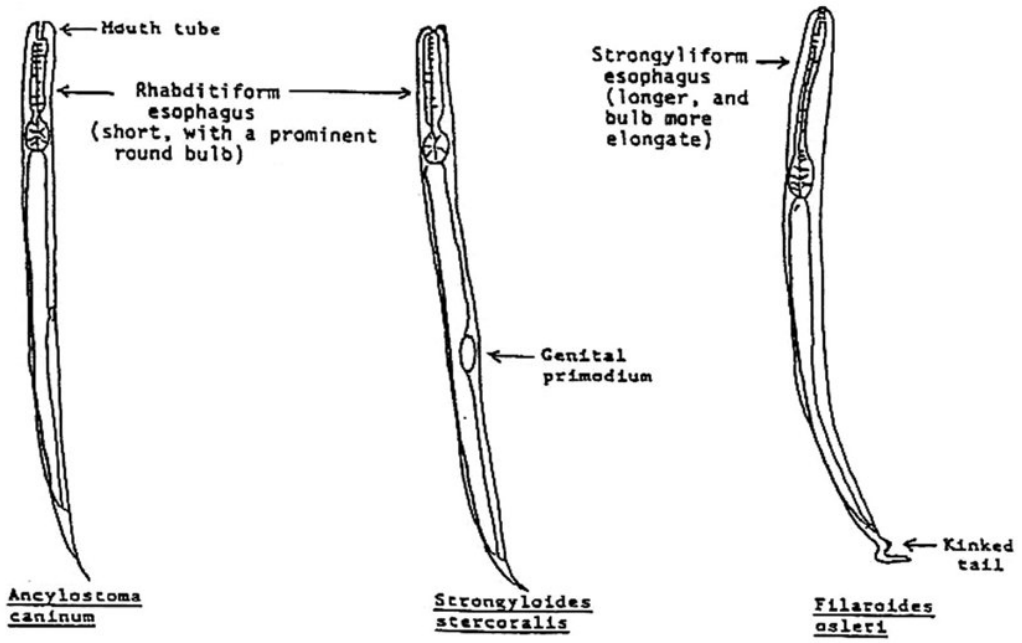
B. If the tail of the L1 is "kinked" then the nematode is *Oslerus (Filaroides)* sp. (or *Aelurostrongylus* sp. if from a cat). If the tail is straight then go on to C.

C. If the L1 lacks a prominent mouth tube, and has a prominent genital rudiment it is *Strongyloides stercoralis.* (You may have to examine several L1s in order to find the one in which the genital rudiment is in such a position that it is visible.)

D. If the L1 with the straight tail has a prominent mouth tube (and no visible genital rudiment) then it is *Ancylostoma* spp. (This assumes that the feces are old.)

Diagram 1.

Primary Diagnostic Features of Nematode Larvae (L1) found in Dog Feces.



Checklist of Objectives

1. Recognize hookworm eggs and the L1 stage in fecal specimens from dogs and cats.
2. Differentiate between the adult hookworms of dogs based on cutting plates or teeth.
3. Recognize typical metastrongyle (lungworm) L1s and the adults (by size and location).
4. Recognize the eggs and L1 of *Strongyloides* spp.
5. Distinguish between and recognize the L1 *of Strongyloides stercoralis, Ancylostoma* sp., and *Oslerus (Filaroides)* sp. (or *Aelurostrongylus* sp. if from a cat) from the feces of dogs.
6. Recognize *Strongyloides* adults (size, long esophagus, location [small intestine] and absence of males).

Laboratory #4 Ascarids, *Oxyuris,* Trichocephalids

Objective: The egg deposited in the feces is the usual diagnostic stage for the worms considered in this laboratory. Therefore, you should be able to identify the eggs of these worms. Sometimes you or your client will notice adult worms in feces or vomitus of infected animals and, therefore, you should also be able to identify the adults of these nematodes (most can easily be recognized by size and characteristic morphology). *Trichinella spiralis* is exceptional in that it does not have eggs or larvae occurring in the feces. For this species the L1 in the muscles is the diagnostic stage.

Ascaridoidea

The ascarids are large nematodes that usually live in the small intestine. All ascarids have three lips around the mouth opening and have no buccal capsule. Species occurring in cats and dogs have prominent cervical alae. Eggs are thick-shelled and unsegmented when passed. They embryonate in feces or fecally contaminated soil. Infection is by ingestion of the embryonated egg, by ingestion of a larva in a paratenic host, or by vertical transmission (in utero or via the milk). Vertical transmission is particularly important among the ascarids of dogs (prenatal) and cats (transmammary).

Pig

Ascaris suum - largest nematode of the pig, up to 40 cm long, a.

Demonstrations of Adults

b. Eggs - bottle #59 (60 x 45 :m, pg. 131 Foreyt). Note: many of the eggs have lost their rough proteinaceous outer layer.

c. Slide #SSB25 is a hematoxylin and eosin-stained section from the lung of a guinea pig, showing migrating *Ascaris suum* larvae. The guinea pig had been experimentally infected 6 days previously. There is little histopathology associated with a primary infection; subsequent infections elicit a strong host response with marked cellular infiltration and granuloma formation around the killed larvae. A similar reaction in the liver produces "milk spots", the gross lesions visible on the liver's surface as white spots.

Horse

Parascaris equorum - largest nematode of the horse (up to 40 cm long), similar to *A. suum* in appearance.

a. Demonstration of Adults (These will be seen in the feces of successfully treated horses).

b. Eggs - bottle #19 (90 to 100 :m, pg. 119 Foreyt). Note: many of the eggs have lost their rough proteinaceous outer layer, and the bottle has been contaminated with *Oxyuris* and *Capillaria* eggs.

Cats and Dogs

Toxocara canis - a large (up to 10 cm) nematode of the dog. Adult worms from dogs may be confused with those of *Toxascaris leonina.* However, their eggs differ greatly and if eggs can be expressed from female adults a positive identification can be made. Males differ in the shape of the tail.

a. Demonstration of Adults - Note the presence and shape of the cervical alae (clear cuticular flanges running along the anterior lateral margins of the worm). (See Diag. #1)

b. Eggs - bottle #23 (90 x 75 :m, pg. 19, Foreyt). **Note:** This bottle contains eggs of: *Toxocara, Toxascaris, Trichuris.*
Using your 40 X objective focus carefully on the surface of the egg and note that the surface is pitted. At 10 X note that the egg contents almost fill the shell cavity.

Toxascaris leonina - an ascarid found in both dogs and cats.

a. Demonstration of Adults - Grossly, the adults are morphologically similar to those of the larger *Toxocara canis* in the dog, but are easily distinguished from *Toxocara cati* of cats by the shape of the cervical alae. In *T. leonina* the alae terminate gradually, merging into the cuticle, giving the anterior end of the worm a "lance-like" appearance (see Diag. #1) compared to the "arrow-head" appearance of *T. cati* (see below). All 3 species have 3 lips.

b. Eggs - bottle #23 (80 x 65 :m, pg. 19 & 47, Foreyt). **Note:** This bottle contains eggs of: *Toxocara, Toxascaris, Trichuris.*
These eggs have a smooth shell (use 40 X objective), are slightly ovoid and the egg's contents do not fill the shell (use 10 X objective).

c. Do a float on the dog feces. The stock is under the hood. Eggs of which species are present?

Toxocara cati - another ascarid of the cat.

a. Demonstration of Adults - The cervical alae of this worm differ from those of *T. leonina,* the other ascarid of cats. The alae are broad and end abruptly, giving the anterior end an "arrow-head" appearance (see Diag. #1).

b. eggs are identical to those of *Toxocara canis* (pg. 47, Foreyt).

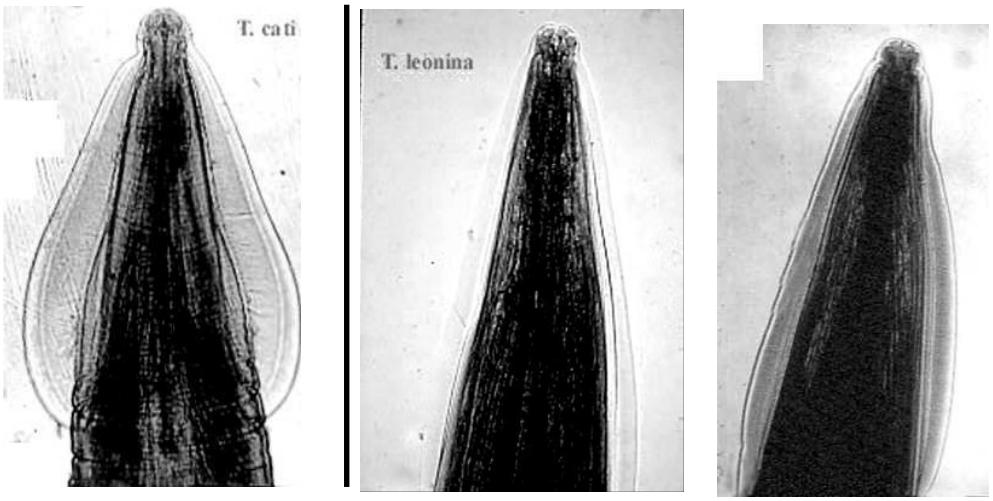
Raccoon and Dog

Baylisascaris procyonis - the ascarid of raccoons, causes an often fatal visceral larva migrans (cerebrospinal nematodiasis) in other animals, including humans and dogs. Some examples are: 1) Fatal CNS disease in a flock of 85 penned Bobwhite Quail. Soil analysis showed 10,000+ eggs in 1500 gm of soil from the floor of the pen, which had been previously used for racoons. 2) A similar outbreak in a flock of 600 chickens raised on the ground was attributed to a wild racoon. Its larvae also occur in man, in whom fatal infections have been reported. This parasite is receiving increasing attention in veterinary medicine and public health. This ascarid can infect dogs, although such infections may be rare. Egg is similar to that of *Toxocara* spp.

a. Demonstration of Adults. Note the size of the adult worms. The adult worms lack cervical alae and are filled with thousands of eggs. Should you treat a "pet" raccoon, warn the client to dispose of the large, easily seen, adults (and the stools) by flushing them down the toilet. This applies to your premises also. Wash cages thoroughly with very hot water taking care that the wash water goes down the drain (steam if possible). Keeping raccoons as pets should be strongly discouraged. (pg. 159, Foreyt)

Diagram #1.

The cervical alae of *Toxocara cati, T. canis,* and *Toxascaris leonina.*
Note: *Baylisascaris procyonis,* a parasite of racoons which sometimes infects dogs, has no cervical alae.



Toxocara cati
alae wide at base.

Toxascaris leonina
alae narrow at base, identical to *Toxocara canis.*

Toxocara canis
alae narrow at base, identical to *Toxascaris leonina.*

Heterakidae

Poultry

Generally speaking, the veterinarian, when working with poultry, is treating the flock, not individual birds. Therefore a diagnosis is normally made by necropsy of the culled sick birds. Helminth parasites are increasingly important as poultry farming returns to nature and birds are raised on the ground.

Ascaridia galli - The largest nematode of poultry. This worm lives in the lumen of the small intestine.

a. Demonstration of Adults. (Size and predilection site are diagnostic)

Heterakis gallinarum - small nematode found in the large intestine and cecum of poultry.

a. Demonstration of adults. These occur in birds raised on the ground. They are one of the few parasitic nematodes known to be a **vector of another parasite,** the protozoan *Histomonas meleagrides.*

Oxyuroidea

The pinworms are nematodes found in the large intestines of their hosts. The name "pinworm" comes from the long pointed tail of the female nematode of some, but not all, species of this family.

Horse

Oxyuris equi

a. Demonstration of Adults

b. eggs - bottle #13 (90 x 42 :m, pg. 117, Foreyt). Also see Demonstration.
Note the operculum (cap) at one end. These eggs may be found in the feces, but since the female worm normally deposits them on the skin of the perianal area, scrapings of this region are more likely to reveal the infection.

Trichocephalids

The common morphological feature of these worms is the presence of a **"stichosome"** which constitutes part of the esophagus. The stichosome is a structure composed of a long slender tube surrounded by a row of large cells (stichocytes).

Mammals

Trichinella spiralis - The causal agent of trichinosis. The migrating larvae (L1) cause the important pathology.

a. Larvae - L1: Note, the adult female gives birth to first-stage larvae, and, after migration these larvae encyst in the muscles.

1. Cross section: Student Slide box #38 (pg. 159, Foreyt) This is a specimen of encysted larvae as may be seen in histopathology. Note the nurse cell.

2. Whole prep - take a very small (!!!) piece of muscle (from a rodent; center bench) and crush it between 2 slides and look for L1 s. (This is a diagnostic technique you can use in the field. It is easier to do this successfully with fresh tissue [i.e., before fixation].)

Dog and Cat

Trichuris vulpis - whipworm of the dog *(T. serrata* the cat whipworm, is very rare). Found in the cecum and large intestine, this nematode gets its name from the long narrow anterior end and the shorter, thicker posterior end, both parts together give the worm the appearance of a whip.

a. Demonstration of Adults. Note the characteristic whip-like appearance.

b. Eggs - Do a float on the dog feces stored under the hood and recover *T. vulpis* eggs (80 x 40 :m, pg. 19, Foreyt). Note the lemon-shape and the plugs at both ends. The egg is usually a light brown in color. At higher magnification (40 X), note the smooth surface of the shell. (These eggs can also be seen in Bottle #23.)

Capillaria aerophila - a lung worm of dogs and cats. The adults are found embedded in the mucosa of the lungs.

a. Eggs - DEMO (70 x 35 :m, pg. 49 Foreyt). The eggs resemble those of *Trichuris* (the whipworm), however they are more cylindrical than whipworm eggs and the plugs may appear to be asymmetrical with respect to the long axis of the egg. The easiest way to distinguish these eggs from whipworm eggs is by the character of the surface. *Capillaria* eggs have a rough surface (vs. the smooth surface of whipworm eggs) - use the 40 X objective to see this.

Capillaria plica - these worms are found in the urinary bladder of cats and dogs, and, thus, the eggs are found in the urine rather than the feces.

 Dioctophymatoidea **Dogs and wild carnivores**

Dioctophyma renale - the kidney worm. This is the largest nematode parasite of domestic animals (60 cm in length) and is found in the kidney (whose tissue it replaces). Eggs are found in the urine. *D. renale* is an occasional parasite of dogs, but an important parasite of minks and other animals farmed for fur.

a. Demonstration of adult. Note the size and the massive kidney destruction.
b. Demonstration of eggs (75 x 50 :m, pg. 34 Foreyt)

Checklist of Objectives
Laboratory #4

1. Be able to identify the eggs of *Toxocara canis, T. cati, Toxascaris leonina, Ascaris suum, Parascaris equorum, Oxyuris equi, Trichuris vulpis, Capillaria* spp., and *Dioctophyma renale.*
   Recall which are found in the feces and which are found in the urine. Also recall which the above can cause larval migrans in man and in animals.

2. Know how to identify the adults of the above nematodes by size and morphology.
   Know how to distinguish between *Baylisascaris procyonis, T. cati* and *T. leonina* (grossly, by the shape of the alae). In lab you will not be expected to distinguish between the adults of *Toxocara canis* and *Toxascaris leonina,* but you will be expected to know how to do so should the need arise. What is the easiest way to do it?

3. Be able to recognize *Trichinella spiralis* by finding the L1 in the muscle. (Why would you not seek the egg or newborn larva of this gut-dwelling parasite in the feces?)

4. Answer the review question.

Laboratory #5

Spirurids and Filariids

Objective: The filariid worms produce motile embryos (microfilariae) that accumulate in the skin or blood awaiting ingestion by an arthropod vector. Parasitologic diagnosis is made by finding these microfilariae either in blood or in skin snips. In today's lab you will learn how to recognize the microfilariae of *Dirofilaria immitis* in the blood of the dog. You will also learn several techniques for concentrating these microfilariae in order to make this diagnostic technique more sensitive. Finally you will have the opportunity to run several configurations of the antigen-capture serologic assay for adult heartworm infection.

