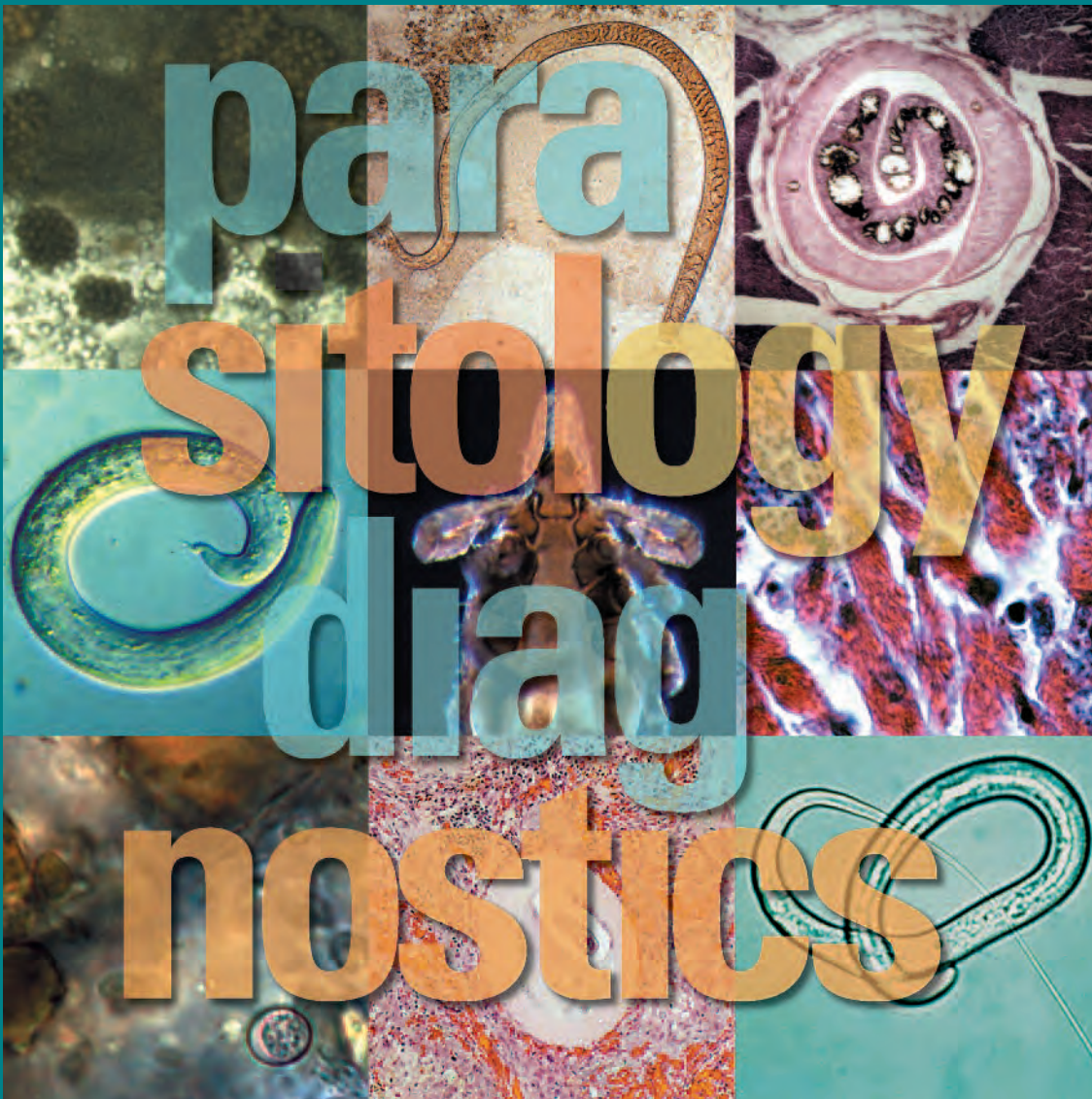


Parasitology: Diagnostics in Dogs and Cats

Dwight D. Bowman, MS, PhD
Elizabeth A. Fogarty, BA

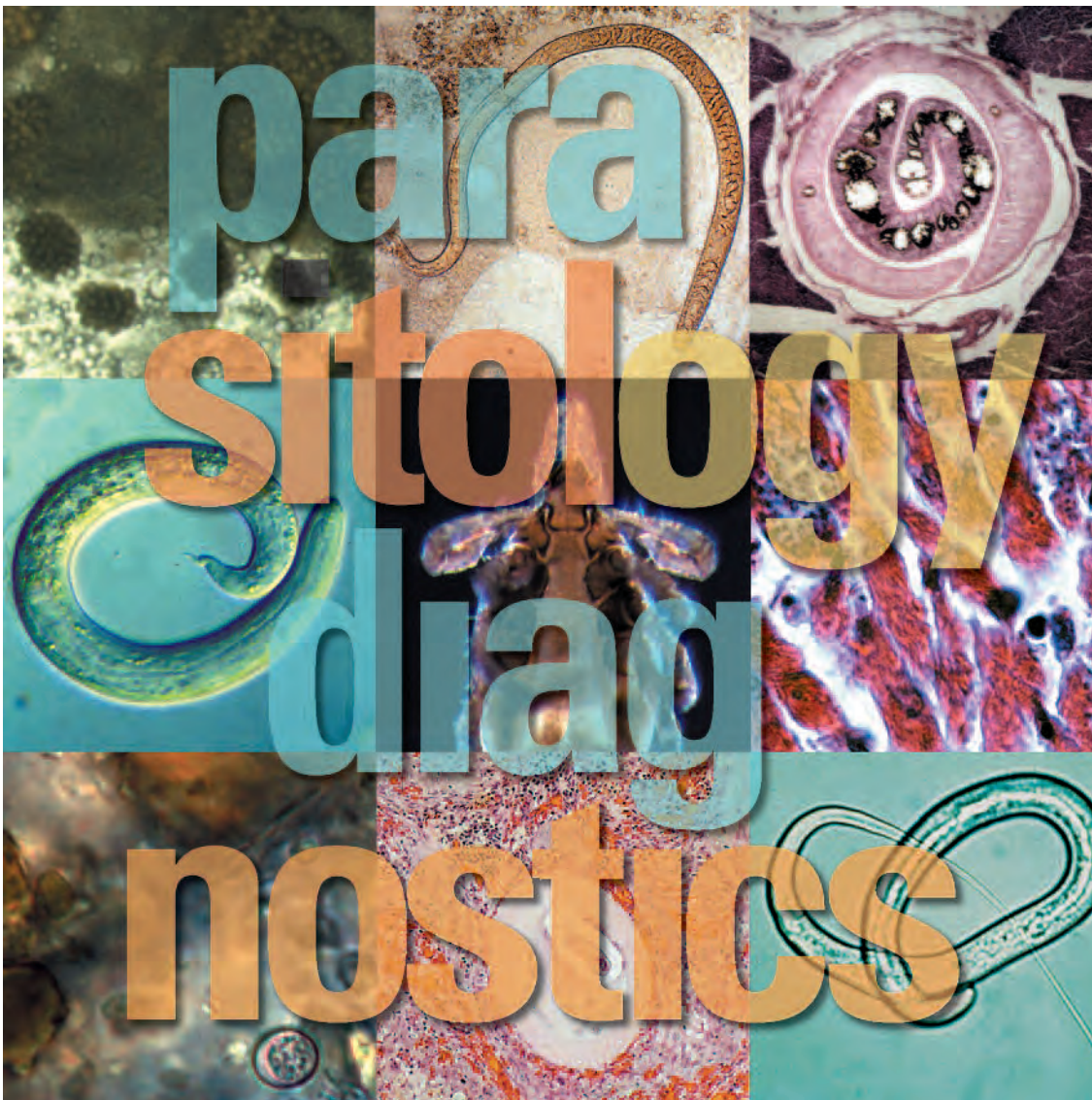


Nestlé PURINA

Clinical Handbook Series

Parasitology: Diagnostics in Dogs and Cats

Dwight D. Bowman, MS, PhD
Elizabeth A. Fogarty, BA



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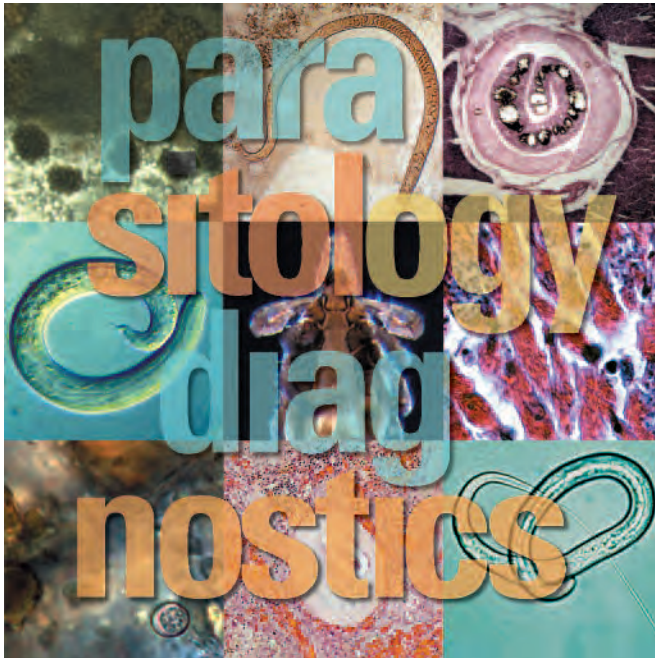
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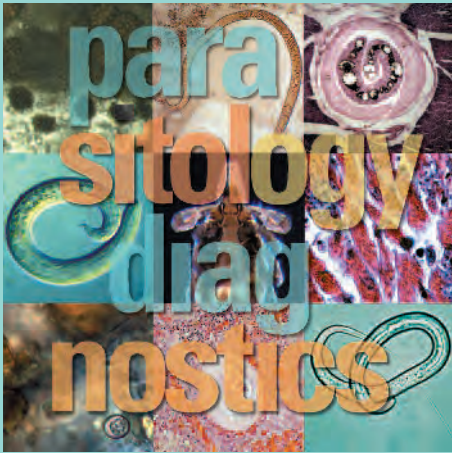
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Veterinarians in clinical practice routinely examine fecal, urine, blood, and tissue specimens for parasites to diagnose parasite infections in dogs and cats. No one single method is available, however, that is appropriate for every parasite. For example, while a direct fecal smear may be the best method for detecting the trophozoites of certain flagellates, it often is not sensitive enough to detect the rare helminth egg that may be present. A stained specimen of urine sediment is probably not the best means of detecting the eggs of different helminths that could be present in the urinary system because there is a good chance that the eggs will not adhere to the slide during staining. Similarly, the staining of slides of tracheal wash material may not be the best means of determining whether lungworm larvae or *Paragonimus* eggs are present in the sample, whereas direct examination of the sediment would be more likely to give positive results. When specimens are preserved, the buoyant density of the eggs will often change, and thus, the same test that works well on fresh feces may be inappropriate for preserved fecal material.

A successful parasitologic diagnosis is of great benefit because it typically means that effective treatment can be implemented. Products are commercially available for the treatment of most parasitic infections, and parasitology thus is one field in which cures are usually straightforward and simple. There are exceptions, such as infections with *Cytauxzoon*, *Cryptosporidium*, or larval *Mesocestoides*, but, for the most part, parasitic infections can be dispatched to nonexistence relatively easily. The correct diagnosis allows the timely administration of a product that will cure the pet of its infection.

There are two types of parasitologic examinations: those performed as part of a routine physical examination and those performed because a specific agent or type of agent is suspected. When fecal analysis is performed as part of a routine physical examination, a technique is chosen that will produce consistent and reliable results for the most common parasites. Special techniques are utilized when there is a need to look for underlying causes of an unexpected condition or to verify a clinical suspicion based on a clinical examination or other diagnostic methods such as radiographs. Specialized methods, such as blood cultures and cultures of skin or bone marrow biopsies for flagellate infections, are sometimes performed by specialty laboratories, and such testing will not be discussed in detail herein because it is assumed that most such samples will be submitted after consultation with the laboratory performing the test.

Parasitology: Diagnostics in Dogs and Cats is divided into three parts. **Part I** covers basic information on the various methods available to clinicians and their laboratories for the examination of fecal, urine, blood and tissue specimens for parasites. The step-by-step procedures, including equipment and reagents needed, are outlined for each of the techniques typically performed in veterinary practice. A general discussion is also included of the more specialized methods that usually are performed by centralized diagnostic laboratories, as

noted above. In **Part II**, case studies are presented, demonstrating the use of the techniques described in Part I in actual clinical cases. Each case study describes the patient's signalment, history, physical examination, initial assessment and presumptive diagnosis, and goes on to discuss the diagnostic plan and outcome. Reference material is provided in **Part III**, including a glossary of terms used in parasitology, suggested reading for further information, and figure and subject indexes.

This handbook is designed to introduce the various methods that are and can be used in the clinical laboratory. It is not meant to be an exhaustive compilation of all possible techniques. The techniques that are presented are described in sufficient detail to allow them to be performed in most laboratories. The usefulness of many of the techniques is illustrated with a series of case histories that present situations in which the different methodologies could assist in a diagnosis. Some of the techniques presented will not necessarily be the preferred method by all laboratories or all teachers of parasitology. Much of the disagreement among parasitologists about the different techniques actually seems to come down to a difference in personal preference and the routine parasites that a parasitologist or laboratory technician might be looking for in a certain geographic area. This handbook also is not an illustrated guide to all parasites that might appear in the feces, blood, urine, or tissues of cats and dogs. Readers are referred to other sources for these details (see Suggested Reading in Part III).



Part I

Chapter 1: Fecal Examination

OVERVIEW

Fecal examinations can be performed for different purposes in veterinary practice. If the fecal examination is part of a pet's routine health check, appropriate tests for this purpose include:

- ❑ The direct fecal smear
- ❑ The zinc sulfate flotation
- ❑ Stationary flotation methods
- ❑ Centrifugal sugar flotation

Other methods, including sedimentation assays and the Baermann apparatus, may be used when the fecal examination is performed to diagnose a suspected parasite infection of a certain type.

Different assays work better in different situations, regardless of how well the test might perform for the diagnosis of an infection. Some laboratories prefer stationary methods for routine examinations. One practitioner, a friend of the authors, sets up a stationary fecal assay at the beginning of the pet's clinical examination using either a fecal specimen supplied by the owner or one collected during the examination. Set-up takes only a few seconds, and when the examination is completed, in about 10 or 15 minutes, he is ready to examine the specimen microscopically in front of the owner. This practitioner feels that this method engages the owner with the treatment that is recommended for the pet. He knows his parasitology well and acknowledges that he might possibly miss some parasites as part of his diagnosis: however, he feels that the routine helminths and coccidia that might be present will be detected and does not worry about less-common parasites that might be overlooked in an otherwise-healthy dog or cat. Other practitioners do not plan on having the results of the examination completed at the time of the pet's visit; rather, they use callbacks to inform the client as to the results of the test. In these cases, other analytical methods may be used or the technical staff may use several different assays.

Fresh Versus Fixed Samples

In veterinary medicine, fresh fecal samples, rather than fixed samples, usually are processed for routine examina-

tion. In contrast, in human medicine, the danger of infection of laboratory personnel with agents present in the samples has led to most samples being fixed prior to submission. Infectious agents, such as hepatitis A, and other pathogens such as the human amoeba, *Entamoeba histolytica*, usually are not present in canine and feline samples; thus, in veterinary medicine, samples are still processed without being fixed initially. One advantage of examination of fresh preparations is that living organisms can often be visualized through their movement. The disadvantage is that without fixation, some protozoa may decompose if the sample is not examined soon after it is received. The eggs and cysts in human fecal samples that are fixed in formalin do not have the same buoyancy characteristics as they do when they are not fixed in formalin. Thus, sedimentation methods are used much more commonly in human medicine laboratories than in veterinary laboratories. Sedimentation methods, as described in texts on human parasitology and later in this chapter, are useful methods but they tend to require more time in the examination of the specimen. Thus, flotation methods are often preferred for routine diagnostics using fresh feces.

Specialized Tests

Methods are now available that detect antigens of different parasites in feces. The two parasites most commonly detected with this method are *Giardia* and *Cryptosporidium*. These tests were developed for human infections and are not yet being directly marketed for canine and feline fecal specimens. Several animal diagnostic laboratories routinely use these tests, however, and the tests seem to provide adequate results based on in-house verification of their comparison to more standard diagnostic methods. These tests are relatively expensive, and often require the purchase of sufficient material to run many samples. For these reasons, these tests are often performed in centralized diagnostic laboratories. Similarly, the methods that label pathogens with fluorescent antibodies also tend to be used only by centralized laboratories with fluorescence microscopes. Fecal antigen tests are discussed further at the end of this chapter.

DIRECT FECAL SMEAR

The quickest method for detecting a parasite infection is the direct saline fecal smear. The term *smear* is a bit of a misnomer because there is actually no smearing of the feces. Instead, a small quantity of feces, about enough to fit on the end of a wooden applicator stick, is dabbed lightly about in a drop of saline on a microscope slide (*Figure 1.1*). With dog and cat feces, there is a good chance that there will be large bits of grain from the dried food the pet has been fed; these bits can be dragged out of the way using the end of the applicator. Dog and cat feces often absorb the moisture from the drop on the slide, so it may be necessary to add a bit more saline. Applying the drop to the applicator stick and allowing it to run onto the slide with the feces is one of the best ways to do this.

There is usually no reason to make these preparations using water. The point of the direct smear is to visualize living trophozoites, and these stages will often be lysed if the preparation is made with water rather than saline. Of course, if it is the only medium available, water can allow the clinician to take a quick look for high concentrations of eggs or cysts that may be detectable in water samples.

Once the material has been dabbed about on the slide until there is a homogeneous preparation with large chunks removed, a coverslip can be placed on the preparation (*Figure 1.2*). The slide should first be scanned with a 10× or 20× objective (with training, all the parasites of dogs and cats can be visualized with a 10× objective). Objects of interest can then be examined more closely using the high-dry (40×) objective. The use of an oil-immersion lens on wet mounts usually is unsatisfactory because the preparation is too thick or because the material under the slide moves from the pressure of the lens on the coverslip's surface. Oil-immersion observation is a possibility, but only if the slide is first sealed with fingernail polish or melted paraffin. In human parasitology, the preparations are often stained with iodine, which tends to make the nuclei in the trophozoites easier to visualize and which stains glycogen granules in some of the cysts and trophozoites. In veterinary medicine, most of the species of amebae and other protozoan parasites that are of importance in human medicine are usually not observed, so the use of iodine is typically neither needed nor helpful.

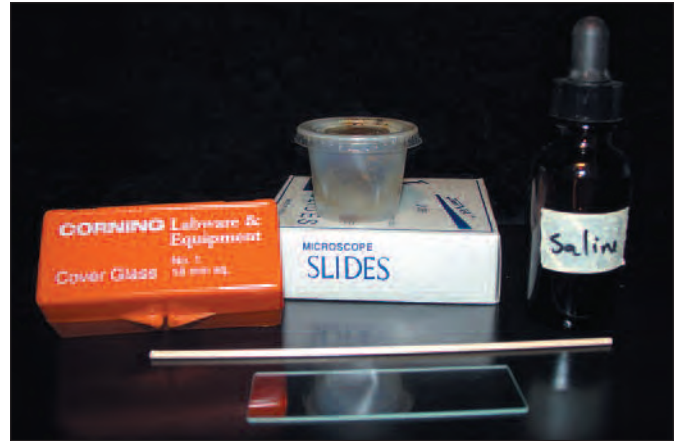


Figure 1.1. The materials needed for a direct smear include a 22 × 22-mm (or 18 × 18-mm) cover glass, microscope slide, applicator stick, feces, and saline. It is always best to make the preparation in saline, rather than water, so that living trophozoites will remain viable and motile.

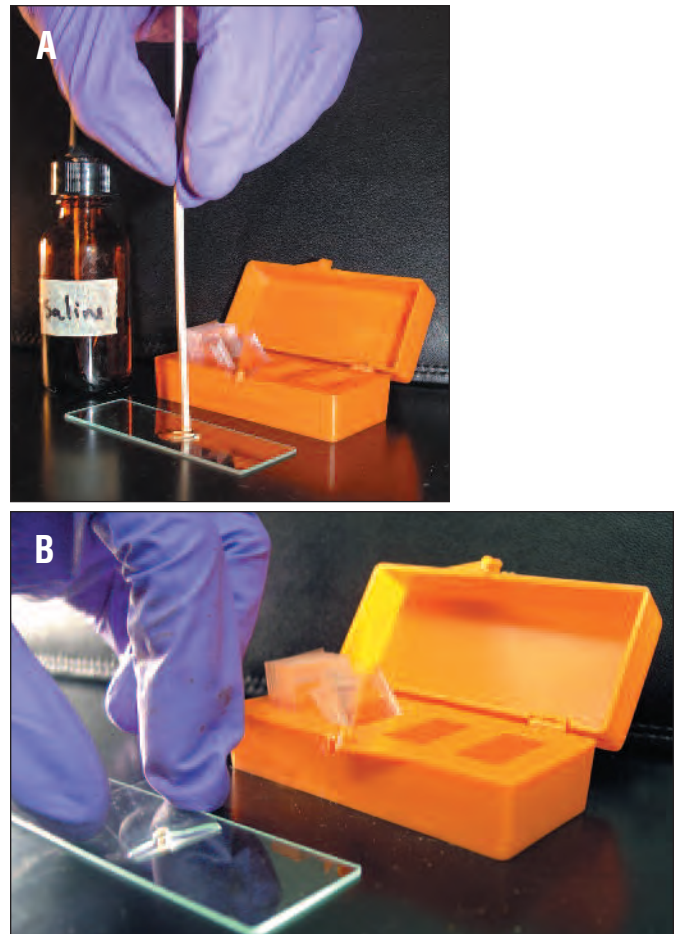


Figure 1.2. When preparing the direct smear, it is important to put a small amount of saline on the slide and then to add a small quantity of feces, about the size of the end of the applicator stick, about 2 mm². The feces is then mixed with the saline and daubed about until a relatively clear mixture is formed (A). If there are particulates present, especially common when animals are fed dried foods, the particulates can be pushed to one side of the drop carefully with the edge of the coverslip before it is lowered onto the preparation (B).

ZINC SULFATE FLOTATION

One of the best means of observing the cysts of *Giardia* and various eggs of parasites is with the zinc sulfate flotation. This technique has the advantage of being fast and relatively easy to perform. The current availability of premade zinc sulfate solution makes the task less onerous because one does not have to deal with the preparation of this reagent. An inexpensive alternative to zinc sulfate is magnesium sulfate (Epsom salts). The only disadvantages to Epsom salts are that the solution is a little more viscous and it tends to crystallize a little more rapidly during observation (Figure 1.3).

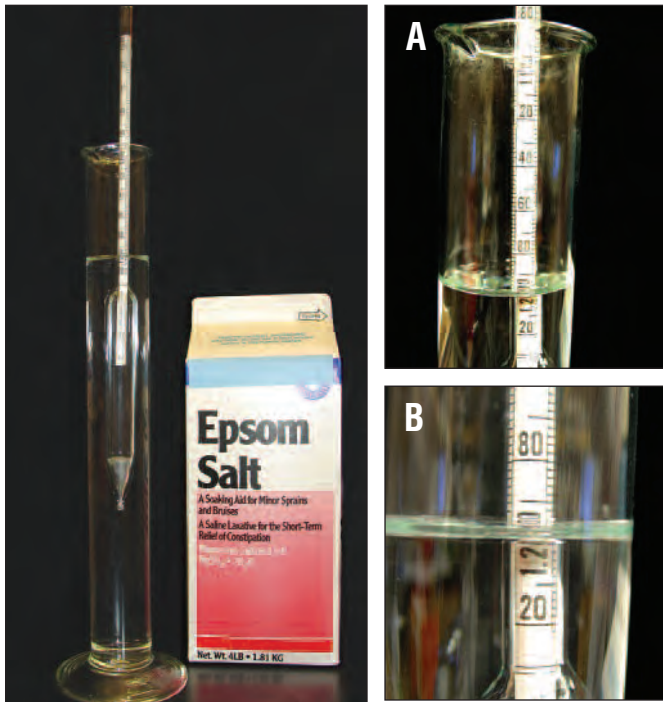


Figure 1.3. Whether using zinc or magnesium sulfate, or sodium nitrate, it is always best to check the specific gravity with a hydrometer. Sodium nitrate can be purchased in a dry form in a premeasured quantity for stationary flotations. Zinc sulfate can be purchased as a liquid with a specific gravity of 1.18. Magnesium sulfate is probably the least expensive of the flotation solutions, but some Epsom salts (although food grade and approved for use as a laxative) can be slightly discolored or contain a few particulates when in solutions of specific gravity 1.2; the choice of another brand will often resolve these problems. (A) This hydrometer has a scale from 1.000 (the specific gravity of water) to 1.220. (B) In this image, the meniscus is at 1.200, between the numbers 80 (1.180) and 20 (1.220). This hydrometer would be inappropriate for checking a heavy sugar solution.

When performed properly, the zinc sulfate centrifugation can be performed rapidly. Using a centrifuge capable of holding six tubes at a time (Figure 1.4), it is easy for one person to process and examine 60 or so samples an hour with this method. The secret to rapid analysis is to not use a coverslip during the observation phase of the examina-



Figure 1.4. When picking up the sample from the top of the centrifuge tube, it should be carefully pushed straight down through the top of the liquid and then pulled straight up. Occasionally the material on the top of the tube may be so thick that it is more like mud than a meniscus. In these cases, some of the material can be transferred from the side of the loop to the slide.

