

## Bursate Nematodes

(The Trichostrongyles, Strongyles and Hookworms)

### Introduction:

The bursate nematodes (also known as strongyle-type nematodes because of their eggs), are a group of nematodes in which the males have a copulatory bursa at their posterior end which wraps around the female during mating. All these worms live in the intestine of their hosts and have "strongyle-type" eggs (see the photo to the right). This type of egg has a thin shell, and an 8 to 16 cell morula visible inside (as they are passed in the feces). Hookworms are bursate worms in which the anterior end is bent 90 degrees, putting the mouth on the side of a worm instead of at the front. Hookworms live in the small intestine of their hosts.



### 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. In dogs and cats the presence of a strongyle-type egg in the stool indicates an infection with a hookworm. Since there are several hookworms with similar sized eggs (some of which are of public health importance), the parasitologist must examine the mouthparts of the adult worms to determine which species is present

### Checklist of Objectives:

Be able to do the following techniques:

- McMaster egg count to determine the EPG.
- Fecal Egg Count Reduction Test

Be able to identify the following:

- A typical "strongyle-type" egg (in a dog or cat this indicates an infection with either *Ancylostoma* or *Uncinaria*).

- *Nematodirus* spp. egg
- *Haemonchus* Adult (Largest abomasal nematode)
- *Ostertagia* Adult (abomasal nematode - middle in size)
- *Trichostrongylus* Adult (Smallest abomasal nematode)
- *Ollulanus* Adult (from stomach of a cat)
- *Strongylus vulgaris* Adult
- Large Strongyle Adult (generic, from horse)
- Small Strongyle Adult (generic, from horse)
- *Ancylostoma caninum* and *A. tubaeforme* (by size, location and host)

### At the Bench

- 1.) *Ancylostoma* eggs: Do a fecal float to recover *Ancylostoma caninum* ova from dog feces. (60 x 40 µm, pg. 23, Foreyt)
  
- 2.) *Ancylostoma caninum* adult: Look at student slide box slide #29. Without using the microscope get a feeling for the size of the worm. Hookworms will be the only nematodes that are this size and found in the small intestine of dogs and cats. *A. tubaeforme* is identical to *A. caninum* but is only found in cats. *Uncinaria stenocephala* is somewhat smaller than the *Ancylostoma* spp. If the specimen on slide 29 is mounted with the ventral side up, you will be able to see the buccal cavity with the characteristic “teeth” using the 10X lens of your microscope (see figure 1).

### 3) The McMaster counting slide and its use in the Fecal Egg Count Reduction Test (FECRT).

Estimate the number of strongyle type eggs per gram of feces using the McMaster Egg Counting method (see Lab 1 pg 13). Fecal samples (2 grams, pre-weighed for you) for use with this technique are provided on the benches. The fecal samples came from individual animals in a herd, both before and after anthelmintic treatment. You will be using your counts and those of your classmates in the Case Study that accompanies this lab.

**Note: You can make one flask of the diluted feces and everyone at the bench can use it to do a McMaster count, just be sure to shake it very well before filling your McMaster slide chambers.**

Enter your counts on the web page given in the Case Study by tomorrow afternoon. All counts for each sample will be averaged and the mean counts for each animal before and after treatment, will be emailed to the class by Monday.

With the advent of multi-drug resistance in many of the GI nematodes of sheep and goats (and in some areas, llamas and cattle) a fecal egg count is becoming a necessity in the management of ruminant herd health. Drug resistance can be defined by the **Fecal Egg Count Reduction Test (FECRT)**. This is just 2 fecal egg counts, one done before drug treatment and the other done post-treatment (the time post-treatment varies with drug used -

see Lab 2 appendix). A reduction of less than 85% indicates the worms are resistant to the drug used. In general this is done at the herd level to reduce the variation due to physical problems with the count and/or drug treatments that would be seen in individual animals. The value of 85% was somewhat arbitrarily chosen, but is generally accepted by the experts (although you will sometimes see higher or lower values being used, depending on why the test is being run). The percent reduction can be calculated from the following equation:

$$(1 - (\text{Count post-treatment}/\text{count pre-treatment})) \times 100 = \% \text{ reduction}$$

