Comparison of Common Fecal Flotation Techniques for the Recovery of Parasite Eggs and Oocysts*

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CLINICAL RELEVANCE

A variety of procedures are available to detect parasite eggs or oocysts in feces. This study compared the efficacy of simple flotation, a commercial assay, and various centrifugation techniques and three common flotation solutions. Results indicate that centrifugation consistently recovered more eggs than other methods. Proper technique is critical, including ensuring that the specific gravity of the flotation solution is correct and allowing the sample to stand for a sufficient amount of time before examining the coverslip. Because of the zoonotic health risks of many companion animal parasites, veterinarians and their staff should better utilize fecal examinations in their routine diagnostic plan.

INTRODUCTION

To ensure the health and well-being of pet dogs and cats, coprologic examinations for parasite eggs and oocysts are an important part of the daily routine for most veterinary practices. Many different procedures and techniques are used, each with its own advantages and limitations. Direct fecal smears are useful for detecting motile protozoa, and sedimentation examinations are useful 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. The methods most frequently used to recover parasite eggs and oocysts are flotation techniques that rely on the differences in the specific gravity (SG) of the egg(s), fecal debris, and flotation solution.

The SG of most parasite eggs is between 1.05 and 1.23.[1] For parasite eggs to float, the SG of the flotation solution must be greater than that of the eggs. Ideally, all helminth eggs and protozoan oocysts would float and still maintain their morphologic integrity while fecal debris would sink in the chosen flotation solution. Flotation

*Publication of this article is being funded by an educational grant from Bayer Animal Health, Shawnee Mission, KS.

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). Common flotation solutions include saturated sodium chloride (NaCl; SG 1.18), sugar (Sheather's solution; SG 1.27 to 1.33), sodium nitrate ($NaNO_3$; SG 1.18 to 1.20), magnesium sulfate ($MgSO_4$; SG 1.20), and zinc sulfate ($ZnSO_4$; SG 1.20). These solutions are effective, easy to make or commercially available, and relatively inexpensive.

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 then 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 is removed from the top 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. Several commercial apparatuses that use a screen to prevent debris from floating to the top are variations of the simple shell vial technique.

A further refinement of the flotation technique involves centrifugation to spin down the debris and allow the eggs to float to the surface of the solution where they can be recovered. If a fixed-head centrifuge is used, the centrifuge tubes cannot be filled completely and thus should be removed from the centrifuge after spinning and placed vertically in a test tube rack. If a swing-head centrifuge is used, the tubes can be filled to a slight positive meniscus and covered with 18- or 22-mm^2 coverslips before spinning. When tubes are spun with coverslips in place, care should be taken not to open the centrifuge before it stops spinning, or the coverslips can shift and ruin the preparation. Veterinary hospitals usually use one or more of these methods based on cost, ease of use, availability of hardware, or simply tradition.

Flotation Solutions for Helminth Ova[a]

Magnesium Sulfate ($MgSO_4$; SG 1.20)
450 g $MgSO_4$
1,000 ml tap water

Zinc Sulfate ($ZnSO_4$; SG 1.18–1.20)
331 g $ZnSO_4$
1,000 ml warm tap water

Sodium Nitrate Solution ($NaNO_3$; SG 1.18–1.20)
338 g $NaNO_3$
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.

[a]Check 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.

The purpose of this study was to compare the relative efficacies of simple flotation and centrifugation procedures and three commonly used flotation solutions in recovering common helminth eggs and oocysts from canine feces.

MATERIALS AND METHODS

Several trials were run to evaluate and compare different flotation techniques and flota-

Standard Centrifugation Fecal Examination Technique

1. Weigh out 2–5 g of feces.
2. Mix feces with approximately 10 ml flotation solution.
3. Pour mixture through a tea strainer into a beaker or fecal cup.
4. Pour strained solution into a 15-ml centrifuge tube.
5. Fill tube with flotation solution until a slight positive meniscus forms.[a,b]
6. Place a coverslip on the tube, and put the tube in the centrifuge.[b]
7. Make sure the centrifuge is balanced.
8. Centrifuge at 1,200 rpm (280 ×*g*) for **5 minutes**.
9. Remove the tube and let it stand for **10 minutes**.[c]
10. Remove the coverslip and place on a glass slide. Systematically examine the entire area under the coverslip at 100 diameters (i.e., 10× magnification). You may wish to use the 40× objective lens to confirm the diagnosis and make measurements; however, *with practice, most parasites can be identified at 100 diameters.*