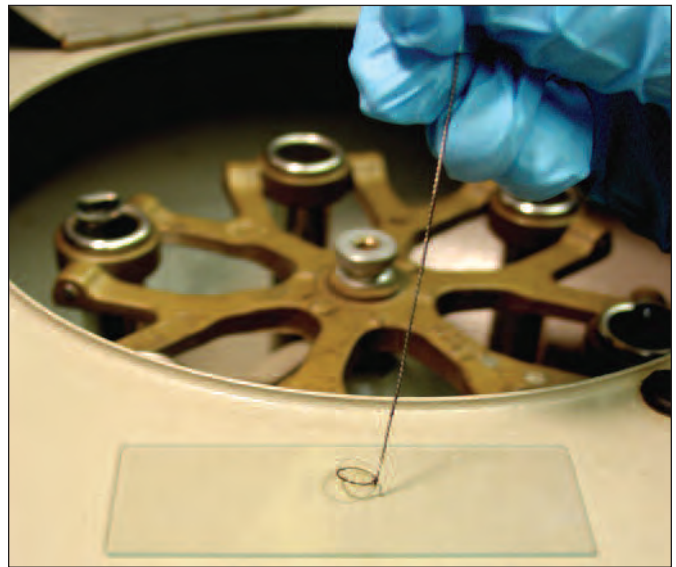


Figure 1.5. When the loop is transferred to the microscope slide, it can be examined as the simple small loopful or under a coverslip. Looking at one or two loopfuls has the advantage that the specimen is not spread out under a larger area as happens with a coverslip. The disadvantage of examining a loopful is that it may dry out if the examiner takes too long during the observation.

tion. Once one has gone to the trouble of concentrating the cysts and eggs into a small 5-mm circle, why spread it out under a 22 × 22-mm coverslip? Performing the test without a coverslip requires that the samples be examined rapidly, before they dry out and the crystals formed as the zinc sulfate goes out of solution make observation of the samples impossible (Figure 1.5). Thus, one can only prepare the number of samples that one is capable of reading at one sit-

ting. With six samples, three loopfuls can be placed on each slide, so only two slides are required for each six samples. If more time is required, a loop of water or a drop of saline can be placed on the slide before the loop from the tube is added. If a coverslip is ultimately needed, a drop of water can be added to the material on the slide, and then a coverslip applied. The loop will work best if it is flamed before each used (*Figure 1.6*). The flaming ensures that there is no carry over between samples. As the loop is flamed, there can be a buildup of zinc sulfate and fecal material on the loop so that it will become crusted. The loop can be cleaned with a few dips in water, scraping with an applicator stick, and further flaming. It is important that the loop be made with flame-resistant wire, and also that it be thin enough to work well. A typical microbiology loop for agar plates has wire that is too thick, and the opening in the loop is too small.

The cysts of *Giardia* will float on the surface of the zinc sulfate. Often, when present at the edges of the material on the slide, they will appear rather pink due to the spherical aberration caused by the curvature of the drop on the slide. Eggs will also be obvious on the slide. As the slide dries, the cysts will collapse. One drawback of the zinc sulfate method is that it does not detect heavier eggs. Thus, the



Figure 1.6. The loop used to pick up a specimen from the top of a tube is finer than the typical microbiology loop with a somewhat larger diameter. It is important that the loop be made from wire that is resistant to flame, or it will melt when it is flamed. The flaming seems to help the loop collect the sample from the meniscus of the centrifuged tube. Occasionally the loop will accumulate a crust of dried material from the feces and flaming that can be carefully broken off the wire using the end of an applicator stick.

ZINC SULFATE FLOTATION

MATERIALS

- ❑ Centrifuge: general bench-top type with horizontal, swinging-bucket rotor, and shields for 13 × 100-mm tubes
- ❑ Compound binocular microscope: should have at least 10× and 40× objectives with 10× eyepieces
- ❑ Paper cups, unwaxed, or equivalent 3- to 4-oz. cup
- ❑ Two-ply cheesecloth
- ❑ Hydrometer: specific gravity range of 1.000 to 1.300
- ❑ Plastic wash bottles
- ❑ Round-bottom centrifuge tubes, glass, 13 × 100 mm
- ❑ Wooden applicator sticks
- ❑ Microscope slides: glass, 3 × 1 inches
- ❑ Bunsen burner, alcohol lamp, or cigarette lighter
- ❑ Wire loop, about 28-gauge, 4- to 5-mm loop
- ❑ Vortex mixer
- ❑ Tap water

REAGENTS

- ❑ Zinc sulfate solution, specific gravity 1.18 (about 33 g of crystals in 100 mL water). Check specific gravity with hydrometer and adjust if needed. Zinc sulfate can be purchased already made at a specific gravity of 1.18

PROCEDURE

1. Comminute about 1 g of fecal specimen in 5 mL of water in a paper cup. This can be achieved by manually mixing with two applicator sticks. If the applicators are held by the thumb and forefinger, a flicking action of the second and third fingers on the sticks produces an effective mixing action.
2. Filter fecal suspension through two layers of gauze into a second paper cup, washing with a small volume of water.
3. Pour filtrate into test tube.
4. Spin for 1 minute at 800g.
5. Decant supernatant.

6. Add about 3 mL of zinc sulfate solution and resuspended using applicator sticks and vortex mixer.
7. Fill tube to within 1 cm of rim and centrifuge again at about 800g for 1 minute.
8. Without removing the tube from the centrifuge and using a freshly flamed wire loop, remove 1 or 2 loops from the center of the surface film and place on a slide bearing the specimen number.
9. Examine with a compound microscope using 100× and 400× magnifications.



eggs of taeniid tapeworms and *Diphyllobothrium* and *Spirometra*, most trematodes, and some nematodes, eg, *Trichuris vulpis*, are likely not to be observed in the sample with this method.

STATIONARY FLOTATION METHODS

Stationary flotation is a means by which parasites' eggs and cysts can be floated to the surface of a liquid medium. The process is simple to perform and can be set up using either in-house materials or with several commercially available kits, such as the Fecalizer® (EVSCO Pharmaceuticals) or Ovassay® (Synbiotics Corporation). These methods all work on the same principle. The eggs are heavier than water, but much of the material in the fecal matter is heavier than the eggs. Thus, the feces is mixed with a solution that has a buoyant density that is greater than the eggs but less than the other material in the feces. Trial and error has shown that a specific gravity of 1.2 is about the correct density to cause good separation between the eggs and debris in the fecal sample. A solution of material that weighs 1.2 times that of an equal volume of water has a specific gravity of 1.2; thus, 10 mL of water

weighs 10 grams and 10 mL of a solution with a specific gravity of 1.2 weighs 12 grams.

Common reagents that are used in these preparations include table salt (NaCl), zinc sulfate, magnesium sulfate, and sodium nitrate. The first routinely used medium for this procedure was saturated table salt (brine), which has a specific gravity of about 1.2. The separation is not usually as good as when centrifugation is used, but it is usually adequate to get a fairly clean sample. The sodium nitrate solution has the disadvantage that it seems to crystallize on the slide slightly faster than the other solutions. The magnesium sulfate solution has a viscosity that is slightly higher than that of the other three solutions, and thus it may be that the eggs float to the surface slower in this material than in the other three solutions.

The methods in the various prepared tests (eg, Fecalizer and Ovassay) work on the same principle. They differ from the method described in that the sample holder also functions as a sieve. In the Fecalizer, the tube in which the flotation occurs also serves as a sample collection device in which the bottom of the insert provides a means of measuring the amount of feces that is introduced into the tube.

STATIONARY FLOTATION

MATERIALS

- ❑ Compound binocular microscope: should have at least 10× and 40× objectives with 10× eyepieces
- ❑ Paper cups, unwaxed, or equivalent 3- to 4-oz. cup
- ❑ Two-ply cheesecloth
- ❑ Hydrometer: specific gravity range of 1.000 to 1.300
- ❑ Plastic wash bottle
- ❑ Round-bottom centrifuge tubes, glass, 16 × 100 mm
- ❑ Test tube rack
- ❑ Wooden applicator sticks
- ❑ Microscope slides
- ❑ Coverslips, glass, 18 × 18 mm
- ❑ Vortex mixer

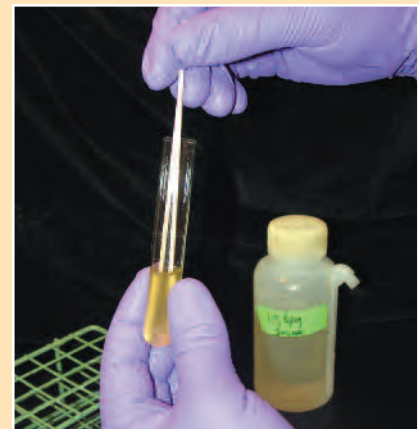
REAGENTS

- ❑ Flotation solution: Saturated sodium chloride (brine); zinc sulfate, specific gravity 1.18; magnesium sulfate, specific gravity 1.2; or sodium nitrate, specific gravity 1.2

PROCEDURE

1. Comminute about 1 g of fecal specimen in 5 mL of flotation solution in a paper cup. This can be achieved by manually mixing with two applicator sticks. If the applicators are held by the thumb and forefinger, a flicking action of the second and third fingers on the sticks produces an effective mixing action.
2. Filter fecal suspension through two layers of gauze into a second paper cup, washing with a small volume of flotation solution.
3. Pour filtrate into test tube, place tube in rack.

4. Fill tube with flotation solution until a slight meniscus of liquid is budding over surface.
5. Place a coverslip on top of the tube.
6. Let tube with coverslip stand for 10 to 15 minutes, then remove coverslip and place on microscope slide.
7. Examine with a compound microscope using 100× and 400× magnifications.



Fecalyzer

The Fecalyzer is a commonly used flotation device in many veterinary practices (*Figure 1.7*). The green insert has a portion at the bottom that the client can insert into the pet's feces to collect a premeasured quantity of feces. The insert can then be placed in the white snap-top vial and brought to the clinic for examination. The flotation solution, typically sodium nitrate solution at a specific gravity of 1.2, is added to the white chamber, the green insert is reinserted and mixed by rotation with the flotation solution. The insert is then filled with the solution so that a meniscus appears at the top, and a 22 × 22-mm coverslip is placed on the surface of the meniscus. After approximately 5 to 10 minutes, the coverslip is removed and placed on a glass slide for examination. The green insert contains a screened portion that prevents larger particles from floating to the surface and interfering with the microscopic examination of the specimen.



Figure 1.7. The Fecalyzer is a stationary flotation device that has been developed for the collection and processing of fecal samples. The internal portion can be used to collect a portion of feces of appropriate size by inserting the end into the feces. The sample can then be placed into the outer container and brought into the clinic. When it is time to examine the sample, flotation medium can be added and the sample mixed by twisting the insert inside the holder. The insert is then filled with flotation solution so that a positive meniscus is formed. A sieve in the insert prevents large particles from floating to the surface. A 22 × 22-mm coverslip is then placed on the top of the tube, and it is allowed to sit for 5 to 10 minutes. The coverslip is then removed and placed on a microscope slide to determine what parasites have floated to the surface.



Figure 1.8. The Ovassay is a fecal collection and processing device used to perform a stationary fecal flotation. The internal device can be used to collect a sample of appropriate size by stabbing it into the feces. The insert can then be placed into the holder and brought to the clinic. When it is time to process the sample, flotation medium can be added to the tube, and the feces is mixed with the medium by twirling the insert in the holder. The insert is then filled with flotation medium until a positive meniscus is formed. A 22 × 22-mm coverslip is then placed on the meniscus, and the device is allowed to stand for 5 to 10 minutes. The coverslip is then removed and transferred to a microscope slide to identify those parasite stages that have floated to the surface.

Ovassay

The Ovassay Plus Kit is very similar to the Fecalyzer in design and concept (*Figure 1.8*). Again, feces is added to a central insert and mixed with flotation solution, typically zinc sulfate. The device filter is seated in the device, and a positive meniscus is created by the addition of more flotation solution. A microscope coverslip is added to the top of the device, and then the material is allowed to float for approximately 10 minutes. Again, the central insert has a screen that prevents larger particles from floating up against the coverslip and interfering with microscopic observation.

As with the Fecalyzer, the stationary flotation has the distinct advantage that it does not require a centrifuge for

processing. The disadvantage is that the method does not allow for maximal recovery of parasite eggs and cysts from the fecal sample. For routine diagnoses, however, both assays are useful and can be helpful in most clinic situations.

CENTRIFUGAL SUGAR FLOTATION

Veterinary parasitologists for the most part prefer a centrifugal flotation method over stationary methods. Different workers prefer different flotation media, but one method that is used commonly is the centrifugal sugar flotation method. This method has the advantage that it will float heavier eggs than the methods using the various salt solutions (eg, magnesium sulfate, zinc sulfate, sodium chloride, or sodium nitrate.) The sugar solution can work with stationary flotation, but often eggs and cysts will be distorted by the osmotic pressure caused by the sugar, and the high

viscosity of the solution requires that the eggs remain in the solution for a longer time before they work their way to the surface of the stationary tube. This drawback has been overcome by the use of centrifugation. The sugar solution has the disadvantage that it can attract flies and cockroaches. When working at outdoor sites, the flies can become quite frustrating as they land and feed on the edges of the coverslips on the final sample. Cockroaches are often present in laboratories where they feed on the spillage or drops of leftover samples or on material left in centrifuge tubes or in centrifuges.

For most species of parasite eggs, centrifugal sugar flotation is a very easy to use and highly successful method. However, eggs of trematodes, such as those of *Paragonimus* and *Alaria*, will often collapse or open and be cleared of their internal content, which can sometimes make them more difficult to recognize.

This method is excellent for the demonstration of oocysts of *Cryptosporidium* species. With the microscopes typically used in most practices, the interaction between the optical properties of the solution and the oocysts causes the oocysts to appear as small pink to red dots floating on the surface of the sugar just under the coverslip. When research-grade microscopes are used, however, the color of the oocysts is often not observed because the corrections for spherical aberration in the objective lenses will often correct the property that causes the oocysts to appear pinkish.

Another advantage of the sugar flotation procedure is that the slides can be examined for some period of time after they are prepared. If the slides are kept from drying out (by placing them on a damp paper towel in a petri dish) and they are kept refrigerated, many of the eggs can be observed quite successfully for a few days. If the slides are placed in a slide folder and kept cold, they can even be shipped on ice packs for assistance in diagnosis, often with no problems in the viewing of the contained parasites. The oocysts of *Cryptosporidium* tend to collapse after a half hour or so and disappear; the *Giardia* cysts that are present will often appear collapsed and require skill to identify even in fresh slide preparations; and in samples that have set awhile the eggs of hookworms may embryonate, which makes them rather clear and more difficult to observe. The hard-shelled eggs and coccidial oocysts, however, typically can be seen quite well for several days.

The solution used for the flotation is a saturated sugar solution that has a specific gravity of approximately 1.3.

The flotation solution is made by dissolving cane sugar into water to the point of saturation (*Figure 1.9*). Often, the process can be hurried by producing the mixture using heat, where the water is warmed prior to the addition of the sugar or during the process. A friend typically makes the sugar solution on a double boiler where the sugar is added to the heated water. If heat is used, the specific gravity needs to be verified after the solution has been allowed to cool. If the solution is heated during production, a large amount of crystals may form as the solution cools. These can be left in the stock bottle. After the solution is made, it is necessary to add a preservative to keep mold from growing in the sugar water. Two different preservatives are used most often, formalin and phenol (carboxylic acid), typically adding either about 5 mL of 37% formaldehyde or 5 mL of liquefied phenol to each liter. The sugar solution can also



Figure 1.9. When preparing sugar for flotation, typically about 900 g (2 lbs) is needed for every 700 mL of water (need about 1300 g/L). It will be necessary to slowly add the sugar to the water and dissolve it by stirring. Often the water can be warmed to help in the dissolution of the sugar, but it should be cooled before the specific gravity is checked with a hydrometer. Some find it very useful to prepare the sugar solution in a double boiler just like when making candy. It is a good idea when the product has cooled to add a small quantity of formalin (10 mL/L) to prevent mold from growing in the medium.

be preserved by autoclaving and freezing, but autoclaving can cause caramelization of the sugar and may darken its

appearance. The centrifugal sugar flotation procedure is described in the figures below (*Figures 1.10–1.13*).

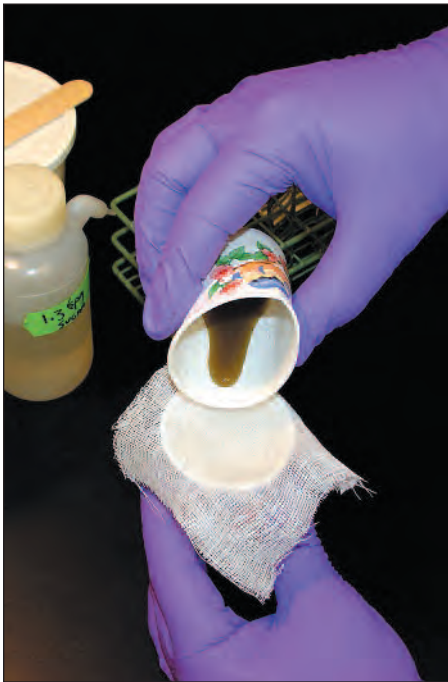


Figure 1.10. Mix the feces in a small cup and then pour it through some gauze to remove the particulates before spinning it down and mixing it with sugar. Some practitioners skip the sieving stage, but if skipped, the particulates in the final sample can make it very hard to examine under the microscope.



Figure 1.12. After the centrifuge comes to a complete stop, the coverslip can be lifted directly from the top of the tube.

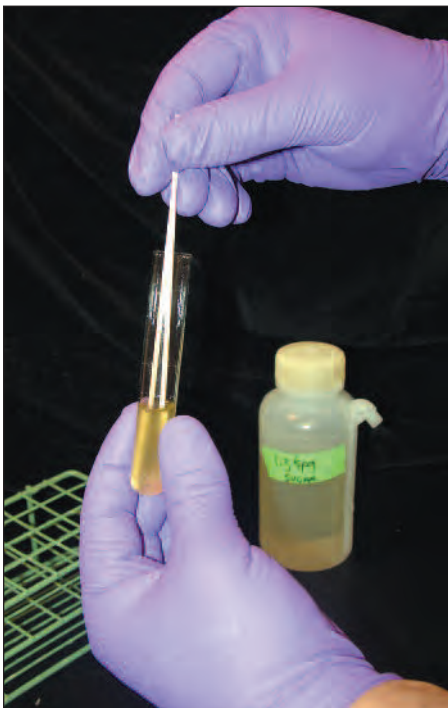


Figure 1.11. After the water is decanted from the centrifuged sieved sediment, sugar solution (about 3 to 4 mL) should be added and the sediment suspended in the sugar solution. It is important that the sample be well mixed, and this is easiest to perform using two applicator sticks. A vortex mixer can be used if sticks are inserted in the samples, but a large number of air bubbles can be created if care is not used.

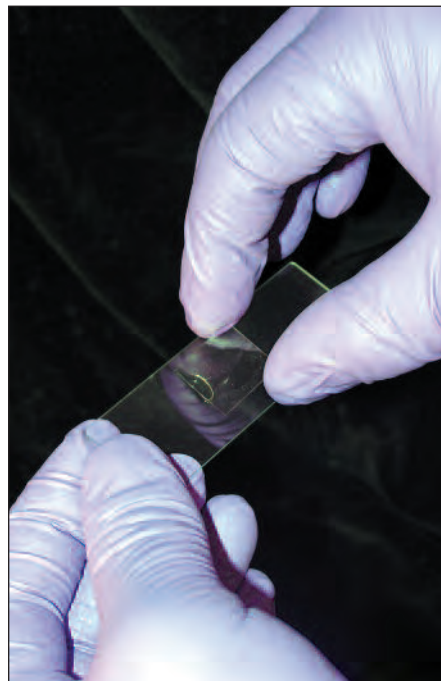


Figure 1.13. The coverslip should be carefully placed on a slide to capture as few air bubbles as possible.

CENTRIFUGAL SUGAR FLOTATION

MATERIALS

- ❑ Centrifuge: general bench-top type with horizontal rotor and shields for 16 × 100-mm tubes
- ❑ Compound binocular microscope: should have at least 10× and 40× objectives with 10× eyepieces
- ❑ Paper cups, unwaxed, or equivalent 3- to 4-oz. cup
- ❑ Two-ply cheesecloth
- ❑ Hydrometer: specific gravity range of 1.000 to 1.300
- ❑ Plastic wash bottles
- ❑ Round-bottom, centrifuge tube, glass, 16 × 100 mm
- ❑ Wooden applicator sticks
- ❑ Microscope slides
- ❑ Cover glasses: 18 × 18 mm
- ❑ Marking pen
- ❑ Tap water
- ❑ Magnetic stirrer

REAGENTS

- ❑ Sugar flotation solution: slowly add approximately 1000 g of pure, granulated sugar to 1000 mL of distilled water in a 4 L beaker on a magnetic stirrer. Check the specific gravity (it should be 1.300; this is basically a saturated solution). Adjust if needed by adding more water or more sugar.

PROCEDURE

1. Using applicator sticks (two usually work better than one) put about 1 g of feces into paper cup.
2. Add approximately 8 mL of tap water and disperse the feces in the water vigorously with the applicator sticks. Feces may not readily break apart; if so, let sample sit for a short period of time to allow softening.
3. Sieve the fecal slurry by pouring through cheesecloth into a second paper cup.
4. Pour the sieved slurry from the second cup into centrifuge test tube.
5. Centrifuge at 800g for 1 minute.
6. Decant supernatant.
7. Add sugar flotation solution to approximately 1/4 full and resuspend the pellet vigorously with two applicator sticks.
8. After placing tube in centrifuge, slightly overfill the tube with sugar solution so there is a slight bulging (meniscus) of the surface film above the rim of the tube.
9. Add a coverslip to the tube's surface.
10. Load and balance the centrifuge (be sure to balance with another sugar filled tube). Centrifuge at 800g for 10 minutes.
11. Remove the coverslip by lifting straight up so that a drop adheres to it. Drop coverslip onto a slide, and examine using microscope.



SEDIMENTATION ASSAYS

Veterinary medicine makes very little use of the different sedimentation assays for the following reasons. The more common parasites are typically detected using the flotation methods; there is usually no need to fix canine and feline specimens (as discussed earlier, this changes the permeability of the eggs and cysts, and hence their buoyant densities); and many of the trematodes and amebae in human stools are not present in samples from dogs and cats. Thus, the benefits of sedimentation do not usually outweigh its detriments, which include the larger amount of material that often must be examined, the difficulty in seeing the various protozoa when they are spread out in this larger sample without the aid of spherical aberration, and the amount of extra steps required in processing. The samples do have the advantage that they can be preserved so that part or all of the sample can be observed at a later date, but

in most veterinary practices, clinicians simply do not have the time to come back later and re-examine slides.

The most basic sedimentation procedure is just the simple sieving of the fecal material in water or saline followed by sedimentation in a tube without centrifugation. A large percentage of the bacteria will stay in solution and the heavier eggs will sink to the bottom of the tube. The trouble is that there is usually a large amount of material still to examine. However, the procedure is better than nothing and costs basically nothing to set up. Many of the eggs are very heavy compared with water, and thus they will often settle out in a relatively short period of time.

A procedure that has caught on in human medicine is the formalin/ethyl acetate sedimentation procedure (*Figure 1.14*). This method is also useful in veterinary medicine because it is capable of finding almost anything that might be in a fecal sample. The problem again is that there is often a great deal of material to examine. The process

cleans up samples that have a lot of fiber or a lot of fat in them, but this unfortunately is not that common in samples from dogs and cats. The acid/ethyl acetate sedimentation is a sister technique to the formalin/ethyl acetate sedimentation. For this technique, fresh feces is suspended in an acid solution, typically dilute hydrochloric acid (made at a ratio of 40 parts concentrated HCl to 60 parts water). The remainder of the technique is the same as for the formalin/ethyl acetate sedimentation method. The acid/ethyl acetate method tends to provide a cleaner sample than that made with formalin, but it does not work for protozoan cysts. However, some practitioners prefer to use the acid/ethyl acetate method for fecal specimens collected from wild life.

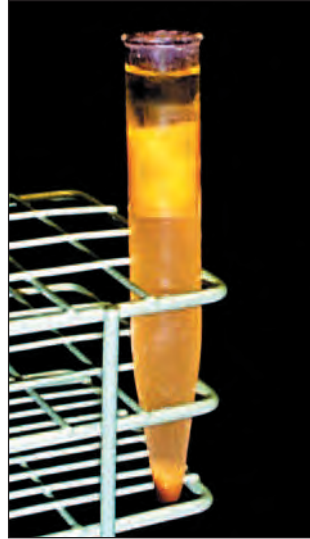


Figure 1.14. When the ethyl acetate is mixed with the fecal water, formalin, or acid mixture and then centrifuged, a layer will form between the organic solvent and the aqueous phase. It is necessary to dislodge this plug with an applicator stick before the tube is decanted. Care should be taken to carefully dislodge the material from the walls of the tube so that it will not fall back into the pellet when it is decanted. If the pellet is in acid alcohol, it is probably desirable to resuspend it in water before it is placed on a slide; otherwise it tends not to form a puddle but instead rapidly spreads to the edges of the slide and beyond.

