

LABORATORY

1

INTRODUCTION TO THE PARASITOLOGY LABORATORY

Introduction

This lab is an introduction to some techniques used by veterinarians to detect eggs, cysts, and larvae of parasites in the feces of animals. The proper use of the microscope is vital to these techniques .

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.

At the Bench

A. Use of the microscope for fecal exams:

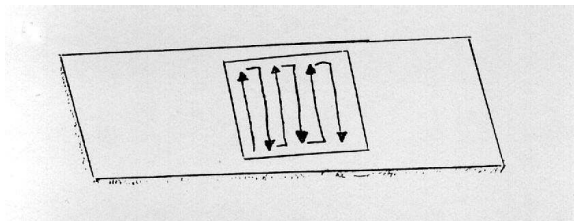
The following tips will help you adjust to using your microscope for the examination of fecal samples for parasites.

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 closed down 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 3 techniques, making use of the samples of dog feces at your place. (These fecal samples contain eggs of a nematode parasite.) **Record your results (# of eggs per coverslip) on the DATA SHEET (pg 6) and enter your data into the web site by 1 PM tomorrow.**

1. **Passive Saturated salt flotation** - There are numerous devices for doing this type of flotation now in use in local veterinary hospitals. see instructions on Techniques Pgs. 2, and 3 .
2. **Zinc Sulfate Centrifugal Flotation Technique** - see instructions on Techniques Pg. 4 and 5.
3. **Direct Wet Mount** - see instructions on Techniques Pg. 1.

The methods for examining feces covered in this laboratory are also covered in Foreyt's "Veterinary Parasitology Reference Manual" pp. 1 - 10, and in Zajac and Conboy (2006) "Veterinary Clinical Parasitology, 7th Ed." pp. 3 - 24 (as well as in earlier editions).

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. When collecting horse or ruminant feces that might be in transit for a while before reaching the lab, collect it in a “zip lock” plastic bag and carefully remove as much air as possible before sealing it. This will keep it anaerobic and prevent the eggs in it from developing and possibly hatching.
- 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. (Do not fix samples intended for use with the Baermann technique.)
 - III. Horse and ruminant fecal sample collected in a zip lock bag as described in “1b” can remain at room temperature for up to 5 days.
- 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 consistency, frank or digested 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 page 5. Step-by-step directions for performing the various methods can be found in the Techniques section at the back of this book.

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

a. Clinical signs suggest parasitism, but initial fecal exam was negative. (The infection may be pre-patent or just patent with low numbers of the diagnostic stage. One fecal exam will find about 72% of infections, while 3 fecal exams will find > 95% of infections.) Repeat in 2 or 3 days. Repeat for a total of 3 times within 7 to 10 days; if no parasites are seen after 3 examinations it is likely the animal is not infected.

b. Following specific therapy of a parasitic infection, have owner submit a fecal specimen 1 - 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.)

Demonstrations

Checklist material

Parasites from various groups of invertebrates are shown in the demonstrations. Be sure you are able to put an unknown parasite into one of the following: Protozoa, Nematoda, Trematoda, Cestoda, Acanthocephala, Insecta, Arachnidia.

Checklist of Objectives:

- Be able to run a passive fecal flotation.
- Be able to run a centrifugational fecal flotation.
- Be able to make a “wet mount” slide.
- Be able to examine a slide made from any of the above techniques.
- Be able explain what the various fecal examination techniques are best suited for and their problems (Table Pg 5).
- Be able to explain how a specimen is collected and processed for parasitology, as well as the timing of repeat fecal exams.
- Have an appreciation of the general characteristics of the various phyla to which parasites belong (i.e. be able to identify an organism as a protozoan, fluke, tapeworm, nematode or an arthropod).
- Enter the data you collected into the proper area on the parasitology Course Materials (Learn) web site.

COMPARISON OF FECAL EXAMINATION TECHNIQUES

Technique	Best Used For:	Problems
Zinc Sulfate Centrifugal Flotation	Good choice for standard fecal examinations. Only technique for <i>Giardia</i> cysts and best technique for <i>Trichuris</i> eggs. Will, in most cases, recover nematode larvae.	Trematode, Pseudophyllidean tapeworm and <i>Physaloptera</i> 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 crenated to identify. Saturated sugar solutions tend to leave a sticky mess.
Saturated Sugar Centrifugal Flotation	Considered the Gold Standard for fecal examinations as the higher specific gravity of this solution will float more eggs. However, <i>Giardia</i> cysts will crenate and thus require special attention to identify.	
Passive flotation: Saturated sucrose, saturated salt (sodium chloride or sodium nitrate), or zinc sulfate	Standard technique used in many veterinary clinics. Will miss most <i>Giardia</i> cases and many of the mild whipworm cases.	All the problems mentioned above, plus: Nematode larvae and <i>Giardia</i> 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 Wet Mount	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.

LAB 1 DATA SHEET

This sheet is for your records and should remain in your lab manual. Enter your data into the web site (address will be given in lab) by tomorrow.

- 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 DOG FECAL SAMPLE

	Egg Counts	Time needed to do procedure
Passive Float (Saturated Salt) (in commercial device)	_____	_____
ZnSO₄ Centrifugal flotation	_____	_____
Direct Wet Mount	_____	_____