[a]**Do not** overfill the tube. Doing so will cause some of the floating eggs to be forced down the side of the tube when the coverslip is placed.
[b]Steps 5 and 6 are done only if the centrifuge has a swinging bucket rotor (swing-head). If the centrifuge has a fixed angle head (fixed-head), the tube is spun without being completely filled. After centrifuging, the tube is moved to a test tube rack and filled with flotation solution until a slight positive meniscus forms; a coverslip is then placed on the tube, and the tube is allowed to stand for an additional 10 minutes before the coverslip is removed and examined.
[c]Step 9 was not done in the first and second series of experiments.

tion solutions in their ability to recover common helminth eggs from canine feces. All centrifugations were done at 280 ×*g*.

Role of the Specific Gravity of a Fecal Solution and Comparison of Ovassay and Swing-Head Centrifuge

In the first series of experiments, the ability of two methods to recover *Toxocara canis, Ancylostoma caninum,* and *Trichuris vulpis* eggs from canine feces was compared: The 15-minute Ovassay (Symbiotics) method using $ZnSO_4$ solutions having SGs of 1.1 and 1.2 was compared with the 5-minute swing-head centrifugation method (see box above) using the same $ZnSO_4$ solutions as well as a sugar solution with SG adjusted to 1.2. These experiments were designed to demonstrate the importance of exercising care in preparing flotation solutions to obtain proper SG. The best way to ensure that a flotation solution has the proper SG is to test it with a hydrometer calibrated to measure in the range desired (we used the Specific Gravity Hydrometer, Fisher Scientific, St. Louis, MO). Hydrometers are available to measure SGs from 1.0 to 1.4; a hydrometer used to test urine SG will not work. Fecal samples from each of three dogs having mixed infections of *T. canis, A. caninum,* and *T. vulpis* were thoroughly combined, and replicate 2-g samples were weighed out. The data presented are the mean parasite egg counts of three 2-g samples.

Comparison of Simple Flotation and Swing-Head Centrifuge

The second set of experiments compared the number of eggs recovered using $NaNO_3$ and sugar solutions in the simple flotation technique and the 5-minute swing-head centrifuge technique. The classic simple flotation technique involves placing a small amount of feces

in a cylindric container (usually a shell vial), adding flotation solution, mixing thoroughly, and allowing the preparation to stand for specified times (5, 10, 15, and 20 minutes) to allow the eggs to rise to the surface. To compare results of simple flotation and centrifugation methods, 15-ml polystyrene centrifuge tubes (product no. 889-205004, Oxford Labware, St. Louis, MO) were used for both techniques to keep the height of the column constant. SG of the $NaNO_3$ solution was adjusted to 1.2 and that of Sheather's sugar solution was adjusted to 1.27; SGs were confirmed with a hydrometer. Feces were collected from three dogs harboring *T. canis, A. caninum,* and *T. vulpis* and thoroughly mixed; forty-eight 2-g samples were removed. The data presented are mean parasite egg counts of three 2-g samples obtained with sugar and $NaNO_3$.

Comparison of Time to Examination and Parasite Egg Recovery

A third series of experiments was conducted to determine whether more parasite eggs could be recovered if tubes were allowed to sit undisturbed for 10 minutes after samples were centrifuged. In these experiments, 2-g samples of feces were obtained as described previously, mixed with 1.20-SG $NaNO_3$, and centrifuged at 280 ×*g* for 5 minutes in a swing-head centrifuge (see box on page 17). Coverslips were either removed and examined immediately after the centrifuge stopped spinning or were left undisturbed while the tubes sat for an additional 10 minutes; coverslips were then removed and examined. The data presented are mean parasite egg counts of three 2-g samples.