ACID / ETHYL ACETATE SEDIMENTATION

MATERIALS

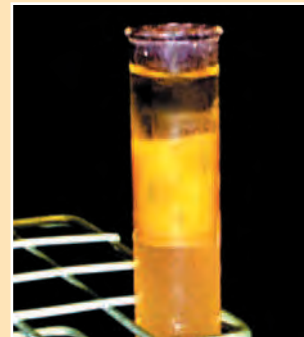
- ❑ Centrifuge: general bench-top type with horizontal rotor and shields for 16 × 100-mm tubes
- ❑ Compound binocular microscope: should have at least 10× and 40× objectives with 10× eyepieces
- ❑ Paper cups, unwaxed, or equivalent 3- to 4-oz. cup
- ❑ Two-ply cheesecloth
- ❑ Hydrometer: specific gravity range of 1.000 to 1.300
- ❑ Plastic wash bottles
- ❑ Conical, centrifuge tube, glass, 15 mL
- ❑ Rubber stopper
- ❑ Wooden applicator sticks
- ❑ Microscope slides
- ❑ Cover glasses: 18 × 18 mm

REAGENTS

- ❑ Ethyl acetate
- ❑ Dilute HCl: 40 mL concentrated HCl in 60 mL water

PROCEDURE

1. Using applicator sticks (two usually work better than one) put about 1 g of feces into paper cup.
2. Add approximately 8 mL of tap water and disperse the feces in the water vigorously with the applicator sticks. Feces may not readily break apart; if so, let sample sit for a short period to allow softening.
3. Sieve the fecal slurry by pouring through cheesecloth into a second paper cup.
4. Pour the sieved slurry from the second cup into centrifuge test tube.
5. Centrifuge at 800g for 1 minute.
6. Decant supernatant.
7. Add about 8 mL of acid solution mixing with applicator stick.
8. Add about 5 mL of ethyl acetate, stopper, and shake vigorously holding stopper in place.
9. Remove stopper, load and balance the centrifuge (be sure to balance with another tube of similar weight). Centrifuge at 800g for 2 minutes.
10. There will be a plug of material at the site of the acid/ethyl acetate interface. Ring along glass wall with applicator stick, and decant ethyl acetate, plug, and acid solution.
11. Examine sediment for the presence of eggs.



BAERMANN APPARATUS

There are occasions in veterinary medicine when nematode infections are suspected that produce larvae rather than eggs that are passed in the feces. In dogs, this occurs in infections with *Crenosoma vulpis*, *Filaroides birthingi*, *Filaroides osleri*, and *Strongyloides stercoralis*. In cats, *Aelurostrongylus abstrusus* and *Strongyloides felis* (found only in the South Pacific) are about the only infections that result in the shedding of larvae in the feces. There may be the rare addition to this list, but these represent the majority of cases in which the stage that is passed in the feces is a larva. *F. birthingi* and *F. osleri* have larvae that tend not to move much; if either of these are the suspected agent of observed lung signs, there is a good chance that the use of a Baermann apparatus will add nothing to the diagnosis. For these two larvae, the best means of finding the larvae is with a zinc sulfate flotation.

For the other species, the Baermann apparatus (Figure 1.15) will increase the opportunity of finding the larvae that may be relatively sparsely distributed throughout a fecal sample. Once the larvae are found, they must be differentiated, but that is actually a relatively easy task. Also, one must be careful about the age of the feces used to set up the apparatus because hookworm eggs are capable of hatching and confusing the diagnosis. For the most part, however, the Baermann method is a useful technique for verifying an infection with any of these species of nematodes.



Figure 1.15. This device consists of a funnel attached to a piece of rubber tubing with a clamp. Fecal material is placed in a piece of gauze or a tea strainer and is suspended in water in the funnel. Larvae migrate out of the feces and into the water. With time, the larvae will settle to the bottom of the tube and can be harvested either by placing a drop out of the tip onto a slide or by spinning the contents down in a centrifuge tube and examining the sediment.

BAERMANN APPARATUS

MATERIALS

- ❑ Compound binocular microscope: should have at least 10× and 40× objectives with 10× eyepieces
- ❑ Funnel: 5 or 6 inches in diameter
- ❑ Centrifuge: general bench-top type with horizontal rotor and shields for 16 × 100-mm tubes
- ❑ Ring stand and funnel support
- ❑ Tea strainer or equivalent support screen
- ❑ Piece of thin-walled tubing, 3 or 4 inches long
- ❑ Eye-dropper without the rubber bulb: inserted in one end of the thin-walled tubing; the other end is attached to the funnel
- ❑ Tubing clamp

- ❑ Conical 15 mL centrifuge tube
- ❑ Tongue depressors
- ❑ Cheesecloth
- ❑ Microscope slides
- ❑ Cover glasses

REAGENTS

- ❑ Water

PROCEDURE

1. Using the tongue depressor, break apart 5 to 15 g of feces and place it in the tea strainer (It may be easier to spread the material on cheesecloth and place this in the strainer, or if no strainer is available, a bag made of the cheesecloth can be suspended by a string to the top of the funnel.)

2. Fill the funnel with lukewarm tap water; jiggle the tube and clamp to remove any trapped air bubbles.
3. If a large number of active larvae, such as is often the case with *Aelurostrongylus*, are present, a few drops can be examined after an hour or so, but it may be necessary to allow the apparatus to sit overnight.
4. Fill the centrifuge tube from the bottom by opening the clamp (REMEMBER, *Strongyloides* third-stage larvae can penetrate the skin!)
5. Centrifuge and examine under the microscope.

FECAL ANTIGEN TESTS

Tests are now available for detecting the antigens of *Cryptosporidium* and *Giardia* when they are present in fecal samples. These tests have the advantage that they remove the need for the reliance on microscopy. A major drawback, however, is that they currently are relatively expensive to perform. Thus, when running a positive and a negative control, a single test can be very expensive. However, there is every reason to think that these types of tests will be more and more commonly performed for the diagnosis of these relatively hard to find pathogens.

The current tests are run in the typical fashion of an enzyme-linked immunosorbent assay (ELISA) plate or as solid-phase immunoassays. It is much more likely that veterinarians will perform the solid-phase assays (Figure 1.16) because they are designed for use with single patients.



Figure 1.16. This is an example of a solid-phase immunoassay designed to be used with a single sample (ProSpect® Giardia/Cryptosporidium Rapid Format). The test is similar to those developed for detecting various antigens and antibodies in blood or serum samples. The diluted feces is applied to the membrane, and then after a series of steps, a colorimetric change will occur in the membrane with spots that will darken if the antigen is present in the feces. Such tests have been developed for detecting the antigens of *Giardia* and *Cryptosporidium*. Photo compliments of REMEL Inc.

Diagnostic laboratories are much more likely to utilize the ELISA plates (Figure 1.17) because they are more likely to batch process samples and to have many samples to run at one time.

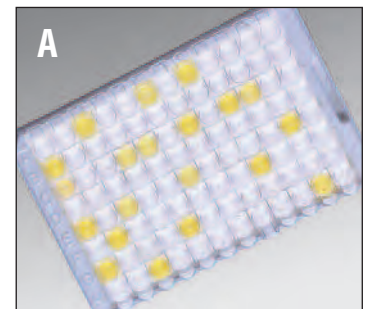
Examples of these assays are the various ProSpect® assays (Remel) for *Giardia* and *Cryptosporidium*.

FECAL CULTURE METHODS

The only fecal culture method for protozoans likely to be performed by most veterinary practices is the recently introduced InPouch™ culture system for trichomonads (BioMed Diagnostics)(Figure 1.18). This system has small plastic pouches containing media that can be inoculated with fecal swabs and the protozoans are grown at room temperature. The pouch has proven to be very successful in the isolation



Figure 1.17. This is an ELISA that has been used routinely to detect antigens of *Cryptosporidium* in fecal material (ProSpect® Cryptosporidium Microplate Assay; a similar assay is available for *Giardia*). The cost of the plates makes the test very expensive, so it is routinely used only in centralized diagnostic laboratories. The sample is typically run with a positive and negative control sample. The results can be read either visually or by using an ELISA reader. In the test shown (A), the positive wells turn yellow at the end of the test, and the negative wells remain clear. The intensity of the color can be used as a rough approximation as to the intensity of the infection. Photos compliments of REMEL Inc.



of trichomonads from the feces of cats with diarrhea.

The kit used for the detection of organisms in feline feces was originally designed for the detection of *Tritrichomonas foetus* from bovine vaginal samples and is still only marketed for this use. Fortunately, the process works well for the fecal samples without a great overgrowth of bacteria to make the samples unreadable. Once the sample has been incubated in the pouch, it can be examined with a microscope directly through the wall of the pouch using a device provided by the manufacturer that flattens and spreads the pouch for examination. If trichomonads are present, they can either be removed with a pipette for further examination with the microscope or for passage to other media. This is the first kit that makes the culture of specimens for this pathogen relatively easy for in-house testing.



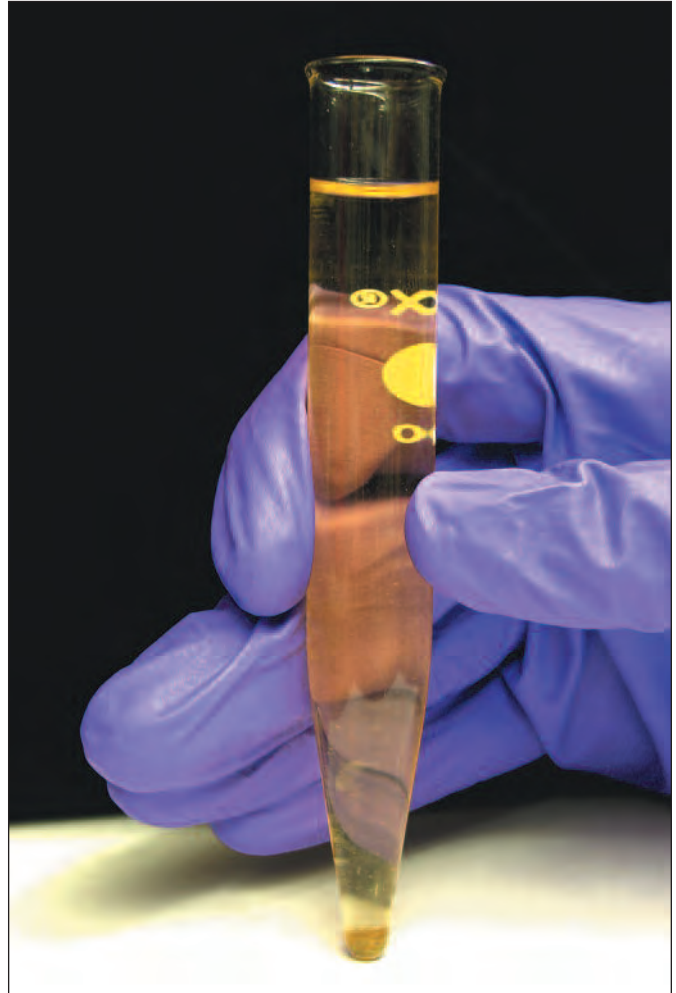
Figure 1.18. Until recently, there were no methods that would routinely be applied to the culture of organisms in a veterinary practice. The development and application of the in-pouch assay for trichomonad infections in cats seems to be a method that could be used more routinely. The method was originally developed for the culture of the sexually transmitted trichomonads of the urogenital tract of cattle and people, but the cattle kit seems to work for the culture of trichomonads present in cat feces. *Photo compliments of BioMed Diagnostics, Inc.*

Chapter 2: Urine Specimens

The examination of urine specimens for parasites is relatively easy compared with fecal analysis. There are very few parasites of the urinary tract that commonly occur in dogs and cats; these include the capillarids, *Pearsonema plica* and *P. feliscati*. In general, no other parasite eggs are routinely found in the urine of dogs and cats. On rare occasions, dogs become infected with the giant kidney worm, *Diocotophyme renale*, which produces eggs found in the urine. In very rare cases, cats have been found to have typically free-living nematodes, *Pelodera strongyloides*, multiplying within the urine of the bladder. In areas where the prevalence of infection with *Dirofilaria immitis* is high, it is possible to find microfilariae within the urine of dogs.

The particulates in the urine should be concentrated by centrifugal sedimentation, and the sediment should be washed with saline (*Figure 2.1*). The sediment can then be examined in a wet preparation for the presence of any of these parasite stages.

Figure 2.1 Simple centrifugal sedimentation followed by examination of the pellet after decanting will usually provide the best chance of success for parasite recovery and identification.



Chapter 3: Blood Samples

Blood can be examined for parasites using several methods, the choice of which depends to a great extent on the goal of the examination. When looking for living organisms such as microfilariae or trypanosomes that are expected to be alive and present in relatively large numbers, the best choice is putting a drop of fresh blood on a slide and examining it for wriggling organisms.

In *Babesia* and *Cytauxzoon* infections, the stages in the blood are typically not moving except within the blood cells they parasitize. Thus, detection of these parasites is usually by the examination of stained blood films. These parasites are best seen when the blood is stained with a Romanovsky stain, such as Giemsa, although adequate results can often be obtained using routine blood-staining methods.

For filarial infections in which the microfilariae are found in the blood, methods have been developed for concentrating the microfilariae by lysing the red blood cells. The two methods that routinely utilize this approach are the Knott's test and the membrane technique. The development of many on-site patient-side antigen and antibody tests has resulted in blood being examined on many occasions by direct application of whole blood, plasma, or serum to various devices that are then examined through various antigen or antibody capture and visualization technologies. These various antigen and antibody detection kits for use with blood are undergoing very rapid development, improvement, and changes.

WET MOUNT FOR MICROFILARIAE AND TRYPANOSOMES

Placing a small drop of blood under a microscope provides a rapid means of diagnosing infections with trypanosomes or filarioid nematodes if there are sufficient stages circulating in the blood at the time of examination (*Figure 3.1*). In those parts of the world where heartworm is very common, the examination of a wet mount prepared with a small amount of blood on a coverslip will often be a very sensitive and inexpensive method for detecting infections. With the low prevalence and the many dogs on heartworm preventatives in much of the United States, however,

the wet mount is often not a highly productive technique. Trypanosomes are only rarely found in dogs in most areas of the world and *Trypanosoma cruzi* is often present in the blood at very low levels. Thus, use of the wet mount for diagnosis of these infections is also often not worthwhile. It is extremely gratifying, however, when heartworm is expected and the examination of a drop of blood reveals a large number of wriggling larvae on the slide.

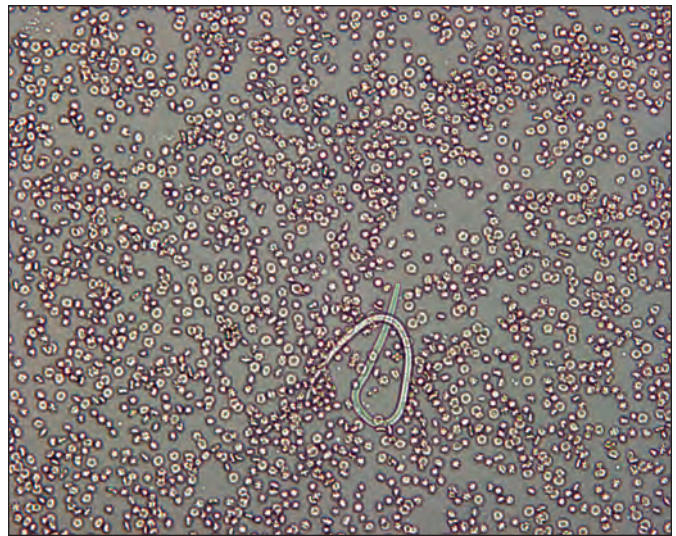


Figure 3.1. Placing a small drop of blood on a slide is an excellent way to find microfilariae if they are present in any number. There is nothing more rewarding than preparing the slides and seeing wriggling microfilariae. It is also a fairly good way to distinguish the microfilariae of *Dirofilaria immitis* (shown here) from those of *Dipetalonema reconditum*; the latter are capable of propelling themselves along easily in the blood while the heartworm microfilariae tend to just thrash about. An even more exciting find is coming across something like a trypanosome in one of these preparations.

KNOTT'S TECHNIQUE

The Knott's technique is a method for the examination of a larger quantity of blood for microfilariae than is possible with the wet mount (typically, 1 mL of blood for the Knott's technique versus 0.02 mL with the wet mount). The technique is based on the fact that hypo-osmotic solutions will cause the lysis of red blood cells without affecting the microfilariae. The test has routinely been performed using

2% formalin to lyse the red blood cells and fix the microfilariae for later examination (*Figure 3.2*). The typical mistakes made by beginners are making the 2% formalin in saline rather than water and using 10% formalin rather than 2% formalin; both mistakes cause the red blood cells to be fixed rather than lysed, which defeats the purpose of the test. After the cells are lysed, the red blood cell ghosts, the white blood cells, and any microfilariae that are present are concentrated using centrifugation (*Figure 3.3*).

Typically in these samples, the microfilariae are stained with methylene blue; either a drop is added to the pellet (it is best to use a very small drop or there will be a lot of precipitate in a very dark blue sample) or the 2% formalin is made containing the diluted stain.

It is not always necessary to add formalin or stain if the examiner knows what he or she is looking for in the preparation. The red blood cells can be lysed with water, the tube centrifuged, and the pellet resuspended in a small

amount of saline, which will produce a pellet that can be examined for microfilariae. The only problem is that the microfilariae will not be stained and will appear solely as small thin vermiform lines with one rounded end and one very pointed end (*Figure 3.4*). The diameter of the microfilaria is about the same as a red blood cell. The microfilariae are easier to identify, however, when methylene blue stain is added (*Figure 3.5*).



Figure 3.2. Blood, 1 mL, either fresh, EDTA-treated, or heparin-treated, is transferred to 10 mL of 2% formalin, which causes the red blood cells to lyse.



Figure 3.3. After the sample has been allowed to stand for 5 to 10 minutes to allow the red blood cells to lyse and to give the microfilariae time to be fixed by the formalin, the tube is centrifuged to collect white blood cells, the red blood cell ghosts, and the microfilariae into the pellet. A small quantity of methylene blue stain is often added to the pellet to help in the visualization of the microfilariae.



Figure 3.4. The pellet can be examined unstained and the microfilariae can be identified as thin hair-like structures with pointed tails. In fact, the assay can be run without using formalin and the microfilariae will still be wriggling if the preparation is examined soon after the pellet is formed. The test was originally developed for looking at samples days to weeks after they were collected in the field, and the stain was added so that various different types of microfilariae commonly occurring in human blood could be distinguished.

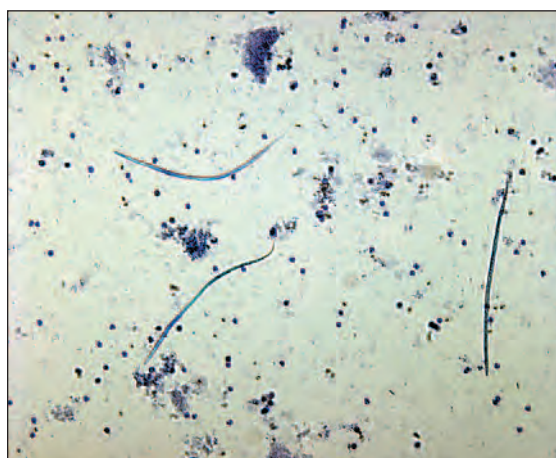


Figure 3.5. With the added methylene blue stain, the numerous nuclei within the fixed microfilariae are stained blue, making the microfilariae easier to identify. Care should be taken not to add too much stain or the sample will become too dark to examine easily.

MEMBRANE TECHNIQUES

Another means of examining blood for microfilariae is the use of a membrane filter (Difil-Test®; EVSCO Pharmaceuticals). With this method, the blood cells are lysed much in the same fashion as they are for the Knott's test. Then, instead of the sample being concentrated using centrifugation, the lysed blood is forced through a membrane filter. The filter is then taken and placed on a microscope slide under a coverslip for examination. The test can be very sensitive and captures all of the microfilariae in the milliliter of blood on the membrane. Again, as with the Knott's technique, the microfilariae can be examined either with or without staining.

ANTIGEN TEST KITS

The antigen tests are typically capable of discriminating between infections with *Dirofilaria immitis* and *Dipetalonema reconditum*, and this makes the need for the microscopic differentiation of these two blood-dwelling microfilariae of less importance than a few years ago. These tests can sometimes produce false-negative results when the burden of

adult worm heartworms is quite low but also sometimes when very high numbers of circulating microfilariae are present. Thus, in dogs with unknown histories, it is always best to perform both a test for microfilariae and an antigen detection test. It must also be remembered that some 20% of dogs with heartworms have "occult" infections, ie, infection without circulating microfilariae. In these cases and in dogs that have been receiving avermectin preventatives, the antigen detection tests are the preferred means of determining whether a dog has a heartworm infection.

STAINED BLOOD SMEAR

Routine blood smears stained by most laboratories are capable of revealing parasites when present. These methods are the most reliable means of diagnosing babesiosis or cytauxzoonosis, but antigen tests being developed for babesiosis that are likely to supplant the routine blood smear for this purpose. These tests will also work well for trypanosomiasis, but often there are insufficient protozoa present, making diagnosis difficult.

Chapter 4: Tissue Specimens

SKIN SCRAPING

A skin scraping to detect the mites *Sarcoptes scabiei*, *Notoedres cati*, or *Demodex* species is best performed using a scalpel and a small quantity of mineral oil (Figure 4.1). For *Sarcoptes* and *Notoedres*, it is necessary to scrape rather deeply, perhaps drawing a small quantity of blood from the site of scraping. The crusts can sometimes be rather thick, and it may be necessary to break them apart on the slide using fine forceps or a pair of stainless-steel dental probes (Figure 4.2). After the material is teased apart, a coverslip can be added and the preparation examined under the microscope. Often the mites will be moving, making them relatively easy to spot.



Figure 4.1. The materials required for performing a skin scraping are mineral oil, forceps, scalpel, microscope slide, and coverslip.

The method for the examination of ceruminous material from ears of animals with expected ear mites is similar. The adults of *Sarcoptes* and *Notoedres* are much smaller than *Otodectes*, so it is necessary to look for these parasites with a bit more care.

SKIN SMEAR AND BONE MARROW ASPIRATES

Routine Diff-Quik staining of cells from skin and from bone marrow aspirates can demonstrate the amastigote stages of trypanosomes and *Leishmania*. The careful staining of these preparations with Giemsa or other hematology stains will improve the definition of the parasites within the host cells in which they are found.

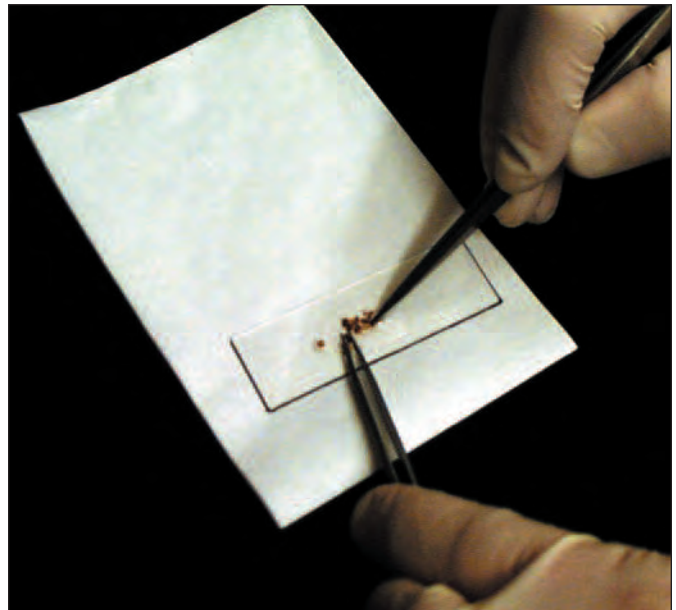


Figure 4.2. When performing the skin scraping, it may be necessary to scrape until a bit of blood oozes from the site of the scraping. This is especially true when dealing with the deeper-dwelling *Sarcoptes scabiei*. The scalpel blade is dipped in the mineral oil and then used to scrape the skin. Some of the crusts may be quite hard, and this may require that they be teased apart with forceps before the coverslip is applied to the slide with the material.

Part II

Chapter 5: Parasites Detected in Feces—Case Studies

CASE 1

Signalment. Two-month-old female mixed-breed dog
History. Adopted two weeks previously from a local shelter, the puppy had received initial vaccinations and had been dewormed with pyrantel pamoate (Nemex®; Pfizer). The puppy had been alert and healthy, but over the last two days had become lethargic and anorectic, and had not been observed to defecate during that period. Feces observed near the anus were dark and soft; no liquid stools had been observed by the owners.

Physical Examination. The puppy was depressed, but otherwise in good body condition. The bowel loops were felt to be thickened and markedly palpable. The mucous membranes appeared somewhat pale, suggestive of mild anemia.

Initial Assessment. This puppy's history and the presence of the thickened bowel loops led the clinician to suspect an infection with perinatally acquired helminths, the most typical rule-outs being hookworms, roundworms, or both. The thickened bowel loops were suggestive of roundworm (*Toxocara canis*) infection, but the slight anemia was suggestive of hookworm (*Ancylostoma caninum*) infection.