4) Examine the worms found in the 4 dishes on the bench. The nematodes in the dishes (separated for you by species) might be found in a sample of the abomasum's contents. You should identify the species 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 (it has a bursa) and because of its size it probably is *Haemonchus contortus*.**

The number of adult *Haemonchus contortus* in a sheep or goat can qualitatively be estimated by the use of the FAMACHA eye chart (see DEMO) which measures the level of anemia in the animal. The greater the level of anemia, the greater the number of *H. contortus* (i.e. blood loss is directly related to worm number).

Take a worm from the *Haemonchus* male and female dishes and examine them 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.)

5) Examine the stained section (Student Slide Box #30) which show the buccal capsule of a *Strongylus* sp. attached to the large intestinal wall of a horse.

## Demonstrations

### Checklist Material:

1.) Strongyle-type egg: This is the type of egg produced by most bursate nematodes. It is basically oval in shape (although it may be pointed at one or both ends in some species) with a clear thin shell. It will contain an embryo of 8 or more cells (the number of cells depends on how long it has been out of the host). The strongyle-type eggs of large animal parasites will measure 77 x 34  $\mu\text{m}$  to 100 x 50  $\mu\text{m}$  depending on the species. Hookworm eggs - The “hookworms” are bursate nematodes and thus have “strongyle”-type eggs. Hookworm eggs usually measure 60 to 70  $\mu\text{m}$  long. Hookworms are the only common strongyle-type worms found in dogs and cats in the USA.

2.) *Nematodirus* spp. egg: This egg is similar to the other strongyle-type eggs, but is much larger measuring 175 x 75  $\mu\text{m}$  (*N. battus*) to 200 x 90  $\mu\text{m}$  (*N. filicollis*) and thus easily recognizable to genus and even to species.

3.) The abomasal nematodes of ruminants: *Haemonchus*, *Ostertagia*, *Trichostrongylus*.

These nematodes are displayed together on a separate bench (see above). You should be able to identify them by their size and the fact that they were removed from the abomasum.

4.) *Ollulanus tricuspis* - this is the stomach worm of cats and pigs. This small nematode (~1 mm) is ovoviviparous (gives birth to larva rather than eggs, the eggs hatch in the uterus) and transmission is via vomiting. They feed on the stomach wall producing ulcers which leads to the vomiting, the next host then eats the vomit containing the L3. Normally no stage will pass in the feces, so a diagnosis is made by finding the adults or larvae in the vomit.

The Large Strongyles of Horses : separate them first by size - *S. vulgaris* is much smaller than the other two. Parasitologists can separate *S. edentatus* from *S. equinus* by the number of teeth in the buccal cavity, but you will not be asked to do so.

5.) *Strongylus vulgaris* - the smallest (1.5-2.5 cm) of the 3 species of *Strongylus* (the “large strongyles”) 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 dorsal, ear-shaped teeth.

6.) *S. edentatus* (2.5 to 4.5 cm) No teeth in the buccal capsule.

7.) *S. equinus* (2.5 to 5.0 cm) 3 cone-shaped teeth in the buccal capsule

**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.

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

9.) *Ancylostoma caninum* adult: These small worms (2-3 cm) are found attached to the wall of the small intestine of dogs. They are the only bursate worms you are likely to see in the intestine of dogs in the US. The adult worm can suck 0.2 ml of blood per day.

### **Other bursate nematode parasites:**

#### Ruminants

The following worms may be found in the small intestine of ruminants:

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.

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 (**see checklist demos or pg. 83, Foreyt**).