You will also learn to recognize the adults *of Dirofilaria, Spirocerca,* and *Setaria,* all of which may be found at necropsy.

Spirurids

Physaloptera spp.

This nematode is common in the stomachs of raccoons and opossums. Dogs and cats are occasionally infected. The eggs may not float in a standard saturated salt flotation so diagnosis is usually made by identifying the adult worm after it has been vomited or seen during endoscopy.

A. Adults **DEMO** - Adults recovered from the stomach of an opossum. Note the body size, lack of cervical alae and presence of a "collar" at the anterior end. The most often vomited nematode by both dogs and cats is *Toxocara* spp., be sure you can tell the difference between *Toxocara* and *Physaloptera.*

Spirocerca lupi

This nematode is found in nodules in the esophageal or, less frequently, gastric lining of dogs. Diagnosis is based on radiographic imaging of nodules or on presence of distinctive eggs in feces.

B. Adults **DEMO** - Adults recovered from esophageal nodule. Note the spirally coiled tails of the male worm.

C. Eggs Bottle # 90 - Make a wet preparation and note the very small size (30 - 37 µm x 11 - 15 µm) and parallel-sided appearance. When passed in the feces eggs *Spirocerca* contain a coiled 1st stage larva. Students have remarked that this combination of characters gives *Spirocerca* eggs the appearance of tiny paper clips!

C. Pathology **DEMO** - Esophageal nodule.

D. Radiology **DEMO** - Esophageal nodule.

Gongylonema spp.

The adults of these spirurid worms are found embedded in the mucosa of the upper portion of the gastrointestinal tract of many host species. Ruminants are the preferred hosts.

A. Adults in the esophagus of a cow. **DEMO.**

Dracunculus insignis

In North America, the adults of this nematode are found in the subcutaneous tissues of raccoons and, occasionally, dogs and cats. In tropical Africa and Asia, a close relative, *Dracunculus medinensis,* causes Guinea Worm disease.

A. Adults and L1 **DEMOs.**

Filariids

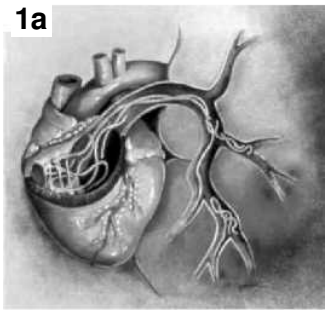
These are long, slender, whitish nematodes without lips. They dwell in the tissues or tissue spaces of the vertebrate host. Fertile females are viviparous and "give birth" to actively motile vermiform (worm like) embryos called **microfilariae.** These microfilariae are found in peripheral tissues, e.g. the skin or peripheral blood circulation, where they are liable to be picked up by hematophagous arthropod vectors. Parasitological diagnosis of filarial infection is by demonstration of microfilariae in blood or skin biopsies.

A. *Dirofilaria immitis* - Heartworm

As adults these nematodes live in the pulmonary arteries of dogs, cats, ferrets and seals. Microfilariae are in the peripheral circulation and are used for parasitologic diagnosis.

1. a) Adults **DEMO** - Size, shape and location in the pulmonary arteries and right heart at necropsy are sufficient to identify these nematodes. (Fig. 1a)

   b) Adults in Pulmonary Artery - **SSB** #35 - Find the pulmonary artery with cross sections of adult worms. Note the villus-like projections of the arterial endothelium. (Fig. 1b)



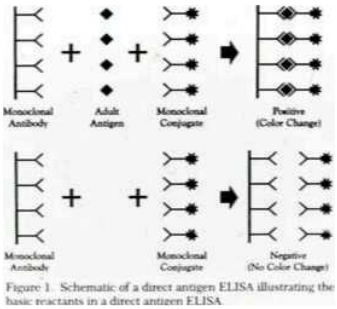


Figure 1. Schematic of a direct antigen ELISA illustrating the basic reactants in a direct antigen ELISA.

2. Serodiagnosis - Over the past two decades, serological methods for detection of *Dirofilaria immitis* infection have been developed. These provide quick, accurate and, sometimes, semi-quantitative methods of diagnosing infection with adult heartworms without microscopic examination of blood. These methods are increasingly regarded as the primary screening tests for heartworm diagnosis. They are also valuable for diagnosing occult heartworm infection in which adult heartworms and possibly heartworm disease are present, but no circulating microfilariae are detected. The most recent commercial versions of these involve detection of circulating antigen originating from the adult female worm by the general mechanism shown in Figure 2. Representatives of these commercial tests are provided for you to run on the canine sera provided. Details of this exercise will be given in the introduction to lab.

Figure 2

QUESTION: Current serodiagnostic methods for feline heartworm are based on detection of circulating anti-heartworm antibodies rather than antigen from adult female worms. What is the reason for this difference?

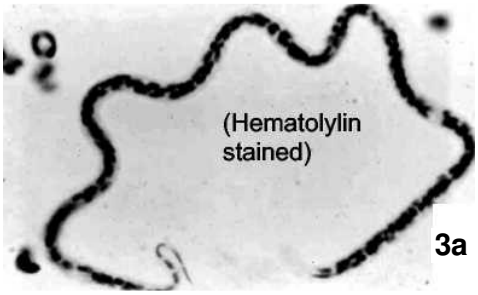
3. Microfilariae - These long-lived embryos are found in the peripheral blood of infected dogs, wild canids and, rarely, felids including domestic cats (Fig 3 a). Samples of dilute blood from a *D. immitis* infected dog are provided for you on the center bench. Resuspend the contents of one of these tubes and perform the following observations:

a. Make a wet preparation (one drop of blood on a slide with a cover slip). Observe under the microscope for movement of microfilariae.

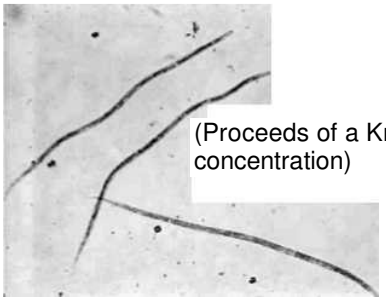
b. Perform the modified Knott technique as outlined in the appendix. **(Note:** because this blood is dilute, you will not see a pellet of buffy coat cells as large as would be expected from centrifugation of a whole blood lysate.)

c. Perform the filtration technique as outlined in the appendix.

Note: Novice diagnosticians commonly mistake artifacts such as dust, cotton fibers or strands of fibrin for microfilariae. Microfilariae of *D. immitis* are quite uniform in size and shape measuring approximately 310 µm in length with sharply pointed tails and blunt anterior ends (Fig. 3b).



3a



3b

QUESTION: What are the relative advantages and disadvantages of the three techniques for detecting blood-borne microfilariae?

4. Development of *Dirofilaria immitis* in its mosquito vector. **DEMO.** *D. immitis* undergoes a required developmental sequence in a susceptible mosquito. This demonstration illustrates this sequence.

a. Females of *Aedes aegypti* feeding on blood containing microfilariae *of Dirofilaria immitis* via an artificial membrane feeding apparatus.

b. Digestive tract (Fig. 4) of a blood engorged *Ae. aegypti.*

c. Gut contents of female *Ae. aegypti* showing microfilariae of *D. immitis.*

d. Larvae of *D. immitis* from the malpighian tubules (Fig. 4) 6 days after infection.

e. Third-stage (infective) larvae in mouthparts (labial sheath) and malpighian tubules 13 days after infection.

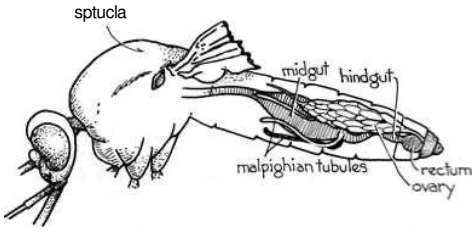


Figure 4

5. View the video on heartworm disease (running time approx. 25 min).

B. *Dipetalonema reconditum*

This non-pathogenic filaria is found in the subcutaneous tissues of the dog and is transmitted by fleas. Its microfilariae are located in the peripheral blood and thus can confound the diagnosis of *D. immitis* infection based solely on presence of microfilariae *per se.* However, the antigen-capture serologic tests we have discussed are specific for *Dirofilaria* and will not cross react with *Dipetalonema.* Therefore, with serodiagnosis increasingly becoming the first line of heartworm diagnosis, this confounding factor is less problematic. *Dipetalonema* infection would be on the list of differential diagnoses in the relatively rare case of a healthy, microfilaria positive dog without circulating heartworm antigen.

QUESTION: What would be another differential diagnosis in such an animal?

In such rare cases, microfilariae may be referred to a specialist for identification. The following material on identifying *Dipetalonema* microfilariae is provided for your information, but it is not listed among the objectives of this lab exercise. Differing morphological characters (see table in the appendix) and differential acid phosphatase staining patterns provide the specialist with a means of distinguishing microfilariae *of Dirofilaria* and *Dipetalonema.*

1. Microfilaria **DEMO-** This is a specimen from a Knott test. Note the differences in size and in the shape of the anterior ends especially.

2. Microfilaria **DEMO** - This is a specimen subjected to a histochemical stain for acid phosphatase activity (red areas). Compare the *Dipetalonema* microfilariae with those of *Dirofilaria.* Note that *Dirofilaria* has two discrete loci of staining, and *Dipetalonema* stains intensely throughout the length of the worm.

C. *Onchocerca lienalis*

In large animals, adult onchocercid worms usually live in the large ligaments, and microfilariae migrate through the skin. While *O. lienalis* is essentially non-pathogenic, microfilariae of a closely related parasite in horses, *O. cervicalis,* may cause a pruritic, nonseasonal dermatitis. *O. volvulus* causes onchocerciasis in humans, a leading cause of blindness in endemic areas.

1. Microfilaria **DEMO** - Observe the microfilariae migrating out of the skin biopsy provided. This illustrates the method for parasitological diagnosis of onchocercid infections.

D. *Setaria equina*

These nematodes are usually harmless and live in the peritoneal and pleural cavities of horses. They are transmitted by mosquitoes.

2. Adults **DEMO** - These are long slender worms. Their size, shape and location are sufficient basis for identification. Microfilariae would be found in the peripheral blood.

Checklist of Objectives

Are you:

3. able to identify microfilariae in a wet preparation and in the proceeds of the Knott concentration and filtration techniques (You are responsible for knowing how to perform the relevant diagnostic techniques.),

4. familiar with the theory and design of the various antigen capture assays for adult heartworm infection,

5. able to identify the adults of *Dirofilaria, Spirocerca, Physaloptera* and *Setaria* by their morphologies and locations in the host and

6. able to answer the review question?

APPENDIX

LABORATORY METHODS FOR DIAGNOSIS OF CANINE HEARTWORM INFECTION BY THE DEMONSTRATION OF MICROFILARIAE

There are several reasons for using one of the concentration techniques in the laboratory examination of dog blood for microfilariae. Probably the main reason for using a concentration method vs. the direct smear is that more than 25% of the positive cases may be missed if the direct smear is the only method used. Secondly, a concentration method that kills the microfilariae allows easy differentiation between *Dirofilaria immitis* and *Dipetalonema reconditum.*

The two acceptable concentration methods most commonly employed in practitioners' laboratories are:

A.Modified Knott Method (Knott J. A method for making microfilarial surveys on day blood. *Trans Roy Soc Trop MedHyg* 1939;33:191-196.)

1.Add 1 ml freshly-drawn blood to 9 ml 2% formalin (aqueous) in a centrifuge tube.

2.Mix well to lyse red blood cells.

3.Centrifuge for 5 minutes at 1500 rpm.

4.Pour off supernatant fluid. Note: Invert the tube completely when decanting the supernatant. Remember, the blood sample you are using is dilute so you won't see a large pellet.

5.Add a drop of 0.1% aqueous methylene blue. (Adjust the amount to suit yourself; it stains the microfilariae blue and makes them much easier to see.) Then stir or mix up the sediment in the bottom of the tube.

6.Mix again and place a drop of the stained mixture on a microscope slide and add a cover slip.

7. Examine under a microscope.

Microfilariae of:	***Dirofilaria immitis***	***Dipetalonema reconditum***
Numbers	May exceed 2×10^4 ml^{-1}	Usually < 103_{ml}-1
Length	> 300 microns	< 300 microns
Width	6.7 - 6.9 microns	4.7 - 5.8 microns
Anterior End	slightly tapered (cone on a cylinder)	blunt (hemisphere on a cylinder)
Posterior End	straight (usually; may vary)	hooked (usually; may vary)

NOTE: As a further modification, a microfilaria count can be made if a measured amount of the stained mixture is counted. Although it is only a generality, *D. immitis* microfilaremias are often characterized by having high concentrations of microfilariae, whereas *D. reconditum* microfilariae are often found in low concentrations.

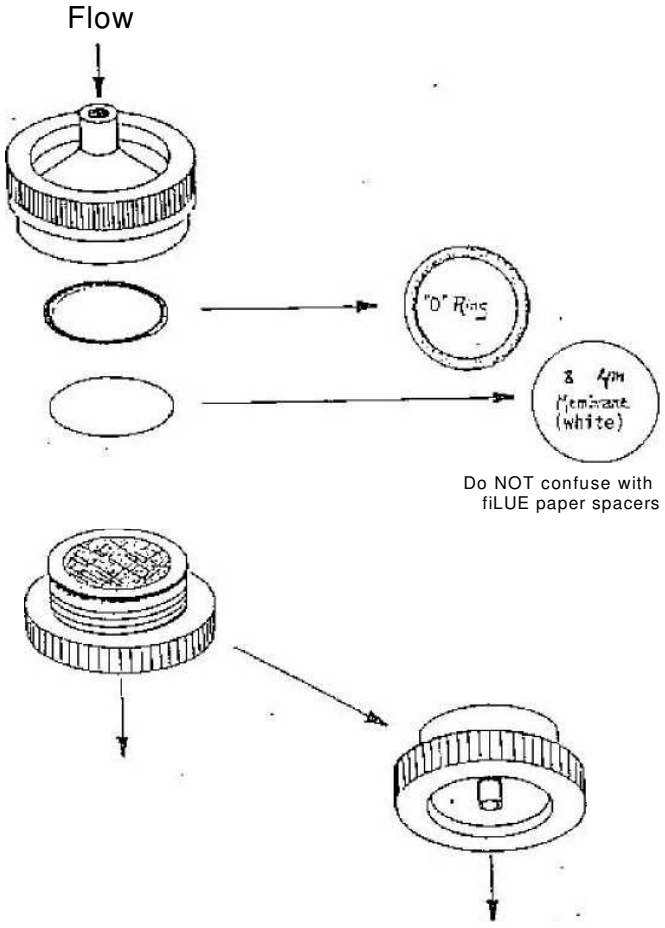
Filtration Method

1. Collect a 1 ml blood sample into EDTA or heparin and add to 10 ml lysing solution within a syringe. Mix thoroughly. (Lysing solution consists of 5.0 ml Triton X-100, 8.0 grams $NaCO_3$,1 liter water.)

2. Attach syringe to a filter unit (see drawing). The lysed blood solution is pushed through an 8 μm pore filter membrane.

3. Remove the filter from the filter holder, place it on a microscope slide and add one drop of 1:10,000 Methylene Blue Stain. Cover filter with a cover glass and examine under microscope.

MilHpore Filter Unit



C. Miscellaneous

It is frequently difficult to distinguish microfilariae of *D. immitis* from microfilariae of *D. reconditum* using the morphologic characteristics outlined above. More definitive techniques for differentiation are available, but they are not usually practical for routine use in the practitioner's laboratory.

The first technique employs a histochemical (acid phosphatase) stain of microfilariae. *D. immitis* stain positive in certain zones only and *D. reconditum* stain over the entire microfilariae. SeeJ. *Am. Vet. Med. Assoc.* 158:601-605, 1971 or consult a parasitologist.

The second technique exploits the fact that D. reconditum microfilariae have a cephalic hook and D. immitis microfilariae do not. Again, since this technique requires good microscopic capability, it may not be suited for routine use. See *Proc. Helminthol. Soc. Wash.* 32(1): 15-20, 1965, or Bowman's Georgi's *Parasitology for Veterinarians* or consult a parasitologist.

