Virtual Microscopy Workshop for Sheep and Goat Producers
(or, how to count parasites under the microscope!!!)

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Objective: *equip producers with knowledge and skills to make good management decisions about internal parasites including how & when to take fecal samples, how to perform a Fecal Egg Count (FEC) and how to use a microscope*.

- Using the microscope for fecal egg counts (FEC) - Workshop activities: collecting good samples (when and how); steps to prepare samples for viewing & counting eggs; using the microscope; counting eggs & calculating infection; keeping records; using records in management decisions
- Is a microscope in my farm's future? Will it pay to have a microscope?

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"This material is based upon work supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, through the Northeast Sustainable Agriculture Research and Education program under subaward number LNE14-337."

We encourage sheep and goat producers to contact Dr. Weber at jaweber@maine.edu if they have any questions.

Modified McMaster Fecal Egg Count – Standard Sensitivity

Materials:

- disposable gloves
- saturated sodium nitrate (Fecasol soln.)
- graduated beaker
- tongue depressor or weigh spatula
- balance with sensitivity of 0.1 mg
- cheesecloth (gauze 3 x 3's work well)
- disposable plastic pipet
- McMasters egg counting slide **
- paper towels
- compound microscope.

Notes:

1. A fresh fecal sample should be collected. For pooled samples, take small amounts from 10 animals, if possible. Keep sample tightly wrapped in plastic to exclude air.
2. If a larval culture is also being completed, set it up before refrigeration.

Procedure:

1. Weigh out 2g of feces in a small beaker or paper cup.
2. Add 28 ml. of sodium nitrate flotation solution (q.s. to 30 ml) to feces, mix well.
3. Strain through 1 or 2 layers of cheesecloth (or tea strainer), mix well. Squeeze out as much fluid as possible from cheesecloth ball.
4. Immediately withdraw about 1 ml of the suspension with a pipet or syringe and fill both counting chambers of the McMaster slide. Work quickly, stirring as you draw up fluid. Let stand for 1-2 minutes to allow eggs to float to top. If visible air bubbles are present, remove the fluid and refill.

Hints:

- Steps 3 and 4 should be done at same time without letting sample sit between steps
- Once chambers are filled, step 3 can be started for the next sample
- Once filled, the chambers can set for 60 min before counting without causing problems.
- Count all eggs inside of grid areas (greater than 2/3 of egg inside grid) using low power (10x) objective. Focus on the top layer, which contains the very small air bubbles (small black circles). Count both chambers. Count eggs from Trichistrongylids (oval shaped, ~ 80 microns long), Tapeworms (irregular to square, less than 70 microns), Strongyloides (oval with larvae w/I egg), ~ 50 microns long), and Nematodirus (oval, >150 microns).

Total egg count (both chambers of McMasters slide) x 50 = EPG (eggs per gram).

Each slide has two chambers, each of which holds a volume of 0.15 mL. Two chambers hold 0.3 ml of fecal mixture, which is 1/100th of the total volume of 30 ml. The number of eggs counted must be multiplied by 100 to calculate the total number of eggs in the sample. However, since you began with 2g of feces, you must multiply by 100 and then divide by 2 to yield eggs per gram.

*(** While we cannot recommend specific vendors for the McMaster's slides used for parasite egg counts, I will tell you that you can find several sources by doing a Google search on "Two-Chambered McMaster Standard Slide".)*

Proper Microscope Use

Microscopes must be properly adjusted to visualize blood cells. Following are some guidelines for cleaning, adjusting and using the microscopes in this lab. Most microscopes have a pair of 10X eyepieces and three to four objectives (4x, 10x, 40x and sometimes 100x). These objectives are mounted in a turret, and each can be rotated to line up with the eyepieces. The total magnification of an eyepiece / objective pair equals Eyepiece Magnification x Objective Magnification). For example a 10x eyepiece and a 4x objectives will produce a magnification power of 40x.

All lenses on the microscope may be cleaned using specially marked lens paper and 70% isopropyl alcohol. Never use rags or paper towels! The light source can also be cleaned (only when microscope is unplugged!) using an alcohol-dampened lens paper. Pre-packaged, disposable optical lens cleaning cloths are available in optical stores and pharmacies.