#### Swine

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

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

#### Poultry

A. *Syngamus trachea* - Occurs in the trachea of turkey, goose, fowl and various wild birds. The sexes are found in permanent copulation, hence the "Y"-shaped appearance of the worm pair. Male 2 to 6 mm Female 5 to 20 mm

Equine

Parasitologists will separate the large strongyles based on the characteristics of their buccal cavity.

- A. *Strongylus vulgaris* - (1.5-2.5 cm) Two dorsal, ear-shaped teeth.
- B. *S. edentatus* (2.5 to 4.5 cm) No teeth in the buccal capsule.
- C. *S. equinus* (2.5 to 5.0 cm) Three cone-shaped teeth in the buccal capsule

Horse large intestinal contents:

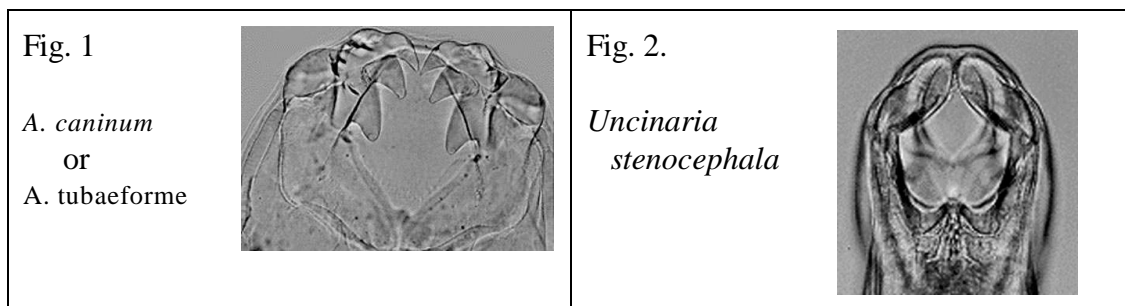
Material contained in this tray, was removed from the large intestine of an equine at post-mortem. **Notice the difference in size between the large and small strongyles.** The large strongyles can be differentiated by size and the number of “teeth” in the buccal capsule.

The following worms may be found: *Strongylus vulgaris*, *S. edentatus*, *S. equinus*, *Cyathostoma* sp. and other small strongyles, and *Anoplocephala perfoliata* (a tapeworm which will we cover in Lab 7).

