

Gastrointestinal Parasites

The Practice Guide to Accurate Diagnosis and Treatment

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Gastrointestinal Parasites: The Practice Guide to Accurate Diagnosis and Treatment 3

Michael W. Dryden, Patricia A. Payne, Robert K. Ridley, and Victorene E. Smith

Standard Centrifugation Fecal Examination: A Step-by-Step Guide 14

Commentary: A Strategic Approach to Diagnosing and Treating Gastrointestinal Parasites 15

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Gastrointestinal Parasites

The Practice Guide to Accurate Diagnosis and Treatment

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To ensure the health and well-being of pet dogs and cats, coprologic examinations for parasite eggs, oocysts, and cysts are an important part of the daily routine for most veterinary practices. Although a fecal examination is considered a routine procedure in many clinics, it has been our experience that often little thought is given to performing the procedure correctly.

Many different procedures and techniques are used, each with its own advantages and limitations. For example, direct fecal smears are useful for detecting motile protozoa, whereas sedimentation examinations are more suitable for recovering heavy (e.g., *Physaloptera* spp) or operculated (e.g., fluke) eggs that do not float well because of the hypertonic effects exerted by the flotation solution. However, the methods used most frequently to recover parasite eggs, oocysts, and cysts are flotation techniques, which rely on the differences in the specific gravity (SG) of the egg(s), fecal debris, and flotation solution.

For parasite eggs to float, the SG of the flotation solution must be greater than that of the eggs. Flotation solutions are made by adding a measured amount of salt or sugar to a specific amount of water to produce a solution with the desired SG (see box, right); such solutions are effective, easy to make or commercially available, and relatively inexpensive. It is important to ensure that the flotation solution used has the proper SG, which is best accomplished by using a hydrometer calibrated to measure in the desired range. Hydrometers used to measure urine SG do not cover the required SG range needed for fecal examinations.

Flotation procedures vary from the simple to the complex. The simplest procedure involves

mixing a small amount of feces with flotation solution in a cylinder (shell vial or centrifuge tube) and adding solution until the cylinder is nearly full. The preparation is then allowed to stand until the eggs float to the top, and a sample from the top is removed to a microscope slide using a tool such as a wire loop, straw, needle hub, or glass rod. A refinement of this method involves filling the cylinder until a slight positive meniscus is formed and placing a glass coverslip over it. Again, the cylinder is allowed to stand until the eggs have had time to float to the top, and the coverslip is then removed to a microscope slide and examined. A further refinement of the flotation technique uses a centrifuge to spin down the debris and accelerate the movement of eggs to the surface of the solution where they can be recovered. Veterinary hospitals usually use one or more of these methods based on cost, ease of use, availability of hardware, or simply tradition.

Although coprologic examinations should be performed by veterinarians or trained veterinary technicians, the assignment is commonly given to the newest staff

Flotation Solutions for Helminth Ova^a

Magnesium Sulfate (MgSO₄; SG 1.20)

450 g MgSO₄
 1,000 ml tap water

Zinc Sulfate (ZnSO₄; SG 1.18–1.20)

331 g ZnSO₄
 1,000 ml warm tap water

Sodium Nitrate Solution (NaNO₃; SG 1.18–1.20)

338 g NaNO₃
 1,000 ml tap water

Saturated Salt (NaCl; SG 1.18–1.20)

350 g NaCl
 1,000 ml tap water

Modified Sheather's Solution (SG 1.27)

454 g granulated sugar
 355 ml tap water
 6 ml formaldehyde

Dissolve sugar and water in the top of a double boiler or with gentle heat. If solution is not clear, filter it through coarse filter paper.

^aCheck specific gravity (SG) with a hydrometer that has a range compatible with the solution being tested. Hydrometers with ranges of 1.000–1.400 are available.

member, often with very little instruction or emphasis on the importance of the task provided. Accurate evaluation of fecal samples is important and must be taken seriously by all members of the clinical practice. Not only is pet health at stake, but several of the parasites that may be recovered and identified are potentially zoonotic, such

as *Toxocara canis*, *Toxocara cati*, *Ancylostoma caninum*, *Giardia* spp, *Cryptosporidium parvum*, and *Toxoplasma gondii*.

Giardiasis in particular is a diagnostic dilemma. We agree that *Giardia* is one of the most commonly misdiagnosed, underdiagnosed, and overdiagnosed parasites. Based on the numerous phone calls and emails we receive, we realize that many veterinary practices find it difficult to diagnose giardiasis using fecal examinations. Many pseudoparasites such as yeasts (Figure 1), plant remnants, and debris have been mistaken for these tiny organisms. Identification of *Giardia* cysts is further compromised because microscopes used in private practice are often not equipped with micrometers that can allow measurement of cysts that are as small as 8 to 12 × 7 to 10 μm (Figure 2). Cysts are shed intermittently, and repeated fecal analyses may be needed before cysts are recovered in a sample.^{1,2} *Giardia* cysts are delicate and deteriorate rapidly in fecal flotation solutions; if a flotation solution other than zinc sulfate (ZnSO₄) is used, the cysts may be distorted (Figure 3). In many clinics, the only technique used to detect *Giardia* is the direct smear, but the trophozoites are fragile and

are often found only in very fresh, diarrhetic feces. Finally, because of the difficulty in detecting *Giardia* spp, veterinarians have told us that they often rely on a patient's response to metronidazole treatment as a presumptive diagnosis.

This article describes the findings of two recent studies: (1) an evaluation of the efficacy of various fecal flotation methods and solutions in detecting common helminth eggs from canine feces³ and (2) a separate investigation of methods to recover *Giardia* cysts, including the SNAP *Giardia* Test Kit (Idexx Laboratories) and a centrifugation fecal flotation technique using ZnSO₄ and Sheather's sugar solution.⁴ An illustrated step-by-step guide provides detailed recommendations for performing routine parasite diagnostic procedures in the clinical setting.

LABORATORY EVALUATION OF COMMON FECAL FLOTATION TECHNIQUES

Trials were conducted to evaluate and compare the ability of different flotation techniques and solutions to recover common helminth eggs from canine feces. Unless stated otherwise, fecal samples used in these studies were prepared by collecting feces from each of three dogs known to have mixed infections of *T. canis*, *A. caninum*, and *Trichuris vulpis*; the samples were thoroughly combined and replicate 2-g samples weighed out. Swing-head centrifugations were performed as illustrated in the step-by-step guide on page 14, and all centrifugations were done at 280 ×g.