 Laboratory #6 TREMATODES

Objectives:
Many trematode eggs do not float in the routine solutions used in practice and, therefore, the first indication of a trematode infection may come at necropsy. Thus, you should be able to identify the adult flukes by their size and location in the host.

Phylum PLATYHELMINTHES
Class Trematoda

Subclass **Monogenea**

Gyrodactlyus sp. (Fish - Free-living Life cycle)

DEMO - Adult. The adults of this monogenean are ectoparasites offish.

Subclass **Digenea**

Flukes of Large Animals

Fasciola hepatica (Sheep - Snail - Vegetation Life cycle)

These flukes live in the bile ducts.

A. Eggs - bottle #11 - These large eggs (140 x 75 :m) have an operculum at one end. These eggs do not float in a standard saturated salt solution. **(Pg. 83, Foreyt)**

B. Adults - Student Slide box #12 - Note the size and shape of this trematode. This specimen is stained and thus the internal organs are visible. The caecae *of Fasciola* are highly branched.
Diagram 2 shows a drawing of the internal anatomy of a digenean trematode. This is for your information only, you will not be tested on the internal anatomy of the trematodes in the laboratory portion of this course.

DEMOS - unstained adults - leaf shaped with conical anterior end.

C. Larval stages - **DEMOS** of cercaria and metacercaria. Diagram 2 shows drawings of the internal anatomy *of Fasciola hepatica* larvae. This is for your information only.

Fascioloides magna (Deer - Snail - Vegetation Life cycle)

A parasite of deer which causes an extensive amount of hepatic pathology in sheep, but little in cattle.

A. Adults - **DEMO** - note the large size of this worm.

Dicrocoelium dendriticum (Sheep - Snail - Ant Life cycle)
These small (1 cm) flukes are found in the bile ducts.

A. Adults - Student Slide box #16 - stained to show internal organs - note the simple caecae.
DEMO - note the size and shape of these worms, you can't confuse them with *F. hepatica* the other fluke found in the bile ducts of sheep.

B. Eggs - Student Slide box #17 - These eggs are small (45 x 30 :m), dark brown, and contain a miracidium when passed in the feces.

Paramphistomum cervi (Sheep, Cattle - Snail - Vegetation Life cycle)

The rumen fluke. small, conical trematode lives in the rumen of sheep and cattle.

A. Adults **-DEMO-** Note the shape, these worms are not flat like other trematodes, also they have a large posterior sucker.

Flukes of Small Mammals

Paragonimus kellicotti (Small Mammal-Snail-Crayfish Life cycle)

The lung fluke of dogs and cats. Adults are usually found in pairs of fibrous cysts within the lungs.

A. Eggs **-DEMO** - (100 x 50 *:m)* (pg. 27, Foreyt).
Note the operculum surrounded by a collar at one end. These eggs may be found either in the feces or in the sputum.

B. Adults - **DEMO** of a related species: *P. westermanii.*
Note the size and shape of this lung fluke.

Heterobilharzia americana (Dog [Raccoon] - Snail Life cycle)

A. Adults - **DEMO** of a related species *Schistosoma mansoni.*
Note that the sexes are separate in this family of digenean trematodes.

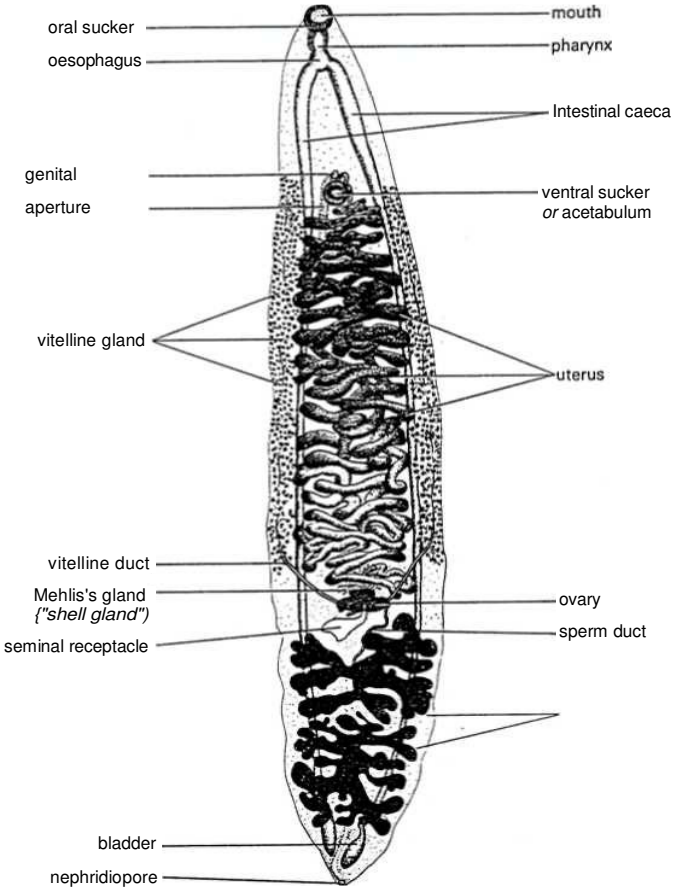
Checklist of Objectives

1. Be able to recognize the eggs of *Fasciola hepatica, Dicrocoelium dendriticum,* and *Paragonimus kellicotti.*

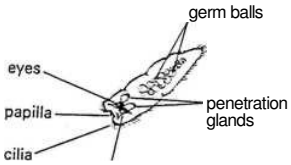
2. Be able to identify the adults *of Fasciola hepatica, Dicrocoelium dendriticum, Fascioloides magna and Paramphistomum cervi* (by size, shape and location within the host).

3. Answer the review question.

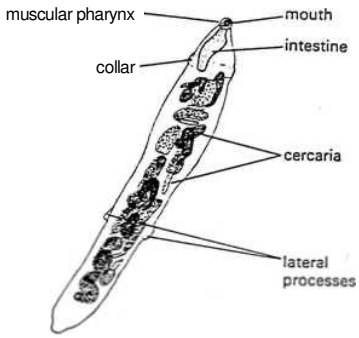
brain



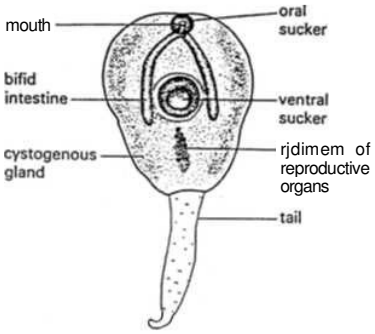
Digenean Trematode



Miracidium of Fasciola



Redia of Fasciola



Cercaria of Fasciola

DIAGRAM 1.

These diagrams are here for your information only. You will not be tested on the anatomy of these stages.

Diagram 2.

CESTODES AND ACANTHOCEPHALANS

Objectives: Because the cyclophyllidean tapeworms shed gravid proglottids, you must be able to recognize both the proglottid and the eggs expressed from it in order to diagnose the infection. Although in many cases the drug used to kill the adult tapeworms works against many cestode species it is important to identify which tapeworm you are dealing with, as the intermediate host will be different and, therefore, the control measures will differ for each cestode.

The acanthocephalans are parasites found in a variety of animals, however they are not very important in domestic animals in the United States. In this lab we will present you with the basic structure of the adult acanthocephalan (using the thorny-headed worm of swine as our model) so you will be able to recognize parasites of this phylum if you should ever come across them.

Phylum PLATYHELMINTHES
Class Cestoda

Tapeworms of Small Animals

Diphyllobothrium latum ([Dog, cat, mink, seal, human] - Cyclops - Fresh water fish Life cycle)

This Pseudophyllidean tapeworm utilizes a crustacean as the first intermediate host and fresh water fish as the second intermediate host and paratenic hosts.

A. Adults - **DEMO** - Notice the large size of this worm and the typical pseudophyllidean segments.

B. Eggs - **DEMO** - Typical pseudophyllidean eggs. They look like Trematode eggs (60 X 45 and may not float in most common flotation solutions.

Spirometra mansonoides (Cat - Cyclops - Frog or Water snake Life cycle)

This pseudophyllidean tapeworm utilizes a crustacean and a vertebrate (esp. frogs and water snakes) as intermediate hosts.

A. Adults - **DEMO** - Notice the typical pseudophyllidean segments.

B. Eggs - Bottle #144 (60x35 :m); unembryonated in the feces.
Note: These eggs will not float in most common flotation solutions.

Mesocestoides (Dog, Cat - Arthropod - Vertebrate Life cycle)

This tapeworm utilizes a mite as the first intermediate host and various vertebrates (including dogs and cats) as the 2nd intermediate host. The adult worms are found in the small intestine of dogs in cats.

A. Gravid proglottid - **DEMO** - note the paruterine organ full of eggs. These proglottids are often "club" shaped.

Dipylidium caninum (Dog, Cat - Flea Life cycle)

The adult of this cestode is found in the small intestine of dogs (and sometimes children) while the cysticercoid is found in fleas or chewing lice.

A. Adults - Student Slide box #5 and 6

Scolex (SSB #5) Note: The 4 suckers and armed rostellum.(i.e. has hooks on it).

Proglottids (SSB #6) Note: The bi-convex shape ("cucumber seed"), the duplicated reproductive organs and two lateral genital pores.

B. Eggs - Student Slide box #4 **(pg. 29, Foreyt)**

The egg packets have been expressed from gravid proglottid.
Note: Each egg packet contains up to 20 eggs, and within each is an onchosphere bearing 3 pairs of hooks. Take a proglottid from the dish on the center bench. Place the segment on a slide with a drop of water, place a second slide on top of the segment and apply gentle pressure to straighten it out and flatten it without crushing it. Note the shape and look for the two genital pores (if the proglottid is not gravid these features will be all you have to identify the cestode). Now apply more pressure and crush the proglottid between the two slides and look for the characteristic egg packets that will be released. Note also the calcareous granules found in the parenchyma: these are characteristic of cestodes. Also note the six hooks in the onchosphere (embryo), a common feature of all cyclophyllidean tapeworm eggs. (Proglottids dry out quickly, so the client may present you with "Sesame-seed"-like objects. These can be re-hydrated by soaking in water for a few minutes and then crushed to release the eggs.)

Taenia sp. (Dog, Cat - Small Mammal or Human - Livestock Life cycle)

Tapeworms of this genus are found in the small intestine of carnivores and the larval stage is found in various tissues of the mammals that serve as the prey of the carnivore.

A. Eggs - *T. saginata* - bottle #14 **(pg. 29, Foreyt)**
All eggs of Taeniid tapeworms *(Taenia, Echinococcus)* look alike. They are round (30-35:m) and have a striated embryophore (shell). Note the six hooks in the onchosphere (embryo), a common feature of all cyclophyllidean tapeworm eggs.

B. Adults - mature proglottids Student-Slide box #1
Note: The reproductive organs within the proglottids and the lateral, irregularly alternating genital pores. Diagram 1 shows a drawing of the internal anatomy of a mature proglottid. This is for your information only.

Scolex - Student Slide box #2 (Also see Diagram 1. Note: parts are labeled for your information only.) Note the 4
suckers; is there a rostellum?

Gravid proglottids Student-Slide box #3

Note: The branched uterus, full of eggs (the number of branches is characteristic of the individual species of *Taenia).* (Also see Diagram 1. Note: parts are labeled for your information only.)

C. Larva (metacestode) - **DEMOS.**
The cysticercus (bladder worm) is a relatively small, fluid filled cyst which contains the inverted protoscolex.

Echinococcus granulosus (Dog - Sheep Life cycle)

The adult of this small (3 to 4 segments) taeniid tapeworm inhabits the small intestine of dogs, the metacestode (the hydatid cyst) is found in sheep (usually in the liver). This tapeworm is a public health concern as man can be an accidental intermediate host.

A. Adults - Student Slide box #9
Note the small size; this worm consists of the scolex with 4 suckers and an armed rostellum, an immature proglottid, a mature proglottid containing the genital organs, and a terminal gravid proglottid filled with eggs. Diagram 1 shows a drawing of the internal anatomy of *Echinococcus.* This is for your information only.

Remember: Because the eggs in the proglottid are fully embryonated and infectious for humans, the feces of dogs suspected of being infected must be handled with caution. Lab ware, etc., must be sterilized after use or disposed of safely.

B. Larva (metacestode) - **Demo** of hydatid cysts. These fluid-filled cysts contain many protoscolices and smaller cysts (brood capsules).

Tapeworms of Large Animals

Moniezia expansa (Sheep - Mite Life cycle)

A. Eggs**-DEMO (pg. 83, Foreyt)**
These eggs (56 to 67 :m) are triangular in shape and contain an onchosphere surrounded by a pyriform apparatus (a pear-shaped structure).

B. Adults - Student Slide Box #7 - mature proglottid
Note: The segments are broader than long, and there are duplicated sets of reproductive organs.

DEMO - The scolex is unarmed and has 4 suckers. Proglottids are broader than long.

Anoplocephala sp. (Horse - Mite Life cycle)

Eggs of these tapeworms of horses resemble those *of Moniezia.* The intermediate host is a forage mite (a type of free-living mite).

A. Adults:

Anoplocephala perfoliata - in tray on center bench.
Note the lappets, one behind each sucker, the short strobila with proglottids broader than long, and the lack of a rostellum (not easily seen).

A. magna - **DEMO** - Similar to *A. perfoliata* but lacks the lappets under the suckers and is larger in size.

Phylum ACANTHOCEPHALA

This phylum was briefly covered in lecture. The worms in this phylum are known as thorny-headed worms and are parasites of the digestive tract of vertebrates. Many different species are found in wildlife, but only one acanthocephalan is a parasite of domestic livestock (pigs).

Macracanthorhynchus hirudinaceus (Swine - Junebug Grub Life cycle)

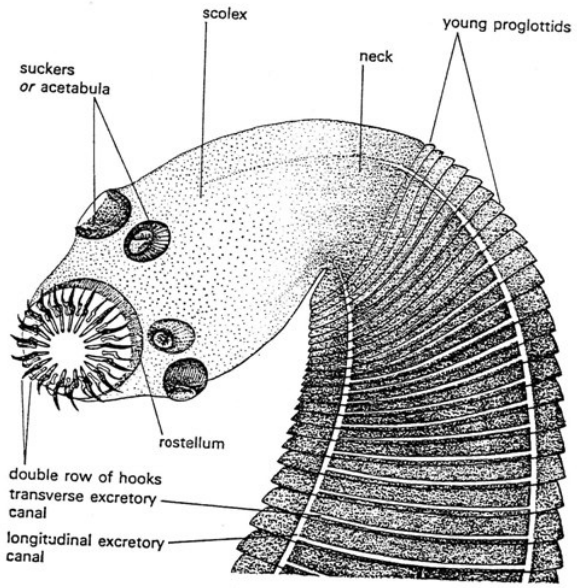
Thorny-headed worm of pigs.

A. Adults - **DEMO** - note the spiny proboscis at the anterior end which gives this worm its name. Be able to tell this worm from *Ascaris suum.*

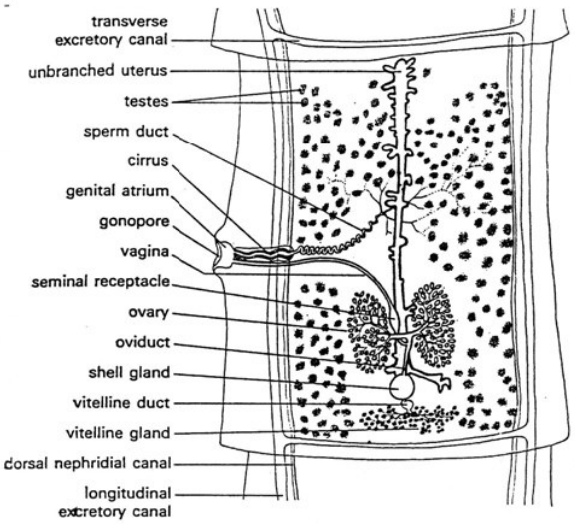
B. Eggs - **DEMO** - Note the multiple layers of shell.