Presumptive Diagnosis. Severe nematode infection, probably roundworms

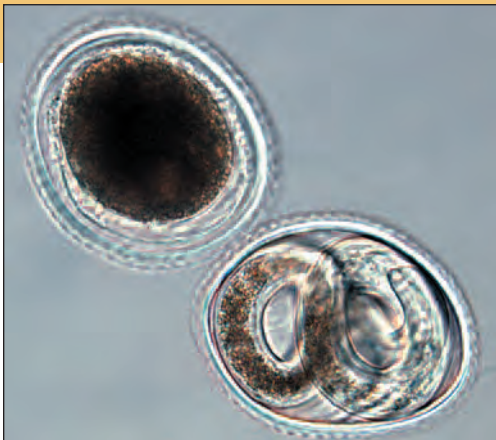


Figure 5.1. The egg of *Toxocara canis* is a good benchmark egg to remember for size; it is slightly larger than the long-axis length of *Ancylostoma caninum* and has a diameter that is about the same as the long-axis length of the eggs of *Trichuris vulpis* and *Uncinaria stenocephala*. Under the 10× objective of most diagnostic scopes the distance across the field of view is about 1.8 to 2 mm, or 1800 to 2000 μm. The egg of *T. canis* is

about 80 μm in diameter (*T. cati* is slightly smaller in diameter, being around 70 μm in diameter), so under 10×, about 22 to 25 of the eggs of *T. canis* would fit across the field of view. Under the 40× objective, the field of view is about 450 to 500 μm, so only 6 or so of these eggs would fit in a line along the field of view. The other good frame of reference is the cysts of *Giardia canis* or *G. cati*. These cysts are about 10 μm long or about one tenth the diameter of a *T. canis* egg. Thus, under the 40× objective, as many as 60 *Giardia* cysts could be seen lined up across the field of view.

Diagnostic Plan

Collect a fecal sample for simple examination for parasite eggs. A small portion of feces was collected per rectum and a stationary sodium nitrate flotation was performed. After 10 minutes, the sample was examined and found to contain numerous *T. canis* eggs.

Diagnosis: *Toxocara canis*

Several other tests could have been performed that also would have recovered the *T. canis* eggs. A centrifugal flotation test probably would have recovered eggs in situations with low egg counts in which the stationary flotation might have been negative. On the other hand, with a large number of eggs present, it is likely that a direct smear of the feces would have been as successful as any of the concentration methods at revealing the eggs.

Outcome

The puppy was treated with praziquantel/pyrantel pamoate/febantel (Drontal® Plus; Bayer) and passed a large number of worms. The previous treatment with pyrantel pamoate had probably been successful in removing the majority of adult worms present at that time, but it was to be expected that additional worms had still migrated to and developed in the intestine from sequestered sites in the liver and lung where they had been since before the puppy's birth. Plans were made to start the puppy on a routine heartworm preventative that included control of roundworm infections.

The owners were informed that there was a good chance that the areas of the backyard where the puppy had defecated were very likely to be contaminated with large numbers of *T. canis* eggs. The puppy had been purchased as a companion for their children, so the owners were warned that there was a need to try to clean up the environment by either removing or covering the contaminated soil or somehow preventing access to the contaminated site to reduce the chances of zoonotic transmission.

CASE 2

Signalment. Two-year-old (approximately) male, Labrador retriever/German shepherd mix

History. The owners had adopted the dog from a shelter in Illinois and brought it to the veterinary hospital for a neutering with a coupon from the shelter and to begin its routine pet health program. Nothing else was known about the dog's previous background.

Physical Examination. The dog appeared to be perfectly healthy except for some scarring on the right ear. The appetite had been noticed to be good and stools had been observed to be normal. There was no sign of ear mites, and the skin appeared normal. Slight gingivitis suggested that the dog should have a dental cleaning and begin routine dental care.

Initial Assessment. The dog was young and healthy, but had a virtually unknown history. The owners wanted to begin routine care for their pet, including heartworm prevention and routine vaccinations. The owners had other dogs and were known to be in good compliance with recommended treatments and scheduling of visits for those pets.

Presumptive diagnosis. Normal dog

Diagnostic Plan

The dog needed to be examined for a potential heartworm infection before beginning preventative therapy. Because the history was not known, a routine fecal examination was performed using a sugar flotation procedure. A routine hemogram was also performed.

The hemogram was normal and the heartworm tests, antigen and Knott's, were both negative. The fecal examination revealed eggs of *Toxocara canis*, *Ancylostoma caninum*, *Uncinaria stenocephala*, *Trichuris vulpis*, and a round egg with a thick roughened brown shell that looked a bit like *T. canis*. Further examination of the sample and comparison of the eggs to images in texts and on the Internet revealed that it was the egg of *Baylisascaris procyonis*. *Baylisascaris procyonis* is typically a parasite of raccoons, but infections with the adult forms of this parasite have been found in dogs, especially in the Midwestern United States.

Diagnosis: *Baylisascaris procyonis* and other intestinal nematodes

Again, as with the *T. canis* eggs in Case 1, any of the routine concentration methods would probably have revealed the infection in this dog. However, the tests that utilize sodium nitrate, zinc sulfate, and magnesium sulfate have the disadvantage that the slides tend to dry out and the flotation solution crystallizes rather rapidly. Thus, it is difficult to preserve the slide for very long when something unusual is found. For most of the helminth eggs (other than the Diphylobothriid tapeworms and many of the larger fluke eggs), the slide prepared for a sugar flotation can be examined with care often for up to a week after it is made. Care needs to be taken to keep the slide in a humid chamber and in the refrigerator when the sample is not being examined. It is best to let the sample and its container warm to room temperature before examination because otherwise condensation on the coverslip will initially prevent the viewing of the material under the coverslip; however, this condensation will clear with the application of some patience. Such slides, if immobilized, can be shipped in the mail for examination by others. It must be remembered that if the slide stays at room temperature too long the hookworm eggs will begin to

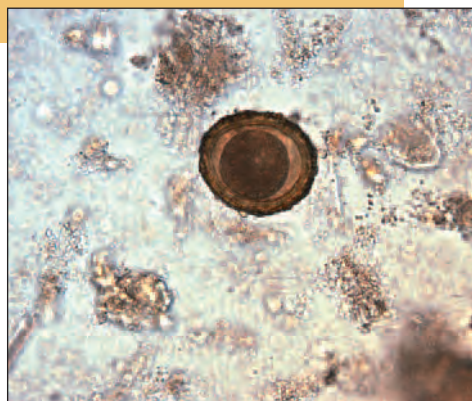


Figure 5.2. Egg of *Baylisascaris procyonis* in dog feces. This egg is slightly smaller than the eggs of *Toxocara canis* and *Toxascaris leonina*, has a thick brown shell, and is typically passed containing a single cell. The roughened exterior of the eggshell is not patterned like the eggshell of *T. canis* and is rough and dark compared with the eggshell of *T. leonina*.

develop. Initially, they will become very clear as the larva forms, but ultimately they may hatch. Thus, although the slides can be read for several days after they are made, it is always best to examine them the day they are made if at all possible.

Outcome

The dog was treated with fenbendazole (Panacur®; Intervet) for three days, which cleared all of the intestinal nematodes. The dog was then started on a heartworm preventative. The feces were checked two weeks after treatment and no eggs were found.

Baylisascaris procyonis has been responsible for severe neurologic disease and the death of several children in the United States. It is believed that these cases have been related to children having contracted large numbers of eggs from soil contaminated with raccoon feces. Neurologic disease has also been recorded in more than 100 species of birds and mammals that have ingested the eggs of this parasite. Raccoons typically defecate in the same place over

and over again at sites that are called “raccoon latrines.” Thus, disease in children tends to occur when they somehow eat soil from one of these sites or place articles or fingers in their mouths that have been in contact with the soil or other ground cover present in these sites.

As more and more dogs are infected, the concern is that there may be more cases of human infections with this parasite. The dog is an indiscriminate defecator compared with raccoons, and they will cause a wider dispersal of the eggs in their feces in areas that are more commonly frequented by people. It is not known for certain how dogs become infected with this parasite, but it is believed that they become infected by eating rodents or other animals that have ingested the eggs much in the same fashion that adult raccoons become infected.

The egg of *B. procyonis* is as resistant as those of *T. canis*, so cleaning up the soil is a very difficult task. The eggs of these parasites can probably survive in the soil in most climates for several years.

CASE 3

Signalment. Three-year-old spayed female domestic shorthair cat

History. The cat's owner reported that the cat is a healthy indoor-outdoor cat. However, the owner had found large numbers of poppy-seed-like balls in the cat's bed and bedding. The owner brought both the cat and the many small seed-like objects into the office for examination, and also supplied a fecal sample for examination. The owner stated that otherwise the cat appeared normal and healthy.

Physical Examination. The cat appeared bright and alert, and had a gentle disposition. Otoscopic examination revealed normal ear canals. The cat appeared to have a bit of alopecia on its back, and signs of flea dirt were present, although no fleas were found.

Initial Assessment. This appeared to be a normal healthy cat without any untoward signs of infection or dermal lesions except for a small area on the back. It was thought that the cat might have a flea infestation, although no fleas were seen and the owner reported that none had been seen at home either. The cat has not been on a flea control program.

Presumptive Diagnosis. The clinician suspected that the cat was infected with a tapeworm and that the seed-like material represented dried proglottids. Another possibility was that the small seed-like material represented a form of some plant or free-living insect that may have found its way into the bedding by accident and was not a disease-causing agent.



Figure 5.3. Dried segments of *Dipylidium caninum* that appear similar to small round seeds about the same size as sesame seeds. (Specimen courtesy of Dr. Jeanne Baines)

Diagnostic Plan

A routine stationary fecal flotation was prepared from the fecal sample provided. The “seeds” were also examined. A couple of the “seeds” were placed in a small quantity of tap water.

The sodium nitrate flotation was negative. The examination of the “seeds” revealed hard, yellow, rather nondescript balls. The seeds that were soaked in water, however, slowly expanded and unfolded to present themselves as tapeworm segments. Examination of the segments closely revealed that they were the segments of the tapeworm *Dipylidium caninum*, which is recognizable by the contained egg balls and the paired lateral reproductive openings.

Diagnosis: *Dipylidium caninum*

Flotation solutions often will not diagnose infections with tapeworms because the individual eggs of the taeniid tapeworms and the egg balls of *Dipylidium caninum* are too heavy for most solutions other than sugar and, therefore, the eggs do not float to the surface of the medium.

In Canada, the northern Midwestern United States, and parts of Europe, there is concern that cats and dogs might be shedding taeniid tapeworm eggs that are in reality the eggs of *Echinococcus multilocularis*. This is a very significant zoonotic hepatic infection of people that will cause death if it is not treated. The natural final host is typically the fox,

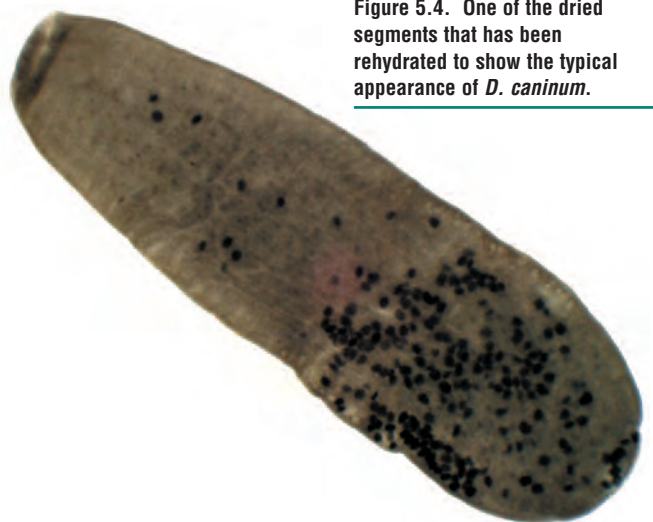


Figure 5.4. One of the dried segments that has been rehydrated to show the typical appearance of *D. caninum*.

and the intermediate host is a small rodent. If dogs and cats hunt, there is the possibility that they could be infected with this parasite. Thus, if eggs of taeniid tapeworms are seen in the feces but taeniid segments are never observed, there should be concern that the infection is *Echinococcus*. Although the treatment of the cat will be the same, *E. multilocularis* infects people but *Taenia taeniaeformis* of the cat almost never does.

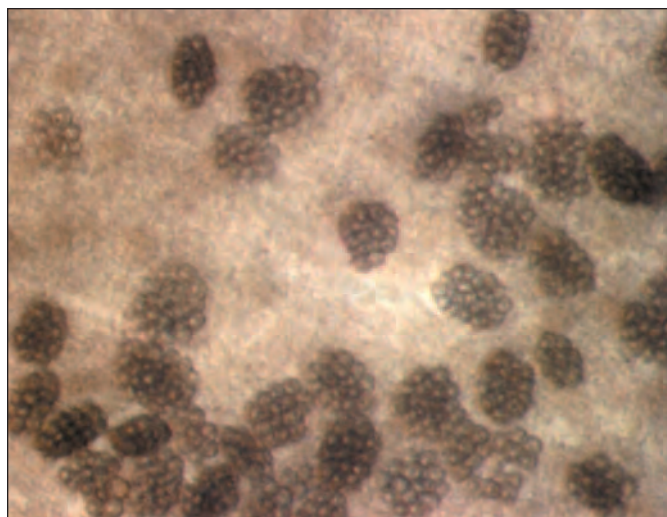


Figure 5.5. Close-up of the segment of *D. caninum* to show the contained egg balls within the segment.

Outcome

The cat was treated uneventfully with praziquantel/pyrantel pamoate (Drontal) to remove any tapeworms that might have been present. The cat was also started on a monthly flea prevention program. The owner was informed that there are likely to be fleas in the household because that is probably how the cat became infected.

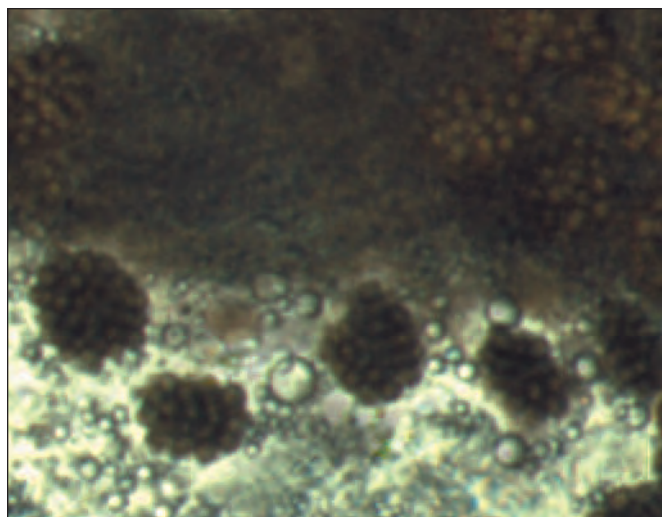


Figure 5.6. The segment has been squashed to release the egg balls (five) into the area next to the segment.



Figure 5.7. Single egg ball viewed using differential interference contrast microscopy to show the appearance of the individual hexacanth organisms within the egg balls.



Figure 5.8. Two individual hexacanth embryos of *D. caninum* in which the larval hooks are evident.

CASE 4

Signalment. Four-year-old intact male domestic shorthair cat

History. This healthy indoor-outdoor cat was brought to the clinic for its routine annual checkup. The cat was on ivermectin (Heartgard® for Cats; Merial) to prevent infections with heartworms, hookworms, and roundworms and was receiving fipronil (Frontline®; Merial) for flea and tick control. The cat was thin and has lost weight since its visit a year ago. The cat was up-to-date on all necessary vaccinations.

Physical Examination. The cat appeared to be normal on examination. The mucous membranes were pale and it was suspected that the cat was somewhat anemic.

Initial Assessment. There was concern over the loss of weight in this otherwise healthy cat. The possible anemia was also considered worrisome because of its potential association with some serious condition related to the weight loss. A hemogram and a routine fecal examination were performed. If nothing abnormal is found, a radiograph will be the next course of action.

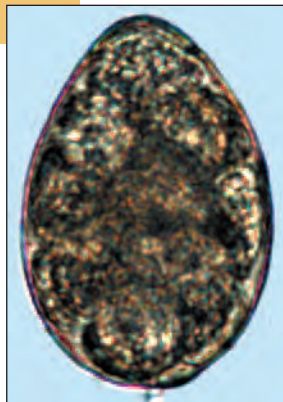
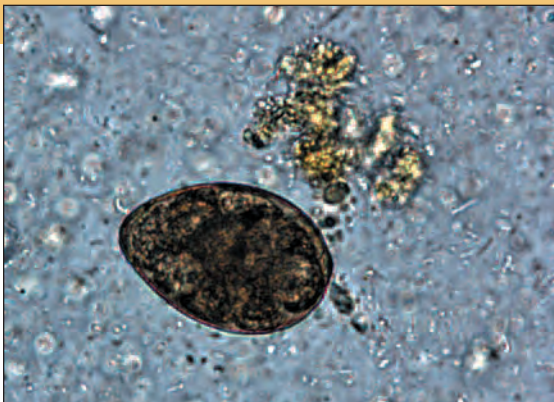
Presumptive Diagnosis. Unexplained weight loss, concern over possible tumor or some other underlying syndrome related to the weight loss and anemia

Diagnostic Plan

The hemogram was performed and cat was found to have a macrocytic anemia with decreased hemoglobin. The fecal examination revealed the egg of what appeared to be either a fluke or a diphylobothriid cestode. Careful examination of the eggs present in the feces determined that it was the egg of *Spirometra mansonioides*. This tapeworm egg is morphologically very similar to the egg of the lung fluke *Paragonimus kellicotti*, but the egg of *Spirometra* is typically slightly less brown and has a slightly thinner shell. Also, the operculum is often less well demarcated than is the case with *Paragonimus*.

Diagnosis: *Spirometra mansonioides*

The eggs of the tapeworms *Spirometra* and *Diphylobothrium*, along with the flukes *Paragonimus kellicotti* and *Alaria marcianae*, will often float in sucrose solution, but they will also often rupture and/or collapse onto themselves, which makes them somewhat more difficult to identify. The problem is that these are relatively heavy eggs compared with the eggs of the more common nematodes (eg, *Toxocara* and *Ancylostoma*), and they will often not float in the routine media that usually do an excellent job with the more common nematodes. Unfortunately, the ranges of *Spirometra* and *Paragonimus* overlap in much of the United States, and this helps make the diagnosis a bit more difficult.



Outcome

The cat was treated with a subcutaneous inoculation of praziquantel (Droncit®; Bayer) given at 6× the routine dose for *Taenia* species and *Dipylidium caninum*. After treatment, the cat improved markedly. The lost weight was rapidly recovered and a repeat hemogram was normal.

Figure 5.9. The egg of this cestode, *Spirometra mansonioides*, looks like a trematode egg. On this specimen, the difficult-to-discern operculum is on the more pointed end of the egg. Some specimens will appear to be more elongate or to have ends more similar in size. The eggs are similar in size to those of *Paragonimus* species and sometimes several specimens will have to be examined before a diagnosis can be confirmed.

CASE 5

Signalment. Two-month-old female domestic shorthair kitten

History. This kitten was presented to the clinic for routine vaccinations and a routine health check. The owner had noticed that the kitten's stools had been soft for the last few days, but the kitten did not seem to be affected in any other way.

Physical Examination. The kitten appeared to be normal, and was bright and alert. The body temperature was slightly elevated (39.5°C). Palpation of the abdomen revealed bowel loops that appeared to be thickened, but not remarkably. Otoscopic and ophthalmoscopic examinations were normal. A routine fecal examination was performed.

Initial Assessment. This was a relatively normal kitten with possible infectious bowel disease of unknown etiology. The kitten had received its first set of vaccinations and was being seen for the purpose of being given its second vaccinations for feline viral rhinotracheitis (FVR), panleukopenia (FPV), and feline calicivirus. Because of the thickened bowel loops and loose stool, it was decided that a routine fecal examination would be performed. Samples were also taken for bacterial culture of the feces.

Presumptive Diagnosis. Enteritis of unknown etiology

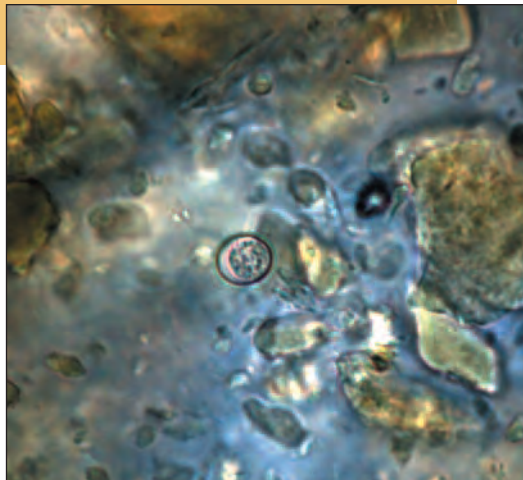


Figure 5.10. A single unsporulated oocyst of *Toxoplasma gondii* can be seen in the center of this 40× objective image of a sugar flotation of cat feces. In fecal samples that are aged slightly, the central sporoblast may have divided into two separate sporocysts.

Diagnostic Plan

The bacterial culture of the feces and a complete blood count (CBC) were both normal. A fecal sample was examined by centrifugal sucrose flotation, and numerous small oocysts were found to be present in the stool. It was determined that the cat was shedding oocysts that were morphologically indistinguishable from those of *Toxoplasma gondii*.

Cats can shed three oocysts that are very small and indistinguishable. In addition to oocysts of *T. gondii*, cats also can shed oocysts of *Hammondia hammondi* or *Besnoitia darlingi*. Because infections with *T. gondii* are by far the most common of these species to be found in cats, it is most likely that the shed oocysts are those of *T. gondii*.

Diagnosis: *Toxoplasma gondii*

It was deemed unnecessary to perform serology for toxoplasmosis on this kitten because it would probably supply little additional information. There is no information on how much cross-reaction exists between serologic tests for these three species in the different assays used to detect antibody to *Toxoplasma* in cat sera. In addition, the kitten has probably only been recently infected. After the ingestion of oocysts, cats will not shed oocysts until about 3 weeks after they are infected, and will typically only shed oocysts for a few days. The young age of this cat indicates that it probably acquired its infection by the ingestion of oocysts when it was about 1 month old rather than by the ingestion of an infected mouse or bird. After the ingestion of an intermediate host with bradyzoites, the kitten would begin to shed oocysts 3 to 4 days after the mouse was eaten.

Outcome

The owners were informed that the cat was infected with *Toxoplasma gondii* and was shedding the oocysts in its feces. They were cautioned to carefully dispose of the litter in the litter pan at home and to disinfect the pan with very hot water. It was recommended that the kitten be housed in the clinic for the next several days so that the feces could be carefully sequestered from the environment and so that additional fecal samples could be collected to verify the negative status of the cat before being sent home. The cat was kept in the clinic for 4 days. Its fecal examination then revealed no oocysts, and it was sent home.

On the next visit when the kitten was 12 weeks of age, it was carefully examined for any signs of disseminated toxoplasmosis (eg, fever, ophthalmoscopic changes, thickened bowel loops), and the kitten appeared completely normal.

CASE 6

Signalment. One-year-old female Abyssinian cat

History. The cat has had recurrent bouts of soft to liquid stools over the past several months.

Repeated fecal flotation examinations have not yielded any parasitic agents and bacterial cultures have been negative. The cat is receiving selamectin (Revolution[®]; Pfizer) monthly for heartworm, intestinal worms, and flea control. The cat has been treated on two occasions empirically for enteric intestinal infections, once with a course of metronidazole and once with a course of amoxicillin, without having any effect on outcome. The cat has been placed on a specially formulated hypoallergenic diet, but this also did not ameliorate the signs.

Physical Examination. The cat appears bright, alert, and apparently in good health. The only sign appears to be the various periods of soft to liquid stool that occur sporadically.

Initial Assessment. This is a continuing cause of concern for the client. The cat, however, appears healthy otherwise and has no other signs of disease. The blood work that has been performed has remained normal and there has never been fever recorded during any of the visits.



Figure 5.11. Trophozoite of a trichomonad as viewed under differential interference microscopy showing the undulating membrane, axostyle projecting from the posterior end, and a hint of the anteriorly directed flagella. In fresh preparations, these organisms are most easily detected by their swimming motion.

Diagnostic Plan

Continue to workup the animal for allergies and consider possible referral or consultation to develop a form of intervention that is curative.

During the examination, the cat defecated a small quantity of liquid feces. Using this material, a direct smear was prepared in a small quantity of saline. The sample was noted to be teeming with small flagellated organisms.

Diagnosis: Infection with trichomonads

Infections with trichomonads would not be detected in fecal flotations; the parasites would be destroyed by the hyperosmotic flotation solution and would not float to the surface either in the stationary float or in the centrifugal float. The direct smear is the easiest way to make the diagnosis because when the organisms move, they are easy to find.

Outcome

The trichomoniasis was treated with a course of metronidazole combined with enrofloxacin (Baytril[®]; Bayer). After 2 weeks, it appeared that the consistency of the feces improved and overall, the cat appeared to be cured. Once the stools became firmer, the organisms were not seen in further fecal examinations. The cat was maintained on its hypoallergenic diet and this also seemed to assist in maintaining proper fecal consistency. Fecal culture would be a possibility to verify the negative status of the cat.

Without very careful work, it is almost impossible to determine which trichomonad species is causing the disease in cats. One group believes the parasite to be a species of *Pentatrichomonas* that occurs in humans and dogs. More recently, it has been suggested that the trichomonad that causes disease in cats is actually *Tritrichomonas fetus*, the same organism that causes abortion in cattle. Until recently, these trichomonads in cats were considered to be commensal organisms that caused no disease to cats, but when cats developed diarrhea, they were shed in the feces and detectable. It now seems that these protozoa may be able to cause disease in their own right.

CASE 7

Signalment. Six-year-old spayed female beagle

History. This dog has been a regular patient for its entire life, and it has been virtually healthy and happy throughout. This visit is no exception; the dog seems its normal self without any complaints.

Physical Examination. Normal; as expected this dog appears to be in perfect health.

Initial Assessment. The dog is healthy, without any signs of disease.