Role of Specific Gravity

The first series of experiments compared the ability of the 15-minute Ovassay (Synbiotics) and the 5-minute swing-head centrifugation technique to recover *T. canis*, *A. caninum*, and *T. vulpis*. The Ovassay was performed using ZnSO₄ solutions with SGs of 1.1 and 1.2; the centrifugation method used the same ZnSO₄ solutions as well as a sugar solution with an SG of 1.2.

Hookworm (*A. caninum*) eggs (SG 1.0559⁵) readily floated using the Ovassay method and 1.1-SG ZnSO₄ solution, but only one ascarid (*T. canis*) egg (SG 1.0900) and no whipworm (*T. vulpis*) eggs (SG 1.1453) were recovered. When the SG of the ZnSO₄ solution was raised to 1.2, *T. vulpis* and *T. canis* eggs were recovered with the Ovassay, albeit in fewer numbers than with the centrifugation method using either

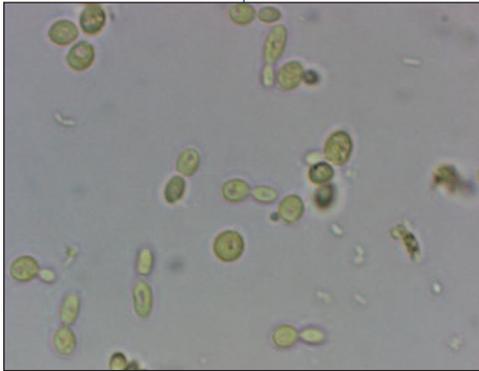


FIGURE 1. Yeasts (12–15 μm) recovered in a fecal examination and incorrectly identified as *Giardia* cysts in a referring veterinary practice.

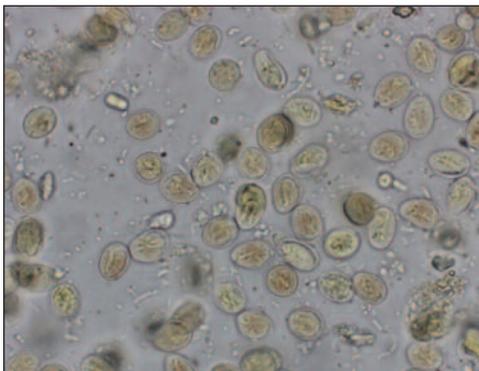


FIGURE 2. *Giardia* cysts stained with Lugol's iodine recovered using 1.18-SG ZnSO₄ in a swing-head centrifuge.



FIGURE 3. *Giardia* cysts recovered using 1.27-SG Sheather's sugar solution in a swing-head centrifuge.

ZnSO₄ or sugar. For all three parasites, the centrifugation method exhibited significantly higher fecal counts compared with the Ovassay method (Table 1). For *A. caninum*, no differences were found between the 1.2-SG ZnSO₄ and sugar solutions using the centrifugation method. Significantly higher *T. vulpis* egg counts were obtained from the sugar solution than the zinc solution. In addition, both *T. vulpis* and *T. canis* fecal egg counts were significantly higher when the SG of the solution was 1.2 compared with 1.1.

Simple Flotation versus Swing-Head Centrifugation

The second set of experiments compared the number of eggs recovered using NaNO₃ and sugar solutions in the simple flotation and swing-head centrifugation techniques. For the simple flotation, a small amount of feces was placed in a centrifuge tube, flotation solution was added and mixed thoroughly, and the preparation was allowed to stand for 5, 10, 15, or 20 minutes while the eggs rose to the surface. To ensure consistency, 15-ml polystyrene centrifuge tubes (product no. 889-205004, Oxford Labware, St. Louis) were used for both techniques. The SG of the NaNO₃ solution was

adjusted to 1.2 and that of Sheather's sugar solution to 1.27; SGs were confirmed with a hydrometer. Forty-eight 2-g fecal samples were evaluated.

Accurate evaluation of fecal samples is important and must be taken seriously by all members of the clinical practice.

For all three parasites, the centrifugation method using 1.27-SG Sheather's sugar solution resulted in significantly higher fecal egg counts than the simple standing flotation method, regardless of the time interval (Table 2). No significant differences in fecal egg counts were shown between the time intervals within the simple flotation method.

For *A. caninum*, the centrifugation method using 1.2-SG NaNO₃ solution resulted in significantly higher fecal egg counts than the simple flotation method with a 5- or 10-minute stand time (Table 3); when the stand time was increased to 15 and 20 minutes, the simple flotation method and cen-

TABLE 1. Comparison of the Mean Counts of *A. caninum*, *T. vulpis*, and *T. canis* Eggs Recovered from Three 2-g Fecal Samples Using the Ovassay and Centrifugation Methods in ZnSO₄ (SG 1.1 and 1.2) or Sugar (SG 1.2)

Parasite	Ovassay (ZnSO ₄ ; SG 1.1)	Centrifugation (ZnSO ₄ ; SG 1.1)	Ovassay (ZnSO ₄ ; SG 1.2)	Centrifugation (ZnSO ₄ ; SG 1.2)	Centrifugation (Sugar; SG 1.2)
<i>A. caninum</i>	680 ^a	1,365 ^b	782 ^a	1,475 ^b	1,598 ^b
<i>T. vulpis</i>	0 ^a	0 ^a	2.7 ^b	7.3 ^c	11.6 ^d
<i>T. canis</i>	0.3 ^a	1 ^a	46.7 ^b	147.7 ^c	158.3 ^c

^{a,b,c,d}Within each row, different letters indicate a statistically significant difference ($P < .05$).

TABLE 2. Comparison of 5-Minute Swing-Head Centrifugation and 5-, 10-, 15-, and 20-Minute Simple Standing Flotations for Recovering *A. caninum*, *T. vulpis*, and *T. canis* Eggs Using 1.27-SG Sheather's Sugar Solution (Mean Egg Counts from Three 2-g Fecal Samples)

Parasite	5-Min Swing-Head Centrifugation	Simple Standing Flotation			
		5-Min	10-Min	15-Min	20-Min
<i>A. caninum</i>	52.3 ^a	1.7 ^b	2.7 ^b	5.3 ^b	5.3 ^b
<i>T. vulpis</i>	9 ^a	0.7 ^{b,*}	0.7 ^{b,*}	1.7 ^b	1.7 ^b
<i>T. canis</i>	293.3 ^a	22.3 ^b	17.3 ^b	20 ^b	21 ^b

^{a,b}Within each row, different letters indicate a statistically significant difference ($P < .05$).