Checklist of Objectives

1. Be able to identify the eggs of *Echinococcus* and *Taenia* spp., *Dipylidium caninum,* and the Anoplocephalids.
2. Be able to identify the proglottids of *Taenia* spp., *Dipylidium caninum,* and *Mesocestoides* sp. and the Pseudophyllidean tapeworms.
3. Be able to tell *Anoplocephala magna* adults from *A. perfoliata* adults (size and presence or absence of lappets).
4. Be able to tell a pseudophyllidean tapeworm from a cyclophyllidean tapeworm.
5. Be able to recognize the adult *of Macracanthorhynchus hirudinaceus* (or other acanthocephalans). Hint: Look for the proboscis.
6. Answer the review question.

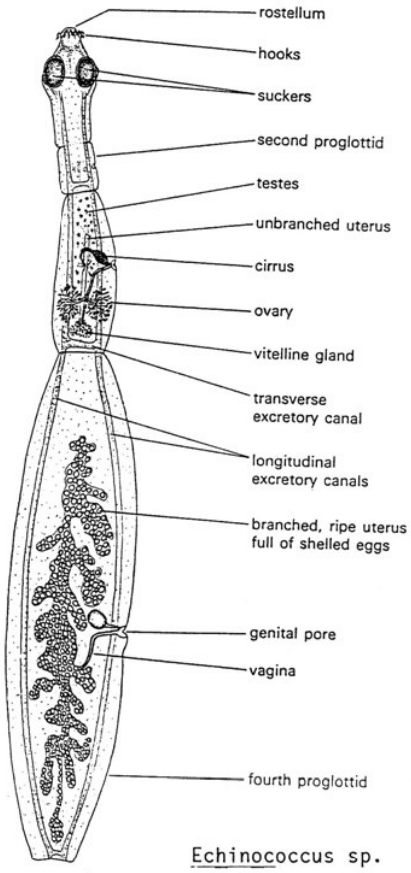


Anterior End of a
Cyclophyllidean tapeworm

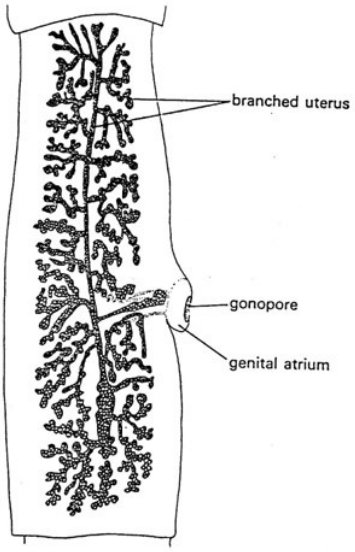


Mature proglottid of a
Cyclophyllidean tapeworm

DIAGRAM 1.
These diagrams are here for your information only.
You will not be tested on the anatomy of these stages.



Echinococcus sp.



Gravid proglottid of Taenia sp.

Laboratory #8

The Arachnids

Objectives: The order Acarina of the class Arachnida includes the ticks and mites and thus, many important ectoparasites of domestic animals. On completing this exercise, we would like for you to 1.) be able to recognize on sight the important **families** of burrowing and non-burrowing mite parasites, 2.) be able to recognize on sight the tick families Ixodidae and Argasidae (the hard and soft ticks respectively) and 3.) be familiar enough with the morphological characters of ticks to use the pictorial key (Figure 7) to identify a tick specimen to the **genus** level.

THE MITES

NON-BURROWING MITES

Family Dermanyssidae

These are tick-like mites with an ovoid body shape. In life, they use their long legs to move about both on the host and in its nest or bedding.

a. *Ornithonyssus sylviarum,* the northern fowl mite. Bottle #E203.

Remember that this mite has a "lair ectoparasitic" life history. The sample you are studying was taken from birds in the Philadelphia area and contains eggs, larvae, nymphs and adults. Characters are evident in the generalized mite diagram (Figure 1).

Note:

i. Eggs are large, oval and dark in c

ii. Larvae have only 3 pairs of legs.

Adult characters:

iii. Oval and tick-like in appearance

iv. 4 pairs of legs with suckers at the ends

v. Coxae (basal leg segments) are evenly spaced on the body.

vi. Mouthparts adapted for sucking

b. *Pneumonyssus caninum* - **DEMO** This mite lives in the nasal cavity and sinuses of dogs. Note its tick-like apparance with ovoid body and 4 pairs of legs with claws on the pretarsi (distal leg segments).

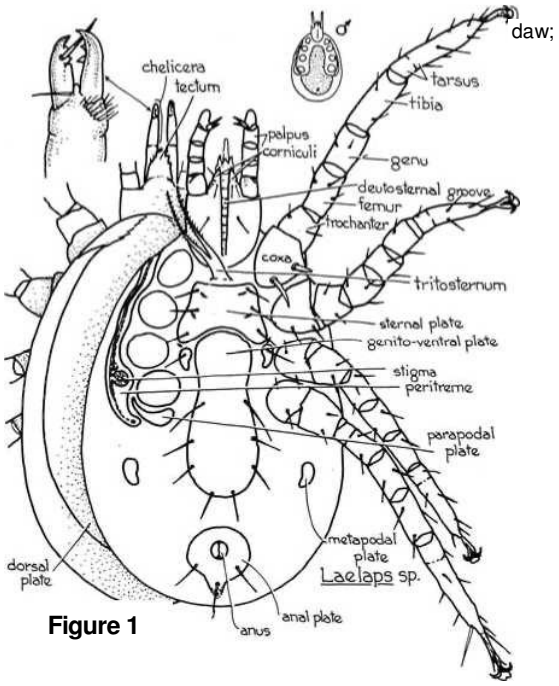


Figure 1

c. *Dermanyssus gallinae* - Student Slide #91 (Foreyt, pg. 150) This is the chicken mite, also called the "red mite" of poultry. The "red mite" is a lair ectoparasite, visiting the birds only to feed. The "red" in its name refers to the mite's color when engorged with blood. This mite may also attack mammals if birds are not available.

Fam. Chyletidae

a. *Chyletiellaparasitivorax* - DEMO (Foreyt, pg. 39). This is the "rabbit mite". Other members of this genus can be found on dogs and cats.

Note: The body has a "waist"; the legs end in combs, and the large palpi have pincers on their ends.

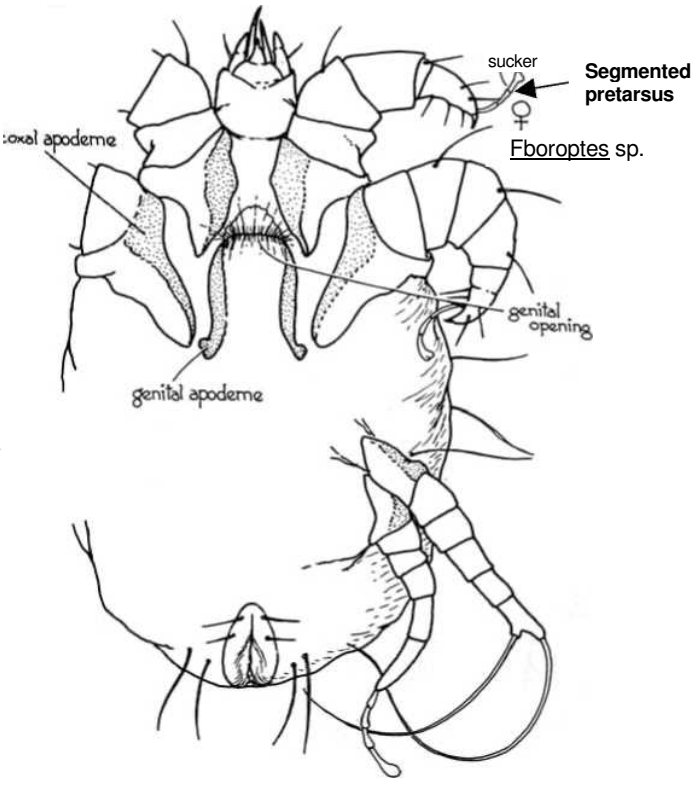
Fam. Psoroptidae

a. *Psoroptes ovis* - DEMO and Student Slide #87 (Foreyt, pg 99) Found on sheep and cattle, this is the cause of "sheep scab" (Psoroptic mange). Other members of the genus cause mange in horses and rabbits. Note the elongate legs (compared to *Sarcoptes).* Legs I, II, and IV bear a segmented pretarsus (Figure 2).

b. *Chorioptes bovis* - DEMO (Foreyt, pg. 99) This mite is found on sheep, cattle, goats and horses. It resembles *Psoroptes* sp. Since it causes little disease in sheep it must be distinguished from *Psoroptes.* Note the pretarsi are short and unsegmented, and there are suckers on legs I, II and III.

c. *Otodectes cyanotis* — DEMO (Foreyt, pg. 39) This is the most common mite ectoparasite of dogs and cats, and it normally lives in the ear. It resembles *Psoroptes* and *Chorioptes* in its general appearance (body-shape and legs). The pretarsi are unsegmented.

Figure 2



BURROWING MITES

Fam. Sarcoptidae

a. *Sarcoptes scabei* - **DEMO** and student Slide #88 **(Foreyt pg** 38). Host-adapted physiologic races of this mite species are found on all domestic animals as well as on humans. It causes sarcoptic mange (or "scabies" in humans). Note the small size and the globular body shape with very short legs. The coxae of legs II and III are widely separated. In contrast to *Psoroptes,* the pretarsi of legs I and II are in the form of simple (unsegmented) stalk with terminal suckers. There are long trailing setae or hairs (Figure 3).

b. *Notoedres cati* - **DEMO (Foreyt, pg. 54).** This mange mite of the cat is similar in appearance to *Sarcoptes* but is smaller. *Sarcoptes* is rare on cats.

c. *Knemidocoptes* - **DEMO.** The "scaly-leg" mite of poultry. This mite also resembles *Sarcoptes* in shape but the legs have claw-like structures instead of suckers *(Sarcoptes* is not found on poultry).

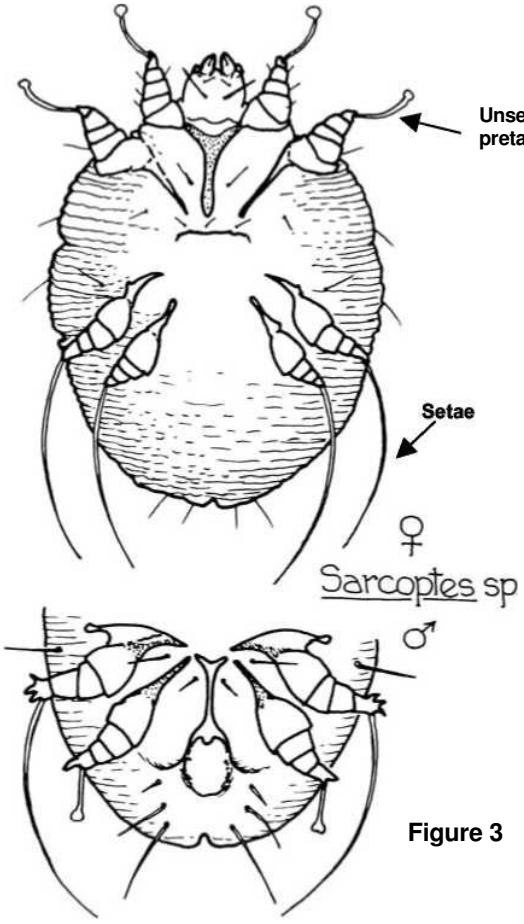


Figure 3

Fam. Demodicidae

a. *Demodex canis* - **DEMO** (and Student Slide #90). **(Foreyt pg.** 38). This is the ubiquitous follicular mite of dogs. Note the elongate shape of the body and the 4 pairs of stumpy legs (Figure 4). This mite, although usually a harmless commensal organism, can cause mange (demodectic mange) especially in immuno-compromised animals. Student slide #90 is a section of skin showing the effects of demodectic mange.

Figure 4

THE TICKS

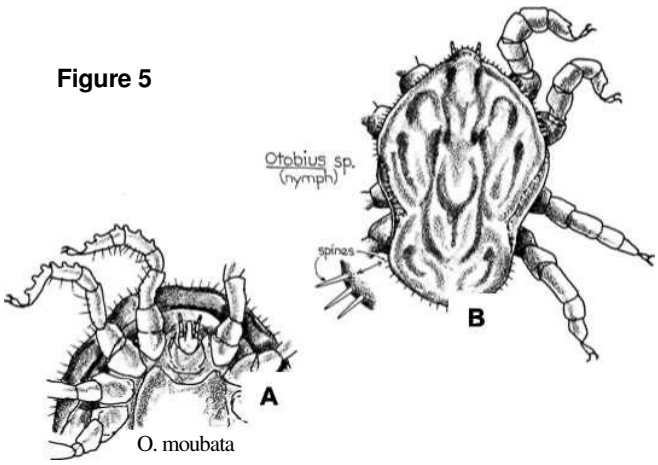
Family Argasidae - The Soft Ticks

Ticks of this family lack the scutum (the hard shield-like plate on the dorsal surface) and have a leathery cuticle. The mouthparts are not visible from the dorsal side (Figure 5), being recessed ventrally. These ticks feed moderately and often, and, therefore, they do not engorge to the extent seen in the hard ticks.

a. *Argas persicus* - The fowl tick - **DEMO (Foreyt, pg. 150)** Note the oval shape of the body, the well-defined lateral margin and the ventrally located mouthparts (Fig. 5A)

b. *Otobius megnini* - The spinose ear tick - **DEMO** These spiny soft ticks are found primarily in the ears of dogs. Note the ventral mouthparts and the spines on the dorsal surface (Fig. 5B).

Figure 5



O. moubata

Family Ixodidae - The Hard Ticks

These ticks possess a rigid, chitinous scutum on their dorsal surface, and their mouthparts appear at the anterior end of the body when viewed from the dorsal aspect (Figure 6).

The aim of this part of the lab is for you to become familiar enough with the structures of these ticks (detailed in Figure 6) to use the pictorial key provided in your handout to identify unknown specimens to the genus level. **Note:** Many of the alternatives highlighted in the key may be seen in the demonstrations.

a. *Ixodes scapularis* - The deer tick (AKA: the black-legged tick) - **DEMO** This is the vector of *Borrelia burgdorferi* (the agent of Lyme disease) in the eastern and Midwestern United States

Note the following about I. *scapularis.*

i. The elongate mouthparts with tips or palpi converging (in the female).
ii. The preanal groove (characteristic of genus).
iii. The inornate (plain brown) scutum
iv. The prominent, posteriorly-directed spine on coxa I

b. *Amblyomma americanum* - The Lone Star Tick - **DEMO** This is an ornate tick. The male has variegated white pattern on its back, while the female has a single white spot ("lone star") on its scutum. Note the long mouthparts and the eyes on the lateral margin of the scutum. Ticks' eyes are simply translucent patches of cuticle overlying photoreceptors.

c. *Dermacentor variabilis* - The American Dog Tick - SSB #96 and **DEMO** This three-host tick is also ornate with the scutum more or less covered with irregular white markings. The larval and nymphal stages of this tick are found on rodents and other small mammals and the adults on a variety of middle-sized to large mammals including dogs and humans.

Note also the rectangular basis capitulum and the festoons on the posterior margin (Figure 6).

d. *Rhipicephalus sanguineus* - The Brown Dog Tick - SSB #95 This inornate, three-host tick is another common parasite of dogs. All of its life stages occur on dogs. Note that the basis capitulum is laterally produced (roughly hexagonal in shape). There are eyes, and festoons may be visible along the posterior margin of the body.