Comparison of Swing- and Fixed-Head Centrifuge Techniques

The fourth series of experiments compared swing- and fixed-head centrifuge techniques (see box on page 17). Fecal samples (2 g) were obtained as described previously. When a fixed-head centrifuge was used, approximately 10 ml of flotation solution (sugar or $NaNO_3$) 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 added, and the preparation was allowed to stand for 10 minutes before coverslips were removed to a glass slide and examined. When the swing-head method was used, flotation solution was added until a slight positive meniscus formed and a coverslip was placed. The covered tube was placed in centrifuge and spun at 280 ×*g* for 5 minutes. After being centrifuged, the tubes were placed in a test tube rack and left undisturbed for an additional 10 minutes. The data presented are mean parasite egg counts of three 2-g samples.

When the Ovassay method with 1.1-SG $ZnSO_4$ solution was used, only hookworm eggs readily floated.

Veterinary Student Evaluation of Egg and Oocyst Recovery Techniques

The fifth series of experiments was conducted to provide second-year veterinary students with the opportunity to evaluate various fecal techniques. From fall 2000 to fall 2004, students were given a short visual presentation on how to perform the direct smear, Ovassay, and swing-head centrifugation techniques. Stu-

dents were also given written directions on conducting the swing-head centrifugation technique (see box on page 17) and the directions that accompany the Ovassay kit. The centrifugation technique included a 5-minute spin followed by a 10-minute rest before coverslips were moved to a glass slide, whereas the Ovassay was allowed to sit for 15 minutes. Both the Ovassay and centrifugation techniques were conducted using Sheather's sugar solution with an SG of 1.23 to 1.27. 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, and the slide was covered with a glass coverslip. Such smears must be thin enough to read newsprint through them.

Students collected 5-g samples from cat and dog feces known to contain parasite eggs. No quantification of egg or oocyst numbers was conducted before the students evaluated the samples. The Ovassay and centrifugation technique were performed using 2-g samples. Students conducted each of the three techniques on a given sample. Slides were systematically examined, and results were recorded as 0, 1 to 10, 11 to 50, or more than 50 eggs or oocysts/slide. Results are presented only for samples evaluated by 10 or more students.

Statistical Analysis

An analysis of variance (ANOVA) using the actual fecal egg counts for each of the test methods was used for each "series" of experiments. Initially, each specific combination (defined using method, solution, SG, time, swing- or fixed-head centrifuge, etc.) was classified as a unique overall method and compared within each "series." In addition, each "series" provided an alternate method of analysis, using method, solution, SG, time of centrifugation, time before removing coverslip, and/or swing- or fixed-head centrifuge as factors in the ANOVA.

RESULTS

When the Ovassay method with 1.1-SG $ZnSO_4$ solution was used, hookworm (*A. caninum*) eggs (SG 1.0559[1]) readily floated; however, only one ascarid (*T. canis*) egg (SG 1.0900) was recovered from one of three samples, and no whipworm (*T. vulpis*) eggs (SG 1.1453) were recovered from canine feces. This points out the necessity for using care in weighing the salts and measuring water when preparing flotation solutions and for assuring proper SG by testing the solution with an SG hydrometer. When the SG of the salt solution ($ZnSO_4$) was raised to the usual 1.2, *T. vulpis* and *T. canis* eggs were recovered in the Ovassay but in fewer numbers than with the centrifugation method using either $ZnSO_4$ or sugar (Table 1). For all three parasites, the centrifugation method exhibited significantly higher fecal egg counts compared with the Ovassay method (Table 1). For *A. caninum,* no differences were found between the 1.2-SG $ZnSO_4$ and sugar solutions using the centrifugation method. Significantly higher *T. vulpis* egg counts were obtained from the sugar solution compared with 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.

The centrifugation method exhibited significantly higher fecal egg counts compared with the Ovassay method.