*Two of the three samples tested were negative for *Trichuris* sp.

Table 3. Comparison of 5-Minute Swing-Head Centrifugation and 5-, 10-, 15-, and 20-Minute Simple Standing Flotations for Recovering *A. caninum*, *T. vulpis*, and *T. canis* Eggs Using 1.20-SG NaNO₃ (Mean Egg Counts from Three 2-g Fecal Samples)

Parasite	5-Min Swing-Head Centrifugation	Simple Standing Flotation			
		5-Min	10-Min	15-Min	20-Min
<i>A. caninum</i>	23.7 ^a	7.3 ^c	13.7 ^{b,c}	21.7 ^{a,b}	19.3 ^{a,b}
<i>T. vulpis</i>	2.3 ^a	0.7 ^{a,*}	0.7 ^{a,*}	1.7 ^a	2.0 ^a
<i>T. canis</i>	262 ^a	46.3 ^b	53 ^b	74.3 ^b	76.7 ^b

^{a,b,c} Within each row, different letters indicate a statistically significant difference ($P < .05$).

*Two of the three samples tested were negative for *Trichuris* sp.

trifugation method recovered significantly similar numbers of eggs. Relatively few *T. vulpis* eggs were retrieved using any of the methods, but the 5- and 10-minute simple floats missed eggs in two of three samples. Centrifugation recovered significantly more *T. canis* eggs than any of the flotation methods (Table 3).

Evaluation of Time to Examination

A third series of experiments evaluated whether more parasite eggs could be recovered if tubes were allowed to sit undisturbed for 10 minutes after samples were centrifuged. Fecal samples were mixed with 1.20-SG NaNO₃ and centrifuged. Coverslips were

Comparison of Swing- and Fixed-Head Centrifugation Techniques

The fourth series of experiments compared swing- and fixed-head centrifugation techniques. For the fixed-head centrifugation technique, approximately 10 ml of flotation solution (sugar or NaNO₃) was added to 2 g of feces, the slurry was mixed thoroughly, and more solution was added until the level in the tube was within 1 cm from the top; the tube was then centrifuged at 280 ×g for 5 minutes. After being centrifuged, the tubes were placed vertically in a test tube rack, flotation solution was added until a slight positive meniscus formed, a coverslip was placed, and the preparation was allowed to stand for 10 minutes before coverslips were removed to a glass slide and examined. The swing-head method was performed as described in the step-by-step guide.

In general, *A. caninum* fecal counts were not significantly different between the swing-head centrifuge and the fixed-head method (Table 5). No significant differences were found between centrifuge types for *T. canis* fecal counts.

Table 4. Comparison of Two Coverslip Examination Protocols: Immediate Removal and Examination versus Waiting 10 Minutes (Mean Egg Counts from Three 2-g Fecal Samples)*

Parasite	Immediate Examination	Examination after Sample Sat for 10 Min
<i>A. caninum</i>	8.3 ^a	24.0 ^b
<i>T. vulpis</i>	3.7 ^a	5.3 ^a
<i>T. canis</i>	135.7 ^a	262.7 ^b

*Fecal samples were spun for 5 minutes in a swing-head centrifuge using 1.2-SG NaNO₃ solution.

^{a,b} Within each row, different letters indicate a statistically significant difference ($P < .05$).

removed and examined either immediately after the centrifuge stopped spinning or after the tubes sat undisturbed for an additional 10 minutes.

A. caninum and *T. canis* fecal egg counts were significantly higher when samples were allowed to sit for 10 minutes after being centrifuged (Table 4). The lack of a difference between the two methods in obtaining *T. vulpis* eggs is likely a reflection of the overall low egg counts.

STUDENT EVALUATION OF COMMON FECAL FLOTATION TECHNIQUES

For each of 5 years (2000–2004), second-year veterinary students were given a short visual presentation on how to perform the direct smear, Ovassay, and swing-head centrifugation techniques. For these procedures, students collected 5-g samples from cat and dog feces known to contain parasite eggs, and each sample was tested using all three techniques.

For the direct smear, a small sample of feces was placed on a glass slide and mixed with a drop or two of saline; the mixture was then spread thinly over the slide (thin enough to read newsprint through it), and the slide was covered with a glass coverslip. The Ovassay and

Table 5. Comparison of the Numbers of *A. caninum* and *T. canis* Eggs Recovered by Swing- and Fixed-Head Centrifugation Methods in 1.20-SG ZnSO₄ or 1.27-SG Sheather's Sugar Solution (Mean Egg Counts from Three 2-g Fecal Samples)

Parasite	1.20-SG ZnSO ₄		1.27-SG Sheather's Sugar Solution	
	Swing-Head Centrifuge	Fixed-Head Centrifuge	Swing-Head Centrifuge	Fixed-Head Centrifuge
<i>A. caninum</i>	137.7 ^{a,b}	111.7 ^a	210.3 ^b	176.3 ^{a,b}
<i>T. canis</i>	35.3 ^a	36.3 ^a	49.3 ^a	38.7 ^a

^{a,b}Within each row, different letters indicate a statistically significant difference ($P < .05$).

Table 6. Combined Results of Student Evaluations

Parasite	No. of Known Positive Samples	Failure of Direct Smear to Detect Eggs/Oocysts	False-Negative Results		Percentage of Samples from Which >50 Eggs or Oocysts/Slide Were Recovered	
			Ovassay	Centrifugation	Ovassay	Centrifugation
Hookworm (<i>A. caninum</i>)	206	72.82%	4.85%	0.97%	36.41%	74.76%
Ascarid (<i>T. canis</i> or <i>T. cati</i>)	171	85.38%	25.88%	10.53%	1.18%	42.69%
Whipworm (<i>T. vulpis</i>)	203	92.61%	32.02%	4.93%	2.96%	23.65%
Tapeworm (<i>Taenia</i> spp)	26	96.15%	76.92%	11.54%	0.0%	15.38%
Coccidia (<i>Isospora</i> sp)	53	94.34%	50.94%	5.66%	0.0%	33.96%

centrifugation techniques were conducted using 2-g fecal samples and Sheather's sugar solution with an SG of 1.23 to 1.27.