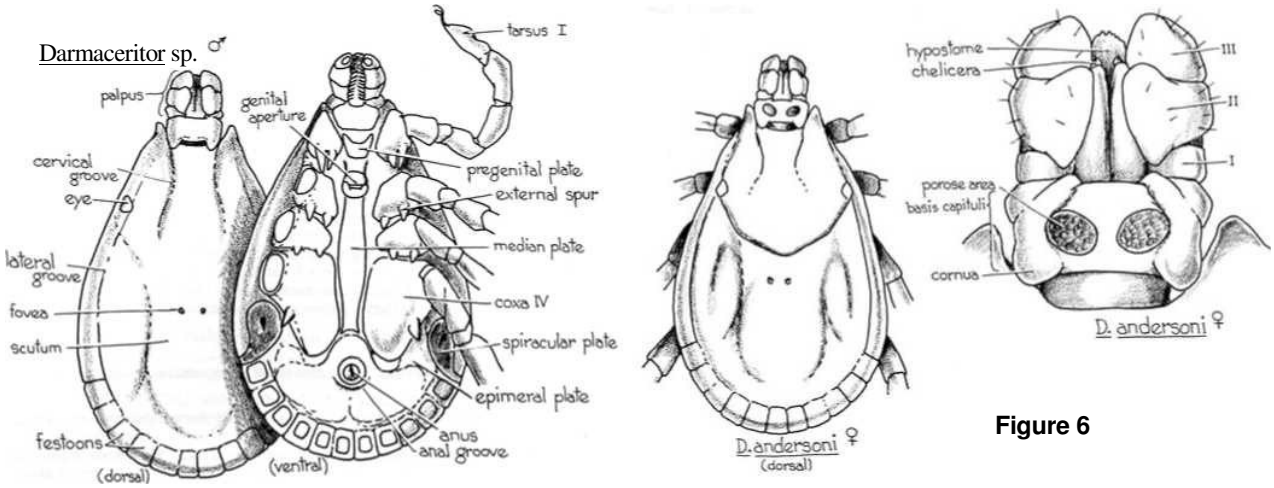


Figure 6

See also SSB #93, a larval tick. Note that there are 3 and not 4 pairs of legs.

Video - Identification of ticks and mites (approx. 15 min.). **Tick keying**

exercise

There are unidentified ticks on the center bench. Take one and try your hand at keying it to the genus level with the pictorial key (Figure 7). (Please return the specimen as soon as you are finished).

Hint: Don't mistake an engorged hard tick for a soft tick.

Checklist of Objectives:

Can you:

1. recognize the families Dermanyssidae, Chyletidae and Psoroptidae of non-burrowing mites,

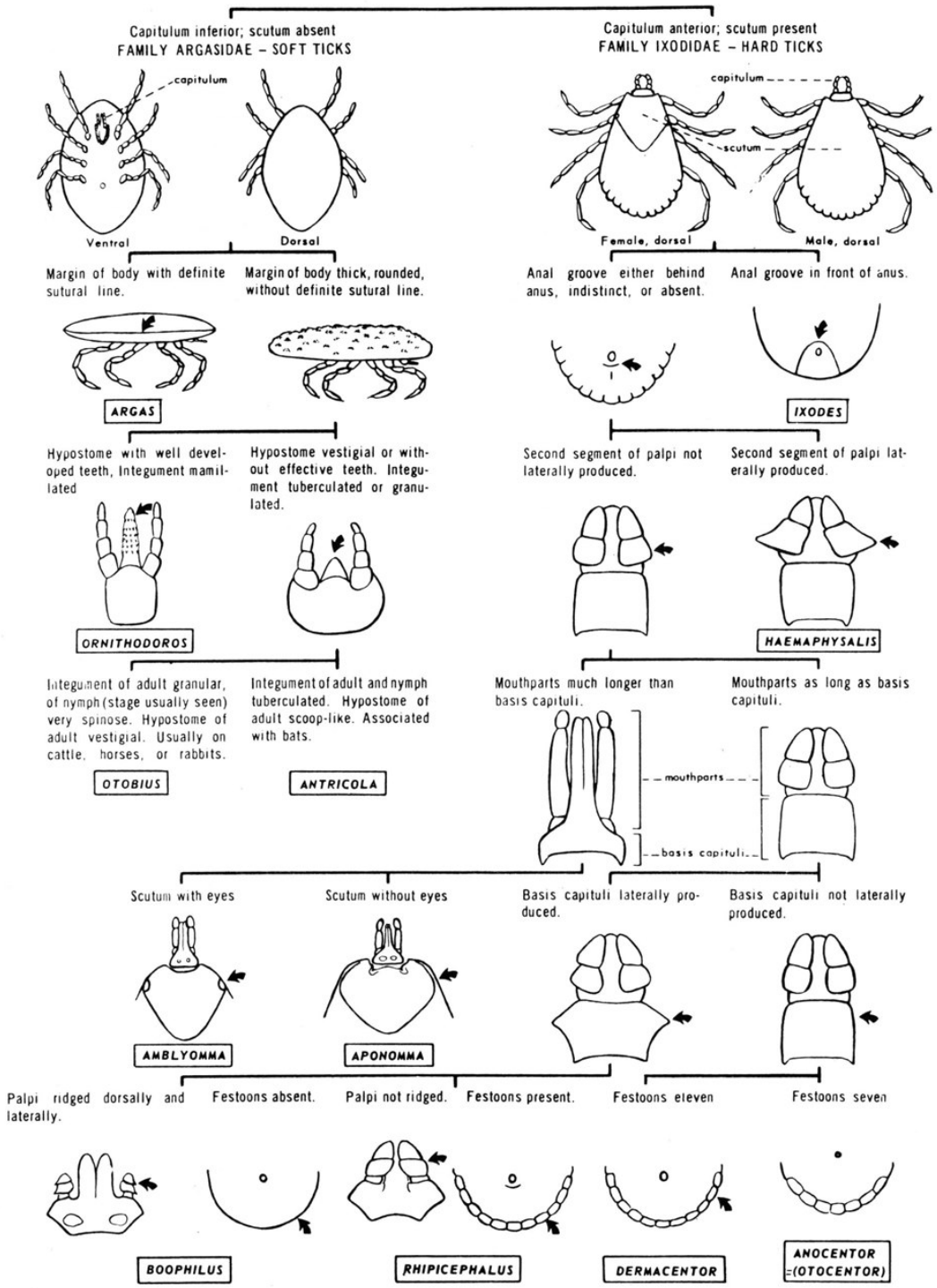
2. recognize the families Sarcoptidae and Demodicidae of burrowing mites,

3. use the pictorial key provided to identify an unknown tick specimen to the genus level,

4. answer the review question?

Figure 7.

PICTORIAL KEY TO GENERA OF ADULT TICKS



visit: www.kashvet.org

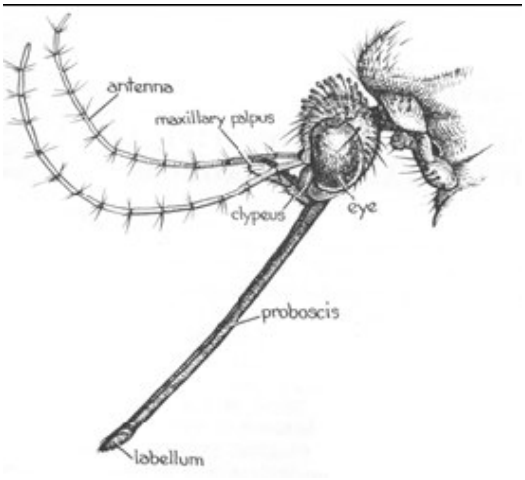
Laboratory #9 Insects of

Veterinary Importance

Objectives: The insects have a profound impact on human and animal health both as transmitters of pathogens, as the agents of diseases of the skin and other tissues and as sources of blood loss and annoyance. This lab is designed to help you diagnose infestations with the major groups of insects of veterinary importance. In a few instances you may be asked to recognize a specimen on site, but in the majority of instances you should strive to learn the morphologies of the different groups to the extent that you can use the keys printed in the lab handout to make the identification.

The specific objectives of this lab session are:

1. to get an intuitive feel for the mosquitoes and their various life stages,
2. to be able to recognize the family Tabanidae (suborder Brachycera) on sight,
3. to be able to use the posterior spiracles mature larvae to identify muscoid flies,
4. to be able to recognize *Melophagus ovinus* on sight,
5. to be familiar enough with flea morphology to use the pictorial key (Fig. 7) to make an identification,
6. to differentiate on sight the chewing and sucking lice as well as the suborders Amblycera and Ischnocera



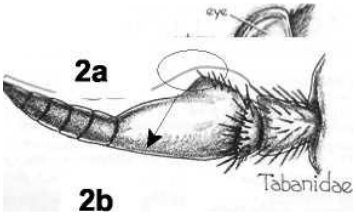
THE ORDER DIPTERA

Suborder Nematocera (the "long-horned" flies)

1. Fam. Culicidae (Mosquitoes). Mosquitoes are tiny delicate flies with long multisegmented antennae (Fig. 1). Their larval and pupal stages are aquatic, and the females of most species require a meal of vertebrate blood to initiate egg development. Mosquitoes constitute a source of blood loss and annoyance but more importantly act as vectors of some important pathogens of vertebrate animals. Glance at the Lucite block museum mounts in the **DEMO** to get a general impression of the appearance of the adults and immature stages of mosquitoes.

2. Fam. Simuliidae (Black flies). Black flies constitute a serious cause of blood loss and annoyance to humans and domestic animals. They also transmit a few pathogens of veterinary importance. Give the **DEMO** a brief look just to get a feeling for the general morphology of these tiny flies. Note that immatures are also aquatic but, unlike mosquitoes, usually live in fast moving streams.

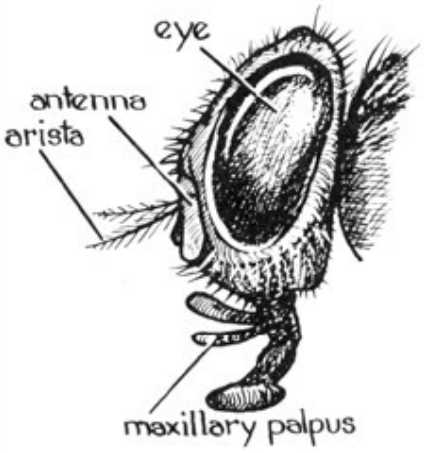
Suborder Brachycera (the "short-horned" flies)



This group includes the horse flies and deer flies. A good example is the horse fly, Tabanus sp. Seen in the **DEMO.** Note

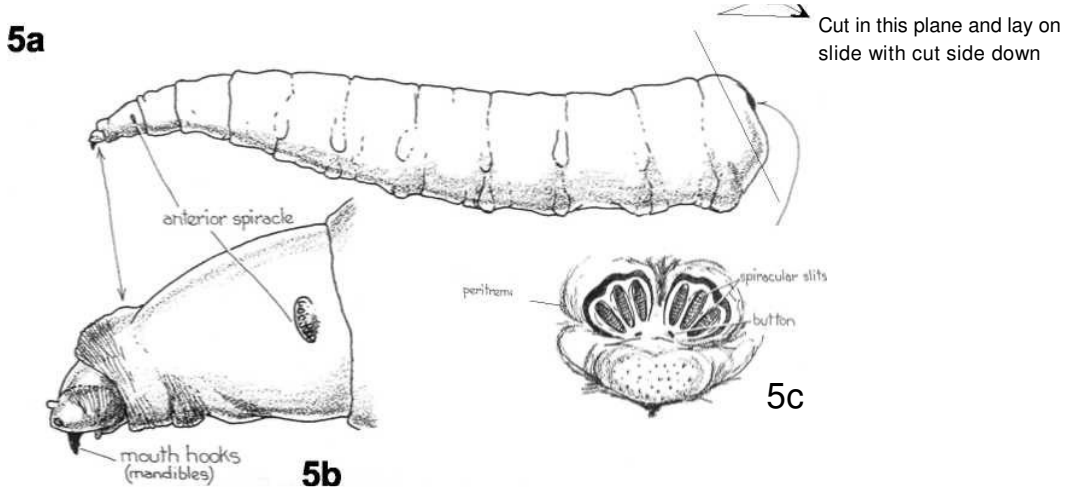
the overall size and morphology of the antenna of the adult fly (Fig 2a,b) and the general shape of the larva.

Suborder Cyclorrhapha (the "muscoid" flies)



The morphology of flies in this suborder is typified by the house fly, *Musca domestica.* Therefore, as a group they are sometimes referred to as "muscoid" flies. Antennae of adult cyclorrhaphans are reduced to a club-like structure, lying flush with the frons or "face" of the fly and bearing a feather-like chemosensory structure called the arista at its tip (Fig. 3.).
Cyclorrhaphan flies may be serious pests in intensive indoor rearing facilities such as poultry ad dairy barns. They are usually seen clinically as mature third-instar (-stage) larvae infesting the tissues of living animals either as obligatory or facultative parasites.
1. *Musca* sp. And *Lucilia* sp. and other muscoid flies are sometimes involved in facultative myiasis. Note the anterior spiracles (a stalk with 5-8 papillae in *Musca,* fig. 5a, b), the mouth hooks (Figs. 5a, b) and the paired posterior spiracles (5c). Compare the shapes of the spiracular slits and peritremes *of Musca* and *Lucilia.* (See Figure 4 as an aid.) This type of morphological variation can be used to identify otherwise rather featureless fly larvae as in 2 below.

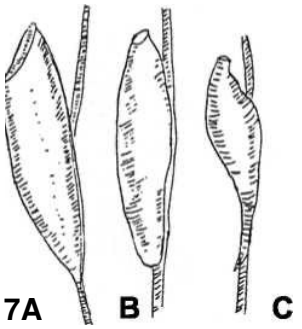
4a *Musca* 4b *Lucilia*



2. **Identification exercise.** Refer to Fig. 5 and locate the posterior end of one of the muscoid fly larvae provided in the dishes on the center bench. Use a scalpel blade to cut off a thin section, containing the spiracles, from the posterior end of a larva.. The slice you make should be thin

enough to transmit a little light but thick enough to include the posterior spiracles. Transfer the resulting slice, cut side down, to a microscope slide and view the spiracles with your compound scope. Use the shapes of the spiracular slits, the overall shape of the peritreme and placement of the button relative to the other structures as diagnostic characters to identify the larva to genus using the key provided in Fig. 6 (on pg. 7).

3. *Gasterophilus* sp. Recall from lecture that the larvae of these flies are obligatory parasites in the stomachs of horses. In most species, the eggs are attached to the hair coat of the host. These eggs may be identified based on their shape. Examine the *Gasterophilus* eggs in your slide box (SSb #70). Use the diagram provided (Fig. 7) to determine the species of these eggs. Look at the DEMOS of larval *Gasterophilis in situ* and also identify the specimens provided using the characteristics of the spines on the larval integument as pictured in Figure 8. (Also, **Foreyt, pg. 119).**



8A G. *nasalis* B G. *intestinalis* C G. *haemorrhoidalis*

4. *Oestrus ovis* **DEMO.** This third-stage larva is the only such parasite to be found in the nasal cavities of sheep. Note the characters highlighted in the DEMO.

5. *Cuterebra* sp. **DEMO.** This parasite causes cutaneous myiasis in rodents, rabbits and, occasionally, in dogs and cats. Note the spiny integument in thethird-stage larva in the DEMO. **(Foreyt, pg. 37)**

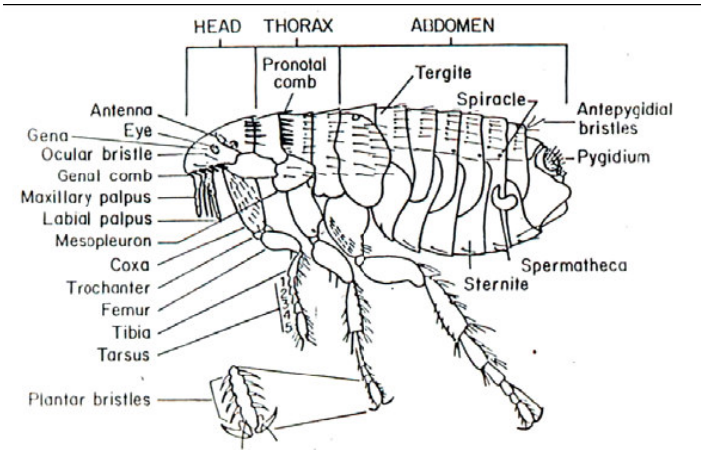
6. *Hypoderma* sp. **DEMO.** This fly larva causes cutaneous myiasis in livestock. Note the characters in the DEMO. You will not be responsible for differentiating the two species.