Presumptive diagnosis. Normal 6-year checkup

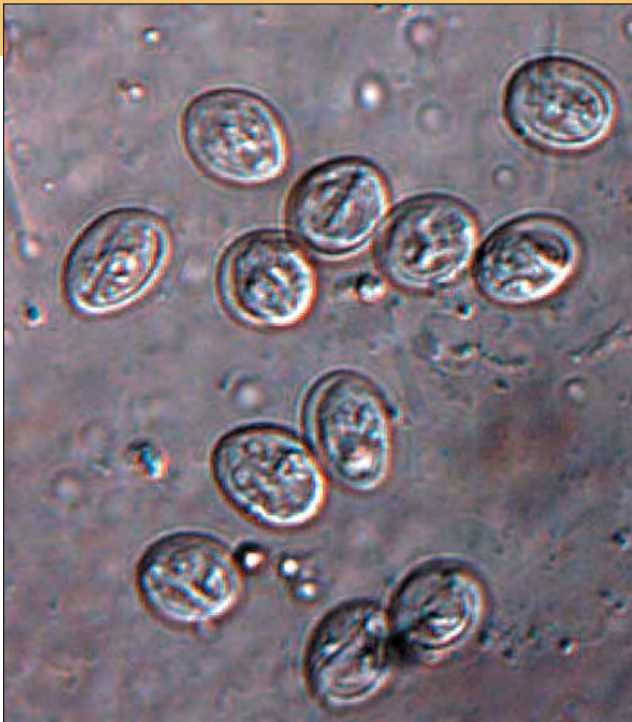


Figure 5.12. Cysts of *Giardia canis* recovered in a zinc sulfate centrifugal flotation

Diagnostic Plan

Because this was a routine annual examination, a fecal examination and the every-other-year heartworm antigen test were performed. The antigen test was negative, but the fecal flotation revealed numerous cysts of *Giardia canis*.

Diagnosis: *Giardia canis*

The method used for this diagnosis was a centrifugal sugar flotation. Using this method, the cysts are often collapsed, or the cytoplasm of the organisms within the cyst may be caved in away from the cyst wall on one end of the cyst, giving the overall appearance of crescentic bodies on one side of a clear oval. An easier and more rapid means of diagnosis of *Giardia* cysts is the use of the zinc sulfate centrifugal flotation method. This is a very rapid method, because the two centrifugations are very short and there is very little material to examine in the looped sample. Using this method, the cysts typically do not collapse. It must be remembered that with *Giardia*, if the dog has diarrhea, there is a very good chance that cysts will not be present in the feces and diagnosis will have to be based either on finding the trophozoites in a direct smear or by the use of an antigen-capture enzyme-linked immunosorbent assay (ELISA) or equivalent antigen-capture test.

It is unclear whether the infection was missed previously or was newly acquired. In either case, the dog does not appear to have any signs of the infection.

Outcome

The outcome is complicated by the fact that *Giardia canis* is a potentially zoonotic agent. The owners have grandchildren and wanted the dog treated. A 3-day course of fenbendazole (Panacur) was given, and the feces were checked again 5 days after the last treatment. The feces were again positive. The dog was then given a second course of fenbendazole treatment that was extended for 5 days. Five days after the last treatment the dog's feces were again examined and found to be negative.

These cases can seemingly bounce around for extended periods and it is unclear how good immunity is relative to the prevention of infection, although it seems fairly good relative to the prevention of diarrhea in infected animals. Because this is a potential zoonosis, treatment is more or less mandated by the parasite's presence. The problem is that it may become almost impossible to clear the dog of the infection, either because the drugs do not cause clearance or because the dog is constantly re-infecting itself. If the owners are truly concerned about the zoonotic potential, it would probably be worthwhile to administer the vaccine that has been developed and which should prevent infection.

CASE 8

Signalment. Five nine-week-old domestic shorthair kittens

History. The owner reported that the litter of kittens had been vaccinated on schedule, had been observed to have roundworms, and had been treated with praziquantel/ pyrantel pamoate (Drontal). During the last week, the kittens have developed soft feces, and one of the five kittens has had fairly severe diarrhea.

Physical Examination. The kittens had temperatures and respiration rates within normal limits. The one kitten that had had diarrhea appeared somewhat dehydrated. The feces that had been expelled did not contain any signs of blood and were rather light colored.

Initial Assessment. The signs were consistent with small bowel diarrhea. The light color of the stool indicated that there was not significant bleeding or hemorrhage of the mucosa in the small intestine. The kittens had all been vaccinated for panleukopenia virus.

Presumptive Diagnosis. Possible gastroenteritis. Fecal samples were taken for examination for *Giardia* cysts and *Isoospora* oocysts.

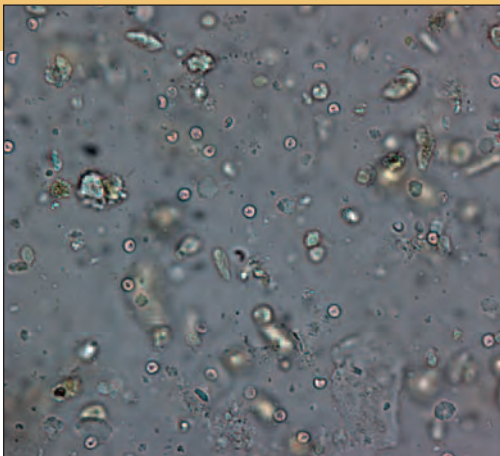


Figure 5.13. The oocysts of *Cryptosporidium canis*, *C. felis*, *C. parvum*, and *C. hominis* are morphologically indistinguishable. The oocysts in sugar flotations can be seen to have a slight pink coloration caused by the spherical aberration present in most inexpensive lenses used on diagnostic microscopes. With the highly corrected optics present on most research-grade microscopes, this pink coloration tends to disappear.



Figure 5.14. This is a view of two oocysts using an oil immersion 100 \times lens and differential interference microscopy to show the sporozoites and residual body present within each oocyst.

Diagnostic Plan

A direct smear from the stool of the kitten with the worst diarrhea was not noted to contain any cysts of *Giardia felis*. A sugar flotation revealed no *Isoospora* oocysts, although very small oocysts consistent with a diagnosis of *Cryptosporidium felis* were identified. A fecal sample from each kitten was submitted to a laboratory for additional testing for cryptosporidiosis to verify the diagnosis.

Diagnosis: *Cryptosporidium felis*

The oocysts seen in the sugar flotation were small and appeared to float under the sugar solution, just underneath the coverslip. The oocysts appeared to have a slight pink color when examined with the microscope. Using the 10 \times objective, the oocysts appeared as small pink spots on the slide. After about 15 minutes or so, the oocysts will collapse and become relatively hard to recognize.

The laboratory reported that all fecal samples were positive for the antigen of *Cryptosporidium*. Thus, all five kittens were likely to be shedding oocysts in their feces.

Outcome

There is no treatment that will stop the shedding of the oocysts of *Cryptosporidium* in the feces of various hosts; thus, the only therapy that could be given to the kittens was supportive. Fluids were administered to the one dehydrated kitten, and the others were closely monitored by the owner to verify that they did not also develop signs of dehydration. A fecal sample was collected from the other five adult cats from the same household, and these were found to be negative for *Cryptosporidium* antigen. Fecal samples collected from the five kittens two weeks after the first visit were normal and did not contain oocysts or antigen.

The owners were informed that this was a potentially zoonotic agent, although it is not known how commonly the feline parasite is transmitted to people. It was suggested to the owners that they clean the litter box frequently and wash their hands regularly. It was pointed out that there was little risk that the oocysts posed a potential of giving disease to the other older cats in the household unless they had some underlying immunosuppressive disorder, and all the older cats were known to be healthy.

CASE 9

Signalment. Four-year-old intact male domestic longhair cat

History. The cat is a healthy indoor/outdoor hunter who strays from home often for several days, often bringing home “trophies” in the way of small carcasses that often seem to wind up on the foot of the bed. The owner religiously applies fipronil (Frontline) on a monthly basis and has not seen any ticks although they are common in the area. The cat has been a fairly regular patient seen on an annual or semi-annual basis, and was treated a year ago for what appeared to be a bite wound. This time the owner has noticed the development of a progressively worsening cough. The signs were relatively innocuous at first, with the occasional cough and sneeze. However, these signs had become progressively worse and now were accompanied by a mucopurulent discharge from nose on some occasions.

Physical Examination. The cat had harsh lung sound and was dyspneic. There were crusts around the nose from the discharge. The temperature was not significantly elevated and the cat had only minimal signs of distress.

Initial Assessment. A Diff-Quik–stained smear of the material revealed a large number of neutrophils with engulfed bacteria and a number of eosinophils.

Experience had shown that cats in this area with this history might sometimes be infected with lungworm.

Presumptive Diagnosis.

Verminous pneumonitis with potential secondary bacterial infection.



Figure 5.15. Larva recovered from the Baermann funnel. These larvae tend to be highly motile and easy to recover with the Baermann method. The larvae are characterized by their long esophagus and the typical hooked end of the tail.

Diagnostic Plan

Radiographic images were performed of the chest that showed marked diffuse interstitial infiltration of the lungs. A Baermann apparatus was set up to examine the feces, and two hours later, many active larvae were observed. The larvae were identified as the larvae of *Aelurostrongylus abstrusus* by the dorsal-spined tail.

Diagnosis: *Aelurostrongylus abstrusus*

Aelurostrongylus is one of the few larval nematodes found in the feces of a cat, and the only one that is commonly found. When larvae are found, a quick examination of the larval spine will often confirm the identification.

Outcome

The cat was treated with a 5-day course of fenbendazole (Panacur) and was also given enrofloxacin (Baytril) to treat the concurrent bacterial infection. Two weeks after treatment, the Baermann funnel technique revealed that there were still larvae present in the stool. It was felt that perhaps these were larvae still maturing from eggs in the tissues, so treatment was withheld and a second sample was examined in another two weeks that was negative for larvae. A follow-up radiograph 6-months later and a further Baermann funnel analysis revealed no signs of the prior infection.

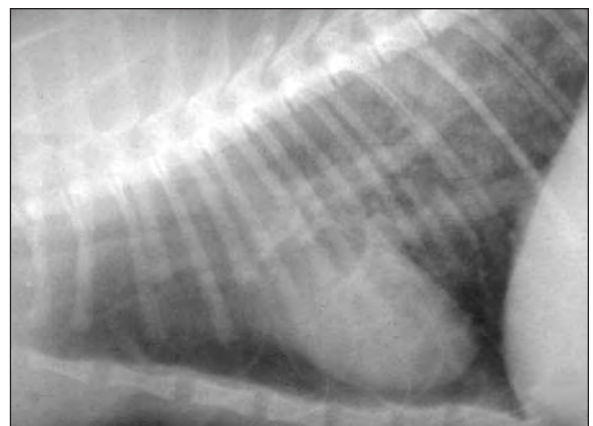


Figure 5.16. Radiograph of the lungs of the cat showing the interstitial patchy infiltrates that are characteristic of very heavy infections with this parasite.

CASE 10

Signalment. Six-year-old intact male Irish wolfhound
History. This dog lives on a large farm, is allowed to roam the farm freely, and consistently comes home at night and sleeps in the family room. The dog was initially noted to be snorting some when sleeping and later appeared to be developing a significant cough. Although he had remained active and continued to roam, the dog seemed to be becoming less active than in the past. The dog had been on consistent heartworm prevention since birth, and 6 months ago, a routine fecal examination was negative for parasite eggs and cysts.

Physical Examination. The dog was bright, alert, and actively playful. The dog was without fever and the CBC and chemistry panel were relatively normal. The dog was noted to be wheezing and it was decided to examine the dog via bronchoscopy.

Initial Assessment. The dog was anesthetized and examined with the bronchoscope. At the various bifurcations of the trachea and bronchi, nodules were detected, some of which were more than a centimeter in diameter. It was estimated that there were probably more than 50 of these nodules that were visible using the bronchoscope. Several of the nodules were excised; one was submitted for histopathology and one was examined grossly in saline. The one that was examined grossly was determined to be a mass of small wriggling worms.

Presumptive Diagnosis. *Filaroides osleri*



Figure 5.17. Larva of *Filaroides osleri* (also known as *Oslerus osleri*) recovered from a centrifugal zinc sulfate flotation. These larvae are not very active, and they are typically not recovered in any number from Baermann funnels.



Figure 5.18. Nodule of *F. osleri* removed by resection during visualization with an endotracheal tube.



Figure 5.19. Histologic section through a resected nodule showing sections through the large number of worms coiled within the nodule.

Diagnostic Plan

Submit the removed nodule for histopathology.

Diagnosis: *Filaroides osleri*

After the discovery of the nodules, a fecal examination was performed using a zinc sulfate centrifugal flotation that proved to be positive for large numbers of larvae of *Filaroides osleri*. The fecal flotation performed previously had been a stationary sodium nitrate float, and it was assumed that this had caused the larvae to be missed.

Outcome

The dog was given a 10-day-course of fenbendazole (Panacur). At the same time, the majority of nodules were removed by excision using the bronchoscope. After three weeks, a zinc sulfate centrifugal flotation revealed that there were still numerous larvae in the feces. The lack of clearance of the infection following treatment with fenbendazole led to a second treatment with a course of levamisole. Another zinc sulfate centrifugal flotation on feces from the dog three weeks after treatment cessation again revealed larvae in the feces. A second course of levamisole cleared the dog's feces of the parasite.

CASE 11

Signalment. Six-month-old intact male Labrador retriever

History. A healthy puppy was presented with the complaint of diarrhea. The dog had received routine heartworm preventatives since near birth.

Physical Examination. There were no significant findings; the CBC and chemistry panel were normal. A routine fecal examination using centrifugal zinc sulfate flotation revealed larvae in the feces.

Initial Assessment. The diarrhea was caused by an infection with *Strongyloides stercoralis*. Larvae in the feces were identified by the shape of the esophagus and the presence of a large genital primordium.

Presumptive Diagnosis.
Strongyloides stercoralis infection



Figure 5.20. This is the first-stage, rhabditiform larva of *Strongyloides stercoralis*. The characteristics of note are the short esophagus that has three distinct portions, the cuticle-lined buccal space, and the very large genital primordium that occurs between the intestine and the ventral body wall just posteriad to mid body. The larvae are fairly motile and can be recovered using a Baermann funnel.

Diagnostic Plan

The fact that this dog was positive for *S. stercoralis*, that the infection is transmitted both through skin penetration and via transmammary infection, and that the dog had recently been purchased from a large supplier of puppies where it lived in close association with a number of other dogs suggested that the infection could be present in other puppies from the same facility. The facility's veterinarian was contacted and provided feces from some of the other dogs. These fecal samples were examined using the Baermann funnel technique to increase the sensitivity of diagnosis. Using the Baermann funnel method, five of seven of the fecal samples that were examined from dogs of all ages and two different litters were found to be positive for the infection.

Diagnosis: *Strongyloides stercoralis* infection in this puppy and others from the same facility

Outcome

The puppy was treated at the time of its clinic visit with a therapeutic subcutaneous inoculation of ivermectin. A fecal sample collected two weeks after treatment and examined using the Baermann funnel did not contain larvae.

After the fecal samples from the facility also showed the presence of *Strongyloides stercoralis* larvae, all dogs in the facility were treated with a therapeutic dose of ivermectin. Two weeks after treatment, an additional set of fecal samples were collected and examined for the presence of larvae using the Baermann funnel method. At this time, the feces of all dogs were found to be negative for larvae.

The need for treatment was compounded by the fact that *S. stercoralis* is a known zoonotic pathogen that is transmissible to humans. Thus, it was essential that the owners and the source facility be made aware of the zoonotic potential of this parasite. The stages in the soil are fairly short-lived, and thus a good thorough cleaning of the environment and the drying of any soil or runs would very likely clear the premises of any potentially threatening larvae in the environment.

CASE 12

Signalment. Six-year-old intact male domestic shorthair cat

History. This indoor-outdoor cat recently developed diarrhea that consisted of puddles of bloody mucoid feces in the litter box. These signs had persisted for about 1 week. The cat had a history of blockage and had been on a special diet for dissolution of struvite crystals. The owner suspected that the cat remained an avid hunter.

Physical Examination. The cat was somewhat lethargic and depressed. There was perhaps a slight increase in rectal temperature. Hematology and clinical chemistries were relatively normal, with a possible increase in lactate dehydrogenase and slight elevation in circulating leukocytes.

Initial Assessment. Examination of the feces by flotation revealed a few cysts of *Giardia felis*. In the direct smear of the feces, small nematodes were noted. The worms seemed fairly large, 1 to 2 mm long, and did not look like the larvae of *Aelurostrongylus abstrusus*, the cat lungworm.

Presumptive Diagnosis. Infection with a nematode, presentation suggestive of *Strongyloides* species, with large numbers of larvae being passed in the feces. The cat was started on a 10-day course of fenbendazole.



Figure 5.21. (Above) Adult male *Trichinella spiralis* recovered from the feces. The male also has a stichosome esophagus that can be seen extending from the head (towards the left) back towards the middle of the body. The male does not have spicules like many nematodes, but instead has a pair of large papillae-like structures that aid in clasping the female during copulation (one of these is visible on the posterior end toward the right)

Figure 5.22. (Right) Adult female *T. spiralis* recovered from the feces. The worm can be noted to contain the typical stichosome esophagus towards the anterior end (on the left) and to have its body filled with small prelarvae from the level of midbody toward the tail (on the right).

Diagnostic Plan

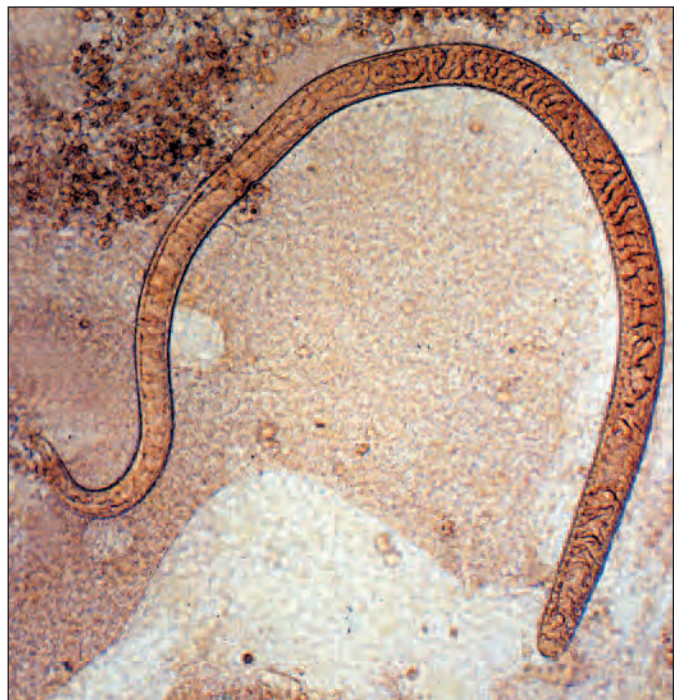
Consultation was sought on the worms recovered from the fecal specimen.

Diagnosis: *Trichinella spiralis*

Outcome

The 10-day course of fenbendazole was completed. As expected, the diarrhea subsided as the intestinal phase of the infection waned. It was uncertain, however, whether the fenbendazole would have hindered the migration of prelarvae to the muscles of the cat. About three weeks after presentation, the cat seemed to have a moderate eosinophilia that subsided after another month. At about this same time, the cat appeared to show signs of erratic behavior, bouts of hyperactivity, hiding, and seemingly extra long periods of staring into space.

Six months later, the cat developed recurrent urinary elimination problems. The problem was found to be due to the continued accumulation of struvite calculi within the urinary bladder despite of the special dietary regimen. Radiographs revealed that the calculi were relatively large and were deemed to require surgical removal. It was



decided that at the same time the calculi were removed that the cat would be neutered. It was also decided that histologic sections of muscles would be examined to verify the success of the fenbendazole therapy.

The histopathology on the muscle tissue removed at surgery revealed the presence of a few typical *Trichinella* cysts within the muscles of the cat. The fact that the cat had no additional signs of infection suggested that there was no need to administer any further treatment for the infection.

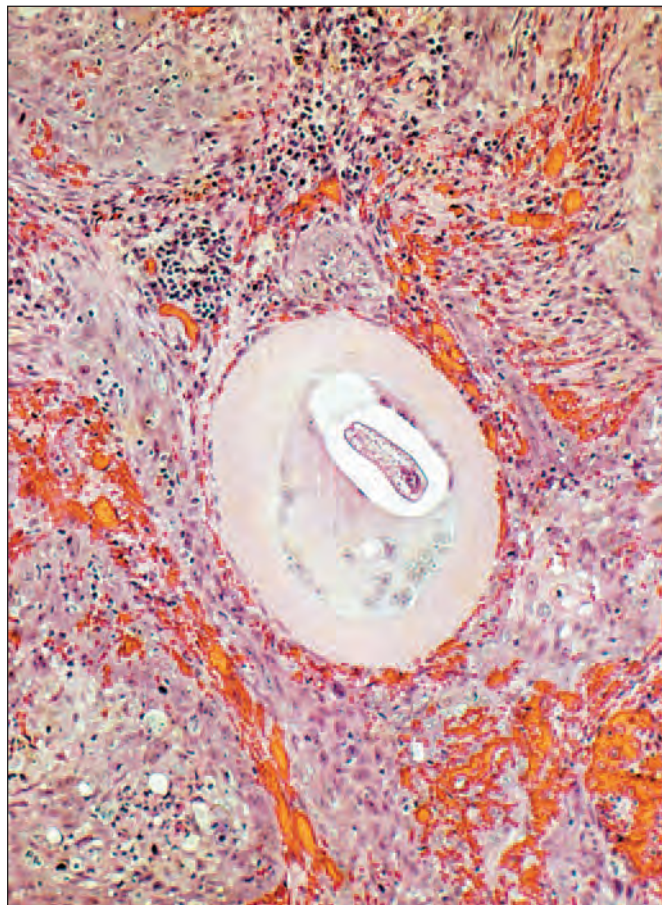


Figure 5.23. Section through the muscle of a cat showing a muscle cell infected with a first-stage larva of *T. spiralis*.

CASE 13

Signalment. Two-year-old spayed female domestic shorthair cat

History. The cat's owners had moved from Ann Arbor, Michigan to Seattle, Washington just 2 months ago. The cat has developed a serious cough, appears dyspneic, anorexic, and has lost weight over the last two weeks.

Physical Examination. The cat was found to have harsh lung sounds. There did not appear to be any upper airway involvement. A CBC revealed slightly elevated eosinophils, but otherwise the panel was normal.

Initial Assessment. Verminous pneumonitis, most likely heartworm

Presumptive Diagnosis. Heartworm disease

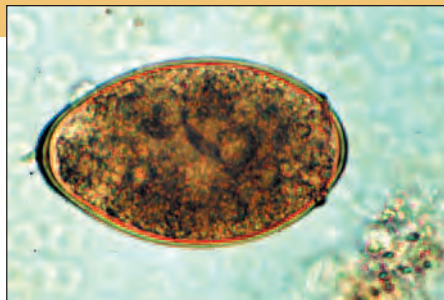


Figure 5.24. Egg of *Paragonimus kellicotti*. Note the seated operculum and the abopercular bump or spine-like structure on the opposite end of the egg.

Diagnostic Plan

Based on the signs, heartworm was given a fairly high status on the rule-out list. Thus, both heartworm antigen and antibody tests were performed, and both were negative for heartworm. The fecal examination revealed the presence of roundworm eggs, the eggs of the capillarid *Eucoleus aerophilus*, and the eggs of the trematode *Paragonimus kellicotti*.

The decision was made to obtain radiographs in order to verify the presence of *P. kellicotti* in the lungs and to verify the negative heartworm status of the cat. It was not expected that the *E. aerophilus* infection would produce any signs consistent with radiographic changes.

Diagnosis: *Paragonimus kellicotti*

Outcome

The radiographs revealed a cavitation lesion in the right caudal lobe of the lung. There was also consolidation of the lungs as disclosed by interstitial opacities. It was expected that the cat had been infected while still in Michigan. The progressively worsening of the signs was considered to be the reaction within the lungs to the eggs deposited within the tissues.

It was decided that the cat would be treated with praziquantel (Droncit) for the lung flukes, and praziquantel was administered for 3 days at 50 mg/kg. Radiographs were taken 1 week after treatment and the lesions appeared to have worsened, presumably because the worms within the nodule had died. Two weeks later, the cat was started on a 7-day course of fenbendazole (Panacur) at 50 mg/kg with the intention of clearing the capillarid infection and ensuring the death of the lung flukes.

A fecal examination and radiographs taken two months after the initial presentation revealed no signs of the lung fluke infection.



Figure 5.25. Radiograph (ventral view) showing multiple patchy infiltrates in the right caudal lobe, one of which is cavitory.



Figure 5.26. Radiograph (lateral view) showing extensive infiltrate with a cavitory lesion in the right caudal lobe. The cavitory lesion probably contains a pair of adult trematodes. The infiltrate is probably due to a reaction to the eggs in the tissues.

Chapter 6: Parasites Detected in Urine—Case Study

CASE 14

Signalment. Five-year-old intact male poodle

History. The owner had noticed that the dog was straining during urination and that there were a few drops of blood in the urine. It was also noticed that the dog was urinating more frequently. The appetite of the dog was slightly reduced over prior weeks.

Physical Examination. The dog seemed in general good health and had a rectal temperature that was only slightly increased over normal. The dog was obviously uncomfortable upon palpation and had an enlarged and turgid bladder. Palpation of the bladder revealed that it was painful. The dog was sedated, and the passage of a urinary catheter was uneventful and produced a volume of urine. There did not appear to be any significant signs of uroliths, and it was decided that a radiograph was not warranted.

Initial Assessment. The urine was noted to contain a number of red blood cells and a few white cells. The examination of the sediment revealed a large number of the eggs of the capillarid nematode, *Pearsonema plica*.

Presumptive Diagnosis. It was decided that the dog was suffering from urethral obstruction secondary to sterile cystitis that was likely caused by the infection of the bladder wall by *Pearsonema plica*.