In total, students evaluated 206 fecal samples known to contain hookworm (*A. caninum*) eggs, 171 samples known to contain ascarid (*T. canis* or *T. cati*) eggs, 203 samples known to contain whipworm (*T. vulpis*) eggs, 26 samples known to contain tapeworm (*Taenia* spp) eggs, and 53 samples known to contain coccidia (*Isospora* spp) oocysts. When all data were combined, the direct smear technique failed to detect hookworm eggs 72.82% of the time, ascarid eggs 85.38% of the time, whipworm eggs 92.61% of the time, tapeworm eggs 96.15% of the time, and coccidia oocysts 94.34% of the time. The Ovassay and centrifugation techniques yielded false-negative results 4.85% and 0.97% of the time, respectively, for hookworm eggs, 25.88% and 10.53% of the time for ascarid eggs, 32.02% and 4.93% of the time for whipworm eggs, 76.92% and 11.54% for *Taenia* spp eggs, and 50.94% and 5.66% of the time for *Isospora* oocysts (Table 6).

■ VETERINARIAN AND VETERINARY TECHNICIAN EVALUATION OF COMMON FECAL FLOTATION TECHNIQUES

Another demonstration of the superiority of centrifugation as a flotation method to

detect parasites is provided by the results of a study conducted during a “wet lab” at the 2005 Central Veterinary Conference.⁴ Participants, which included veterinarians and registered veterinary technicians, were given a fecal sample from naturally parasitized dogs. The sample was a pooled sample from several dogs and contained eggs of *A. caninum*, *Eucoleus boehmi*, *Taenia* spp, *T. canis*, and *T. vulpis*; oocysts of *Cystoisospora* spp; and *Giardia* cysts.

We agree that Giardia is one of the most commonly misdiagnosed, underdiagnosed, and overdiagnosed parasites.

Each participant collected two “quarter-sized” samples from the feces and evaluated them using the 15-minute Ovassay, the swing-head centrifugation technique, and the Snap *Giardia* Test Kit (*Giardia* findings are discussed separately; see page 9). Participants were given a short lecture before the wet lab on how to conduct the various fecal examination techniques, provided written instructions, and shown color images of all parasite eggs, oocysts, and cysts that were in the fecal sample.

Table 7. Veterinarian and Veterinary Technician Comparison of Ovassay and Centrifugation Techniques for Recovery of Parasite Eggs and Oocysts Using 1.18-SG ZnSO₄ or 1.27-SG Sheather's Sugar Solution^a

PARASITES	GROUP 1—ZnSO ₄ (n = 14)									
	Ovassay					Centrifugation				
	0	1–10	11–50	>50	% Positive	0	1–10	11–50	>50	% Positive
<i>T. canis</i>	0	7	7	0	100.0	0	1	4	9	100.0
<i>T. vulpis</i>	2	9	3	0	85.71	0	2	8	4	100.0
<i>A. caninum</i>	1	12	1	0	92.86	0	4	7	4	100.0
<i>Taenia</i> spp	13	1	0	0	7.69	12	2	0	0	14.29
<i>Eucoleus boehmi</i>	10	4	0	0	28.57	0	9	5	0	100.0
<i>Cystoisospora</i> spp	10	4	0	0	28.57	0	6	8	0	100.0

PARASITES	GROUP 2—Sheather's Sugar Solution (n = 13)									
	Ovassay					Centrifugation				
	0	1–10	11–50	>50	% Positive	0	1–10	11–50	>50	% Positive
<i>T. canis</i>	3	5	5	0	76.92	0	0	4	9	100
<i>T. vulpis</i>	2	7	4	0	84.62	0	0	6	7	100
<i>A. caninum</i>	7	4	2	0	46.15	0	0	7	6	100
<i>Taenia</i> spp	5	8	0	0	61.54	0	3	10	0	100
<i>Eucoleus boehmi</i>	10	3	0	0	23.08	0	4	9	0	100
<i>Cystoisospora</i> spp	7	6	0	0	46.15	0	3	10	0	100

^aParticipants recorded the number of eggs recovered as 0, 1–10, 11–50, or >50 eggs/slide.

The participants were divided into two groups:

- Group 1 used 1.18-SG ZnSO₄ for the flotation solution and added one drop of Lugol's iodine to the slide before placing the coverslip.
- Group 2 conducted fecal examinations using 1.27-SG Sheather's sugar solution.

The data showed that centrifugation with either 1.18-SG ZnSO₄ or 1.27-SG Sheather's sugar solution routinely recovered more eggs and oocysts than the passive Ovassay technique (Table 7). Not only did the centrifugation technique recover more eggs and oocysts, but the participants recorded many more samples as positive with this technique. Only once did the Ovassay technique recover all parasites in all samples; in contrast, only once did the centrifugation technique fail to recover all parasites in all samples. Only two of 14 participants in Group 1 recovered *Taenia* eggs using the centrifugation procedure, whereas all 13 participants in Group 2 recovered *Taenia* eggs using the centrifugation technique.

Although ZnSO₄ has been shown to be an efficient flotation solution and is often used in veterinary practices, findings from this study highlight a potential problem in using 1.18-SG ZnSO₄, even in a centrifugation procedure. Whereas only two of 14 (14.29%) participants using the 1.18-SG ZnSO₄ centrifugation procedure correctly recorded that the sample was positive for *Taenia* eggs, 100% of the participants using the 1.27-SG Sheather's sugar solution recovered *Taenia* eggs from the same sample. This result was not completely unexpected because *Taenia* eggs have an average SG of 1.2251.⁵ This indicates that veterinary practices using 1.18-SG ZnSO₄ as their flotation solution are likely failing to identify some dogs infected with *Taenia* tapeworms and possibly other parasites that shed heavy eggs, such as *Physaloptera* spp, which have eggs with an average SG of 1.2376.⁵ Another investigation that evaluated the SG of a fecal flotation solution indicated that solutions with SGs of 1.22 to 1.35 would be best for routine laboratory use.⁶

■ DETECTING *Giardia* spp

As discussed, *Giardia* cysts are particularly difficult to recover and identify. The cysts are small and fragile, and infected animals shed the cysts intermittently. Several studies have demonstrated that recovery of *Giardia* cysts can best be accomplished using a 1.18-SG ZnSO₄ centrifugation technique,^{1,2,7,8} but a single negative flotation using ZnSO₄ and a swing-head centrifuge does not necessarily rule out *Giardia* infection. In addition, even though this technique recovers and maintains the integrity of *Giardia* cysts more consistently than passive flotation techniques, it does not alleviate the problem of proper cyst identification. In our experience, many practices using the 1.18-SG ZnSO₄ centrifugation technique still struggle with the accurate identification of *Giardia* spp. Several fecal antigen tests have been developed that appear to have high sensitivity in detecting *Giardia* antigen in human feces; however, they do not have the same level of sensitivity in detecting giardiasis in dogs and cats when compared with centrifugation using ZnSO₄.^{2,7,9} Thus, we recently conducted several studies comparing the Snap *Giardia* Test Kit (Figure 4) with the swing-head centrifugation technique using 1.18-SG ZnSO₄.

