Detection of Parasitic Infections by Fecal Examination

The commonly occuring intestinal parasitic infections of cats, dogs, and other animals including people are efficiently detected by microscopic examination of fecal samples. In our laboratory at the University of Tennessee College of Veterinary Medicine parasitic infections in companion animals and domestic livestock are detected by fecal examination. The use of these methods is determined by (1) the parasitic species we hope to identify in the sample and (2) the host species from which the fecal sample originated. In this document we provide a discussion and review of our fecal examination methods. It is our hope that this information will encourage wide-spread adoption of the best practices for detecting parasitic infections in client-owned animals.

Fecal Direct Smear

This technique is used to detect motile parasite stages such as protozoan trophozoites and helminth larvae frequently passed in the semi-formed and loose to fluid feces of animals. The larvae of *Strongyloides stercoralis* are usually associated with diarrhea in young puppies and have zoonotic potential. The trophozoite stages of *Giardia* and several Trichomonad species can be found in the loose stools of many different host species. *Giardia* is generally considered to be a zoonotic parasite and play a causative role in diarrheal disease, however, the clinical significance of the Trichomonad parasites is quite variable depending on it pathogenicity for particular host species. Flotation concentration procedures have the potential to distort the delicate structures of these organisms and obliterate their diagnostic attributes so that accurate identification is impossible. Trophozoites and larvae may be immobilized by dropwise application of dilute Lugol's iodine solution (10.5%) to facilitate microscopic examination of their diagnostic morphology.

Fecal Flotation Methods

Fecal flotation methods levitate the diagnostic products of endoparasitic organisms (eggs, larvae, oocysts, and cysts) in the feces of animals by use of suspension medium with a higher specific gravity than the parasite products. Parasite eggs, cysts, and oocysts are concentrated on the surface of the medium because of their lighter density. The result is a clean preparation for microscopic examination with a minimal amount of distracting fecal debris.

Although 3 different flotation media widely used in veterinary medical practice, Sheather's Sucrose and Zinc Sulfate are the flotation media of choice in our laboratory because of their superior ability to concentrate particular parasite products for diagnostic analysis.

Table 1. Flotation Solutions used in Veterinary Parasitology at the University of Tennessee College of Veterinary Medicine

Flotation Solution	Ingredients and concentration	Specific Gravity
Sheather's Sucrose	454 grams sugar, dissolved in 355 ml water. 6 ml formalin to prevent mold growth	1.275
Zinc Sulfate	350 grams granular zinc sulfate, dissolved in 1000 ml water	1.18
Sodium Nitrate	378 grams granular sodium nitrate, dissolved in 1000 ml water.	1.20

Sheather's Sucrose (sp gravity 1.275) is used preferentially to identify potential infections with *Toxocara* sp, *Ancylostoma* sp., *Trichuris* sp., *Capillaria* sp. and *Isospora* sp. in fecal samples from dogs and cats. In a study conducted in our

laboratory, Sheather's Sucrose was 5 times more efficient for detecting infections with *Trichuris* compared to Zinc Sulfate (sp gravity 1.18).

Zinc Sulfate solution (sp gravity 1.18) is used preferentially to identify potential infections with parasitic protozoan species like *Giardia lamblia* and for the recovery of delicate larval stages of lungworm parasites like *Oslerus* and *Filaroides* in dogs, and *Aleurostrongylus* in cats. Studies in our laboratory demonstrated that Zinc Sulfate as a flotation medium for detecting *Giardia* infections is nearly 2 times more reliable than Sheather's Sucrose.