Diagnostic Plan

It was decided that the dog would be treated and the urine examined for the presence of eggs again in a month.

Diagnosis: *Pearsonema plica*

Outcome

The dog was started on a 10-day course of fenbendazole administered as granules with the food at a dosage of 50 mg/kg body weight. There was one more apparent period of blockage that again required the passage of a catheter to provide relief. The dog was then without further signs. The examination of the urine a month later revealed no eggs of the capillarid nematode.



Figure 6.1. Egg of the capillarid nematode *Pearsonema plica*, which is one of the few eggs found in the urine of dogs. The species found in cats is generally considered to be *Pearsonema feliscati*.

Chapter 7: Parasites Detected in Blood—Case Studies

CASE 15

Signalment. Four-year-old intact male beagle

History. The dog was presented with serious respiratory disease. The owners first noticed signs in relation to rapid tiring during exercise. The dog's condition had progressively worsened, and it had had two occasions of syncope. Adopted from a shelter at about one year of age, the dog had lived in northern Colorado ever since its adoption. The dog had not been to the veterinarian recently and received its rabies vaccinations from a community service program.

Physical Examination. The dog had a severe cough and respiratory distress. Ascites was evident within the abdominal cavity. Blood samples showed signs of hemolysis.

Initial Assessment. The dog was in severe distress with significant right heart disease.

Presumptive Diagnosis. Congestive heart failure

Diagnostic Plan

Radiographs and blood examination were obtained. Radiographs of the heart and lungs revealed enlargement of the right side of the heart, the main pulmonary artery, and the pulmonary arteries in the lobes of the lung. There was also blunting and thickening of pulmonary arteries. The antigen test for heartworm was positive and numerous microfilariae were present in the blood films that had been prepared for CBCs. The hemolysis of the blood was suggestive of caval syndrome and ultrasound revealed worms within the caudal vena cava.

Diagnosis: Caval syndrome (*Dirofilaria immitis* infection)

Outcome

Because of the seriousness of the diagnosis of caval syndrome, it was decided that surgical intervention to remove as many as the worms as possible was the only means of saving the dog. The dog was sedated and under local anesthesia a jugular venotomy was performed and worms were removed from the dog using alligator forceps. The dog was carefully monitored over the next few days and recovered well. After a month, the dog was treated with melarsomine dihydrochloride (Immiticide®; Merial) that it tolerated very well. The dog was then started on routine heartworm preventative.

This case was surprising because the dog was living in an area where heartworm is not considered to be present. The dog was probably infected elsewhere before it made its way into the shelter from which it was adopted because of the long-standing nature of the infection.

Figure 7.1. Giemsa-stained microfilaria of *Dirofilaria immitis*.



CASE 16

Signalment. Five-month-old Labrador retriever puppy

History. This puppy had been born normal, but beginning in about the tenth week of life developed paralysis of the hind legs that continued to progress. The front legs also appeared to be somewhat involved, and the puppy has reached the point where it can barely move. One of the other puppies in the litter died soon after birth, but the other puppies in the litter have not shown signs of infection. The mother appears normal.

Physical Examination. The puppy was bright and alert with no apparent signs of pain. There was contracture of the joints in both the hind legs and the front legs.

Initial Assessment. The presentation was of contracture of the joints probably resulting from polyradiculitis.

Presumptive Diagnosis. The presentation was fairly representative of the clinical presentation of neonatal neosporosis.



Figure 7.2. This Labrador retriever puppy was most likely infected in utero by *Neospora caninum*. The signs are evident of the contractures of the limbs that occur when the nerves are damaged in the growing dog. The bandages on the feet indicate the attempt to protect the dog from abrasions as it tries to walk.

Diagnostic Plan

For confirmation, serum was collected and sent to a diagnostic laboratory.

Diagnosis: *Neospora caninum* infection

Outcome

As expected, the results of the serology supported the clinical diagnosis. The owners had already been prepared for the diagnosis of neosporosis and the suggestion of euthanasia. The dog was euthanized. A request was made

that that puppy be submitted for a necropsy and the owners agreed. The histopathology revealed organisms within the nerve fibers.

The owners were informed that cases of neonatal infection with *Neospora caninum* are passed from the bitch to the puppies during repeated pregnancies. Thus, the suggestion was made that the female be spayed and not bred again for fear of having a repetition of the signs observed in this puppy.

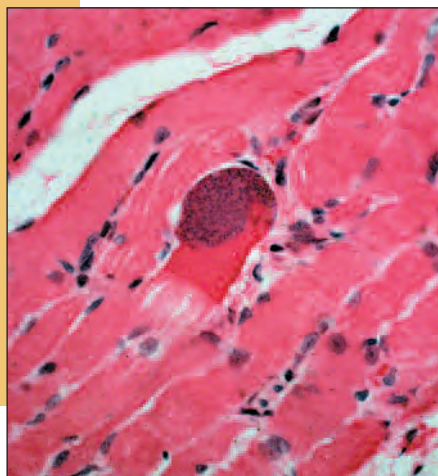


Figure 7.3. Section through muscle showing a cyst in a muscle cell.

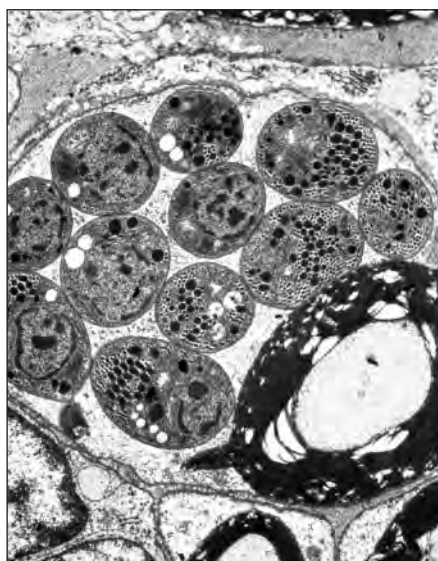


Figure 7.4. Electron micrograph of the organisms in a Schwann cell showing the damage to the myelin sheath (Image courtesy of the late Professor John Cummings).

CASE 17

Signalment. Four-month-old intact male mastiff
History. The 18-kg dog from Oklahoma had become inappetent for 2 weeks prior to referral. During this time the dog had had loose stools, and lost almost a kilogram of body weight. The dog had been treated with several courses of antibiotics including enrofloxacin, trimethoprim sulfa, and chloramphenicol. The dog was current for all vaccinations, and had received prednisone for 3 days just prior to presentation.

Physical Examination. The dog was thin with mild lymphadenopathy. The dog had a slight fever (40°C). Moderate nonregenerative anemia that was normochromic and normocytic was found, as well as a slight lymphopenia. No parasites were detected on fecal examination.

Initial Assessment. Fine-needle aspirates were taken from an enlarged popliteal lymph node. The smears made from the aspirates were stained with Wright-Giemsa stain. The smears contained mature lymphocytes and scattered lymphoblasts. There were numerous extracellular organisms identified as trophozoites of *Trypanosoma cruzi* as identified by their fusiform shape, large posterior kinetoplast, central nucleus, and undulating membrane.

Presumptive Diagnosis.
Trypanosoma cruzi
infection

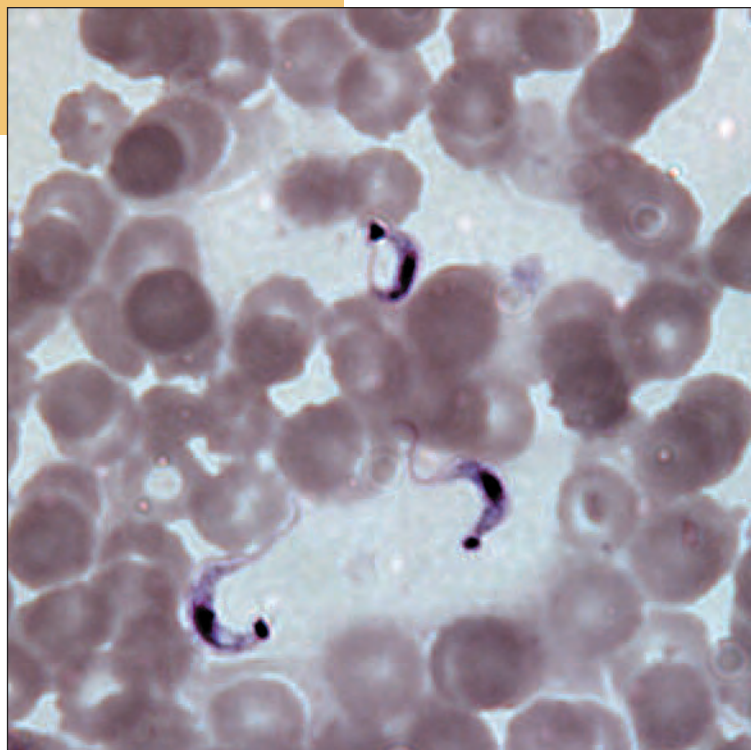


Figure 7.5. This Giemsa-stained blood film shows three trypomastigotes of *Trypanosoma cruzi*, which, unlike those of the African trypanosomes, tend to be “C”-shaped rather than “S”-shaped when seen in blood films. The trypomastigote of *T. cruzi* has a very large kinetoplast at the posterior end that seems to bulge out of the cytoplasm and dividing forms are not seen within the blood.

Diagnostic Plan

Verification of the infection was performed by further testing with cell culture isolation of organisms from additional lymph node aspirates and by serology using an indirect fluorescent antibody test. Both tests verified the result of the initial observation made in the stained aspirate.

Diagnosis: *Trypanosoma cruzi*

Outcome

Because of the poor prognosis for treating this infection in dogs, it was suggested that the owner consider euthanasia for this animal. This was the outcome chosen by the owner, and permission also was obtained to submit the dog for a necropsy and a pathology examination. Histologic examination of the various muscle tissues, including the heart, revealed large numbers of the amastigote stages of the organisms in the tissues.

Typically, human infections are acquired when people rub the feces of the large blood-feeding triatomid bugs into the bite wound caused by the arthropod. In dogs, it is believed that the infection may be acquired by the ingestion of the bugs themselves. When the bugs are crushed within

the mouth of the dog, the organisms will initiate the infection by penetrating the buccal mucosa.

The trypomastigote stages in the blood of dogs are directly infectious to other hosts, including people; thus, the handling of blood from potentially infected animals needs

to be performed with care to ensure that needle sticks are prevented. In most chronic cases, blood-stream stages are rather rare; transmission with blood stages would typically be a problem in blood donor dogs, however, and they should be tested for this potential infection.

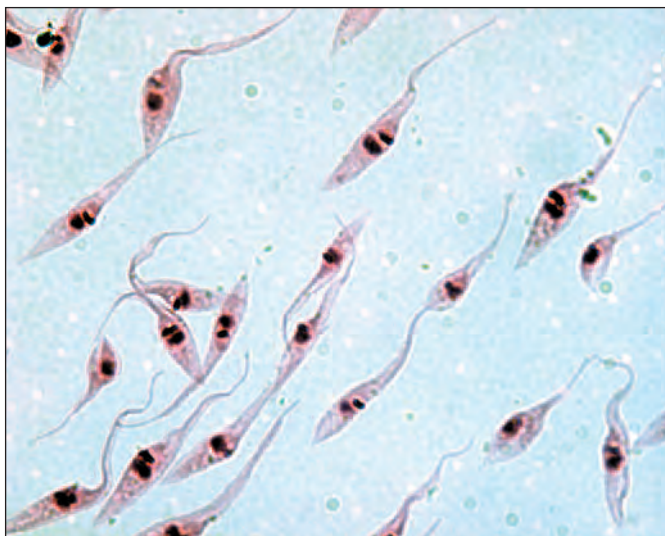


Figure 7.6. This Giemsa-stained preparation shows the promastigote stages of *T. cruzi* that are grown in culture.

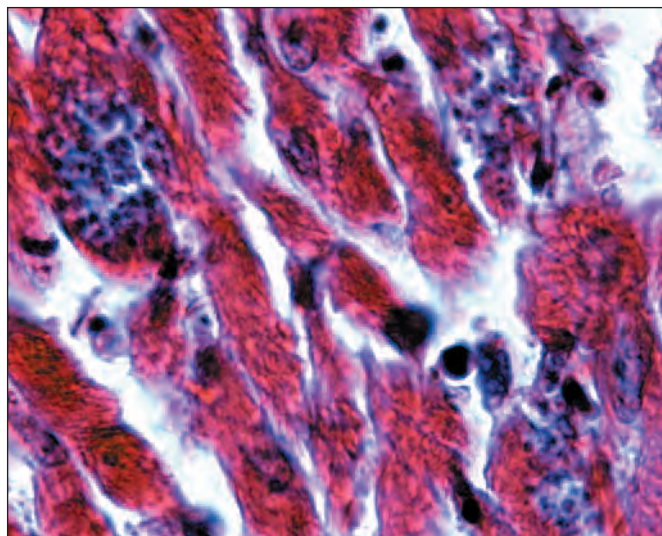


Figure 7.7. This histologic section through muscle tissue shows two nests of amastigotes within muscle fibers.

CASE 18

Signalment. Four-year-old intact male German Shepherd dog

History. The dog had spent the past six months in Greece with its owner's family. Since returning home, the dog had shown signs of anorexia, progressive loss of body weight, and depression. While in Greece, the dog had been healthy, had seen a veterinarian for routine care, and been on routine heartworm preventatives and received monthly imidacloprid (Advantage®; Bayer) for flea prevention. The dog was mainly an indoor dog, going outside only occasionally, mainly on walks in the country with the family. Fecal consistency and frequency of defecation and urination were reported to be normal.

Physical Examination. The dog was noted to have a fever (rectal temperature 40.5°C) and pale mucous membranes. Upon palpation the spleen was found to be enlarged. There were no dermal lesions. The hematocrit was found to be 28%.

Initial Assessment. Given the travel history and signs, it appeared that systemic disease was present that could be related to several infectious agents including *Leishmania*, *Ehrlichia* species, *Rickettsia* species, or *Babesia*.

Presumptive Diagnosis. Infectious disease potentially related to one or several infectious agents that may have been acquired in Greece

Diagnostic Plan

Blood smears were prepared and stained with Diff-Quik and Wright's/Giemsa stain. Serum samples were taken and submitted for serology for canine ehrlichiosis and rickettsiosis. It was decided that further workup for leishmaniasis would await the results of the testing for these other infections. The blood smear revealed merozoites of *Babesia canis* in red blood cells. After several days the results of the serology were returned, and the dog was serologically negative for the other infections.

Diagnosis: *Babesia canis*

Outcome

Treatment was initiated using imidocarb dipropionate (Imizol®; Schering-Plough Animal Health) at 6.6 mg/kg subcutaneously. The treatment was repeated at two weeks (total of two treatments). One month after the first treatment, blood films were again examined and no organisms were demonstrated in the blood smears. By one month after treatment, the spleen was still somewhat enlarged, but the hematocrit was within normal limits.

Because of the signs present, leishmaniasis was also suspected as a potential cause or concomitant infection in this case. After 2 months the dog's condition had markedly improved, but it was decided that it would be best to verify

that the dog was negative for *Leishmania*. Thus, serum was collected and sent to a commercial laboratory (Heska Corp., Fort Collins, CO). The results of the test revealed that the dog was negative for antibodies to *Leishmania*. Six months after treatment for the *Babesia canis*, the dog did not have any signs that might be related to either pathogen.

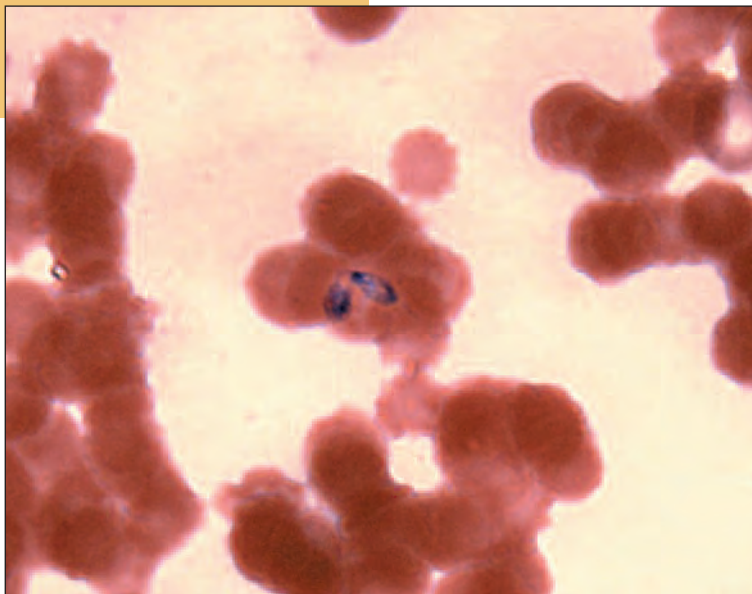


Figure 7.8. Giemsa-stained blood film showing a red blood cell containing a pair of tear-drop shaped *Babesia canis* merozoites in one cell.

CASE 19

Signalment. Five-year-old male Siamese cat

History. The cat was presented with an illness of unknown duration. The owner reported that the cat had disappeared for several days and returned quite ill. After trying to nurse the cat for two days without improvement, the owner brought the cat to the clinic. The cat was an indoor-outdoor cat living in suburbia in southern Arkansas.

Physical Examination. The cat was depressed with a rectal temperature of 37.5°C. At presentation, the cat had severe ascites and icterus and relatively severe respiratory distress. On-site ELISA tests were negative for FIV and FeLV. The stool appeared normal, but the cat had not eaten for the past several days. The urine was dark brown. The hematocrit was 22%.

Blood slides were prepared and stained. Red blood cells contained elements identified at the time as *Haemobartonella*.

Initial Assessment. Intravenous administration of lactated Ringer's solution was started and the cat was given a subcutaneous injection of enrofloxacin.

Presumptive Diagnosis. Systemic infection, haemobartonellosis, or perhaps systemic disease due to perforated intestinal tract or trauma

Diagnostic Plan

Monitor and stabilize.

Outcome

The condition of the cat rapidly deteriorated. Intravenous fluids were administered but the cat continued to develop worsening respiratory distress. The cat died about 48 hours after being admitted to the hospital.

A necropsy was performed and liver, lungs, kidney, and spleen appeared congested. Tissue samples from these organs were submitted to a diagnostic laboratory for possible determination as to cause of death. Histopathology revealed the occlusion of all vessels in the organs submitted with highly enlarged cells containing numerous merozoites of the causative agent, *Cytauxzoon felis*. Reexamination of the stained blood film revealed that the organisms that were present were *C. felis*, not *Haemobartonella felis*.

Diagnosis: *Cytauxzoon felis*

Infections with the tick-transmitted *Cytauxzoon felis*, which naturally exists in bobcats without signs of overt disease, are often fatal to domestic cats. Some domestic cats

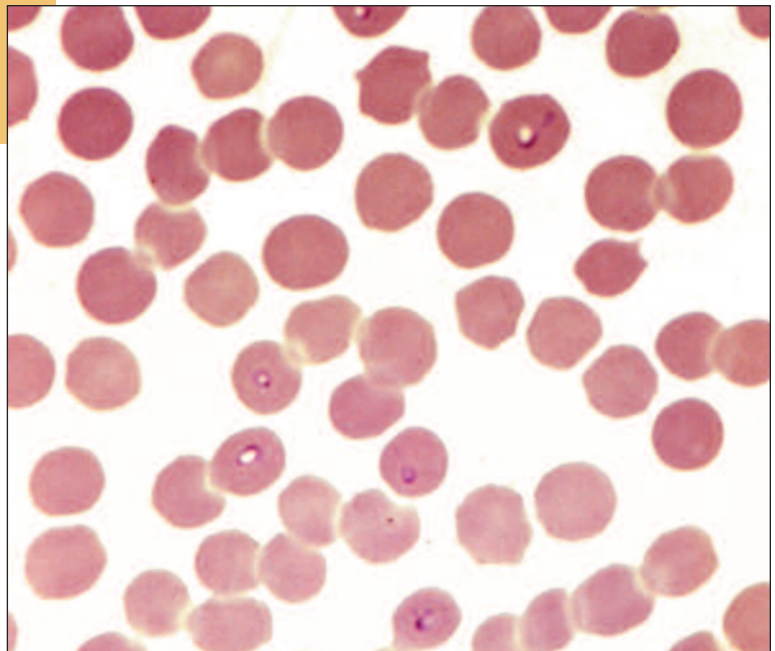


Figure 7.9. Stained blood film showing several red blood cells parasitized by the small ring-shaped trophozoites of *Cytauxzoon felis*.

have survived the infection, however; some of these surviving cats have received treatment (eg, with imidocarb dipropionate [Imizol] or antibiotics) while some received no treatment. It is believed that the isolates may differ in their virulence.

The very small ring stages of *Cytauxzoon* present in the red blood cells of cats are quite small compared with *Babesia canis* of dogs. They resemble the smaller form of

canine babesiosis, *Babesia gibsoni*. Unfortunately, cats that are succumbing to acute disease due to cytauxzoonosis often do not present with stages in red blood cells; the stages in the macrophages that are occluding the blood vessels occur prior to the establishment of the majority of the red cell stages. A bone marrow or lymph node biopsy could perhaps aid in improving the diagnosis of infection with the identification of the hypertrophied macrophages.

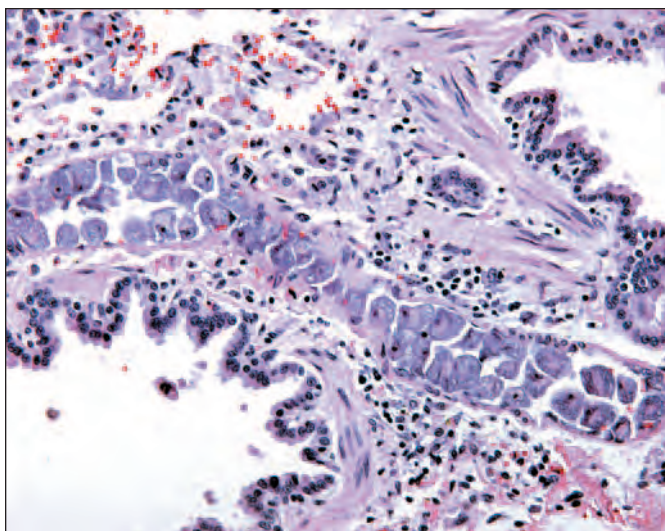


Figure 7.10. Section through the lung tissue of the cat. The image shows a longitudinal section through a blood vessel that has its lumen completely occluded by the large hypertrophied macrophages parasitized by *C. felis*.

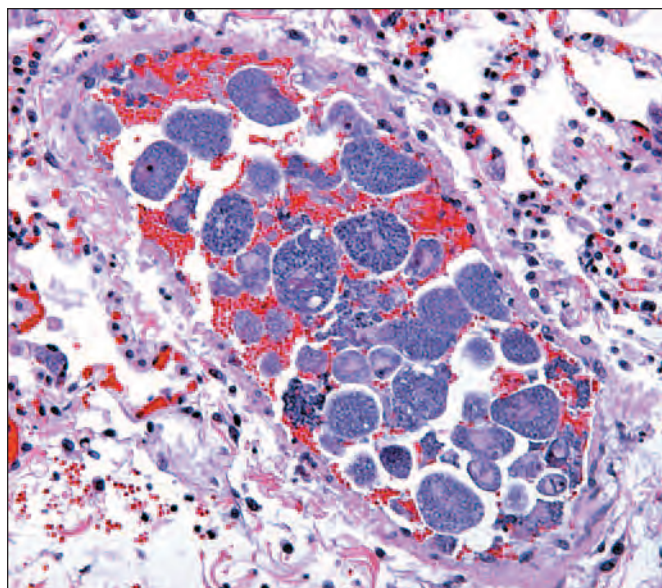


Figure 7.11. Section through the lung tissue of the cat. The image is a cross section of a vessel occluded with the same stages present in the longitudinal section in Figure 7.10.

Chapter 8: Parasites Detected in Tissue Samples— Case Studies

CASE 20

Signalment. Five-year-old spayed female coon hound.

History. About 3 weeks before presentation to the clinic, the dog began to have diarrhea. Otherwise, the dog has been healthy except for some weight loss during the past month. The dog is on routine heartworm prevention and wears flea collars that are changed monthly. Dog recently moved from rural Louisiana to New Orleans.

Physical Examination. The dog appears normal other than being slightly thin. A few thickened bowel loops and some fibrosis of the liver were noted on palpation. There appears to be a slight eosinophilia evident from the bloodwork, and the urine appears to contain increased protein.

Initial Assessment. The dog appears to have enteritis of unknown etiology.

Presumptive Diagnosis. Enteritis caused by viruses (parvovirus, coronavirus), bacteria (colibacillosis, salmonellosis), parasites (whipworms, hookworms, heterobilharziasis), and toxins (foreign material, lead, zinc), hemorrhagic gastroenteritis, intussusception, systemic disease (renal or heart failure)

Diagnostic Plan

Perform a fecal examination, ultrasound scan, and fecal antigen tests for viruses.

Outcome

No viruses were diagnosed and no parasites were detected using the stationary flotation test. A centrifugal zinc sulfate flotation was performed to look for *Giardia*, but no organisms were found. Similarly, the antigen tests for *Giardia* and *Cryptosporidium* were negative. The ultrasound scan revealed slightly thickened bowel loops. Although the dog had been on a routine monthly heartworm preventative that would have removed other nematode infections, the dog was empirically treated with praziquantel/pyrantel pamoate/febantol (Drontal Tablets Plus).

The dog's condition does not change, and the diarrhea and weight loss appear to be worsening. Therefore, an exploratory laparotomy is performed. Upon opening the abdominal cavity, the liver is noted to be firm and mottled. A small biopsy is taken of the liver. Small elongate worm-like objects are noted in the mesenteric veins and one of these is collected.