Veterinary Student Evaluations

During the fall of 2005, 107 second-year veterinary students participated in an exercise on recovering and identifying *Giardia* cysts. They had recently attended a lecture on giardiasis, been given a short visual presentation on identification of *Giardia* cysts, and had previously conducted direct smears and fecal examinations using the swing-head centrifugation technique. The students were also given written instructions on how to use the SNAP *Giardia* Test Kit.

Fecal samples from 116 puppies were provided by a local broker. Each fecal sample was analyzed via direct smear, centrifugation, and the SNAP *Giardia* Test Kit. Samples and techniques were recorded as either positive or negative. The direct smear was performed as described previously, except that a drop of Lugol's iodine was added to the slide before the coverslip was placed.

Almost half (56 of 116) of the puppy fecal samples were recorded as positive for *Giardia*. Only four direct-smear samples were identified as *Giardia* positive, although the data may be artificially low because the fecal samples were

collected several hours before being tested and trophozoites may have been dead at time of examination. Eleven samples that were negative for *Giardia* using the ZnSO₄ centrifugation technique tested *Giardia* positive using the SNAP *Giardia* Test Kit (Table 8). It is unknown how many of these results were true false-negatives on the ZnSO₄ centrifugation technique or false-positives on the SNAP *Giardia* Test Kit.

Findings from the Central Veterinary Conference Wet Lab

In addition to the wet lab evaluations discussed earlier, this study also compared the SNAP *Giardia* Test Kit with the Ovassay and swing-head centrifugation technique using 1.18-SG ZnSO₄

and 1.27-SG Sheather's sugar solution. Even though the participants in the wet lab were told the samples were positive for *Giardia* cysts, recovery and identification of *Giardia* cysts was problematic for the participants regardless of flotation technique used. Only six of 27 participants were able to recover and identify *Giardia* cysts from a known positive sample (Table 9): one participant each using the centrifugation technique with ZnSO₄, the Ovassay with ZnSO₄, and the Ovassay with

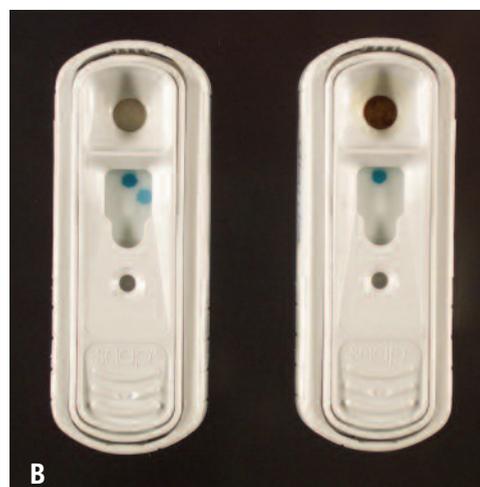


Figure 4. (A) SNAP *Giardia* Test Kit. (B) A positive result is shown on the left and a negative result on the right.

Eleven samples that were negative for *Giardia* using the ZnSO₄ centrifugation technique tested *Giardia* positive using the SNAP *Giardia* Test Kit.

Sheather's sugar solution and three using the centrifugation technique with Sheather's sugar solution. However, all 27 participants obtained a positive result using the SNAP *Giardia* Test Kit.

Table 8. Evaluation by Second-Year Veterinary Students of 1.18-SG ZnSO₄ Centrifugation Fecal Flotations and SNAP *Giardia* Tests to Identify *Giardia* spp in Puppies

Category	SNAP+	ZnSO ₄ +	SNAP+/ ZnSO ₄ -	ZnSO ₄ +/ SNAP-	SNAP+/ ZnSO ₄ +	SNAP-/ ZnSO ₄ -	<i>Giardia</i> + by Combined Methods
No. of samples in each category (total no. of samples tested = 116)	55	45	11	1	44	60	56
Percentage of samples in each category	47.41	38.79	9.48	0.86	37.93	51.72	48.28
Percentage of 56 samples recorded as <i>Giardia</i> positive identified as positive by each specific procedure	98.21	80.36	19.64	1.79	78.57		

Table 9. Number of Samples Identified as *Giardia* Positive by Attendees of the Central Veterinary Conference Wet Lab Using Ovassay and Centrifugation with 1.18-SG Zn SO₄ or 1.27-SG Sheather's Sugar Solution or Using the SNAP *Giardia* Test Kit

Method	No. of Samples Testing Positive
Centrifugation with ZnSO ₄	1
Ovassay with ZnSO ₄	1
Centrifugation with Sheather's Sugar Solution	3
Ovassay with Sheather's Sugar Solution	1
SNAP <i>Giardia</i> Test Kit	27

■ CLINICAL IMPLICATIONS

In today's litigious society, failure to detect a light parasitic infection in a pet, regardless of whether treatment was initiated, could be significant from a legal standpoint. Although lawsuits resulting from ocular larva migrans have usually revolved around failure to initiate appropriate deworming procedures, inappropriate diagnostic methodology could be an issue.

Our results show that centrifugation consistently recovered more eggs than the other techniques, even when comparing a 5-minute centrifugation with a 20-minute simple flotation. Failure to ensure that a flotation solution has the proper SG could result in the practitioner missing a diagnosis of *T. vulpis* or *T. canis*, both of which are pathogenic parasites in dogs. Solutions should be properly prepared following standard formulas (when using bulk sugar or salts) or specific label directions (when hydrating commercial salt solutions). After the

solution has been prepared, it is recommended that the SG be checked with a hydrometer capable of measuring SGs in the desired range. Veterinarians might be well advised to reevaluate their fecal examination protocols or, at the very least, to ensure their flotation solutions are formulated to attain an SG heavy enough to allow *T. vulpis* eggs to float. Spirurid (e.g., *Physaloptera* spp; SG 1.2376³) and tapeworm (e.g., *Taenia* spp; SG 1.2251) eggs are even heavier and require an SG of 1.224 or greater to effectively recover eggs from fecal samples.