**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.

**Other Hookworms:**

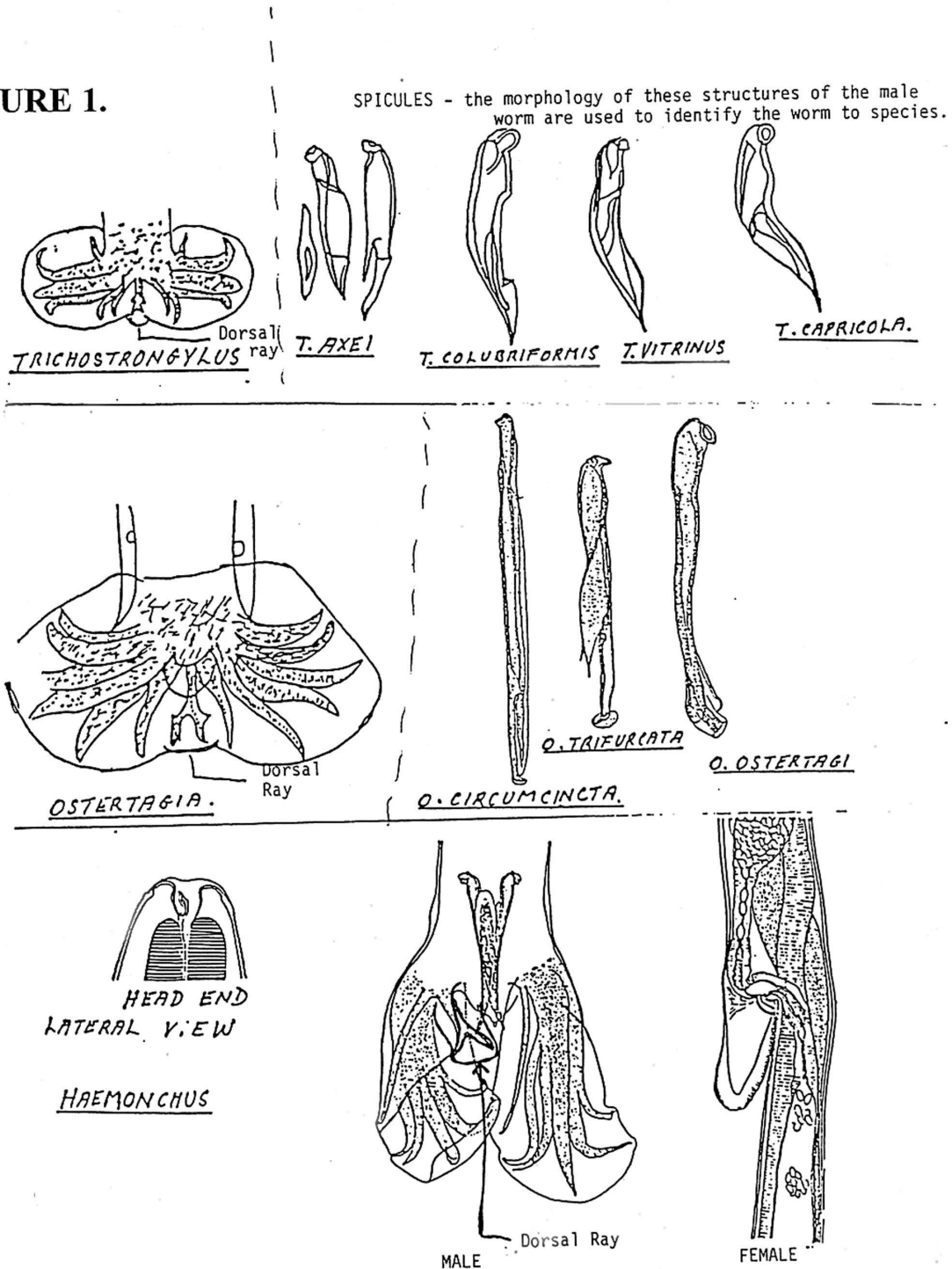
A.) *Uncinaria stenocephala* - wild and domestic canines. Common in Europe and Canada its range extends into the northern U.S. Note the **cutting plates** instead of teeth.(Fig. 2). Its eggs are larger than those of *Ancylostoma* spp. so an observant veterinarian will be able to tell that they are not looking at *Ancylostoma* eggs.



B.) *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, however it is generally found in warmer areas of the world (the Caribbean and Latin America in our hemisphere).

C.) *Bunostomum* sp. - the sheep/cattle hookworm. This large hookworm also has cutting plates in the mouth capsule of the adult worm, similar to *Uncinaria*.

**FIGURE 1.**



## Appendix for Laboratory #6

### 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 drop 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

\* A yearling calf will produce 12,000 gm of feces each day, so at a count of 100 EPG the pasture will receive 1,200,000 eggs per day. (Roberts, et al., 1951, Australian Vet Journal 27:16-18.)

For sheep the above guidelines vary by season: Spring 500 - 1000 EPG is a serious infection, while in the Fall > 2000 EPG is a serious infection.

### EGG LAYING CAPACITY OF SOME NEMATODES

<i>Haemonchus contortus</i>	5000 - 10000 eggs per day
<i>Ostertagia</i> and <i>Trichostrongylus</i> spp.	500 - 2000 eggs per day
<i>Nematodirus filicollis</i>	50 - 250 eggs per day

### SEVERITY OF INFECTION

Fatal effects seldom seen with less than:

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

**Fecal Egg Count Reduction Test** - time needed before 2<sup>nd</sup> count is run:

<u>Drug</u>	<u>Days post-treatment</u>
Levamisole, Pyrantel	7
Benzimidoles	10
Avermectins	14