7. *Melophagus ovinus.* Recall from lecture that this is an atypical fly which has evolved a completely ectoparasitic life history. It ranks as an important parasite of domestic sheep. Examine the adults in your slide box (SSB #68) and in the DEMO.
(Foreyt, Pg. 100)

NOTE: a.) the indistinct segmentation of the abdomen, b.) the strong legs and claws; c.) this is a wingless fly.

THE ORDER SIPHONAPTERA
(Fleas)

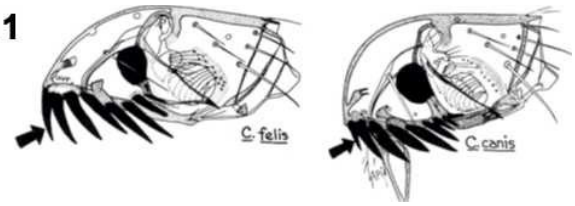
The diagram at the right (Fig. 9) depicts the main landmarks of flea external morphology. The features indicated may be used to identify fleas to species using keys such as the chart in Figure 10 (page 8).



1. *Ctenocephalides felis,* the cat flea (adults, SSB #75) is probably the most common flea seen in both dogs and cats. Its life cycle is typical of most species of flea in that them move about freely in the host hair coat and have a reservoir of immature stages in the environment. In the above prepared speciments observe: **(Foreyt, pp 36-37)**

a. General structure - head, thorax, abdomen; laterally flattened; 3 pairs of legs (Fig. 9).
b. Both genal and pronotal combs present (Fig. 9).
c. *C. canis* is a rare flea and is almost never seen in routine practice. *C. canis* adults (stock slide #1a) have heads which are more bluntly rounded than *C. felis* (Fig. 11). Also, the first tooth ot the genal comb is half the length of the second (Fig 11). NOTE: You may have to focus up and down on the first tooth of *C. felis* in order to appreciate its length.

11

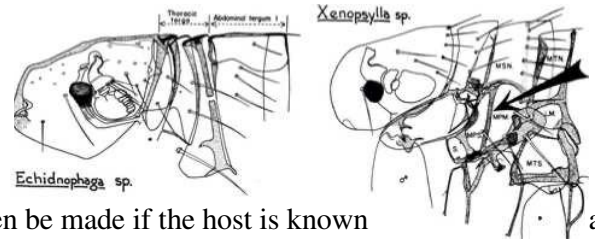


These same features can be seen in the **DEMO.**

2. *Echidnophadga gallinacea,* the sticktight flea of poultry (adults, stock slide #96, center bench). Recall that this flea has an atypical life history in that it remains attached to the skin of the host throughout much of its adult stage. Referring to Figures 9 and 12, examine the preserved specimen and note:

a. the angular head,
b. the absence of ctenidia (combs) and
c. the piercing/sucking mouthparts.

12



ORDER PTHIRAPTERA
(The Lice)

Lice are so host specific that a species diagnosis may often be made if the host is known and the specimen can be assigned to the correct suborder.

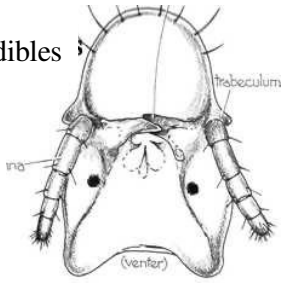
The Chewing Lice (Mallophaga)

Ischnocera

1. *Trichodectes canis,* a common chewing louse of dogs (SSB #77 is *T. equi,* which will serve to convey the morphology). This is a typical chewing louse in the group called Ischnocera so named because of its extended antennae (Figure 13). Note the following features, which exemplify this group in the Mallophaga. **(Foreyt, Pg. 35)**

ADULTS

a. Chewing type mouthparts seen as large opposing mandibles (Fig. 13)
b. General structure - head, thorax and abdomen dorsoventrally flattened.
c. Antennae are visable - 3 segments (Fig 13)
d. 3 pairs of legs, each armed with a strong claw.
e. Palpi not visible. (Fig. 13)



13

EGG

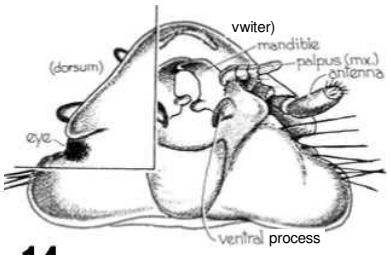
a. The eggs or nits of lice are operculate and are cemented onto the hairs at their bases. When the eggs hatch, the operculum is lost and the larva emerges through the opening. These features are covered in the **DEMO.**

2. *Damalinia* is a common genus of chewing louse in livestock. The example in your slide box is *Damalinia caprae* from goats (adults, SSB #81, eggs SSB #80). These are also in the Ischnocera and show the same general morphological features as *Trichodectes canis.*

Amblycera

1.Menopon gallinae, the shaft louse - a chewing louse of poultry. This louse is an example of the Amblycera which contains many chewing lice of domestic and wild birds. Examine the slide SSB #84 and note:

a. the chewing mouthparts (opposing mandibles) (Fig. 14),
b. the flat broad shape of the head,
c. unlike Ischnocera, the antennae are recessed into lateral depressions on the head. On most of these specimens the palpi are visible on either side of the head (Fig. 14).
d. the dual claws on each leg.

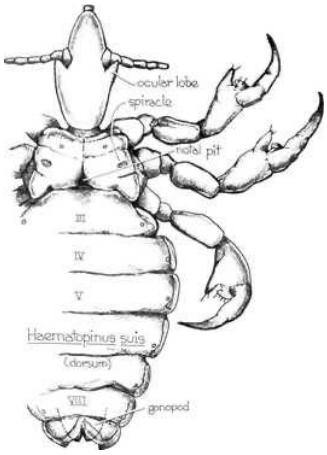


14

These lice are also seen in the **DEMO. Suborder Anoplura (the sucking lice)**

1. *Haematopinus* sp., the hog louse (adults, SSB #83). This important ectoparasite of swine provides a good example of the morphology of the sucking lice. Examine the slide in your slide box and note: **(Fig.** 13 **and Foreyt, pg. 137)**

a. Sucking mouthparts are retracted within the head. They may not be visible in your specimen.
b. The general structure - head, thorax and abdomen are dorsoventrally flattened.
c. The head is narrower than the thorax (unlike the chewing lice).



15

d. The antennae are visible and 5-segmented.
e. There are three pairs of legs each armed with a claw.

2. Linognathus sp. This genus (Foreyt, pg. 35) includes a common sucking louse of dogs, *L. setosus.* A specimen is in your slide box (SSB #78). The general morphology of this genus is similar to that of *Haematopinus.*

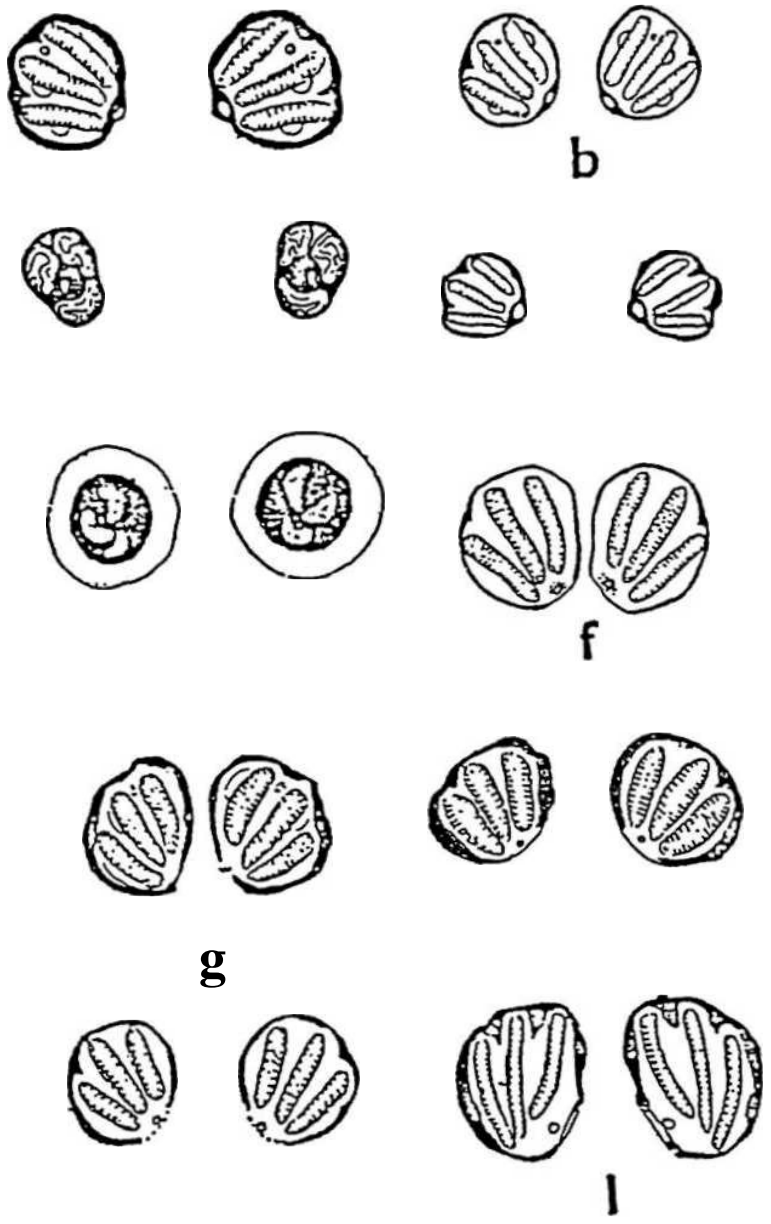
3. Human lice, *Phthirus pubis* and *Pediculus humanus* are on **DEMO.** These lice have the general morphology of the sucking lice; however, note the distinctive shape *of Phthirus pubis.* Remember, these lice are generally very host specific and survive poorly in the environment. The overriding route of transmission is host-to-host contact. Also, because these lice are so host specific, dogs and cats are not able to act as reservoirs for human lice.

4. View the video on identification of fleas and lice (running time approx. 15 min.).

Checklist of Objectives

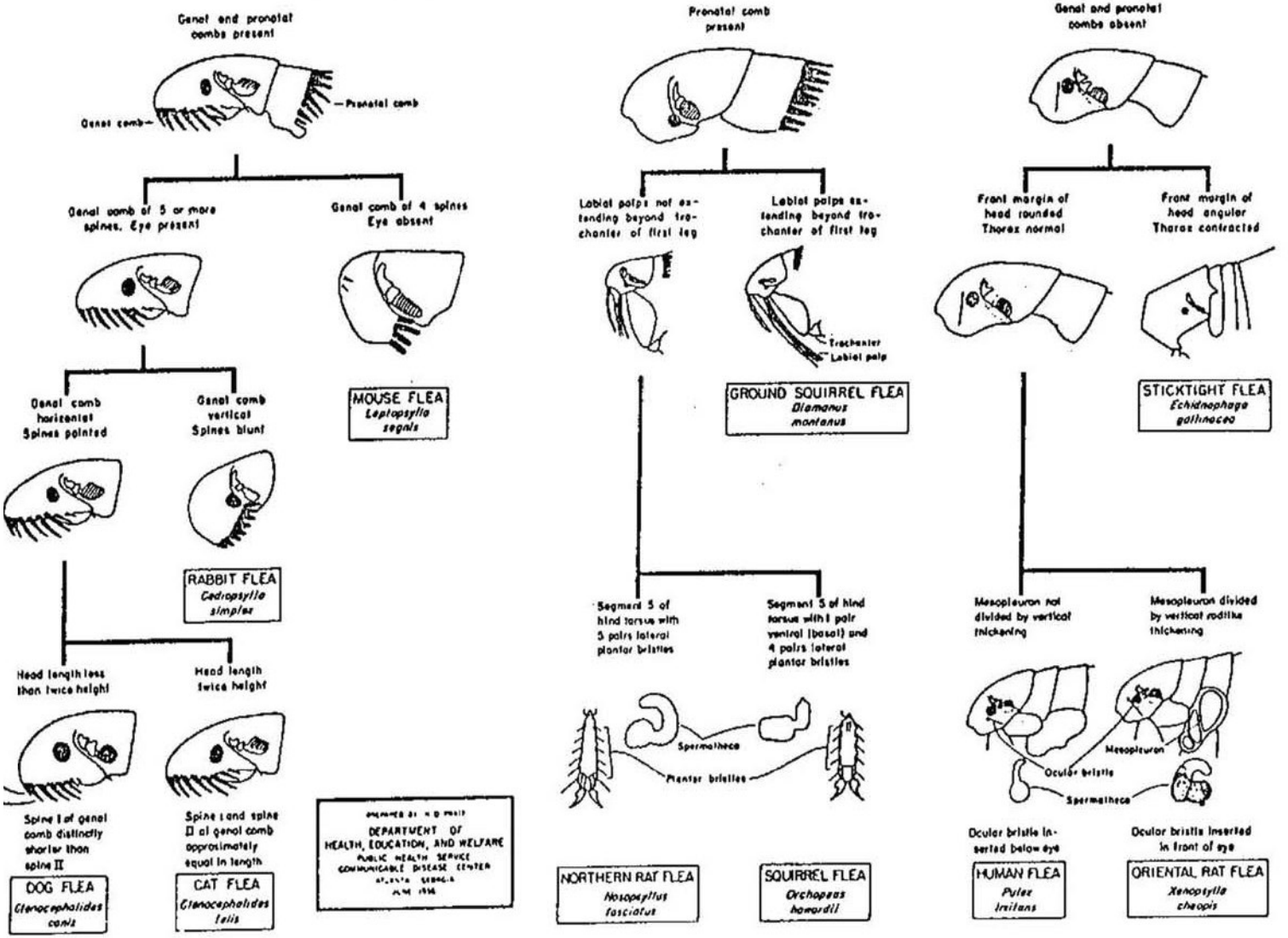
1. Be able to recognize the suborder Brachycera (Fam. Tabanidae) on sight.
2. Be able to prepare posterior spiracles of muscoid fly larvae and make a genus diagnosis based on pictorial keys provided.
3. Be able to recognize *Melophagus ovinus* on sight.
4. Be able to use a pictorial key of the type in the lab handout to identify flea adults to species.
5. Be able to differentiate on sight the chewing and sucking lice.
6. Be able to differentiate on sight the suborders of chewing lice: Amblycera and Ischnocera.
7. Answer the review question(s).

Figure 6.



The posterior spiracles of the larvae of various species of **Cyclorrhapha.** a. *Calliphora erythrocephala* b. *Lucilia sericata* c. *Stomoxys calcitrans* d. *Cynomyia cadaverina* e. *Muscina stabulans* f. *Chrysomyia megacephala* g. *Chrysomyia bezziana* h. *Cochliomyia macellaria* k, *Phormia regitia* 1. *Sarcophaga* sp. (Figures are not drawn to the same scale.)
From Smart, J., 1948

PICTORIAL KEY TO SOME COMMON FLEAS



ure 10.
e also Foreyt, pg 41)

bo

Laboratory #10

The Protozoa

Objectives:

The protozoa are unicellular organisms that are classified on the basis of the organelles used for locomotion.

In this laboratory, you will see many of the parasites that we have discussed in lecture. Some of these you will need to be able to identify because they are parasites commonly seen in practice. Others are shown to assist you in learning their life cycles. There are 4 parts to the laboratory, including (1) live/fresh materials; (2) wet preparations of fixed material; (3) demonstrations and AV; and (4) slides from the student slide box.