Examination of the biopsy material reveals the eggs of the schistosomatid trematode, *Heterobilharzia americana*, in

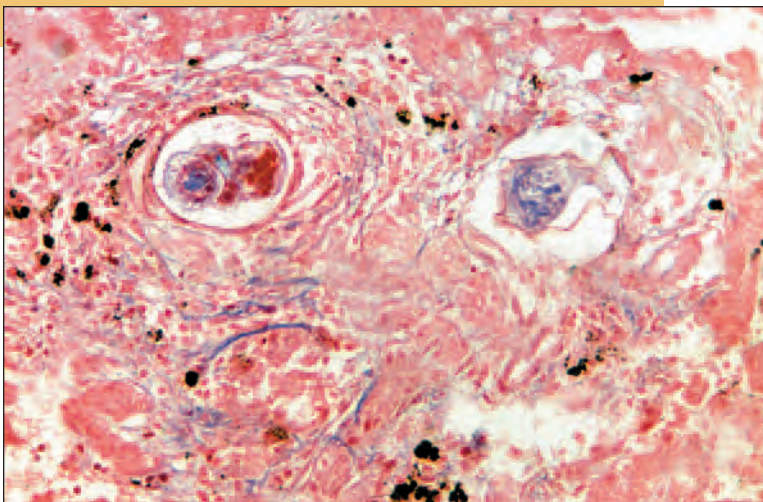


Figure 8.1. Histologic section through a liver biopsy specimen showing two eggs of the trematode *Heterobilharzia americana* in the hepatic tissue.

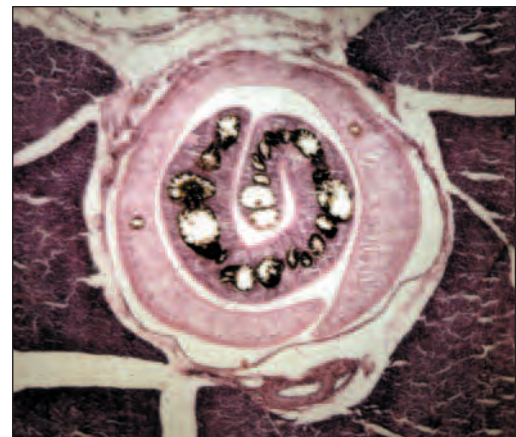


Figure 8.2. Histologic section through a vessel in the liver biopsy specimen showing a cross section through an adult male wrapped around the thinner female worm. The darker areas within the worm are the intestine.

the hepatic tissues. Also, the worm-like object recovered from the mesenteric vein is noted to be an adult pair of flukes.

Diagnosis: *Heterobilharzia americana*

After the diagnosis is made, further examination of the feces using a simple sedimentation of the feces after dilution in saline reveals a large number of the eggs of this trematode species.



Figure 8.3. Adult male and female *H. americana* (stained red) recovered from a mesenteric vein. The larger, thick-bodied male is holding the thinner female within his gynecophoral canal.

The dog was treated as an inpatient with praziquantel at the elevated dose of 50 mg/kg (10 times the dose typically used to treat intestinal parasites). The dog tolerated the treatment well, and within about 6 weeks had returned to normal behavior and weight. The fact that the dog was now living in the city meant that it would probably not be susceptible to a repeat infection from swimming in bayous containing infective cercariae.



Figure 8.4. Egg of *H. americana* recovered using a fecal sedimentation method. Notice that the egg contains a fully developed miracidium and would hatch if it was placed in water instead of saline. Image courtesy of Dr. J. Flowers, North Carolina State University, Raleigh, NC.

CASE 21

Signalment. Nine-year-old intact male domestic shorthair cat

History. The cat, originally from Oklahoma, was presented for alopecia on the pinna of the ears.

Physical Examination. The cat had alopecia that involved about two thirds of the inner pinna and one third of the external pinna on both ears. There was also moderate scaling along the pinnal margins of both ears. In all other aspects, the cat appeared relatively normal. A routine fecal examination was found not to contain any detectable parasite stages. The cat had been receiving ivermectin (Heartgard for Cats) as a heartworm preventative and regular imidacloprid (Advantage) administration for flea control.

Initial Assessment. The cat was considered to have a dermal condition consistent with dermatophytosis, mange, or leishmaniasis.

Presumptive Diagnosis. Infectious dermatitis due possibly to dermatophytes (*Microsporum* or *Trichophyton*), mange (notoedric or demodectic), or leishmaniasis.

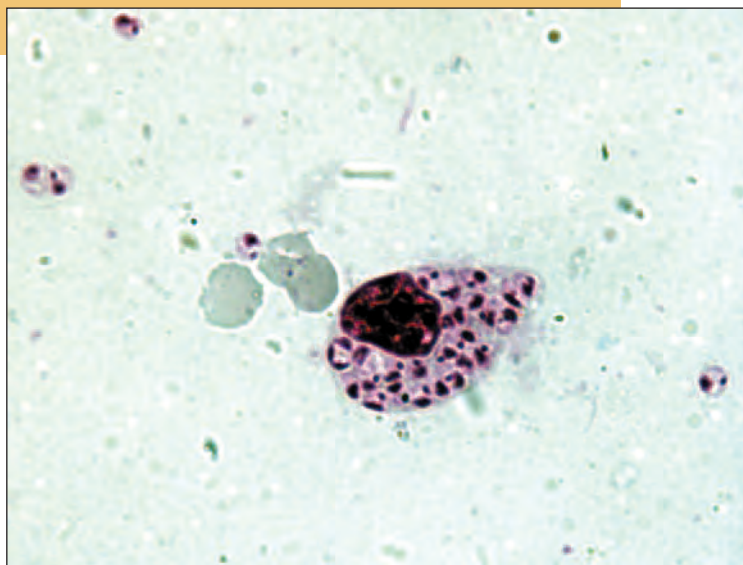


Figure 8.5. This macrophage in a Giemsa-stained preparation from skin shows a macrophage full of the amastigote stage of *Leishmania mexicana*. Morphologically, the amastigotes cannot be distinguished from those of other species of *Leishmania* and when in macrophages cannot be distinguished from those of *Trypanosoma cruzi*.

Diagnostic Plan

The cat was worked up for all three conditions. The ears were examined with a Wood's lamp and no fluorescence was observed. Hairs from the periphery of the lesion were plucked for fungal culture using a dermatophyte test medium and found to be negative when examined 6 days later. Skin scrapings prepared in mineral oil were found to contain neither *Notoedres* nor *Demodex* mites. A Wright-Giemsa-stained smear of a skin scraping revealed numerous cells containing amastigotes leading to a diagnosis of leishmaniasis.

Diagnosis: *Leishmania mexicana*

Outcome

The diagnosis of leishmaniasis was made on the basis of the stained smears from the ear lesions. Visceral leishmaniasis is considered rare in cats, thus the infection was considered to be a cutaneous infection with *Leishmania mexicana*. Potential treatment options consisted of surgically removing the pinna of both ears or experimental therapy with a six-week course of itraconazole. The owner chose to proceed with the excision of the pinna. The surgical removal of the affected tissue was uneventful, and the cat healed without any recurrence of the lesions.

The infection in the cat provided some concern as to potential zoonotic transmission to the owner or personnel in the hospital. This would have had to occur through the direct transmission of the organism into a lesion or open sore on the skin of the person handling the cat. Although the risk was deemed to be small, it was felt that it was possible that the lesions might not clear spontaneously and the threat would be present. Thus, the decision to treat was considered the only option.

CASE 22

Signalment. Seven-year-old intact male Norwich terrier
History. The dog had recent history of alopecia on the face and the back of the knees on the front legs. Some hair loss was also noted on the ears. This has been occurring for the last several months. The dog seemed to be somewhat, but not excessively, pruritic. There has been no significant change in diet and no changes in the house where the dog lives. The dog is up-to-date on vaccinations and receives its 6-month-long heartworm preventative every summer. Two years ago, there had been a flea problem, but the use of milbemycin oxime/lufenuron (Sentinel®; Novartis) in the heartworm prevention schedule had brought the flea problem under control. A fecal examination performed at the time of the last visit, 6 months ago, was negative for any parasites.

Physical Examination. The dog was bright, alert, and found to be normal upon palpation. Alopecia was observed on the muzzle and on the backs of the knees. The ears appeared to be inflamed at their tips. Examination with a Wood's lamp revealed no fluorescence.

Initial Assessment. Dermatologic infection: Potential differentials included demodicosis, sarcoptic mange, ringworm

Presumptive Diagnosis. Dermatologic lesions consistent with dermatophyte or mite infection.



Figure 8.6. Adult female *Sarcoptes scabiei* mite recovered in a skin scraping. The large spines on the body are readily apparent.

Diagnostic Plan

Skin scrapings were performed for diagnosis. Scrapings were stained with Diff-Quik and Gram's stain. Scrapings were also "teased" apart in mineral oil on the slide using fine needles before a coverslip was placed on the material for examination.

Diagnosis: *Sarcoptes scabiei*

Outcome

Sarcoptes scabiei mites (recognized by their round shape and long unsegmented pretarsi) were found in the skin scraping. The dog was treated with a subcutaneous inoculation of ivermectin (200 µg/kg). One month later, the lesions had significantly improved, and the dog was started on monthly selamectin (Revolution) as a heartworm preventative. Within six months, the dog had no further lesions and skin scrapings taken at that time were negative for mites.



Figure 8.7. Adult male *S. scabiei* mite recovered in a skin scraping.

CASE 23

Signalment. Eight-year-old spayed domestic shorthair cat

History. The cat had been adopted from an animal shelter 2 months earlier and had problems with improper urination around the house. The cat was brought into the clinic for a consultation on minimizing the urination problem. A routine physical examination was performed.

Physical Examination. The cat appeared to be normal, and its coat was in good condition. There were enlarged inguinal lymph nodes, and what was suspected to be a pellet from a pellet gun was felt within the right rear thigh; however, the lack of any lesion suggested that this was an old wound. The pinnal-pedal reflex was positive, and the ears were filled with a fine gray-brown grit suggestive of ear mites.

Initial Assessment. Normal cat with behavioral problems related to urination. The lesion from the pellet was considered without need for treatment. The pinnal-pedal reflex and the appearance of the cerumen were highly suggestive of ear mites.

Presumptive Diagnosis. Behavioral problems and potential ear-mite infestation

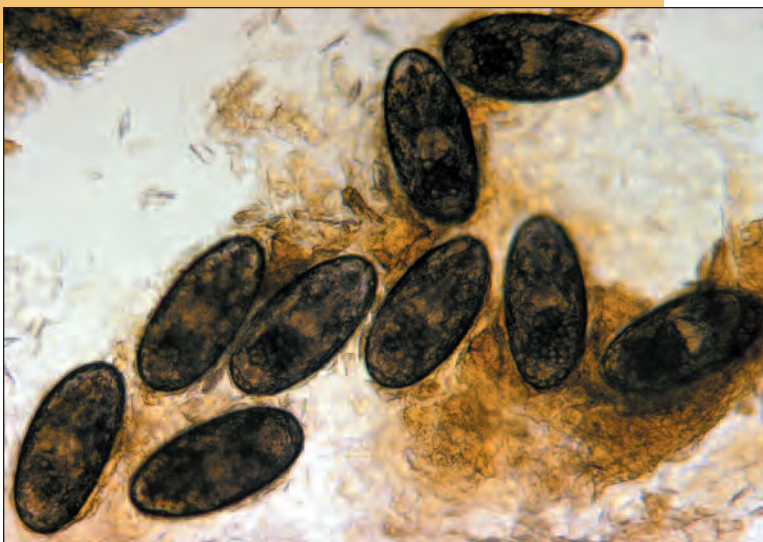


Figure 8.8. Eggs of *Otodectes cynotis* recovered from the ear canal by swabbing. The developing mites can be seen within the eggshells.

Diagnostic Plan

Workup for potential urinary tract infection; examination of the ears for ear mites

Diagnosis: *Otodectes cynotis*

Outcome

The otoscopic examination revealed hundreds of mites in each ear. This was also verified by placing a small quantity of the material on a slide and examination with a microscope. Massaging 1 mL of mineral oil into each ear canal and wiping with cotton swabs cleaned the ears. The condition was then treated with the application of ivermectin (ACAREXX®; Blue Ridge Pharmaceuticals/IDEXX Laboratories) into each ear.

The workup for a potential urinary tract infection did not reveal an underlying infection. The elimination improved with the addition of extra litter boxes, but the problem, although reduced, still remained. Treatment then began on determining whether the cat was dealing with substrate aversion, location aversion, or substrate preference. The owner showed a strong willingness to work through the various tests until the problem was rectified.



Figure 8.9. An adult female *O. cynotis* mite recovered by an ear swab.

CASE 24

Signalment. Four-year-old female hound mix
History. This hound was a regular active member of the household in southern Wisconsin, often accompanying the family to local parks and on camping and canoeing treks. The dog has traveled extensively on different trips to various southern states, has gone on several long trips to the mountains of Colorado, and hiked much of the Appalachian Trail. The dog receives routine veterinary care and has been receiving milbemycin oxime/lufenuron (Sentinel) and fipronil (Frontline) regularly since soon after birth. One week earlier, the dog developed a bump on the inside of its left foreleg. The foreleg lesion developed into a papule that ruptured. The owners thought they could see what might be a white thread or a bit of what might be a tendon in the sore. The dog does not seem to have any other signs of infection.

Physical Examination. The dog appeared to be in good health, with no signs of generalized infection. There was no fever, and there were no significant findings from a CBC and chemistry panel.

Initial Assessment. The dog had a lesion at the elbow that could be consistent with trauma or some type of draining tract lesion. A decision was made to perform Diff-Quik and Gram stains of the site of the lesion. The examination of the slide reveals some polymorphonuclear neutrophils (PMNs) and eosinophils, and a few gram-negative cocci are present on the slides. Some long worm-like structures, about 600 μm in length, were also noted.

Presumptive Diagnosis.
Dracunculus, heartworm, rhabditiis, or some other skin-dwelling nematode parasite



Figure 8.10. Leg of a dog with a protruding female of *Dracunculus insignis*.

Diagnostic Plan

Examine the larvae for morphologic details. The larvae can also be examined in a fresh saline preparation; larvae of *Dracunculus* should have a long and very pointed tail.

Diagnosis: *Dracunculus insignis*

Outcome

It was decided that surgery would be the best approach to removing the worm. The dog was anesthetized and a surgical incision was made along the area of the worm's free end. With careful dissection it was possible to remove the entire worm. The initial concern was that the worm would require an incision to extend a long distance and cause a significant scar. Fortunately, the worm was coiled back upon itself several times, allowing relatively easy removal.

A second potential concern was the possibility that there might be more than one worm within the dog. No second worm was noticed at the site of incision, but there was worry that worms might be present in other tissues. Ivermectin was not administered because it is believed to have little effect against the adults of this parasite. The owners were informed that the dog had been infected by the ingestion of water containing copepods of this nematode, which is typically found in mink or other carnivores. There was very little to no risk that the owners had acquired the same parasite in a similar fashion.



Figure 8.11. Larva of *D. insignis* from the fluid recovered from around the area where the worm was protruding from the tissues of the leg.

CASE 25

Signalment. Four-year-old intact male chocolate Labrador retriever

History. This dog from southern Vermont was examined as part of a routine health examination. The dog was on a routine heartworm preventative and past fecal examinations had been negative. Although usually an indoor pet, the dog does occasionally have access to the grounds around the house, which is on the edge of town.

Physical Examination. The dog seems healthy and fit. During the physical examination an adult tick was removed from the base of the right ear. In all other respects the dog appeared to be normal.

Initial Assessment. Tick infestation

Presumptive Diagnosis. *Ixodes dammini* (or *Ixodes scapularis*)

Diagnostic Plan

The tick was identified as an adult of the genus *Ixodes* by the identification of the preanal groove that is characteristic of this genus. Three questions arise relative to the presence of the tick. First, is the isolated tick a species of *Ixodes dammini* or some other *Ixodes* species, eg, *Ixodes cookei*, that is less likely to vector Lyme disease? Is the tick harboring the Lyme disease agent, *Borrelia burgdorferi*? Third, is the tick sufficiently engorged to have transmitted the agent to the dog?

Out of caution, the dog was started on a course of tetracycline. It also was recommended that the dog be placed on fipronil (Frontline) for tick control. The tick itself was submitted to a local diagnostic laboratory for specific identification and to determine by polymerase chain reaction (PCR) whether it was infected with the spirochetes.



Figure 8.12. An adult female *Ixodes dammini* (*Ixodes scapularis*) tick that has fed for two days.



Figure 8.13. An adult female *I. dammini* tick that has fed for four days.



Figure 8.14. An adult female *I. dammini* tick that has fed for six days.

Outcome

The laboratory reported that the tick was indeed *Ixodes scapularis* and that it had been positive for the Lyme disease agent. The large size of the tick at the time of collection suggested that the tick had been on the dog and feeding for at least several days and would have had time to transmit the agent to the dog. Thus, the course of antibiotics was probably the correct course of action.

Diagnosis: *Ixodes dammini* (*Ixodes scapularis*)

Unlike humans, who are typically infected with Lyme disease by the nymphal ticks, dogs are typically infected with the agent by the adult ticks. Although there are rare

instances of transovarial transmission from the female to the eggs, such transmission is rare, and the larval stages are almost never infected with the agent. It is only when the larvae feed on an infected rodent that they acquire the infection that is then present in the nymphal and adult stages. The tick has to remain attached to the host for more than 24 to 48 hours for the spirochetes to make their way into the salivary glands and through the tick bite into the dog. Thus, if the ticks are removed the same day they are acquired the chances of transmission to the dog are minimal. Care should be taken not to crush the tick during removal because then the spirochetes may be released and enter the bite wound.



Figure 8.15. An unfed nymph of *I. dammini*. This is the stage that usually transmits Lyme disease to humans.



Figure 8.16. An unfed nymph of *I. dammini*; ventral view to show the preanal groove.



Figure 8.17. A just-hatched larva of *I. dammini* and its eggshell. The preanal groove cannot be seen on this dorsal view of the larva.

CASE 26

Signalment. Four-year-old intact domestic shorthair cat

History. The owners have noticed a swelling on the underneath of the cat's right jaw.

Physical Examination. The cat appears fully responsive to handling. There is no sign of pain at the site of the swelling. The swelling appears firm and seems to be somewhat larger than 1 cm in diameter. A close examination of the lesion reveals a small opening in the skin near the center of the lesion.

Presumptive Diagnosis. Possible foreign body or myiasis



Figure 8.18. Lesion in the cheek of a cat from which a *Cuterebra* maggot (bot) was extracted.

Diagnostic Plan

Excision

Outcome

After the application of local anesthetic, a small incision was made at the location of the opening in the skin. At this time, it was noted that there was a living organisms located within the site. The organism was most likely the maggot of a species of *Cuterebra*. The maggot was carefully dissected out of the tissue. The large size and the large black spines identified the maggot as a *Cuterebra*. Other than washing of the wound, no other treatment was administered.

Diagnosis: *Cuterebra* species

This case occurred in northern Arizona, and similar cases occur throughout much of North America. In the northeastern United States, seasonal infections occur mainly in August through September. The lesions can present as in this case or as more serious systemic disease. There appear to be three major forms of presentation, a transitory cough possibly related to the migration of larvae through the lungs, the development of the mature maggot in a warble in the skin, or the unfortunate migration of the maggot within the nervous system, eg, the brain or spinal cord. Occasionally, maggots have been removed from the nasal cavity or from the orbit of the eye.



Figure 8.19. The extracted maggot of the genus *Cuterebra*. The maggot is identified by its large size, rows of black spines, and if examined closely on the posterior end, the characteristic spiracles (not obvious in this illustration at this magnification).

Part III

Glossary of Terms

-A-

Acid/ethyl acetate sedimentation: A diagnostic method in which a slurry is prepared from fresh feces, centrifuged, and suspended in an acid solution (typically dilute HCl). Ethyl acetate is added and, after recentrifugation, the final sediment is examined under a microscope for parasites. See also Sedimentation assays.

Amastigote stages: A stage in the life cycle of many trypanosome protozoa consisting of a round or oval cell with a nucleus, kinetoplast, and basal body but lacking a free-flowing flagellum.

Antigen tests: Diagnostic tests that detect the antigens of certain parasites when they are present in a fecal or blood sample. For fecal samples, solid-phase immunoassays and enzyme-linked immunosorbent assays (ELISAs) are now available for *Cryptosporidium* and *Giardia*. For blood samples, antigen test kits are available for *Dirofilaria immitis* to determine whether a dog has a heartworm infection.

Axostyle: An elongate supporting rod or tubular structure passing through the longitudinal axis of some flagellate protozoa, such as trichomonads. Can be single or multiple, filamentous or rigid, and often projects out the posterior end.

-B-

Baermann apparatus: This method is used to diagnose nematode infections in which larvae, rather than eggs, are shed in the feces. The device consists of a funnel attached to a piece of rubber tubing with a clamp; fecal material is placed in gauze or a tea strainer and suspended in water in the funnel. Larvae migrate out of the feces and settle to the bottom of the tube, where they can be harvested by placing a drop out of the tip onto a slide or by spinning the contents down in a centrifuge tube.

Blood smears: A small sample of blood is applied to a slide for study under a microscope. Typically, the blood is dried on the slide and then fixed and stained using several different methods. In a “thin” blood smear, the goal is to disperse the blood using the edge of another slide so that the smear is only one cell thick.

Bone marrow aspirates: A small volume of bone marrow removed from the pelvis or sternum under general or local anesthesia is examined under a microscope to identify abnormalities in the developing blood cells.

Bradyzoites: A small, comma-shaped form of *Toxoplasma gondii* found in clusters encased in a pseudocyst. Bradyzoites, unlike tachyzoites, tend to be resistant to pepsin digestion and tend to contain stored glycogen.

-C-

Centrifugal sugar flotation: A commonly used diagnostic method for detecting parasite eggs and cysts in a fecal sample. The fecal sample is suspended in water, the slurry is sieved through cheesecloth and centrifuged, and the pellet is resuspended in a sugar solution with specific gravity of about 1.3. A coverslip is placed over the centrifuge tube, and the tube is recentrifuged. The coverslip is then removed and placed on a microscope slide for examination.

Copepods: Tiny aquatic crustacean arthropods that serve as the intermediate host for the parasites *Diphyllobothrium*, *Dracunculus*, and others.

Culture, fecal: A diagnostic method for the detection of protozoans in which culture medium is inoculated with fecal swabs. A test kit is commercially available for the detection of trichomonads using pouches of culture media that grow trichomonads at room temperature.

-D-

Diff-Quik stains: Giemsa-type stains with a simplified methodology that allows faster preparation of a finished product for examination.

Direct fecal smear: A basic diagnostic technique in which a small quantity of feces is dabbed lightly in a drop of saline on a microscope slide.

-E-

Enzyme-linked immunosorbent assay (ELISA): An immunoassay using an antibody labeled with an enzyme marker to diagnose parasite infections. Depending on how the test is configured, the assay can be used to detect circulating antigens or antibodies to specific parasites in the blood of an animal. In veterinary parasitology, ELISA test kits are commercially available for the diagnosis of *Cryptosporidium* and *Giardia* in fecal material. Antibody detection ELISAs are used in commercial laboratories to assess whether animals have been exposed to *Leishmania*, *Toxoplasma*, or heartworms, as well as, many other parasites.

-F-

Fecal smear: A basic diagnostic technique in which a small quantity of feces is dabbed lightly in a drop of saline on a microscope slide.

Filariform esophagus: A form of esophagus found in nematodes that is not divided into three distinct sections. This type of esophagus that occurs in metastrongyloid larvae is named after the type of esophagus found in the filaroid nematodes. (Compare with rhabditiform esophagus).

Fixed samples: Samples treated with a preservative, such as formalin, for preservation.

Flotation methods: Any of a number of procedures for concentrating parasite eggs for more reliable detection than direct examination of fecal samples. A liquid with sufficiently high specific gravity (~1.180 or greater) is used in order to float the eggs to the surface. *See also* Zinc sulfate flotation, Stationary flotation, *and* Centrifugal sugar flotation.

Flagella: A whip-like appendage used for motility on the surface of protozoa. The structure on protozoan cells is often termed an undulipodium by zoologists to differentiate it from the flagella of bacterial cells which are morphologically quite different structures.

Formalin/ethyl acetate sedimentation: This method is similar to the acid/ethyl acetate sedimentation, but substitutes formalin for the acid solution. *See also* Acid/ethyl acetate sedimentation *and* Sedimentation assays.

-G-

Giemsa stain: Compound of methylene blue-eosin and methylene blue used for differential staining of blood smears.

-H-

Hemogram: A written or graphic record of the differential blood count.

Hydrometer: An instrument used to determine the specific gravity of a liquid.

-K-

Knott's technique: A diagnostic technique for the examination of blood for microfilariae based on the fact that hypo-osmotic solutions will cause the lysis of red blood cells without affecting the microfilariae. Formalin (2%) is typically used to lyse the red blood cells and fix the microfilariae for later examination. The sample is

then centrifuged and methylene blue stain can be added to make the microfilariae easier to visualize.

-M-

Magnesium sulfate flotation: A diagnostic method for viewing the eggs of parasites in a fecal sample. The technique is the same as for the zinc sulfate flotation, but magnesium sulfate (Epsom salts), an inexpensive alternative, is used instead of zinc sulfate. *See* Zinc sulfate flotation.

Membrane techniques: A diagnostic technique for examining blood samples for microfilariae. Blood cells are lysed as in the Knott's technique, but instead of centrifugation, the lysed blood is forced through a membrane filter. The filter is then placed on a slide and examined under a microscope.