Fecal flotation using a swing-head centrifuge and a 1.18-SG ZnSO₄ flotation solution should be adequate for the diagnosis of *Giardia* spp by trained personnel,^{1,2,7} but because many clinics do not use centrifugation techniques and cyst shedding is intermittent, it is often necessary to examine several sequential daily samples to ensure accuracy of diagnosis. In fact, it may take three consecutive ZnSO₄ fecal flotations over a 7- to 10-day period to obtain an accurate diagnosis of *Giardia* spp using the centrifugation flotation technique. Of course, this is often impractical in private practice.

Furthermore, even though the ZnSO₄ centrifugation procedure conducted by a well-trained technician has been considered the gold standard for the diagnosis of *Giardia* spp in dogs,^{1,2,7,8} participants in the Central Veterinary Conference wet lab using ZnSO₄ as their flotation solution recorded 13 of 14 samples as cyst negative that were positive on the SNAP *Giardia* Test Kit and were unable to identify cysts even when told that the samples were definitely positive for *Giardia*. Inability to recog-

Summary—What Every Practitioner Should Know about Diagnosing and Treating Gastrointestinal Parasites

Fecal examinations are important in diagnosing internal parasites in dogs and cats. As has been shown in the studies reported here, technique is critical:

- For most parasites, the swing-head centrifugation technique—including centrifuging the tube for 5 minutes and then allowing it to stand for 10 minutes before removing the coverslip—is better able to recover eggs and oocysts than is a direct smear or simple flotation technique.
- Sheather's sugar solution, with a specific gravity of 1.27, is the preferred flotation solution because it will float the heavier parasite eggs, such as *Taenia* spp.
- Some of the salt flotation solutions may be used as a practical alternative, but the specific gravity of any flotation solution should be confirmed with a hydrometer.
- In areas where *Giardia* is a concern, fecal examinations should also include the SNAP *Giardia* fecal antigen Test Kit.
- Once a diagnosis is confirmed, treatment with an appropriate dewormer is indicated. Several anthelmintics are effective for the treatment of *T. vulpis* in dogs, such as febantel, milbemycin oxime, and fenbendazole, whereas *Taenia* spp infections can be treated with praziquantel, epsiprantel, or fenbendazole. With three active ingredients with different modes of action and spectra of activity after a single dose, Drontal Plus (Bayer Animal Health) is an excellent broad-spectrum dewormer. Drontal Plus contains praziquantel to eliminate cestodes (tapeworms), pyrantel pamoate to eliminate hookworms and ascarids, and febantel to eliminate nematode parasites, including whipworms.
- Metronidazole, fenbendazole, and the combination of praziquantel, pyrantel pamoate, and febantel are commonly used for the treatment of giardiasis. We have found the combination of praziquantel, pyrantel pamoate, and febantel (5, 5, and 25 mg/kg, respectively; administered for 3 to 5 days) in Drontal Plus to be very effective for the treatment of giardiasis in dogs.

Because of the zoonotic potential of many internal parasites infecting cats and dogs, the importance of fecal examinations cannot be ignored. All personnel performing fecal examinations should be properly trained, and the attitude that "it's just a fecal" must be eradicated.

nize a few *Giardia* cysts is likely one of the problems encountered by the veterinarians and veterinary technicians who participated in the wet lab. The sample did not contain numerous *Giardia* cysts, and the cysts are smaller than other parasite eggs and oocysts in the sample. As we walked around the room to assist the participants, we observed cysts in almost every sample prepared using either ZnSO₄ or Sheather's sugar solution; many of the participants, however, were unable to recognize the small cysts.

Because cyst shedding is notoriously intermittent, the number of *Giardia* cysts recovered on any given day is likely not a good indicator of the level of infection. In a previous investigation, it was noted that dogs could have almost a 10-fold change in the number of cysts recovered on quantitative fecal examinations conducted 3 days apart.² In that study, the cysts/g of one control dog went from less than 10 to 3,190 within 3 days. In another dog, the cyst/g count increased from 150 to 44,610 within 3 days. The clinical implication of finding only a few *Giardia* cysts on fecal analysis may be no different from finding hundreds. Therefore, fecal examination from dogs or cats

suspected of having giardiasis requires careful microscopic examination in case they are shedding low numbers of cysts.

If giardiasis is on the differential list of a dog or cat with diarrhea, the data indicate that conducting both ZnSO₄ centrifugation fecal examination and a SNAP *Giardia* Test Kit may increase the chances of recording a positive finding. However, it must also be remembered that a single negative examination, even if both tests are conducted simultaneously, does not necessarily rule out giardiasis. Although using the proper *Giardia* cyst recovery technique is important, identification of recovered cysts is critical. In the Central Veterinary Conference wet lab, veterinarians and veterinary technicians had great difficulty identifying cysts even when informed the samples were positive. Proper training of veterinarians and clinical staff to identify *Giardia* cysts is important and would greatly improve diagnostic accuracy. An important point when using the SNAP *Giardia* Test Kit is that even a very slight color change indicates a positive test result.

The major question is, "What procedure or procedures should be conducted for routine

fecal examinations?” Data from the studies discussed here^{3,4} suggest that the swing-head centrifugation technique using 1.27-SG Sheather’s sugar solution is an efficient method of recovering many commonly encountered parasite eggs and oocysts. However, although the sugar solution is effective for many eggs and oocysts, it distorts and/or destroys most *Giardia* cysts, often rendering them unrecognizable to most veterinarians and technicians (Figure 3). In addition, currently used sugar solutions must be mixed on-site and some chemical (e.g., phenol, formalin) must be added to prevent bacterial growth; furthermore, sugar solutions are sticky and can attract flies and ants. Many practices, therefore, use commercially available salt solutions for routine fecal examinations; however, as demonstrated in this investigation, a 1.18-SG ZnSO₄ flotation solution may not be able to float parasite eggs with a higher SG.

