Platyhelminthes and Acanthocephala

Introduction:

The Phylum Platyhelminthes contains both the trematodes and cestodes. The trematodes (flukes) have an incomplete digestive tract (a mouth but no anus) and have no body cavity, the organs being imbedded in the parenchyma of the body. There are 2 subclasses of parasitic flukes: the Monogenea, whose members are parasitic only as adults and the Digenea, whose members have two or more hosts in their life cycle, and the first host is a mollusk.

The Cestodes (tapeworms) have no mouth, as adults they live in the small intestine of their hosts and absorb nutrients through their tegument (external surface). The tapeworm life cycle involves an intermediate host. In many species the larval stages cause more pathology than the adult worms.

The Phylum Acanthocephala contains parasites, which as adults, lack a digestive tract and live in the small intestine of their hosts. As the name suggests, they have a spine-covered proboscis at their anterior end (they are also known as "thorny headed worms"). Although many different species are found in wildlife, only one species is a parasite of domestic livestock (pigs).

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.

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.

The other group of tapeworms, the pseudophyllideans, lay eggs that look like typical trematode eggs.

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.

In this lab we will also present you with the basic structure of the adult acanthocephalan so you will be able to recognize parasites of this phylum if you should ever come across them.
At the Bench

1) *Fasciola hepatica* - Eggs (one slide per bench) - These large eggs (140 x 75 µm) have an operculum at one end. These eggs do not float in a standard saturated salt solution, but may float when using the ZnSO4 centrifugational technique. (Pg. 91, Foreyt)

2) *Dicrocoelium dendriticum* - Eggs - Slide #17 on your bench - These eggs are small (45 x 30 µm), dark brown, and contain a miracidium when passed in the feces.

3) *Dipylidium caninum*

   Slide #5 (RED, background information only) Scolex (the very small segment on the slide that contains the anterior end of the tapeworm) - this is a typical scolex of a cyclophyllidean having 4 suckers and (in this species) an armed (has hooks on it) rostellum (a beak-like extension on the anterior end, it may be extended or retracted to allow the tapeworm to set or release the hooks). The scolex is used to anchor the worm to the gut wall.

   Slide #6 Proglottids - Note the bi-convex shape ("cucumber seed"), the duplicated reproductive organs and two lateral genital pores. Many times the proglottid that you are examining will have dumped its eggs while crawling around the collection vessel, in these cases it is the presence of the 2 lateral genital openings that are the key to identifying this tapeworm.

   Slide #4 Eggs - (pg. 33, Foreyt) The egg packets have been expressed from gravid proglottid. Each egg packet contains up to 20 eggs, and within each is an onchosphere bearing 3 pairs of hooks.

4) *Taenia* sp.

   Slide #2 Scolex - Note the 4 suckers and the rostellum (this may be armed or unarmed, or even missing depending on the species). Also see Diagram 3 (RED, This diagram is for your information only).

   Slide #3 Gravid proglottids - Note the branched uterus, (RED, background information only) full of eggs (the number of branches is characteristic of the individual species of *Taenia*). Note there is only one genital opening, unlike the proglottid of *Dipylidium caninum*. Also see Diagram 3 (RED, This diagram is for your information only).

5) *Echinococcus granulosus*

   Slide #9 Adults - 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 3 (RED, This diagram is for your information only) shows a drawing of the internal anatomy of *Echinococcus*. 
6) Examine the following slides on your bench:

A) *Taenia* sp. Eggs - (pg. 33, Foreyt) All eggs of Taeniid tapeworms (*Taenia, Echinococcus*) look alike. They are round (30-35 μm) and have a striated embryophore (shell). Note the six hooks in the onchosphere (embryo), a common feature of all cyclophyllidean tapeworm eggs.

B) *Moniezia* spp. Eggs - (pg. 91, Foreyt) These eggs (56 to 67 μm) are triangular or square in shape and contain an onchosphere surrounded by a pyriform apparatus (a pear-shaped structure).

Identification of *Dipylidium caninum* proglottids:

Take a proglottid from the dish on the front 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, one on each lateral margin (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.)