Merozoites: A stage in the life cycle of some Protozoa (eg, *Plasmodium*) that develops from a sporozoite.

Microfilariae: The prelarval stage of nematode parasites (Filarioidea) found in blood and tissues.

Miracidium: The ciliated first-stage larva of a trematode. The stage that hatches from the egg.

-O-

Oocyst: A stage in the life cycle of apicomplexan protozoa characterized by an encapsulated zygote representing the fusion of a macrogamete and a microgamete.

Operculum: A lid or covering structure.

-P-

Proglottids: A segment of the body of a tapeworm.

-R-

Rhabditiform esophagus: The form of esophagus found in nematodes in which there are three distinct portions, a muscular anterior portion, a thinner medial portion, and a bulb near the junction with the intestine. The form is named after the free-living rhabditoid nematodes that typically all possess this type of esophagus.

Romanovsky stain: An eosin-methylene blue stain used for blood smears.

-S-

Sedimentation assays: A diagnostic method for detecting eggs of parasites in a fecal sample. Sedimentation assays are used very little in veterinary medicine; flotation methods are preferred because they effectively detect the most common parasites in dogs and cats and because sedimentation assays require the examination of a larger

amount of fecal material. In the acid/ethyl acetate and the formalin/ethyl acetate sedimentation assays, a fecal slurry is prepared and sieved, centrifuged, decanted, and mixed with the acid or formalin solution. Ethyl acetate is added to the tube, which is recentrifuged; the sediment is examined under a microscope.

Skin scraping: A skin scraping is performed to obtain a sample for the detection of external parasites such as the mites *Sarcoptes scabiei*, *Notoedres cati*, and *Demodex* species. The sample is obtained using a scalpel and a small quantity of mineral oil, and the material is then examined under a microscope. Ceruminous material from the ear can be examined in the same manner to look for ear mites.

Skin smear: A basic diagnostic technique in which skin cells are spread across a microscope slide for examination. Staining with Giemsa or other hematologic stains can improve the visualization of parasites, such as the amastigote stages of trypanosomes and *Leishmania*.

Sodium nitrate flotation: A diagnostic method for viewing the eggs of parasites in a fecal sample. The technique is the same as for the zinc sulfate flotation, but sodium nitrate is used instead of zinc sulfate. *See* Zinc sulfate flotation.

Sporoblast: An immature sporocyst of a coccidian parasite.

Sporocyst: A stage in the life cycle of various trematodes that develops from an egg. Unfortunately, the same term is used to describe the diagnostic stages of *Sarcocystis* species that are shed in the feces of carnivores and which represent the stages produced within the oocyst of an apicomplexan protozoan.

Sporozoite: A stage in the life cycle of some apicomplexan Protozoa (eg, *Plasmodium*) that results from the reduction divisions that occur within the oocyst and which are the infective stage that is produced from the fusion of gametes.

Stationary flotation methods: A diagnostic method in which parasite eggs and cysts are floated to the surface of a liquid medium and then examined under a microscope. The solution must have a buoyant density that is greater than that of the eggs but less than that of the other material in the feces in order for separation to occur (ideally, a specific gravity of 1.2). Common reagents used include table salt (brine), zinc sulfate, magnesium sulfate, and sodium nitrate. The process can be performed using in-house materials or commercially available kits.

-T-

Trophozoites: The active, motile feeding stage of a protozoan.

Trypomastigote stages: A stage in the life cycle of certain trypanosomatoid protozoa characterized by a slender elongate body with a kinetoplast, an undulating membrane along the length of the body, and a flagellum emerging from the side of the body.

-V-

Vermiform: Resembling a worm in form or motion.

-W-

Wet mount: A basic diagnostic technique in which a drop of blood is placed on a slide and examined under a microscope. The wet mount is a rapid means of diagnosing infections with trypanosomes or filarioid nematodes if sufficient stages are circulating in the blood at the time of the examination.

-Z-

Zinc sulfate flotation: A fast and relatively easy diagnostic method for viewing the eggs and cysts of parasites in a fecal sample. The sample is suspended in water, filtered through gauze, centrifuged, resuspended in zinc sulfate solution, and recentrifuged. A wire loop is used to pick up the surface layer, which is placed on a slide and examined under a microscope for eggs or protozoan cysts.

Suggested Reading

Ash LR, Orihel TC. *Parasites: A Guide to Laboratory Procedures and Identification*. Chicago: ASCP Press, 1987; 328 pages. *This is an excellent methods book, however, it is designed mainly for human parasites.*

Ash LR, Orihel TC. *Atlas of Human Parasitology*, 4th ed. Chicago: ASCP Press, 1997; 410 pages. *This is a beautifully illustrated atlas of human parasitology and also provides some introduction to each parasite and to diagnostic methods.*

Bowman DD. *Georgis' Parasitology for Veterinarians*, 8th ed. Philadelphia: WB Saunders, 2002; 408 pages. *A general text of veterinary parasitology covering both large animal and small animal parasites.*

Hendrix CM. *Diagnostic Veterinary Parasitology*, 2nd ed. St. Louis: Mosby, 1998; 321 pages. *This book has an excellent and concise description of diagnostic methods for animal parasites.*

Foreyt WJ. *Veterinary Parasitology, Reference Manual*, 5th ed. Ames, IA: Iowa State University Press, 2001; 244 pages. *An excellent guide to veterinary parasitology and diagnosis; contains important information on exotic parasites.*

Garcia, LS. *Diagnostic Medical Parasitology*, 4th ed. Washington, DC: ASM Press, 2001; 1092 pages. *This is an excellent in-depth source on parasites and laboratory methods for the human parasitology laboratory.*

Sloss MW, Kemp RL, Zajac AM. *Veterinary Clinical Parasitology*, 6th ed. Ames, IA: Iowa State University Press, 1994; 208 pages. *No veterinary parasitology or practice can be without this handy manual. An even better new color edition will be published shortly.*

Chapter 1

- Figure 1.1.The materials needed for a direct smear include a 22 × 22 mm (or 18 × 18 mm) cover glass, microscope slide, applicator stick, feces, and saline. It is always best to make the preparation in saline, rather than water, so that living trophozoites will remain viable and motile.
- Figure 1.2.When preparing the direct smear, it is important to put a small amount of saline on the slide and then to add a small quantity of feces, about the size of the end of the applicator stick, about 2 mm². The feces is then mixed with the saline and daubed about until a relatively clear mixture is formed (A). If there are particulates present, especially common when animals are fed dried foods, the particulates can be pushed to one side of the drop carefully with the edge of the coverslip before it is lowered onto the preparation (B).
- Figure 1.3.Whether using zinc or magnesium sulfate, or sodium nitrate, it is always best to check the specific gravity with a hydrometer. Sodium nitrate can be purchased in a dry form in a premeasured quantity for stationary flotations. Zinc sulfate can be purchased as a liquid with a specific gravity of 1.18. Magnesium sulfate is probably the least expensive of the flotation solutions, but some Epsom salts (although food grade and approved for use as a laxative) can be slightly discolored or contain a few particulates when in solutions of specific gravity 1.2; the choice of another brand will often resolve these problems. (A) This hydrometer has a scale from 1.000 (the specific gravity of water) to 1.220. (B) In this image, the meniscus is at 1.200, between the numbers 80 (1.180) and 20 (1.220). This hydrometer would be inappropriate for checking a heavy sugar solution.
- Figure 1.4.When picking up the sample from the top of the centrifuge tube, it should be carefully pushed straight down through the top of the liquid and then pulled straight up. Occasionally the material on the top of the tube may be so thick that it is more like mud than a meniscus. In these cases, some of the material can be transferred from the side of the loop to the slide.
- Figure 1.5.When the loop is transferred to the microscope slide, it can be examined as the simple small loopful or under a coverslip. Looking at one or two loopfuls has the advantage that the specimen is not spread out under a larger area as happens with a coverslip. The disadvantage of examining a loopful is that it may dry out if the examiner takes too long during the observation.
- Figure 1.6.The loop used to pick up a specimen from the top of a tube is finer than the typical microbiological loop with a somewhat larger diameter. It is important that the loop be made from wire that is resistant to flame, or it will melt when it is flamed. The flaming seems to help the loop collect the sample from the meniscus of the centrifuge tube. Occasionally the loop will accumulate a crust of dried material from the feces and flaming that can be carefully broken off the wire using the end of an applicator stick.
- Figure 1.7.The Fecalyzer is a stationary flotation device that has been developed for the collection and processing of fecal samples. The internal portion can be used to collect a portion of feces of appropriate size by inserting the end into the feces. The sample can then be placed into the outer container and brought into the clinic. When it is time to examine the sample, flotation medium can be added and the sample mixed by twisting the insert inside the holder. The insert is then filled with flotation solution so that a positive meniscus is formed. A sieve in the insert prevents large particles from floating to the surface. A 22 × 22-mm coverslip is then placed on the top of the tube, and it is allowed to sit for 5 to 10 minutes. The coverslip is then removed and placed on a microscope slide to determine what parasites have floated to the surface.
- Figure 1.8.The Ovassay is a fecal collection and processing device used to perform a stationary fecal flotation. The internal device can be used to collect a sample of appropriate size by stabbing it into the feces. The insert can then be placed into the holder and brought to the clinic. When it is time to process the sample, flotation medium can be added to the tube, and the feces is mixed with the medium by twirling the insert in the holder. The insert is then filled with flotation medium until a positive meniscus is formed. A 22 × 22-mm coverslip is then placed on the meniscus, and the device is allowed to stand for 5 to 10 minutes. The coverslip is then removed and transferred to a microscope slide to identify those parasite stages that have floated to the surface.
- Figure 1.9.When preparing sugar for flotation, typically about 900 g (2 lbs) is needed for every 700 mL of water (need about 1300 g/L). It will be necessary to slowly add the sugar to the water and dissolve it by stirring. Often the water can be warmed to

help in the dissolution of the sugar, but it should be cooled before the specific gravity is checked with a hydrometer. Some find it very useful to prepare the sugar solution in a double boiler just like when making candy. It is a good idea when the product has cooled to add a small quantity of formalin (10 mL/L) to prevent mold from growing in the medium.

Figure 1.10.Mix the feces in a small cup and then pour it through some gauze to remove the particulates before spinning it down and mixing it with sugar. Some practitioners skip the sieving stage, but if skipped, the particulates in the final sample can make it very hard to examine under the microscope.

Figure 1.11.After the water is decanted from the centrifuged sieved sediment, sugar solution (about 3 to 4 mL) should be added and the sediment suspended in the sugar solution. It is important that the sample be well mixed, and this is easiest to perform using two applicator sticks. A vortex mixer can be used if sticks are inserted in the samples, but a large number of air bubbles can be created if care is not used.

Figure 1.12.After the centrifuge comes to a complete stop, the coverslip can be lifted directly from the top of the tube.

Figure 1.13.The coverslip should be carefully placed on a slide to capture as few air bubbles as possible.

Figure 1.14.When the ethyl acetate is mixed with the fecal water, formalin, or acid mixture and then centrifuged, a layer will form between the organic solvent and the aqueous phase. It is necessary to dislodge this plug with an applicator stick before the tube is decanted. Care should be taken to carefully dislodge the material from the walls of the tube so that it will not fall back into the pellet when it is decanted. If the pellet is in acid alcohol, it is probably desirable to resuspend it in water before it is placed on a slide; otherwise it tends not to form a puddle but instead rapidly spreads to the edges of the slide and beyond.

Figure 1.15.This device consists of a funnel attached to a piece of rubber tubing with a clamp. Fecal material is placed in a piece of gauze or a tea strainer and is suspended in water in the funnel. Larvae migrate out of the feces and into the water. With time, the larvae will settle to the bottom of the tube and can be harvested either by placing a drop out of the tip onto a slide or by spinning the contents down in a centrifuge tube and examining the sediment.

Figure 1.16.This is an example of a solid-phase immunoassay designed to be used with a single sample (ProSpecT® *Giardia/Cryptosporidium* Rapid Format). The test is similar to those developed for detecting various antigens and antibodies in blood

or serum samples. The diluted feces is applied to the membrane, and then after a series of steps, a colorimetric change will occur in the membrane with spots that will darken if the antigen is present in the feces. Such tests have been developed for detecting the antigens of *Giardia* and *Cryptosporidium*.

Figure 1.17.This is an ELISA that has been used routinely to detect antigens of *Cryptosporidium* in fecal material (ProSpecT® *Cryptosporidium* Microplate Assay; a similar assay is available for *Giardia*). The cost of the plates makes the test very expensive, so it is routinely used only in centralized diagnostic laboratories. The sample is typically run with a positive and negative control sample. The results can be read either visually or by using an ELISA reader. In the test shown, the positive wells turn yellow at the end of the test, and the negative wells remain clear. The intensity of the color can be used as a rough approximation as to the intensity of the infection.

Figure 1.18.Until recently, there were no methods that would routinely be applied to the culture of organisms in a veterinary practice. The development and application of the in-pouch assay for trichomonad infections in cats seems to be a method that could be used more routinely. The method was originally developed for the culture of the sexually transmitted trichomonads of the urogenital tract of cattle and people, but the cattle kit seems to work for the culture of trichomonads present in cat feces.

Chapter 2

Figure 2.1.Simple centrifugal sedimentation followed by examination of the pellet after decanting will usually provide the best chance of success for parasite recovery and identification.

Chapter 3

Figure 3.1.Placing a small drop of blood on a slide is an excellent way to find microfilariae if they are present in any number. There is nothing more rewarding than preparing the slides and seeing wriggling microfilariae. It is also a fairly good way to distinguish the microfilariae of *Dirofilaria immitis* (shown in figure) from those of *Dipetalonema reconditum*; the latter are capable of propelling themselves along easily in the blood while the heartworm microfilariae tend to just thrash about. An even more exciting find is coming across something like a trypanosome in one of these preparations.

Figure 3.2.Blood, 1 mL, either fresh, EDTA-treated, or heparin-treated, is transferred to 10 mL of 2% formalin, which causes the red blood cells to lyse.

Figure 3.3.After the sample has been allowed to stand for 5 to 10 minutes to allow the red blood cells to lyse and to give the microfilariae time to be fixed by the formalin, the tube is centrifuged to collect white blood cells, the red blood cell ghosts, and the microfilariae into the pellet. A small quantity of methylene blue stain is often added to the pellet to help in the visualization of the microfilariae.

Figure 3.4.The pellet can be examined unstained and the microfilariae can be identified as thin hair-like structures with pointed tails. In fact, the assay can be run without using formalin and the microfilariae will still be wriggling if the preparation is examined soon after the pellet is formed. The test was originally developed for looking at samples days to weeks after they were collected in the field, and the stain was added so that various different types of microfilariae commonly occurring in human blood could be distinguished.

Figure 3.5.With the added methylene blue stain, the numerous nuclei within the fixed microfilariae are stained blue, making the microfilariae easier to identify. Care should be taken not to add too much stain or the sample will become too dark to examine easily.

Chapter 4

Figure 4.1.The materials required for performing a skin scraping are mineral oil, forceps, scalpel, microscope slide, and coverslip.

Figure 4.2.When performing the skin scraping, it may be necessary to scrape until a bit of blood oozes from the site of the scraping. This is especially true when dealing with the deeper-dwelling *Sarcoptes scabiei*. The scalpel blade is dipped in the mineral oil and then used to scrape the skin. Some of the crusts may be quite hard, and this may require that they be teased apart with forceps before the coverslip is applied to the slide with the material.

Chapter 5

Figure 5.1.The egg of *Toxocara canis* is a good benchmark egg to remember for size; it is slightly larger than the long-axis length of *Ancylostoma caninum* and has a diameter that is about the same as the long-axis length of the eggs of *Trichuris vulpis* and *Uncinaria stenocephala*. Under the 10× objective of most diagnostic scopes the distance across the field of view

is about 1.8 to 2 mm, or 1800 to 2000 μm. The egg of *T. canis* is about 80 μm in diameter (*T. cati* is slightly smaller in diameter, being around 70 μm in diameter), so under 10×, about 22 to 25 of the eggs of *T. canis* would fit across the field of view. Under the 40× objective, the field of view is about 450 to 500 μm, so only 6 or so of these eggs would fit in a line along the field of view. The other good frame of reference is the cysts of *Giardia canis* or *G. cati*. These cysts are about 10 μm long or about one tenth the diameter of a *T. canis* egg. Thus, under the 40× objective, as many as 60 *Giardia* cysts could be seen lined up across the field of view.

Figure 5.2.Egg of *Baylisascaris procyonis* in dog feces. This egg is slightly smaller than the eggs of *Toxocara canis* and *Toxascaris leonina*, has a thick brown shell, and is typically passed containing a single cell. The roughened exterior of the eggshell is not patterned like the eggshell of *T. canis* and is rough and dark compared with the eggshell of *T. leonina*.

Figure 5.3.Dried segments of *Dipylidium caninum* that appear similar to small round seeds about the same size as sesame seeds. (Specimen courtesy of Dr. Jeanne Baines)

Figure 5.4.One of the dried segments that has been rehydrated to show the typical appearance of *D. caninum*.

Figure 5.5.Close-up of the segment of *D. caninum* to show the contained egg balls within the segment.

Figure 5.6.The segment has been squashed to release the egg balls (five) into the area next to the segment.

Figure 5.7.Single egg ball viewed using differential interference contrast microscopy to show the appearance of the individual hexacanth organisms within the egg balls.

Figure 5.8.Two individual hexacanth embryos of *D. caninum* in which the larval hooks are evident.

Figure 5.9.The egg of this cestode, *Spirometra mansonioides*, looks like a trematode egg. On this specimen, the difficult-to-discern operculum is on the more pointed end of the egg. Some specimens will appear to be more elongate or to have ends more similar in size. The eggs are similar in size to those of *Paragonimus* species and sometimes several specimens will have to be examined before a diagnosis can be confirmed.

Figure 5.10.A single unsporulated oocyst of *Toxoplasma gondii* can be seen in the center of this 40× objective image of a sugar flotation of cat feces. In fecal samples that are aged slightly, the central sporoblast may have divided into two separate sporocysts.

- Figure 5.11.Trophozoite of a trichomonad as viewed under differential interference microscopy showing the undulating membrane, axostyle projecting from the posterior end, and a hint of the anteriorly directed flagella. In fresh preparations, these organisms are most easily detected by their swimming motion.
- Figure 5.12.Cysts of *Giardia canis* recovered in a zinc sulfate centrifugal flotation
- Figure 5.13.The oocysts of *Cryptosporidium canis*, *C. felis*, *C. parvum*, and *C. hominis* are morphologically indistinguishable. The oocysts in sugar flotations can be seen to have a slight pink coloration caused by the spherical aberration present in most inexpensive lenses used on diagnostic microscopes. With the highly corrected optics present on most research-grade microscopes, this pink coloration tends to disappear.
- Figure 5.14.This is a view of two oocysts using an oil immersion 100× lens and differential interference microscopy to show the sporozoites and residual body present within each oocyst.
- Figure 5.15.Larva recovered from the Baermann funnel. These larvae tend to be highly motile and easy to recover with the Baermann method. The larvae are characterized by their long esophagus and the typical hooked end of the tail.
- Figure 5.16.Radiograph of the lungs of the cat showing the interstitial patchy infiltrates that are characteristic of very heavy infections with this parasite.
- Figure 5.17.Larva of *Filaroides osleri* (also known as *Oslerus osleri*) recovered from a centrifugal zinc sulfate flotation. These larvae are not very active, and they are typically not recovered in any number from Baermann funnels.
- Figure 5.18.Nodule of *F. osleri* removed by resection during visualization with an endotracheal tube.
- Figure 5.19.Histologic section through a resected nodule showing sections through the large number of worms coiled within the nodule.
- Figure 5.20.This is the first-stage, rhabditiform larva of *Strongyloides stercoralis*. The characteristics of note are the short esophagus that has three distinct portions, the cuticle-lined buccal space, and the very large genital primordium that occurs between the intestine and the ventral body wall just posteriad to mid body. The larvae are fairly motile and can be recovered using a Baermann funnel.
- Figure 5.21.Adult male *Trichinella spiralis* recovered from the feces. The male also has a stichosome esophagus that can be seen extending from the head (towards the left) back towards the middle of the body. The male does not have spicules like many nematodes, but instead has a pair of large papillae-like structures that aid in clasping the female during copulation (one of these is visible on the posterior end toward the right)
- Figure 5.22.Adult female *T. spiralis* recovered from the feces. The worm can be noted to contain the typical stichosome esophagus towards the anterior end (on the left) and to have its body filled with small prelarvae from the level of midbody toward the tail (on the right).
- Figure 5.23.Section through the muscle of a cat showing a muscle cell infected with a first-stage larva of *T. spiralis*.
- Figure 5.24.Egg of *Paragonimus kellicotti*. Note the seated operculum and the abopercular bump or spine-like structure on the opposite end of the egg.
- Figure 5.25.Radiograph (ventral view) showing multiple patchy infiltrates in the right caudal lobe, one of which is cavitory.
- Figure 5.26.Radiograph (lateral view) showing extensive infiltrate with a cavitory lesion in the right caudal lobe. The cavitory lesion probably contains a pair of adult trematodes. The infiltrate is probably due to a reaction to the eggs in the tissues.

Chapter 6

- Figure 6.1.Egg of the capillarid nematode *Pearsonema plica*, which is one of the few eggs found in the urine of dogs. The species found in cats is generally considered to be *Pearsonema felicati*.

Chapter 7

- Figure 7.1.Giemsa-stained microfilaria of *Dirofilaria immitis*.
- Figure 7.2.This Labrador retriever puppy was most likely infected in utero by *Neospora caninum*. The signs are evident of the contractures of the limbs that occur when the nerves are damaged in the growing dog. The bandages on the feet indicate the attempt to protect the dog from abrasions as it tries to walk.
- Figure 7.3.Section through muscle showing a cyst in a muscle cell.
- Figure 7.4.Electron micrograph of the organisms in a Schwann cell showing the damage to the myelin sheath.
- Figure 7.5.This Giemsa-stained blood film shows three trypanomastigotes of *Trypanosoma cruzi*, which, unlike those of the African trypanosomes, tend to be “C”-shaped rather than “S”-shaped when seen in blood films. The trypanomastigote of *T. cruzi* has a very large kinetoplast at the posterior end that seems to bulge out of the cytoplasm and dividing forms are not seen within the blood.

Figure 7.6.This Giemsa-stained preparation shows the promastigote stages of *T. cruzi* that are grown in culture.

Figure 7.7.This histologic section through muscle tissue shows two nests of amastigotes within muscle fibers.

Figure 7.8.Giemsa-stained blood film showing a red blood cell containing a pair of tear-drop shaped *Babesia canis* merozoites in one cell.

Figure 7.9.Stained blood film showing several red blood cells parasitized by the small ring-shaped trophozoites of *Cytauxzoon felis*.

Figure 7.10.Section through the lung tissue of the cat. The image shows a longitudinal section through a blood vessel that has its lumen completely occluded by the large hypertrophied macrophages parasitized by *C. felis*.

Figure 7.11.Section through the lung tissue of the cat. The image is a cross section of a vessel occluded with the same stages present in the longitudinal section in Figure 7.10.

Chapter 8

Figure 8.1.Histologic section through a liver biopsy specimen showing two eggs of the trematode *Heterobilbazia americana* in the hepatic tissue.

Figure 8.2.Histologic section through a vessel in the liver biopsy specimen showing a cross section through an adult male wrapped around the thinner female worm. The darker areas within the worm are the intestine.

Figure 8.3.Adult male and female *H. americana* (stained red) recovered from a mesenteric vein. The larger, thick-bodied male is holding the thinner female within his gynecophoral canal.

Figure 8.4.Egg of *H. americana* recovered using a fecal sedimentation method. Notice that the egg contains a fully developed miracidium and would hatch if it was placed in water instead of saline.

Figure 8.5.This macrophage in a Giemsa-stained preparation from skin shows a macrophage full of the amastigote stage of *Leishmania mexicana*. Morphologically, the amastigotes cannot be distinguished from those of other species of *Leishmania* and when in macrophages cannot be distinguished from those of *Trypanosoma cruzi*.

Figure 8.6.Adult female *Sarcoptes scabiei* mite recovered in a skin scraping. The large spines on the body are readily apparent.

Figure 8.7.Adult male *S. scabiei* mite recovered in a skin scraping.

Figure 8.8.Eggs of *Otodectes cynotis* recovered from the ear canal by swabbing. The developing mites can be seen within the eggshells.

Figure 8.9.An adult female *O. cynotis* mite recovered by an ear swab.

Figure 8.10.Leg of a dog with a protruding female of *Dracunculus insignis*.

Figure 8.11.Larva of *D. insignis* from the fluid recovered from around the area where the worm was protruding from the tissues of the leg.

Figure 8.12.An adult female *Ixodes dammini* (*Ixodes scapularis*) tick that has fed for two days.

Figure 8.13.An adult female *I. dammini* tick that has fed for four days.

Figure 8.14.An adult female *I. dammini* tick that has fed for six days.

Figure 8.15.An unfed nymph of *I. dammini*. This is the stage that usually transmits Lyme disease to humans.

Figure 8.16.An unfed nymph of *I. dammini*; ventral view to show the preanal groove.

Figure 8.17.A just-hatched larva of *I. dammini* and its eggshell. The preanal groove cannot be seen on this dorsal view of the larva.

Figure 8.18.Lesion in the cheek of a cat from which a *Cuterebra* maggot (bot) was extracted.

Figure 8.19.The extracted maggot of the genus *Cuterebra*. The maggot is identified by its large size, rows of black spines, and if examined closely on the posterior end, the characteristic spiracles (not obvious in this illustration at this magnification).

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