For all three parasites, the centrifugation method using 1.27-SG Sheather's sugar solu-

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 $ZnSO_4$ (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)*
A. caninum	680^a	$1{,}365^b$	782^a	$1{,}475^b$	$1{,}598^b$
T. vulpis	0^a	0^a	2.7^b	7.3^c	11.6^d
T. canis	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-Minute Swing-Head Centrifugation*	*Simple Standing Flotation*			
		5-Min	*10-Min*	*15-Min*	*20-Min*
A. caninum	52.3^a	1.7^b	2.7^b	5.3^b	5.3^b
T. vulpis	9^a	$0.7^{b,1}$	$0.7^{b,1}$	1.7^b	1.7^b
T. canis	293.3^a	22.3^b	17.3^b	20^b	21^b

^{a,b,c,d}Within each row, different letters indicate a statistically significant difference ($P < .05$).
[1]Two of the three samples tested were negative for *Trichuris* sp.

tion 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 $NaNO_3$ solution resulted in significantly higher fecal egg counts than the simple flotation method, which was allowed to stand for 5 or 10 minutes (Table 3). The 15- and 20-minute simple flotation methods recovered significantly similar fecal egg counts as compared with the centrifugation method. In this particular sample, relatively few *T. vulpis* eggs were retrieved using any of the methods. With such low numbers of *T. vulpis* eggs, the 5- and 10-minute simple flotations missed eggs in two of three samples. With *T. canis,* significantly more eggs were recovered using the centrifugation method than any of the flotation methods (Table 3). Although a direct comparison between solutions was not conducted, $NaNO_3$ appears to be preferable to sugar when conducting a simple flotation.

A. caninum and *T. canis* fecal egg counts were significantly greater when samples were allowed to sit for 10 minutes after being spun compared with examining the coverslip immediately after centrifugation (Table 4). The statistical comparison of *T. vulpis* fecal egg counts failed to show a difference between the two methods, likely because of the overall low eggs counts.

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 $NaNO_3$ (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*
A. caninum	23.7^{a}	7.3^{c}	$13.7^{b,c}$	$21.7^{a,b}$	$19.3^{a,b}$
T. vulpis	2.3^{a}	$0.7^{a,1}$	$0.7^{a,1}$	1.7^{a}	2.0^{a}
T. canis	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 ($P < .05$).
[1]Two of the three samples tested were negative for *Trichuris* sp.

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*
A. caninum	8.3^{a}	24.0^{b}
T. vulpis	3.7^{a}	5.3^{a}
T. canis	135.7^{a}	262.7^{b}

*Fecal samples were spun for 5 minutes in a swing-head centrifuge using 1.2-SG $NaNo_3$ solution.
^{a,b}Within each row, different letters indicate a statistically significant difference ($P < .05$).

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 $ZnSO_4$ 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*
A. *caninum*	137.7^{ab}	111.7^{a}	210.3^{b}	176.3^{ab}
T. *canis*	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. 2004 Student Comparison of Direct Smear, Ovassay, and Centrifugation Techniques for Recovery of Parasite Eggs and Oocysts Using 1.27-SG Sheather's Sugar Solution (Eggs or Oocysts/Slide*)

	Direct Smear					*Ovassay*				
Parasite	*0*	*1–10*	*11–50*	*>50*	*% Positive*	*0*	*1–10*	*11–50*	*>50*	*% Positive*
Sample 1 (N = 25)										
T. canis	23	2	0	0	8.00	1	12	11	1	96.00
A. caninum	23	2	0	0	8.00	7	14	4	0	72.00
T. vulpis	25	0	0	0	0.00	20	5	0	0	20.00
Sample 2 (N = 26)										
T. cati	26	0	0	0	0.00	22	3	0	0	11.54
Taenia sp	25	1	0	0	3.85	20	6	0	0	23.08
Isospora sp	26	0	0	0	0.00	21	4	1	0	19.23
Sample 3 (N = 14)										
T. canis	13	1	0	0	7.14	0	11	3	0	100.00
A. caninum	12	2	0	0	14.29	0	4	9	1	100.00

*Students recorded number of eggs or oocysts recovered as 0, 1–10, 11–50, or >50 eggs/slide.
N = total number of samples evaluated by students.