**Demonstrations**

**Flukes (Trematodes)**

Subclass *Digenea* (these flukes have a snail as the first intermediate host).

1) *Fasciola hepatica* - Adults - These adult worms live within the bile ducts of their host. Note the size and shape of this trematode.

2) *Fasciola hepatica* - Eggs - These large eggs (140 x 75 μm) have an operculum at one end.

3) *Dicrocoelium dendriticum* - Adults - Note the size (1 cm) and shape of these bile duct worms.

4) *Dicrocoelium dendriticum* - Eggs - These eggs are small (45 x 30 μm), dark brown, and contain a miracidium when passed in the feces.

5) *Fascioloides magna* - Adult - This is a parasite of deer which causes an extensive amount of hepatic pathology in sheep, but little in cattle. Note the large size of this worm. In cattle it will be found within a cyst in the liver. Eggs will not be found in the feces of infected cattle or sheep.
6) *Paramphistomum cervi* - Adults - Note the shape, these worms are not flat like other trematodes, also they have a large posterior sucker. The rumen fluke lives in the rumen of sheep and cattle. The adults are of low pathogenicity, but the larvae can cause damage during the migration through the liver (during the pre-patent phase of the infection).

7) *Paragonimus kellicotti* - Eggs - (100 x 50 μm) (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.

8) *Paragonimus kellicotti* - This is the lung fluke of dogs and cats. Adults are usually found in pairs of fibrous cysts within the lungs. The are not as flattened as other trematodes as you can see in the cross section of the related fluke *P. westermanii*, a parasite of humans.

9) *Heterobilharzia americana* - This is the schistosome of dogs. In the USA, these are usually only seen in dogs from the Gulf Coast. The slide shows the adults of a related species, *Schistosoma mansoni*, a parasite of humans. Note that the sexes are separate in this family of digenean trematodes.

10) *Platynosomum fastosum* - Adults measure 4 to 8 mm long and 1.5 to 2.5 mm wide and are found in the bile and pancreatic ducts of cats (and rarely dogs) in the Southeastern USA and the Caribbean. The second intermediate host is a lizard.

Subclass **Monogenea** (these trematodes have no intermediate host).

11) *Gyrodactylus* sp. - Adult - The adults of this monogenean are ectoparasites of fish. Note the holdfast organ (“haptor” - equipped with hooks) that is used to maintain position on the skin of the fish. These are common parasites of aquarium fish.

12) A trematode life cycle: *Fasciola hepatica*.

We are using this trematode to show you the stages normally seen during the life cycle of a digenean trematode. (See Diagram 2 - RED: The diagram and all the material in this section.)

A) **Eggs** - these eggs pass in the feces of the host and are washed into shallow fresh water where they hatch.

B) **Miracidium** - This is the stage that emerges from the egg. It uses its cilia to swim and its receptors (notably the photoreceptors - note the “eyespots”) to position itself within the water column where it is most likely to come across the specific species of snail that is the first intermediate host. Upon penetration into the snail, the ciliated coat is lost and the miracidium develops into a sporocyst. The large cells in the posterior of the miracidium are the germ cells which will give rise to the offspring of the sporocyst, the **redia**. Diagram 2 shows drawings of the internal anatomy of *Fasciola hepatica* larvae. This is for your information only.
C) **Intermediate hosts** - One of the many snail intermediate hosts suitable for the development of *Fasciola* stages are species of *Lymnaea*.

D) **Redia** - This larval stage is found within the snail and it will asexually multiply to produce many *cercariae*, the next larval stage. Under some circumstances the redia may give rise to a generation of *daughter rediae*, which then produce the cercariae. The redia is characterized by a primitive digestive tract made up of a pharynx and intestine. Note the developing *cercariae* within this redia.

E) **Cercaria** - This stage leaves the snail and swims around until it finds some vegetation to settle on, generally just below the water’s surface. In other trematodes, the cercariae will infect the second intermediate host. Once on the plant, the cercariae of *F. hepatica* will secrete a cyst wall around themselves, transforming themselves into *metacercariae*.