The following live/fresh materials will be provided, each of which will demonstrate different life history stages:

1. Intestines of chickens infected with *Eimeria tenella* (schizonts and merozoites).
2. Hamster intestinal contents containing *Giardia* sp. and *Trichomonas* sp. (trophozoites)
3. Feces containing cysts and/or oocysts for a ZnSO4 flotation. (cysts/oocysts)

Ciliophora - The ciliates

Balantidium coli -(Student Slides #55+56)

This ciliate is a commensal of domestic animals (esp. swine). It can be pathogenic under some conditions. The trophozoite is found in the large intestine where it normally lives in the lumen, but it may invade the intestinal wall producing shallow ulcers. Diagnosis is made by finding the large cysts in the feces.

In Slide #55 trophozoites can be seen in the lumen of a hamster or horse large intestine, in the epithelium. A few are deeper into the tissue. Note the large macronucleus, visible in some of the trophozoites, and the cilia on all the organisms (use your 40 X objective and low light to see the cilia).

In the stained fecal smear (Student Slide #56) note the cysts. They are spherical (40-60 have a hyaline wall, and usually the large macronucleus can be seen within.

Sarcomastigophora - The amoebae and flagellates

Entamoeba histolytica - **DEMO**

This amoeba is one of the few pathogenic amoebae of veterinary importance. It is primarily a parasite of humans and primates, but can occasionally infect other hosts such as the dog. However, cysts are only passed in the feces of humans and primates, so they are the source of infection for domestic animals. Note the characteristic nucleus in the trophozoite. The trophozoite will have 1 nucleus and a feeding vacuole which may contain red blood cells.

E. histolytica presents a diagnostic problem in dogs as only the trophozoite will be passed in the feces. Since a salt float would destroy this stage the only way to see them is to do a direct smear (staining the smear greatly improves your chances of identifying the amoeba).

Giardia sp. - DEMO, Wet prep. and $ZnSO_4$ Float. (Foreyt, pg. 31)

This flagellate is an inhabitant of the small intestine of mammals and birds. The cyst stage is usually found in the feces of infected animals, but in diarrhetic stools the trophozoites can sometimes be seen. To examine stools for the trophozoite you must make a thin direct smear and add a little saline to keep it moist (water may lyse the trophozoites and iodine would kill them making it impossible to see their characteristic motion). The cysts will float in standard salt solutions, but the salt solutions cause osmotic damage to the contents of the cysts. Therefore, a $ZnSO_4$ solution (which is not saturated, thus, less osmotically damaging) is used for flotation.

A) Wet mount - Take a drop of hamster small intestinal contents and place on a slide with a cover slip (no iodine). Look for the characteristic "Falling leaf motion" of the pear-shaped trophozoites (there will be some fast moving *Trichomonas* in this sample in addition to the less highly motile *Giardia).*

B) $ZnSO_4$ Flotation - take a small sample of dog feces and place it through a sieve into a tube full of $ZnSO_4$ solution. Pour the slurry back into a tube and centrifuge for 1 min. Remove the cysts, stain them with a drop of iodine and examine them under the 40X objective. The cysts are small (10-15 :m - just visible at 10X), oval in shape, and contain remnants of the trophozoite organelles (usually the remains of the axostyle can be seen cutting across the long axis of the cyst). (If you have trouble finding *Giardia* cysts, there are several prepared slides on the middle bench, please return them after you use them.)

Trichomonas spp. - Student Slide #42, Wet Mount.

Flagellates of this genus are found in the digestive tract or reproductive tract *(T. foetus)* of mammals and birds. In most cases, *Trichomonas* spp. are normal inhabitants of the digestive tract (esp. the cecum) and are generally considered non-pathogenic. There have been reports of diarrhea in which large numbers of *Trichomonas* sp. have been seen, but these reports haven't shown that these flagellates were responsible for the condition. *T. foetus* is the only proven pathogen of veterinary importance.

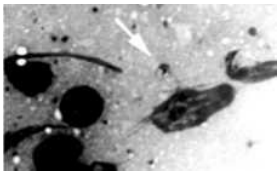
A) Wet Mount - make a wet mount of hamster cecal contents and look for *Trichomonas muris.* At 40X and low light you should be able to see the undulating membrane as a flickering wave on the body of the trophozoite. These flagellates don't form cysts, so the diagnosis depends on identifying the trophozoite. Note how the movement of this organism differs from *Giardia.*

Trypanosoma brucei - Student Slide #39

Trypanosomes are extremely important parasites of domestic animals in Africa and South America. In North America, they are common parasites of birds, in which they may occasionally be pathogenic. *T. brucei* is used in this lab to show the typical morphology of the trypanosomes. This African flagellate is found in the blood and cerebrospinal fluid of mammals. Use your oil lens and observe the trypanosome with its undulating membrane, single anterior flagellum and kinetoplast.

Leishmania donovani - Student Slide #41 and DEMO

Leishmania spp. are common parasites in tropical and sub-tropical areas of the world. They are found in rodents, dogs and humans. At VHUP, we have seen several cases of visceral leishmaniasis in dogs that were brought to this country from the Mediterranean region and the CDC is currently investigating infections in foxhound kennels in the eastern United States. This parasite exists as a flagellated promastigote in the gut of a sand fly or as a non-flagellated amastigote in the macrophages of a mammal. *L. donovani* is found in the macrophages of the bone marrow, liver and spleen. Slide #41 (use your oil lens) is a spleen impression smear. This type of smear is made by cutting the organ and gently blotting on a paper towel to remove excess blood. The cut surface is then touched to a glass slide to leave the impression. The spleen cells rupture due to the excess surface tension and the cell nuclei and amastigotes are left behind. The cell nuclei stain as purple blobs and smears, while the amastigotes show up as small (2-3 :m) circles with a nucleus and kinetoplast.



Amastigote (arrow) in lymph node impression smear. Drawing of an amastigote.

Apicomplexa - The Piroplasma, Haemosporidia, and coccidia.

The Piroplasma

Babesia canis - DEMO and Student Slide #54 (Foreyt, pg. 40)

This small protozoan is found in the red blood cells of dogs and is transmitted by ticks. (It is more often seen in blood obtained from cutaneous capillaries than from systemic venous blood however, the diagnosis is usually based on having an antibody titer to the organism.) They may be round or pear-shaped, 2-5 :m long (a red cell is 7 to 10 :m) and several may be seen in the same red blood cell. Use your oil lens to see these organisms. There is a mouse infected with *Babesia microti* in MDL-12. Make a blood smear from this mouse, stain it, and examine it for *Babesia.*

The Haemosporidia

Hemoproteus sp. - DEMO (Foreyt pg. 140)

Members of this genus are very common in birds and can also be found in reptiles. Schizogony occurs in the tissues and gametocytes are the only stage found in the red blood cells of birds. *Hemoproteus* spp. are generally considered non-pathogenic and are transmitted by blood sucking flies.

Leucocytozoon smithi - DEMO (Foreyt, pg. 148)

Schizogony occurs in the tissues and gametocytes are found in blood cells (usually the white cells) of birds. They distort the host cell and appear more spindle shaped than oval. Two species *(L. smithi* and *L. simondi,* in turkeys and ducks, respectively) are pathogens, but other non-pathogenic species are common in birds.

The Coccidia

The diagnostic stage of this group of parasites is the oocyst which is passed in the feces. One type of oocyst has 2 sporocysts, each containing 4 sporozoites (this type is seen in *Isospora, Sarcocystis,* and *Toxoplasma).* A second type of oocyst contains 4 sporocysts, each with 2 sporozoites (this type is seen in the genus *Eimeria).* In some genera (e.g. *Cryptosporidium)* the oocyst contains only sporozoites (no sporocysts).

Depending on the species, the oocyst may be passed unsporulated and require development in the environment before it is infective, or may be passed in a sporulated form and be immediately infective.

Isospora spp. - **DEMO** (Foreyt, pg. 31) Bottle 199

Parasites of this genus occur in carnivores and omnivores and are very host species specific. This protozoan is an intra-cellular parasite of the epithelial cells of the small intestine. Most species are not highly pathogenic and dogs and cats usually harbor several different species which will differ in size of the oocysts. I. *suis* a common parasite of pigs is an example of a pathogenic species. Oocysts, the diagnostic stage, are passed unsporulated and take 24 to 48 hours to reach infectivity. Oocysts found in the dog and cat range from 12 to 40 :m long. Sporulated oocysts *of Isospora* sp. will contain 2 sporocysts (easily seen) each with 4 sporozoites (not easily seen at 40X).

Eimeria spp. - Student Slide #47, Wet prep. and **DEMO**

The *Eimeria* spp. are common parasites of birds and herbivores. They also are very host specific (most *Eimeria* species are able to infect only one species of host). *Eimeria tenella* causes cecal coccidiosis of chickens. Student Slide #47 is a cross-section of the cecum of an infected chicken. Second generation schizonts (day 5 of infection), each containing many merozoites, can be seen in the sub mucosa. There is hemorrhage in the sub mucosa. The cecal content is primarily blood. In this blood you may see schizonts and sloughed epithelial cells.

Wet prep: Scrape a small amount of material from the damaged mucosa of the chicken's cecum, place it on a slide with some saline and a cover slip. Look for the schizonts and merozoites.

There is a demonstration of the oocysts.

Sarcocystis spp. - Student Slide #50, and **DEMO.** (Foreyt, pg 31), Bottle 181

Sarcocystis spp. have a herbivore - carnivore life cycle, for example, *Sarcocystis cruzi* has a dog-cattle cycle. The dog is the definitive host and the organism divides in the lamina propria of the small intestine. Oocysts sporulate rapidly in the intestine and generally the delicate oocyst wall ruptures while still in the intestine, thus **sporocysts** are passed in the feces. Cattle act as the intermediate host in which *Sarcocystis* is found as **sarcocysts** in the muscles.

Student Slide #50 is a section of infected calf muscle. Note the sarcocysts which contain many banana-shaped **bradyzoites.**

The DEMO contains the diagnostic stage, the sporocyst, of a *Sarcocystis* infection in a dog.

Toxoplasma gondii - **DEMO,** (Foreyt, pg 52) Bottle 182

T. gondii has a cat - mammal/bird cycle, with the intestinal infection occurring only in the definitive host, the cat. Any of a number of mammals or birds on which cats prey may act as non-obligatory intermediate hosts. Other mammals, such as man and dogs, can be infected with the asexual stages of *T. gondii* but are **dead end hosts** (i.e., they are not eaten by the cat). Unsporulated oocysts need 48 hrs to sporulate.

The **DEMO** shows a cross section of a rabbit brain showing cysts containing bradyzoites. (Find the cyst with your 10X lens, and examine it at 40X.)

There is also a DEMO that contains the diagnostic stage of a *T. gondii* infection in a cat, the oocyst. Oocysts are small (10-12 :m) and round. Oocysts of *T. gondii* are shed for only 1 or 2 weeks during the first infection (in young cats) and the cat is then immune to re-infection. Older cats whose immune system is compromised may also shed oocysts.

Cryptosporidium sp. - Student Slide #97 and **DEMO** (Foreyt, pg. 85)

This coccidian can infect a large number of different hosts including both mammals and birds. Since the same species infects both domestic animals and man, this organism is a public health hazard. The diagnostic stage is the small (4 :m) round oocyst containing 4 sporozoites. The size and shape of the oocyst are the same as those of yeast which may also be found in normal or diarrhetic stools. In order to positively identify these oocysts, a special staining technique has been devised. It was found that, like tuberculosis bacteria, these oocysts are acid-fast when stained with an acid-fast stain. That is, they will appear red after staining, while yeast (not acid- fast) will appear blue-green.

Student Slide #97 is an acid fast stain of a direct fecal smear of from a calf. At 40X, you will see the oocysts as small, round red dots - you may have to go to your oil lens to see them better (no internal details will be visible). Both oocysts and yeast will float in a fecal float; therefore, the presence of small, round, organisms in a fecal float would call for the acid-fast stain to confirm the diagnosis.

Video - There is a 15 min. video on a protozoan parasite of cattle.

Checklist of Objectives:

1. Learn to identify the cyst of *Giardia* and the oocysts of *Isospora, Eimeria, Cryptosporidium* and *Toxoplasma* and the sporocyst of *Sarcocystis.*
2. Learn to identify the trophozoite stages of *Giardia* and *Trichomonas.*
3. Learn to identify *Babesia* and *Leucocytozoon* in a blood smear, and *Leishmania* amastigotes in impression smear.

Laboratory #11 Review of the Parasites of Small Animals

Objective: To review the parasites of small animals in a host-oriented manner.

1. The parasite ova and cysts can be found in bottles at the end of your bench and slides can be found in your Student Slide Box. Host - Parasite Lists can be found starting on the next page.

The following lists are not all inclusive, for example the deer tick can be found on many small mammals and birds, but it is just listed under dogs. As you go through the following lists make note of the parasites which can be found in several hosts (i.e. those that are not host species specific). Also note that some of the bottles and slides referred to have eggs or cysts of parasites that look identical to the ones actually in the bottle or on the slide (for example: *Capillaria:* eggs in stool = *C. aerophila,* in urine = *C. plica,* but both look like the eggs in bottle #61).

2. Fecal Examination Techniques:

a) Do a salt flotation on the dog feces in tub #1

b) Do a $ZnSO_4$ flotation on the dog feces in tub #2

3. Blood Examination (Center Bench):
Examine the blood on the center bench for microfilaria using either the Knott's or the Filtration methods.

Dogs

Parasite	Location in Host	Diagnostic Stage*	Lab	Specimen
Spirocerca lupi	esophagus	eggs in feces		B90
Physaloptera spp.	stomach	eggs in feces or adult in vomit		
Dipylidium caninum	small intestine	proglottids (eggs) in feces		SSB 4,5,6
Echinococcus granulosus	small intestine	proglottids (eggs) in feces		SSB9
E. multilocularis	small intestine	proglottids (eggs) in feces		
Taenia ovis, etc.	small intestine	proglottids (eggs) in feces	B	14, SSB 1,2,3
Ancylostoma caninum	small intestine	eggs in feces		SSB 29
A. braziliense	small intestine	eggs in feces		
Strongyloides stercoralis	small intestine	larva in feces		
Toxocara canis	small intestine	eggs in feces		B24
Uncinara stenocephala	small intestine	eggs in feces		
Mesocestoides sp.	small intestine	proglottids in feces		
Giardia	small intestine	cyst in feces		
Sarcocystis	small intestine	sporocyst in feces		B181
Isospora sp.	small intestine	oocysts in feces		B157
Cryptosporidium	small intestine	oocysts in feces		SSB 97
Neospora caninum	sm. intestine, brain	oocysts if in sm. int., serology		
Entamoeba histolytica	large intestine	cyst in feces or trophozoites in diarrhea		
Trichomonas spp.	large intestine	trophozoite in diarrhea		
Trichuris vulpis	large intestine	eggs in feces		B 25, SSB 37
Dirofilaria immitis	pulmonary artery	microfilaria in blood		SSB 34, 35
Filaroides spp.	lungs	larva in feces		B113
Capillaria aerophila	lungs	eggs in feces or sputum		B61
Paragonimus kellicotti	lungs	eggs in feces or sputum		B142
Leishmania donovani	Macrophages	maybe seen in ascites from peritoneal	cavity	SSB
Babesia canis	red blood cells	in RBCs (also serology)		SSB 54
Toxoplasma gondii	brain, other tissues	none (serology)		SSB 49
Capillaria plica	Urinary bladder	eggs in urine		B61
Dioctophyma renale	kidney	eggs in urine		
Pneumonyssus caninum	nasal cavity	adults in nasal swab		
Otobius megnini	ear	adults seen		
Otodectes cyanotis	external ear	adults seen		
Sarcoptes scabiei	skin	adults in skin scrape		SSB 88
Demodex canis	skin	adults in skin scrape		SSB 90
Ixodes scapularis	skin	tick seen on skin		
Dermacentor variabilis	skin	tick seen on skin		SSB 96
Rhipicephalus sanguineus	skin	tick seen on skin		SSB 95
Ctenocephalides sp.	skin	adults on skin		SSB 75
Linognathus setosus	skin	adults on skin		SSB 78
Trichodectes canis	skin and hair	adults on skin		SSB 77
Cuterebra sp.	skin	larva under skin		