■ TREATMENT

Once these updated diagnostic procedures are implemented, it is anticipated that many practices will see a marked increase in the number of positive fecal examinations. Certain parasites such as whipworms in dogs and *Taenia* spp tapeworms in dogs and cats will certainly be diagnosed with much greater frequency, which will necessitate an increase in antiparasitic treatments and follow-up examinations. Several anthelmintics are currently approved for treatment of *T. vulpis* in dogs, including Drontal Plus (Bayer Animal Health; praziquantel, pyrantel pamoate, and febantel), Interceptor (Novartis Animal Health; milbemycin oxime), Sentinel (Novartis Animal

Panacur. Many dogs will have shed numerous *Trichuris* eggs into their premises by the time they are treated, and those eggs may remain viable for years. Therefore, placing these dogs on long-term treatment/preventive therapy with a compound such as milbemycin oxime may be prudent. In addition, appropriate fecal examinations as described previously should be conducted 1 and 3 months after treatment to evaluate initial success and then once or twice annually to check for possible reinfection.

Taenia spp infections can be treated orally with products containing praziquantel, epsiprantel, or fenbendazole. Dogs and cats acquire *Taenia* spp tapeworms after eating metacystode-infected prey or animal carcasses, and thus infections will likely recur unless pets are prohibited from hunting and scavenging.

A positive *Giardia* spp fecal antigen test result necessitates an initial round of treatment even in asymptomatic dogs and cats. Although most animal strains of *Giardia* spp are not infective to immunocompetent human hosts,¹¹ all cases in pets must be treated as potentially zoonotic. Currently, no drugs are approved by the FDA for treatment of giardiasis in dogs and cats. Metronidazole, fenbendazole, and the combination of praziquantel, pyrantel pamoate, and febantel are commonly used. Although metronidazole has historically been the most common extra-label treatment, reports of efficacy failures are common. In addition, metronidazole has other properties that can be both positive and negative when treating clinical diarrhea. Metronidazole is effective against anaerobic infections, has anti-inflammatory properties, and is an effective medication for diarrhea associated with colitis.¹² Therefore, metronidazole therapy often firms up loose stools regardless of the cause of the diarrhea. Veterinarians often report to us that they diagnose giardiasis based on a presumptive identification of cysts or trophozoites and rapid response (cessation of loose stools) to metronidazole therapy. Then, when they stop the therapy, giardiasis (diarrhea) returns. Thinking that this indicates either treatment failure or recurrence of infection, they increase the dose or frequency of administration. However, once therapy ceases, diarrhea might recur yet again. Many of the fecal samples submitted to the Kansas State University Diagnostic Parasitology Laboratory as *Giardia* suspects are often heavily laden with various yeasts but no *Giardia* cysts. Use of metronida-

Only six of 27 participants were able to recover and identify Giardia cysts from a known positive sample.

Health; milbemycin oxime and lufenuron), and Panacur (Intervet; 3-day course of fenbendazole). Because of the long prepatent period (70 to 90 days) of *T. vulpis* and tolerance of immature stages to anthelmintics, treatments should be administered on days 0 and 3 and then again at 4 weeks and 3 months.¹⁰ In our experience, clinical *Trichuris* infections are best treated initially with either Drontal Plus or

zole may therefore lead to a misdiagnosis of giardiasis.

We recommend the use of fenbendazole (50 mg/kg) or the combination of praziquantel, pyrantel pamoate, and febantel (5, 5, and 25 mg/kg, respectively) for 3 to 5 days for the treatment of giardiasis in dogs.^{2,13} It has also been suggested that metronidazole (25 mg/kg bid) may be used in combination with fenbendazole.¹¹ Cats may be treated with either fenbendazole (50 mg/kg sid) or metronidazole (25 mg/kg bid) for 5 days.¹¹ In addition, all dogs and cats in the household should be treated, and animals should be bathed after the last treatment to remove infective cysts from the haircoat.

After treatment, fecal examinations and/or fecal antigen tests should be repeated to determine the success of therapy. Persistently positive fecal antigen test results can confound evaluation of treatment success. Although published data are currently lacking, communication with manufacturers and our experience indicate that the SNAP *Giardia* Test Kit may remain positive in some dogs for 1 to 3 weeks after successful treatment.

CONCLUSION

Because of the inability of 1.18-SG ZnSO₄ flotation solution to consistently recover heavier parasite eggs, it may be prudent for many veterinary practices to conduct routine fecal examinations using 1.27-SG Sheather's sugar solution and a centrifuge. Veterinary practices should also consider the routine use of a hydrometer so that the proper SG of their flotation solution can be assured. If giardiasis is encountered in the practice area, fecal examinations should include an in-clinic *Giardia* soluble fecal antigen test, such as the SNAP *Giardia* Test Kit. The difficulty we noted in the ability of veterinarians, veterinary technicians, and veterinary students to identify *Giardia* cysts in our studies is likely reflective of the situation in many practices. Therefore, the SNAP *Giardia* Test Kit likely will improve a clinic's ability to arrive at a correct diagnosis. In addition, the proper recovery and identification of

parasites should allow for a more targeted therapeutic approach.

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Standard Centrifugation Fecal Examination: A Step-by-Step Guide*



1 Make sure all necessary equipment is on hand: Centrifuge, conical test tubes and rack, flotation solution, applicator sticks, disposable cup, strainer, and microscope.



2 Mix 2 to 5 g of feces with approximately 10 ml of flotation solution in a disposable cup until the consistency is uniform.



3 Strain the mixture.



4 Pour the strained mixture into a 15-ml centrifuge tube.



5 Fill the tube with flotation solution to form a slight positive meniscus; do not overfill the tube.



6 Place a coverslip on top of the tube.



7 Put the tube in the centrifuge, make sure the centrifuge is balanced, and spin at 1,200 rpm ($280 \times g$) for 5 minutes.



8 Remove the tube and let it stand for 10 minutes.



9 Lift the coverslip directly upward and place on a glass microscope slide. Examine the entire area under the coverslip at $10\times$ magnification.[†]

*All images ©2006 Veterinary Learning Systems.

[†]You may wish to use the $40\times$ objective to confirm the diagnosis and make measurements; with practice, however, most parasites can be identified at $10\times$ magnification.

Commentary

A Strategic Approach to Diagnosing and Treating Gastrointestinal Parasites

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As stated in this excellent article by Dryden and colleagues, conducting fecal examinations accurately is a must in veterinary practice. Frequent and accurate fecal examinations are necessary for several reasons: Gastrointestinal (GI) parasites remain common in dogs despite the availability of many excellent broad-spectrum agents;¹ certain GI parasites, such as *Toxocara*, *Ancylostoma*, *Dipylidium*, *Giardia*, *Cryptosporidium*, and *Toxoplasma*, are important zoonotic agents and may require the use of specific parasite control products; and pet owners often do not administer broad-spectrum agents regularly even when instructed to do so by veterinarians. Veterinarians and pet owners may believe that fecal examinations are unnecessary because they think that well cared for pets are not exposed to parasites or they presume the animals are receiving heartworm or flea preventives that have additional broad-spectrum activity against GI parasites. I agree with the authors' admonition that failure to monitor parasite control strategies using appropriate fecal examination procedures can result in potentially serious pet and human health issues.