TABLE 7. 2003 Student Comparison of Direct Smear, Ovassay, and Centrifugation Techniques for Recovery of Parasite Eggs and Oocysts Using 1.27-SG Sheather's Sugar Solution (Eggs or Oocysts/Slide*)

	Direct Smear					*Ovassay*				
Parasite	*0*	*1–10*	*11–50*	*>50*	*% Positive*	*0*	*1–10*	*11–50*	*>50*	*% Positive*
Sample 1 (N = 29)										
A. caninum	27	2	0	0	6.90	0	17	12	0	100.00
T. vulpis	29	0	0	0	0.00	4	25	0	0	86.21
Sample 2 (N = 27)										
T. cati	22	5	0	0	18.52	3	12	12	0	88.89
Isospora sp	24	3	0	0	11.11	6	12	9	0	77.78
Sample 3 (N = 13)										
T. vulpis	13	0	0	0	0.00	7	4	2	0	46.15
Sample 4 (N = 12)										
T. vulpis	10	2	0	0	16.67	2	8	2	0	83.33

*Students recorded number of eggs or oocysts recovered as 0, 1–10, 11–50, or >50 eggs/slide.
N = total number of samples evaluated by students.

Centrifugation				
0	*1–10*	*11–50*	*>50*	*% Positive*
0	0	3	22	100.00
0	2	11	12	100.00
9	16	0	0	64.00
16	9	0	1	38.46
3	17	2	4	88.46
3	11	9	3	88.46
1	3	6	4	92.86
1	0	6	7	92.86

Centrifugation				
0	*1–10*	*11–50*	*>50*	*% Positive*
0	2	11	16	100.00
0	4	18	7	100.00
0	1	8	18	100.00
0	3	9	15	100.00
0	6	4	3	100.00
0	1	6	5	100.00

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

Throughout the period from 2000 to 2004, students evaluated 206 fecal samples known to contain hookworm (*A. caninum*) eggs (Tables 6 to 10). When all hookworm data were combined, the direct smear technique failed to detect hookworm eggs 72.82% of the time. The Ovassay and centrifugation techniques yielded false-negative results 4.85% and 0.97% of the time, respectively, and recovered more than 50 eggs/slide 36.41% and 74.76% of the time, respectively (Tables 6 to 10).

Students evaluated 171 fecal samples known to contain ascarid (*T. canis* or *Toxocara cati*) eggs (Tables 6 to 10). When all ascarid data were combined, the direct smear technique failed to detect ascarid eggs 85.38% of the time. The Ovassay and centrifugation techniques yielded false-negative results 25.88% and 10.53% of the time, respectively, and recovered more than 50 eggs/slide 1.18% and 42.69% of the time, respectively (Tables 6 to 10).

Students evaluated 203 fecal samples known to contain whipworm (*T. vulpis*) eggs (Tables 6 to 10). When all whipworm data were combined, the direct smear technique failed to detect whipworm eggs 92.61% of the time. The Ovassay and centrifugation techniques yielded false-negative results 32.02% and 4.93% of the time, respectively, and recovered more than 50 eggs/slide 2.96% and 23.65% of the time, respectively (Tables 6 to 10).

Students also evaluated 53 fecal samples known to contain tapeworm (*Taenia* sp) eggs and 26 samples known to contain Coccidia (*Isospora* sp) oocysts (Tables 6 and 7). The di-

TABLE 8. 2002 Student Comparison of Direct Smear, Ovassay, and Centrifugation Techniques for Recovery of Parasite Eggs and Oocysts Using 1.27-SG Sheather's Sugar Solution (Eggs or Oocysts/Slide*)

	Direct Smear					*Ovassay*				
Parasite	*0*	*1–10*	*11–50*	*>50*	*% Positive*	*0*	*1–10*	*11–50*	*>50*	*% Positive*
Sample 1 (N = 23)										
A. caninum	20	3	0	0	13.04	0	9	10	4	100.00
T. vulpis	18	5	0	0	21.74	3	11	8	1	86.96
Sample 2 (N = 22)										
T. cati	17	5	0	0	22.73	2	18	2	0	90.91
Sample 3 (N = 10)										
T. canis	9	1	0	0	10.00	5	5	0	0	50.00
T. vulpis	10	0	0	0	0.00	2	8	0	0	80.00
A. caninum	7	3	0	0	30.00	0	1	2	7	100.00

*Students recorded number of eggs or oocysts recovered as 0, 1–10, 11–50, or >50 eggs/slide.
N = total number of samples evaluated by students.