* In living host, serology and biopsy excluded. Where no diagnostic stage is present, serology or biopsy may be appropriate. + B = Bottle on front bench, SSB = Student Slide Box Slide #

Cats

Parasite	Location in Host	Diagnostic Stage	Lab Specimen
Physaloptera spp.	stomach	eggs in feces or adult in vomit	
Dipylidium caninum Taenia taeniaeformis	small intestine	proglottids (eggs) in feces	SSB 4,5,6
Mesocestoides sp.	small intestine	proglottids (eggs) in feces	SSB 1,2,3 B
Spirometra mansonoides	small intestine	proglottids in feces	
Diphyllobothrium latum	small intestine	eggs in feces	B144
Echinococcus multilocularis	small intestine	eggs in feces	
Ancylostoma tubaeforme A. braziliense Toxocara cati	small intestine	eggs in feces	
Toxascaris leonina	small intestine	eggs in feces	
Cryptosporidium spp.	small intestine	eggs in feces	
Toxoplasma gondii Isospora sp. *Giardia*	small intestine	oocyst in feces	SSB 97
Capillaria aerophilia	small intestine	oocyst in feces	B 182, SSB 49
Paragonimus kellicotti	small intestine	oocyst in feces	B157
Aelurostrongylus abstrusus	small intestine	cyst in feces	
Dirofilaria immitis	lungs	eggs in feces or sputum	B61
Capillaria plica Otodectes cyanotis Notoedres cati	lungs	eggs in feces or sputum	B142
Cuterebra sp.	lungs	larva in feces	B157
Ctenocephalides sp.	pulmonary artery	usually none	
Trichinella spiralis	urinary bladder	eggs in urine	B61
	external ear	adult in ear	
	skin	adults in skin scrape	
	skin	larva under skin	
	skin	adults on skin	SSB 75
	muscle	larva (biopsy)	SSB 38

Poultry

Parasite	Location in Host	Diagnostic Stage	Lab Specimen
Capillaria sp.	esophagus, sm. intestine	eggs in feces	B61
Ascaridia galli	small intestine	eggs in feces	
Eimeria sp.	small intestine	oocysts in feces	SSB 47, B 30
Giardia	small intestine	cyst in feces	
Cryptosporidium sp.	small intestine	oocysts in feces	SSB97
Heterakis gallinarum	cecum	eggs in feces	
Syngamus trachea	trachea	eggs in feces	
Echidnophaga gallinacea	skin	adults on skin	
Ornithonyssus sylviarum	skin	adults on skin	BE203
Dermanyssus gallinae	skin	adults in skin	SSB 91
Columbicola columbae	skin	adults in skin	SSB 85
Argas persicus	skin	adults on skin	
Knemidocoptes sp.	skin of legs	adults in skin scrape	
Menopon gallinae	feathers	adults on feathers	SSB 84
Leucocytozoon smithi	blood cells	gametocytes in blood	smear
Haemoproteus columbae	red blood cells	gametocytes in blood	smear SSB 52

Wildlife

Parasite	Location in Host	Diagnostic Stage	Lab Specimen
Physaloptera spp.	stomach	eggs in feces or adult in vomit	
Baylisascaris procyonis	small intestine	eggs in feces	
Giardia	small intestine	cyst in feces	
Cryptosporidium	small intestine	oocyst in feces	**SSB9**
Eimeria sp. or *Isospora* sp.	small intestine or liver	oocyst in feces	
Xenopsylla cheopis	skin	adults on skin	**SSB7**
Cheyletiella parasitivorax	skin	adults on skin	
Ixodes sp.	skin	adults on skin	
Cuterebra sp.	skin	larva under skin	
Dermacentor variabilis	skin	stages on skin	**SSB9**
Trichinella spiralis	muscle	larva in biopsy	**SSB3**

Laboratory #12 Review of the Parasites of Large Animals

Objective:

The objective of this lab is to review the parasites of large animals in a host oriented manner.

1. The following Host-Parasites lists show the bottles at the end of your bench and the slides in your Student Slide Box which should be reviewed in this lab.

Note: These lists are not meant to be totally inclusive, for example *Cryptosporidium* and *Trichinella* can be found in all mammals, and mosquitos or other flies (SSB 73), some lice and ticks are not listed with any particular host. The following are some of the lice and ticks not listed elsewhere:

Parasite	**SSB** #
Damalinia caprae	80+81
Linognathus sp.	78
Psoroptes sp.	88
Psoroptes sp.	88
Boophilus annulatus larvae	93
Dermacentor sp.	96
Rhipicephalus sp.	95

Some Bottles contain eggs that are very similar to the species cited (ex. *Strongyloides papillosus* #49, is almost identical to the eggs of *S. westeri* and *S. ransomi).*

2. Fecal Examinations:
   a) Do a McMaster egg/gm count on the sheep feces found in tub #1.
   b) Do an ethyl-acetate sedimentation on sheep feces found in tub #2.
   c) Do a fecal flotation on the pig feces in tub #3.

3. **DEMOS** - MDL 12

   Demos are arranged by host and predilection site.

   Note: Even if a parasite can be found in more than one host, there will be only one demo. The following are lists of parasites covered in lab. These lists are not meant to be complete.

CATTLE

Parasite	Location in Host	Diagnostic Stage*	Lab	> Specimen+
**Haemonchus*	abomasum	egg in feces		SSB28
**Ostertagia*	abomasum	egg in feces		
*	abomasum or sm. int.	egg in feces		
**Paramphistomum*	*i* rumen	eggs in feces		
* *Cryptosporidium*	sm. intestine	oocysts in feces		SSB97
**Giardia*	sm. intestine	cyst in feces		
**Cooperia*	sm. intestine	egg in feces		
**Nematodirus*	sm. intestine	egg in feces		
**Bunostomum*	sm. intestine	egg in feces		
**Strongyloides*	sm. intestine	larvated eggs in feces		B49
**Moniezia*	sm. intestine	eggs in feces		B 37, SSB 7
**Trichuris*	lg. intestine	eggs in feces		
Chabertia	lg. intestine	egg in feces		
**Eimeria*	cecum, lg. intestine	oocysts in feces		
**Fasciola*	bile ducts	eggs in feces	B	11, SSB 12, 13
**Dicrocoelium*	bile ducts	eggs in feces		SSB 16, 17
**Dictyocaulus*	lungs	larvae in feces		
**Hypoderma*	skin of back	larva in skin		
**Psoroptes*	skin	adults on skin		SSB 88
**Chorioptes*	skin	adults on skin		
**Sarcoptes*	skin	adults in skin scrape		
**Damalinia*	skin	adults on skin or hair		SSB 80, 81
**Trypanosoma*	blood	trypanosome in blood smear		SSB 39
**Taenia*	muscle	none		
**Sarcocystis*	muscle	none		SSB 50
Trichomonas	reproductive	i trophozoites on smear		SSB 42

* Also in other hosts.

** The stage used to diagnose the infection in a living animal, if **none,** biopsy or serology may be used.

\+ B=Bottle, SSB=Student Slide Box slide #

SHEEP AND GOATS

Parasite	Location in Host	Diagnostic Stage	Lab Specimen
**Paramphistomum*	rumen	eggs in feces	
**Haemonchus*	abomasum	egg in feces	SSB28
**Ostertagia*	abomasum	egg in feces	
** Trichostrongylus*	abomasum or sm.	int. egg in feces	
**Cooperia*	sm. intestine	egg in feces	
**Nematodirus*	sm. intestine	egg in feces	
**Bunostomum*	sm. intestine	egg in feces	
** Cryptosporidium*	sm. intestine	oocysts in feces	SSB97
**Giardia*	sm. intestine	cyst in feces	
**Strongyloides*	sm. intestine	larvated eggs in feces	B49
**Moniezia*	sm. intestine	eggs in feces	B 37, SSB 7
**Eimeria*	lg. intestine	oocysts in feces	
**Trichuris*	lg. intestine	eggs in feces	
**Dictyocaulus*	lungs	larvae in feces	
Muellerius	lungs	larvae in feces	SSB31
**Echinococcus*	liver and others	none	
**Dicrocoelium*	bile ducts	eggs in feces	SSB 16, 17
**Fasciola*	bile ducts	eggs in feces	B11, SSB 12, 13
Oestrus	nasal cavities	none	
Melophagus	skin	adult on skin	SSB 68
**Hypoderma*	dermis of back	larva in skin	
**Psoroptes*	skin	adults on skin	SSB 88
**Chorioptes*	skin	adults on skin	
**Damalinia*	skin	adults on skin or hair	SSB 80, 81
**Sarcoptes*	skin	adults in skin scrape	
**Trypanosoma*	blood	trypanosome in blood smear	SSB 39
**Taenia*	muscle	none	
**Sarcocystis*	muscle	none	SSB 50

SWINE

Parasite	Location in Host	Diagnostic Stage	Lab Specimen
**Giardia*	sm. intestine	cysts in feces	
Isospora	sm. intestine	oocysts in feces	
** Cryptosporidium*	sm. intestine	cysts in feces	SSB 97
**Eimeria*	intestine	oocysts in feces	
**Strongyloides*	sm. intestine	eggs in feces	B49
Ascaris	sm. intestine	eggs in feces	B 59, SSB 23
Macracanthorhynchus	sm. intestine	eggs in feces	
**Trichinella*	sm. intestine + muscle	none	SSB 38
Oesophagostomum	lg. intestine	eggs in feces	SSB 27
**Trichuris*	lg. intestine	eggs in feces	
Balantidium	lg. intestine	cysts in feces	SSB 55,56
**Fasciola*	bile ducts	eggs in feces	B 11, SSB 12, 13
Stephanurus	kidney	eggs in urine	
Metastrongylus	lungs	larvated eggs in feces	
**Haematopinus*	skin	adults on skin	SSB 83
**Sarcoptes*	skin	adults in skin scrape	SSB 88

HORSE

Parasite	Location in Host	Diagnostic Stage	Lab Specimen
Gastrophilus	stomach	none	SSB 70, 71
**Giardia*	sm. intestine	cysts in feces	
**Cryptosporidium*	sm. intestine	cysts in feces	SSB 97
Parascaris	sm. intestine	eggs in feces	B 19
Strongyloides	sm. intestine	eggs in feces	B 49
Anoplocephala	sm.+lg. intestine	eggs in feces	
**Eimeria*	intestine	oocyst in feces	
Oxyuris	lg. intestine	eggs on perianal skin	B 13
Strongylus	lg. intestine	eggs in feces	SSB 30
Trichonema	lg. intestine	eggs in feces	SSB 26
Setaria	peritoneal cavity	microfilaria in blood	
**Dictyocaulus*	lungs	larva in feces	
Psoroptes	skin	adults on skin	SSB 87
**Chorioptes*	skin	adults on skin	
**Sarcoptes*	skin	adults in skin-scrape	SSB 88
Dermacentor	skin	adults on skin	
**Damalinia*	skin	adults on skin or hair	
**Haemopinus*	skin	adults on skin or hair	
Trichodectes	skin	adults on skin or hair	
**Onchocerca*	lg. ligaments	microfilaria in skin	
Babesia	blood	trophozoite in RBCs	
**Trypanosome*	blood	trypanosomes in blood	SSB 39

Useful Biological Prefixes and Suffixes

Prefix or suffix	Meaning	Examples
a-	not, without	atypical
ab-	from, away	abnormal
acantho-	spine	Acanthocephala
-ad-	to, toward	dorsad, adhere
albi-	white	albicans
-algia	pain	neuralgia
amyl-	starch	amylopsin
an-	not, without	anhydrous
ana-	up, upon	anabolism
ancylo-	bent	ancylostoma
angio-	vessel	angiocarditis
ante-	before, in front of	a n t e r
anti-	against, opposed to	antitoxin, antibody
apo-	off, from, away from	apoplexy
aqua-	water	aquatic
-ase	designating an enzyme	amylase
auto-	self	autosuggestion
bi-	two, twice	biceps, bifocal
bio-	life	biology
brachy-	short	brachycardia
brady-	slow	bradycardia
calor-	heat	calorimeter
cardia-	heart	bradycardia
-cera	horn	brachycera
-cerca	tail	Onchocerca
cerebro-	brain	cerebrospinal

Prefix or suffix	Meaning	Examples
-chezia	defecate	hematochezia
chrom-	color	chromosome
-cidal	killing	bacteriocidal
corpus-	body	corpuscle
-cyst	bladder	oocyst
-cyte, cyto-	cell	cytoplasm
-dermic	skin	hypodermic
di-	two, twice	dichromic
dia-	through, between	diaphragm
dys-	bad, difficult	dyspepsia
-ectomy	cut out	appendectomy
em-, en-, endo-	in, into	embolism, endoskeleton
-emia	blood	anemia
entero-	intestine	enterokinase
epi-	on, above, upon	epiglottis
erythro-	red	erythrocyte
-fer-	to carry, to transport	afferent, efferent
-fract-	to break	fracture, refraction
-gastro-	stomach	pneumogastric
-gen-	to produce, to begin	genetics, glycogen
-glosso-	tongue	hypoglossal
glyc-	glucose, sugar	glycosuria
-gnath-	jaw, cheek	gnathostoma
-gnosis	knowledge, to know	diagnosis
-graph	to write	cardiograph
-helminth	worm	anthelminthic
hemo-	blood	hemorrhage

Prefix or suffix	Meaning	Examples
hetero-	different, other	heterozygous
homo-	alike, same	homozygous
hydro-	water	hydrolytic
hyper-	over, more than	hypersecretion
hypo-	under, less than	hyposecretion
-iasis	infestation	onchocerciasis
inter-	between, together	intercostal
intra-	within	intrathoracic
ir-	not	irregular
-itis	inflammation	appendicitis
kata-, cata-	down	catabolism
kin-	to move or activate	kinetic
-lac-	milk	lactase, prolactin
leuco-, leuko-	white	leucocyte
-ology	science, knowledge	physiology
lymph-, lympho-	lymph	lymphocyte
-lysin, -lysis, -lytic	dissolve, destroy	hemolysis
macro-	large	macrophage
melan-, melen-	black	melanoma, melena
-meter	measure	manometer
micro-	small	microorganism
mono-	one	monocyte, monosaccharide
myo-	muscle	myosin, myoglobin
nema-	thread	nematode
neur-	relating to nerves	neurilemma
nephr-	kidneys	nephritis
-oid	like	lymphoid, ameboid

Prefix or suffix	Meaning	Examples
-ole	small	bronchiole
-oma	swelling, tumor	sarcoma
oncho-	hook	Onchocerca
onco-	mass, bulk	oncosphere
oo-	egg	oocyst
-opia	sight	myopia, hyperopia
-osis	condition or process	cyanosis, phagocytosis
os-, oste-, osteo-	bone	osteology, osteocyte
ovi-	egg	oviducy, ovipositor
para-	near, by, beside	parathyroid
patho-	disease, suffering	pathology
peri-	around, near	pericardium
phago-	to eat	phagocyte
-phil-	loving	basophil, eosinophil
-plasm-	substance	cytoplasm, plasmolysis
platy-	broad	Platyhelminthes
-pnea	breathing	dyspnea
pneumo-	air, lungs	pneumonia
-ped	foot	pseudoped
poly-	many	polysaccharide
post-	after, behind	postganglionic
pro-	before, giving rise to	proenzyme
proprio-	one's own	proprioceptors
pseudo-	false	pseudoped
psycho-	mind	psychology
pulmo-	lung	pulmonary
-renal	kidney	adrenal

Prefix or suffix	Meaning	Examples
-rrhea	flow	diarrhea
sarco-	flesh, muscle	sarcoplasm
soma-	body	somatic cell
-some	body	chromosome
stoma-	mouth	ancylostoma
-strongyle	cylinder	trichostrongylus
-thrombo-	clot, coagulation	thrombosis
-tode	like	nematode
-tome, -tomy	to cut	tonsillectomy
tricho-	hair-like	trichostrongylus
-trophic	feeding	autotrophic
-tropic	attached to	phototropic
-ule	small	saccule
-uria	pertains to urine	glycosuria
-uris	tail	trichuris
vaso-	pertains to blood vessel	vasodilation