F) **Metacercaria** - This cyst-like larva is the stage infective to the definitive host. It measures about 0.2 mm in diameter. Once ingested by the definitive host, it will excyst in the host’s duodenum. It will penetrate the gut wall and migrate through the body cavity to the liver, eventually arriving in the bile ducts where it matures to the *adult* stage.

G) **Adult** - The adult *F. hepatica* live in the main bile ducts of the host, and *eggs* are laid in the bile ducts. The eggs leave the liver with the bile and travel down the intestine, finally leaving the host with the feces. The immature adults migrating through the liver parenchyma do a great deal of damage and the repaired damage can be seen as fibrous tracts running through the liver. The adult worms destroy the epithelium of the bile ducts and the inflammatory reaction leads to a fibrous thickening of the bile duct walls.

**Tapeworms (Cestodes)**

Cyclophyllidean tapeworms

1) **Taeniid Eggs:** *Echinococcus* spp. and *Taenia* spp. All eggs of Taeniid tapeworms look alike. They are round (35-45 μm) and have a striated embryophore (shell). *Taenia* spp. eggs leave the host in proglottids, while those of *Echinococcus* are normally found free in the feces as the proglottids break apart in the feces.

2) **Taenia** spp. proglottid. These are square or rectangular in shape. The size varies by the age of the worm, but are in the range of 8 - 10 mm long by 4 - 5 mm wide for the common cat and dog species of *Taenia*.

3) **Taenia** sp. -Tapeworms of this genus are found in the small intestine of carnivores (and omnivores -primates) and the larval stage is found in various tissues of the mammals that serve as the prey of the carnivore.

   A. Adult - the size of the adult depends on the species and the age of the worm.
B. Larvae - The cysticercus (“bladder worm”, found in *T. solium*, *T. saginata*, *T. pisiformis*, *T. hydatigena*, and *T. ovis*) is a relatively small, fluid filled cyst which contains the inverted protoscolex. The strobilocercus (*T. taeniaeformis*) looks like a miniature tapeworm with a sac at the end. The coenurus (*T. multiceps* and *T. serialis*) is a fluid filled cysts which contains numerous protoscoleces budding off its inside surface.

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

A. Adult - very small (3 to 5 mm). They insert their scolex into the small intestinal crypts and the end of the worm may not reach past the tips of the villi!

B. Egg - These are identical to those of *Taenia* spp. Remember that 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. Labware, etc., must be sterilized after use or disposed of safely. The proglottids are very small and often over looked in the feces, however they rupture easily and the eggs are often found free in the feces. These eggs, like the eggs of *Taenia* spp. will float in a zinc sulfate centrifugation flotation.

C. Larva - hydatid cysts. These fluid-filled cysts contain many protoscolices and smaller cysts (brood capsules or daughter cysts).

5) *Echinococcus multilocularis* - slightly smaller (2 to 5 mm) than *E. granulosus*.

   Larva - the hydatid cyst of this tapeworm consists of many small chambers (alveolar cyst).

6) *Dipylidium caninum* Egg packet. (pg. 33, Foreyt) The egg packets are too big to float, so the diagnosis is made from the proglottid (which can be identified by the presence of the egg packets).

7) *Dipylidium caninum* proglottid. Note the shape and look for the two genital pores and, if this is a gravid proglottid, you will see egg packets within it.

8) *Dipylidium caninum* - 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

9) Anoplocephalid Eggs - *Anoplocephala* spp. and *Moniezia* spp. Triangular or square eggs. These eggs (56 to 67 μm) contain an onchosphere surrounded by a pyriform apparatus (a pear-shaped structure). The thin walled proglottids tend to rupture in the colon or are broken apart during the preparation of the feces for a fecal exam, thus eggs are many times found on a fecal floatation.
10) *Moniezia expansa* - These very large tapeworms are found in the small intestine of ruminants.