To adjust the microscope

- Turn the light source to about 75% of maximum intensity.
- Set the objective to the 10x power.
- Insert a slide onto the slide holder, then position a specimen directly under the objective. Adjust the inter-ocular distance to fit your eyes.
- Looking through the eyepieces, close your left eye, then turn the focus adjusting knob on the right eyepiece to focus the image with your right eye.
- Close your right eye, then adjust the focus on the left eyepiece until your left eye is focused.
- All microscopes have an adjustable condenser below the stage that focus the light beam on the sample to be imaged. The condenser is adjusted so that its upper surface is slightly below the microscope slide. To fine-tune it, place a Post-It note on the surface of the light source so that a corner of the paper is centered on the light. Next, move the condenser up and down while looking through the eyepieces until the shadow of the paper edge is focused.
- Below the condenser is an adjustable diaphragm. The diaphragm should be opened just enough so that the light border is not visible when looking at the specimen (note that the diaphragm opening increases as you go from high to low magnification). Check to see that the light beam is centered in your field of view. If it is not, then refer to your owner's manual to re-center it.
- Switch to the 40X objective, and refocus, using first the coarse adjustment, then the fine.
- While the 4x, 10x and 40x objectives absorb light that passes through air, the 100x objective is designed to collect light that travels from the microscope slide to the objective through an oil interface. To use the 100X objective, turn the objective turret so that the slide is half way between the 40x and 100x objectives. Place a small drop of immersion oil on the point where light passes through the microscope slide. Slowly set the 100x objective into place. BE CAREFUL NOT TO DRAG ANY OTHER OBJECTIVES THROUGH THIS POOL OF OIL!!!

When finished, remove your microscope slide and clean any oil from the 100x objective.

How to Interpret your Parasite Egg Counts

(Pooled Fecal Egg Counts from Sheep And Goats)

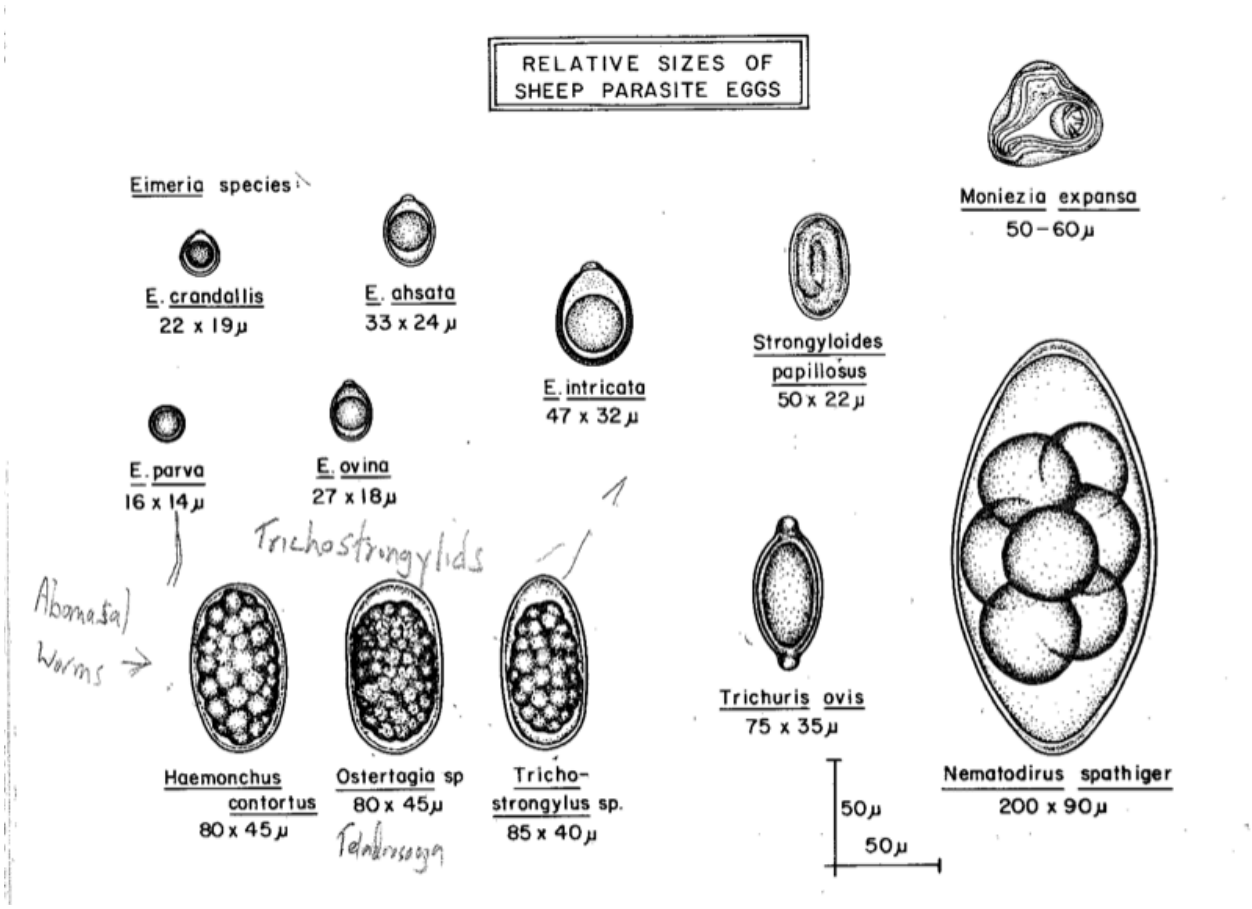
at was measured in your samples:

- Performed a "McMaster's Fecal Egg Count" to quantitate the number of eggs in the manure sample and reported as "eggs per gram" (EPG) of feces
- Provided separate EPG counts for each of the major types of internal parasites: Strongyle worms (*Haemonchus contortus, Teladorsagia spp., Trichostrongylus spp.*), *Nematodirus battus, Strongyloides spp., Trichuris ovis*, *Tapeworm (Moniezia expansa*) and Coccidia (*Eimeria spp.)*
- For samples that had strongyle egg counts greater than 500 EPG, a second assay could be performed using a fluorescent microscope to differentiate the most dangerous strongyle worm (*Haemonchus contortus*) from other strongyle eggs that are visually identical. Contact Dr. Weber at U of Maine for more information.

ır Results: The following chart may be used to record the counts (broken down by species) that were found in your pooled samples. Please e that the absence of a particular species in this single sample does not mean that it is not present on your farm. To help you interpret this ormation, we have also included ranges of EPG's that we consider worrisome, and specific characteristics of each parasite.

Parasite Species	Source of tested manure	Threshold EPG levels	Notes
Haemonchus contortus (Barber Pole Worm)		>1000 adults >400 young	Infestations are associated with anemia and often death, especially in females around the time of giving birth and in the young on summer pasture. Signs are listlessness, pale inner eyelids and bottle jaw. Very seasonal in New England, with huge numbers of infective larvae on summer pastures, especially during hot, humid seasons. To slow the development of dewormer resistance on your farm, treat animals based on FAMACHA testing (animals with scores of 4 and 5 are in danger of dying, and animals with scores of 3+ should be dewormed). The best *Haemonchus* management strategy is to **reduce pasture contamination during Spring**.
Trichostrogylus / ***Teladorsagia*** (other Abomasal Worms)		> 1000 adults >500 young	Generally causes decreased weight gain and diarrhea in all ages of sheep. Teladorsagia can cause permanent damage to the stomach, while Trichostrongylus causes scours, especially in the fall. Egg shedding is high during cool times of the year.
Strongyloides *(*Intestinal Threadworm)		>2000	Most damaging in young animals. Thrives on wet pastures during humid summers. Parasites can infest animals by burrowing into the skin above the hoof, so keep out of muddy paddocks. Ewes and does can pass Strongyloides to their offspring during pregnancy, so newborns can become infected soon after birth!
Eimeria (Coccidia)		>5000 (varies)	Causes watery "scours". Adults are generally resistant, although almost all harbor small numbers of coccidia. Most common in young animals under crowded conditions. Sanitation (clean, dry bedding, clean water, feed off the ground) works best. Diarrhea in young stock is often, but not always, caused by coccidia. Treat with coccidiostats in drinking water or with medicated feeds-follow the guidelines on the label!
Monezia (Tapeworm)		>1000	Only a problem with heavy infestations. Can stunt growth, especially in young animals. Only a few specific dewormer chemicals can kill this parasite, so discuss treatment options with your veterinarian.
Nematodirus (Thread-necked Worm)		> 1000	Most damaging in the spring. Signs are weight loss and diarrhea. Heavy infestations can hurt both adults and young. Eggs easily survive on pastures through winter in the North.
Trichuris ovis (Whipworm)		None	Not a serious parasite, although it is fairly common in sheep and goats, and is often found in mixed infestations.

Data from our 2014 survey suggested that **stocking animals at a low density** per acre of pasture, **use of rotational grazing,** and **selective deworming strategies** based on **FAMACHA** and / or **fecal egg counts** were the most effective tools for keeping the egg counts of *Haemonchus* and other parasites at safe levels.



Line drawings of the ova of typical ovine parasites showing shape and relative size. (from Foreyt, William J. Veterinary Parasitology: Reference Manual (5th Edition; 2002). ISBN # 9781118682265. Wiley Press).