The successful recovery of the different parasite products seen in veterinary practice depends on (1) the density of the parasite eggs/oocysts/cysts, (2) the density and viscosity of the flotation medium, and (3) the interaction of these factors that affect the time it takes for the parasite egg to float to the surface. In some cases the parasite structure (*Giardia)* is too delicate and light to float in a dense medium like Sheather's Sucrose without distortion and collapse because of the severe osmotic pressure. In other cases, if the density of the parasite egg (*Trichuris vulpis*) too closely matches the density of the flotation solution (Zinc Sulfate) it will not effectively levitate (separate) the eggs from the fecal debris. It is for these reasons that the choice of a flotation solution must be matched with the characteristics of particular parasite species for optimal results.

Table 2. Specific Gravity of Parasite Structures Recovered by Flotation Methods

Parasite Species	Specific Gravity
Ancylostoma sp.	1.06
Toxocara sp.	1.09-1.10
Trichuris vulpis	1.15
Taenia sp.	1.22
Isospora sp./*Toxoplasma gondii*	1.11
Giardia sp.	1.05

Fecal Flotation with centrifugation: Yes or No?

Centrifugation has been advocated as an important aid to flotation recovery of diagnostic parasite structures for microscopic examination. The Companion Animal Parasite Council recommends centrifugal flotation as a "Best Practice" for processing all fecal samples in veterinary medical practice. Despite the scientific evidence supporting its use, reluctance to employ the centrifugal flotation technique in routine parasitologic examinations persists.

"Centrifugal fecal flotation requires more tech time than is necessary to achieve the same results" is often cited as reason against its use. Properly applied, fecal samples processed by "passive flotation" with Sheather's Sucrose solution should stand for a minimum of 15 minutes before making the wet mount for microscopic examination. With passive flotation parasite eggs gradually rise to the surface of the flotation medium and separation from the heavier fecal debris is achieved by gravity. In our laboratory, the flotation suspended fecal sample is spun in the centrifuge for 5 minutes to separate the parasite eggs. The heavier fecal debris is forced to the bottom of the centrifuge tube much quicker than by the use of simple gravity. With the centrifuge, the lighter (less dense) parasite eggs are driven to the top of the flotation solution meniscus and the elimination of the heavier (more dense) fecal debris from the surface provides a cleaner preparation for microscopic examination of the because there is less material to obscure the view of the parasite eggs. Centrifugation is especially necessary when the flotation procedure is performed using a viscous solution like Sheather's Sucrose. The downward force created by the centrifugal spinning enhances the buoyancy of the eggs in the viscous solution and

drives them to the surface meniscus where they are concentrated and result in greater parasite recovery. The advantage of using centrifugal flotation is particularly important for detection of canine whipworm infections where the greater density of *Trichuris vulpis* eggs makes them very difficult to recover even under optimal conditions.

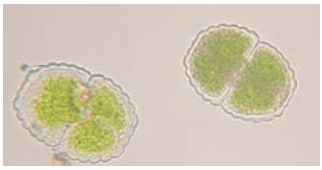
"What about the SNAP® test for detection of *Giardia* sp infections in dogs and cats. Isn't it just as good as a fecal examination?"

The SNAPP® *Giardia* test kit manufactured by Idexx Laboratories is a membrane format ELISA (*Enzyme-linked Immunosorbant Assay)* that detects *Giardia* infections in dogs and cats by developing a blue dot on an antibody coated membrane if the fecal sample contains the *Giardia*-specific antigen secreted by the protozoan parasite living in the animal patient's small intestine. According to the product insert, the sensitivity and specificity of the SNAP test was 95% and 97% when compared to similar immunoassay tests produced by other manufacturers. The ability of the SNAP test to identify *Giardia* in a canine population of known infection status was comparable to the Zinc Sulfate flotation method performed by a trained technician. However, because of the inherent difficulty of identifying *Giardia* from patient fecal samples examined during routine veterinary practice, the SNAP test combined with Zinc Sulfate fecal flotation methods is a useful adjunct for testing animals with a high suspicion of *Giardia* infection.