Common fecal examination procedures used to diagnose GI parasites include direct smear, sedimentation, simple flotation, centrifugal flotation, and fecal ELISA. Dryden et al provide excellent overviews of the comparable performance and strengths and weaknesses of each of these procedures. They also include data from their clinical and teaching laboratories and conference "wet labs" to support their conclusions and recommendations. Several points they raised are worthy of comment. Their results provide the clear message that centrifugal flotation is superior to either direct smear or simple flotation when attempting to recover fecal stages of common GI parasites. They demonstrated significantly increased rates of recovery of roundworm, hookworm, whipworm, tapeworm, and *Physaloptera* ova when using centrifugation. These same results were demonstrated in exercises conducted with veterinarians, veterinary technicians, and students. Results of the studies indicate that centrifugation can be performed with equal accuracy using either a swinging bucket or fixed-angle centrifuge. I also have emphasized these points.² Ova of *Trichuris vulpis* and *Taenia* spp can be especially difficult to

recover by fecal flotation because of their density and their intermittent or erratic passage in feces. Again, the data presented here support the use of centrifugation for greater sensitivity when examining feces for these parasites.

Giardia is a particularly problematic parasite for several reasons. Both trophozoites and cysts are sometimes difficult to recover from feces, even in animals with clinical giardiasis. While it is true that trophozoites are more likely to be observed in diarrheic feces and cysts in formed feces, I have observed exceptions to this claim. Because trophozoites are bound

by a single unit membrane, they are fragile and easily destroyed or damaged by dense flotation solutions. Thus, direct examination of feces (direct smear) may result in better recovery of trophozoites; however, direct smears are less sensitive than centrifugal flotation because of the small amount of feces examined. Cysts, on the other hand, may be difficult to detect because of intermittent passage in feces or because veterinarians or trained staff may not recognize them. The recent introduction of a fecal ELISA for detection of a *Giardia*-specific protein in feces (SNAP *Giardia* Test Kit) has greatly improved our capability to confirm or rule out giardiasis as a potential cause of clinical diarrhea in dogs and cats. Initial data supporting sensitivity and specificity of the test are very encouraging. In the



FIGURE 1. Iodine-stained trophozoite of *Giardia* sp from a direct smear.



FIGURE 2. Iodine-stained cyst of *Giardia* sp from centrifugal flotation in zinc sulfate.

Table 1. Summary of Selected Dewormers

<i>Parasite</i>	<i>Dewormer</i>	<i>Regimen</i>
Ascarids and/or hookworms	Febantel/pyrantel pamoate/praziquantel	Single dose; follow-up doses discretionary
	Pyrantel pamoate praziquantel (cats only)	Single dose; follow-up doses discretionary
	Pyrantel pamoate	Single dose; follow-up doses discretionary
	Fenbendazole	Daily for 3 days; follow-up doses discretionary
	Milbemycin oxime	Single dose followed by monthly administration ^a
	Milbemycin oxime/lufenuron	Single dose followed by monthly administration ^{a,b}
	Ivermectin/pyrantel pamoate Selamectin (cats only)	Single dose followed by monthly administration ^a Single dose followed by monthly administration ^{a,b}
Ascarids, hookworms, and whipworms; whipworms only; or combinations that include whipworms	Febantel/pyrantel pamoate/praziquantel	Single dose; follow-up doses discretionary
	Fenbendazole	Three daily doses; follow-up doses discretionary
	Milbemycin oxime	Single dose followed by monthly administration ^a
	Milbemycin oxime/lufenuron	Single dose followed by monthly administration ^{a,b}
Ascarids, hookworms, whipworms, and tapeworms, or combinations that include whipworms and tapeworms	Febantel/pyrantel pamoate/praziquantel	Single dose; follow-up doses discretionary
Ascarids, hookworms, whipworms, and tapeworms, or combinations that include <i>Giardia</i>	Febantel/pyrantel pamoate/praziquantel	Daily for 3–5 days

^aMonthly administration necessary for effective heartworm prevention.

^bMonthly administration necessary for effective heartworm prevention and flea control.

present publication, the authors provide convincing evidence that a combination of centrifugal flotation using zinc sulfate and fecal ELISA greatly increases our likelihood of positively identifying *Giardia*-positive samples. The use of zinc sulfate is suggested because it causes less distortion to *Giardia* organisms within cysts than does sucrose solution (Figure 1). I find that using iodine to stain either direct smears or fecal flotation preparations enhances the internal structure of both cysts and trophozoites and helps to distinguish *Giardia* cysts from yeasts and plant remnants (Figure 2).

Dr. Dryden and colleagues discuss several broad-spectrum agents that are effective in treating and controlling GI parasites. However, different strategies must be used depending on the parasites recovered. Table 1 summarizes selected dewormers with activities against ascarids, hookworms, whipworms, tapeworms, *Giardia*, and combinations thereof. Some are strategic dewormers; that is, they are used once to remove the target parasites and then at subsequent strategic intervals to eliminate migrating parasites or to maintain the host free of parasites. Others are used monthly for heartworm prevention or flea control and also possess additional activity against certain GI parasites. I agree with the authors' comments on the lack of approved products for treatment of giardiasis and that treatment with metronidazole alone often has

limited success. I concur that efficacy can be improved by using either fenbendazole or a combination of febantel, pyrantel pamoate, and praziquantel. Also, as Dryden and colleagues mention, metronidazole may be used in combination with fenbendazole or febantel, pyrantel pamoate, and praziquantel to achieve enhanced efficacy against *Giardia*; the combination of febantel, pyrantel pamoate, and praziquantel could also prove efficacious against other parasites.

In summary, available data support centrifugal flotation as being the most reliable method of recovering ova and cysts of GI parasites, including tapeworms and whipworms. Use of centrifugal flotation in combination with the Snap *Giardia* Test Kit increases the likelihood of diagnosing *Giardia* infections. Several broad-spectrum agents are available for the treatment and control of major GI parasites. A combination of febantel, pyrantel pamoate, and praziquantel is effective against *Giardia* as well as important nematode and cestode parasites.

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