A. Adults - Large worms with a wide, but short proglottid.

B. Eggs - measure 56 to 67 μm and are triangular in shape. Like other anoplocephalid eggs they contain an onchosphere surrounded by a pyriform apparatus (a pear-shaped structure). *(pg. 91, Foreyt)*

11) *Anoplocephala* sp. Parasites of horses. The intermediate host is a forage mite (a type of free-living mite).

A. Adults:
*Anoplocephala perfoliata* - These short, but wide, tapeworms will be found at necropsy in the colon or just inside the small intestine close to the ileo-cecal junction.

*A. magna* - Similar to *A. perfoliata* but is found in the small intestine and is larger in size.

12) *Mesocestoides* - 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 - note the paruterine organ full of eggs. These proglottids are often "club" shaped and are unusual for a cyclophyllidean tapeworm in that the genital opening is medial.

B. Tetrathyridium - this larval stage will be found in the peritoneal cavity of the intermediate host. Grossly the vast numbers of this asexually multiplying stage resemble “cream of wheat”. Individually they resemble a miniature scolex of the adult worm.

**Pseudophyllidean tapeworms**

13) *Diphyllobothrium latum* - 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 - Notice the large size of this worm and the typical pseudophyllidean segments (short but wide, with the genital opening is medial).

B. Eggs - Typical pseudophyllidean eggs. They look like trematode eggs (60 X 45 μm) and may not float in most common flotation solutions using a passive flotation technique.
14) *Spirometra mansonioides* - This pseudophyllidean tapeworm utilizes a crustacean and a vertebrate (esp. frogs and water snakes) as intermediate hosts.

A. Adults - Notice the typical pseudophyllidean segments. The brown dot in the center of each proglottid is the uterus full of brown eggs.

B. Eggs - (60x35 μm) unembryonated in the feces. These eggs will not float in most common flotation solutions using a passive flotation technique.

**The Spiny Headed Worms (Acanthocephala)**

1) Typical Acanthocephalan Adult - Note the spiny proboscis at the anterior end which gives this worm its name. (See Diagram 1.)

Many different species are found in wildlife, but only one acanthocephalan is a parasite of domestic livestock (pigs).

2) *Macracanthorhynchus hirudinaceus* - “Thorny-headed worm of swine”.

A. Adults - Note the spiny proboscis at the anterior end which gives this worm its name (See diagram 1). The body of this worm is not segmented, the indentations you see are limited to the cuticle. The living worm is much flatter than this preserved specimen.

![Diagram 1. Acanthocephala](image-url)
Checklist of Objectives:

Be able to identify the following trematode eggs:

- any trematode egg  (A brown egg with an operculum is considered a trematode egg or a trematode-like egg.)
- *Fasciola hepatica*  (the operculum, the size and the host should be sufficient to identify this egg).
- *Dicrocoelium dendriticum*  (the operculum, the size and the host should be sufficient to identify this egg).
- *Paragonimus kellicotti*  (the operculum surrounded by a thick ring and the size should be sufficient to identify this egg).

Be able to identify the following cestode eggs:

- *Echinococcus* spp. and *Taenia* spp. (Small (35 - 45 um) brown eggs with striated border)
- *Dipylidium caninum*  (expressed from a proglottid, they will be in packets)
- Anoplocephalids - *Anoplocephala* spp. and *Moniezia* spp. (Triangular or square eggs)

Be able to identify the adults of the following:

- *Fasciola hepatica*  (by size, shape and location within the host).
- *Dicrocoelium dendriticum*  (by size, shape and location within the host).
- *Fascioloides magna*  (by size, shape and location within the host).
- *Paramphistomum* spp.  - (by size, shape and location within the host).
- *Anoplocephala perfoliata*  (by the size, shape and predilection site)
- an acanthocephalan  (a predilection site in the small intestine, and the presence of a anterior proboscis covered with spines coupled with the lack of suckers on the anterior end is enough to identify an adult acanthocephalan.)

Be able to identify the proglottids of:

- *Taenia* spp.  (When gently flattened they are square to rectangular.)
- *Dipylidium caninum*  (When gently flattened they pinch in at the ends - “cucumber seed” shaped.)
Diagram 2. Trematodes.
Diagram 3. Cestodes