Even in cases when *Giardia* is suspected for animals with diarrhea, weight loss, flatulence, etc., a fecal examination is still a good idea because it may indicate infection with other parasites that could be playing a role in the disease picture. In our laboratory, we recommend that fecal samples from *Giardia* suspect animals be examined on 3 consecutive days by Zinc Sulfate flotation with a SNAP test on animals that test negative for cysts in their feces. This protocol is satisfactory for identifying most of the cases when *Giardia* is involved in the production of clinical disease and providing clients and practitioners with high degree of assurance for ruling out *Giardia* as an etiology when the fecal test results are negative.

"Size Matters !" Why it's a good idea to have an ocular micrometer available when conducting fecal examinations.

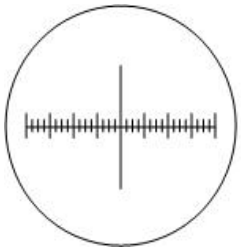
The superior ability of the fecal examination methods we have described makes it possible to concentrate and recover a tremendous variety of microscopic structures from the fecal samples of animals seen in veterinary practice. Parasite eggs, coccida oocysts, protozoan cysts, pollen and mold spores, yeast and mega bacteria, fleas, mites, their eggs, wings, and other anatomical parts have similar density and show up consistently in all fecal samples processed by the fecal flotation methods. The ability of veterinarians, and veterinary technicians to correctly differentiate the diagnostic products of parasites infecting their patients, and the other microscopic stuff "just passing through" the bowel is greatly enhanced by using an ocular micrometer to measure and compare the parasite eggs, oocysts, and other things found on the fecal exam with the descriptions found in textbooks, tables, charts, internet web pages, and other informational resources.



Plant cells (45µm) from fecal flotation @ UTCVM Parasitology Service Laboratory

Accurate measurement is especially important for correctly differentiating oocysts of *Toxoplasma gondii* from those of *Isospora rivolta* in fecal samples from cats. *T. gondii* oocysts are only 10μm in diameter (about ½ the size of *I. rivolta)* and zoonotic to humans.

The ocular micrometer is a glass disk with a ruled scale that is fitted into the microscope eyepiece and used to measure the size of magnified objects. The actual units of the micrometer are arbitrary so calibration to a measurement scale (usually microns) is necessary. This is a one-time task that is easily performed by a technician when the microscope receives its periodic servicing and maintenance. Ocular micrometers are relatively inexpensive and can be purchased online from optical supply sources.



Best Practice Recommendations from the
Diagnostic Parasitology Service Laboratory
University of Tennessee College of Veterinary Medicine

1. Fecal examinations should be conducted on all animals during each veterinary medical appointment to identify parasitic infections that may affect the health of the animal patient and its owners.
2. The Fecal Direct Smear is indicated for any animal presenting with diarrhea or soft-mushy stools. Technicians should be observant for *Giardia* trophozoites and *Strongyloides stercoralis* larvae when they occur in the loose fecal samples from canine patients, especially young puppies. These parasites are capable of zoonotic transmission in households and require additional recommendations from the Veterinarian.
3. All animals, regardless of age, that present with a history of diarrhea, soft stools, flatulence, and weight loss should be suspected of infection with *Giarida*. A fecal flotation with Zinc Sulfate (specific gravity 1.18) is indicated for these patients. If the fecal sample is negative at the initial examination, we recommend assay of the sample with the SNAP® *Giardia* (Idexx Laboratories). Additional samples can be collected on 3 consecutive days and examined by a combination of Zinc Sulfate flotation and SNAP assay if infection is still suspected and other diagnostic efforts for viral and bacterial etiologies are also negative.
4. A fecal flotation with Sheather's Sucrose (specific gravity 1.27) should be performed on all animals, regardless of age, as part of every routine veterinary health visit. This examination is especially important for revealing sub-clinical parasitic infections that may indicate lapses in prophylactic measures such as monthly deworming with heartworm-intestinal helminth combination products, or persistent environmental exposure in fecally contaminated yards, neighborhoods, and designated pet recreation parks.