TABLE 9. 2001 Student Comparison of Direct Smear, Ovassay, and Centrifugation Techniques for Recovery of Parasite Eggs and Oocysts Using 1.27-SG Sheather's Sugar Solution (Eggs or Oocysts/Slide*)

	Direct Smear					*Ovassay*				
Parasite	*0*	*1–10*	*11–50*	*>50*	*% Positive*	*0*	*1–10*	*11–50*	*>50*	*% Positive*
Sample 1 (N = 28)										
A. caninum	16	10	2	0	42.86	1	1	5	21	96.43
T. vulpis	26	2	0	0	0.07	4	17	7	0	85.71
Sample 2 (N = 23)										
T. cati	16	7	0	0	30.43	8	12	2	1	56.52

*Students recorded number of eggs or oocysts recovered as 0, 1–10, 11–50, or >50 eggs/slide.
N = total number of samples evaluated by students.

rect smear technique failed to detect tapeworm eggs 96.15% of the time. The Ovassay and centrifugation techniques yielded false-negative results for *Taenia* sp eggs 76.92% and 11.54% of the time, respectively (Table 6). When the two sets of Coccidia data were combined, the direct smear technique failed to detect Coccidia oocysts 94.34% of the time. The Ovassay and centrifugation techniques yielded false-negative results for *Isospora* sp oocysts 50.94% and 5.66% of the time, respectively (Tables 6 and 7).

Centrifugation				
0	*1–10*	*11–50*	*>50*	*% Positive*
1	1	3	18	95.65
1	3	7	12	95.65
0	0	12	10	100.00
0	5	5	0	100.00
0	0	9	1	100.00
0	0	0	10	100.00

Centrifugation				
0	*1–10*	*11–50*	*>50*	*% Positive*
0	1	1	26	100.00
0	7	20	1	100.00
0	8	5	10	100.00

DISCUSSION

Alcaino and Baker[2] found that when the numbers of eggs were small, centrifugation using sodium dichromate (SG 1.35) recovered *Trichuris ovis* eggs that a sodium nitrate noncentrifugation method failed to recover. By exchanging flotation solutions, they determined the difference was a function of the method, not the solution. The difference in the number of eggs recovered by the sodium dichromate centrifugation method (SDCF) compared with the noncentrifugation fecal flotation (FF) method was expressed as an SDCF/FF index. The SDCF/FF index was 2.4 for strongylate (e.g., hookworm) eggs, 3.2 for ascaridate (e.g., *Toxocara* and *Toxascaris*) eggs, and 6.0 for trichurate (whipworm) eggs.[2]

T. canis continues to cause human disease, even though its pathogenic potential has been recognized for nearly 50 years.[3] A single female ascarid passes an estimated 200,000 eggs/day via feces, so environmental contamination builds up very quickly. However, even in moderate to heavy infections, egg shedding is not always constant, and few eggs might be present in the specimen obtained for examination. When puppies ingest infective *T. canis* eggs, the eggs hatch in the stomach and migrate through the liver and lungs before maturing and becoming patent in the small intestine. However, because humans are abnormal hosts, the larvae migrate through the viscera (visceral larva migrans [VLM]) and often the eyes (ocular larva migrans [OLM]).[4,5] *T. canis* in humans has also presented as eosinophilic ascites and gastroenteritis.[6] This public health risk for zoonotic disease should be of sufficient importance to advise veterinarians to use the most sensitive diagnostic method available to detect *T. canis* and to treat even light infections.[7]

A. caninum wanders subcutaneously, at least to some extent, in the human host, causing cutaneous larva migrans (CLM),[8–11] and has more recently been implicated in human eosinophilic enteritis.[12,13] Immature hookworms being recovered from the ileum and cecum of humans[12] is a finding that necessitates further explanation by veterinarians when clients ask whether their

TABLE 10. 2000 Student Comparison of Direct Smear, Ovassay, and Centrifugation Techniques for Recovery of Parasite Eggs and Oocysts Using 1.27-SG Sheather's Sugar Solution (Eggs or Oocysts/Slide*)

	Direct Smear					*Ovassay*				
Parasite	*0*	*1–10*	*11–50*	*>50*	*% Positive*	*0*	*1–10*	*11–50*	*>50*	*% Positive*
Sample 1 (N = 77)										
A. caninum	45	20	10	2	41.56	2	10	23	42	97.40
Sample 2 (N = 24)										
T. canis	20	4	0	0	16.67	3	16	5	0	87.50
Sample 3 (N = 63)										
T. vulpis	57	5	1	0	9.52	23	27	8	5	63.49

*Students recorded number of eggs or oocysts recovered as 0, 1–10, 11–50, or >50 eggs/slide.
N = total number of samples evaluated by students.

children can become infected with canine hookworms.

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

Practitioners have told us that the reasons they use commercial fecal kits or a simple flotation method instead of centrifugation are that the former cost less to run and take less time. However, our results show that centrifugation consistently recovered more eggs than either of the other techniques, even when comparing a 5-minute centrifugation with a 20-minute simple flotation. Also, examining the coverslip before allowing the sample to stand for 15 minutes when using the simple flotation technique and a solution with an appropriate SG could result in a missed diagnosis of *T. vulpis.*

Failure to ensure that a prepared flotation solution has the proper SG could result in a missed diagnosis of either *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 (see box on page 16) 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.

While the sugar solution was very effective in the centrifugation methods, it consistently recovered fewer parasite eggs than did $NaNO_3$ when the simple flotation method was used. The increased viscosity of the sugar solution might impede egg recovery in a simple flotation. Examining the coverslip before all the eggs in the sample have had a chance to rise to the surface might result in a missed diagnosis or alter a clinical impression if far fewer eggs are recovered. Veterinarians might be well advised to reevaluate their fecal examination protocols or, at the very least, to be sure their flota-

	Centrifugation			
0	*1–10*	*11–50*	*>50*	*% Positive*
0	1	11	65	100.00
1	2	13	8	95.83
0	14	30	19	100.00

tion solutions are formulated to attain an SG heavy enough to allow *T. vulpis* eggs to float. Spirurid (e.g. *Physaloptera* sp; SG 1.2376[1]) and tapeworm (e.g., *Taenia* sp; SG 1.2251) eggs are even heavier and require an SG of 1.24 or greater to effectively recover eggs from fecal samples.

The student-generated data were consistent with data from the previous studies. Of interest was that at times there was a considerable range of egg counts recorded for a particular sample using the same technique. Some of this variability might be explained by uneven distribution of eggs or oocysts within a sample or poor technique—this was the first time some students had conducted these particular procedures. Even with some inherent variability in the various methods, the students determined that the centrifugation technique was more efficient in recovering parasite eggs and oocysts than the commercial passive flotation assay.

All fecal samples used in these evaluations were from naturally parasitized dogs and cats. The level of natural parasitism and corresponding fecal egg or oocyst counts would therefore vary among the different parasites. Thus, no comparison was conducted of the fecal egg or oocyst counts between different parasite species. In addition, only a few eggs or oocysts of a particular parasite were recovered in some of the evaluations. Higher numbers of eggs or oocysts in those samples might have altered some results.

CONCLUSION

Proper techniques are imperative for the accurate diagnosis of intestinal parasites in pets. Veterinarians and their staff should reevaluate their attitude of "it's only a fecal" and better utilize these important techniques in their routine diagnostic plan.

ACKNOWLEDGMENTS

We thank Ann Melli, Department of Pathology, Microbiology, and Immunology, College of Veterinary Medicine, University of California, Davis, for her important contributions to this project. We also thank Terry Settje, BA, Biostatistician, Pharmaceutical R&D, Bayer Animal Health, Shawnee, KS, for statistical evaluation of the data. Thanks also to the second-year veterinary students at Kansas State University for their efforts in conducting the fecal techniques comparison laboratory.

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