

# Clinical Diagnostic Parasitology

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To ensure the health and well-being of pet dogs and cats, examination of feces for parasite eggs, oocysts, and cysts 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, oocysts, and cysts 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.<sup>1</sup> 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 cysts and oocysts would float and still maintain their morphologic integrity while fecal debris would sink in the chosen flotation solution. 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. Common flotation solutions include saturated sodium chloride (NaCl; SG 1.18), sugar (Sheather's solution; SG 1.27 to 1.33), sodium nitrate (NaNO<sub>3</sub>; SG 1.18 to 1.2), magnesium sulfate (MgSO<sub>4</sub>; SG 1.2), and zinc sulfate (ZnSO<sub>4</sub>; SG 1.2). 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 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. Several commercial apparatuses that use a screen to retain 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-angle centrifuge head 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<sup>2</sup> coverslips before centrifuging. 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.

The Ovassay method with 1.1-SG ZnSO<sub>4</sub> solution readily floats, hookworm (*A. caninum*) eggs (SG 1.0559<sup>1</sup>); however, ascarid (*T. canis*) eggs (SG 1.0900) may not be recovered and whipworm (*T. vulpis*) eggs (SG 1.1453) are virtually impossible to float with such a solution.<sup>2</sup> 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<sub>4</sub>) is raised to 1.2, *T. vulpis*, and *T. canis* eggs are recovered in the Ovassay but in fewer numbers than with a centrifugation method using either ZnSO<sub>4</sub> or sugar. A centrifugation method will recover significantly higher fecal counts compared with the Ovassay method.

For *A. caninum*, a centrifugation method using 1.2-SG NaNO<sub>3</sub> solution results in significantly higher fecal egg counts than the simple flotation method, which is allowed to stand for 5 or 10 minutes.<sup>2</sup> A 15- or 20-minute simple flotation method recovers significantly similar fecal counts as compared with the centrifugation method. With low numbers of *T. vulpis* eggs the 5' and 10' simple floats can miss eggs in 2 out of 3 samples.

Over the past decade a number of studies have been conducted to evaluate and compare the performance of various fecal diagnostic techniques.<sup>2-9</sup>

From 2000 to 2004, students at KSU evaluated 206 fecal samples known to contain hookworm (*A. caninum*) eggs.<sup>2</sup> 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.<sup>2</sup>

Students evaluated 171 fecal samples known to contain ascarid (*T. canis* or *T. cati*) eggs. 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.<sup>2</sup>

Students evaluated 203 fecal samples known to contain whipworm (*T. vulpis*) eggs. When all whipworm data was 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.<sup>2</sup>

Students also evaluated 53 fecal samples known to contain tapeworm (*Taenia* sp) oocysts and 26 samples known to contain coccidia (*Isospora* sp) oocysts. The direct smear technique failed to detect tapeworm eggs 96.15% of the time. The Ovassay and centrifugation techniques yielded false-negative results 76.92% and 11.54% of the time, respectively. 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 50.94% and 5.66% of the time, respectively.<sup>2</sup>

Evaluations of centrifugation fecal techniques and IDEXX SNAP® Giardia fecal antigen test kits of puppy fecal samples by 2<sup>nd</sup> year veterinary students showed that almost half (56/116) of the fecal samples were recorded as positive for Giardia. The direct smear technique detected the fewest number of positives with students recording only 4 positive samples. This data may be artificially low since the fecals were collected several hours prior to laboratory and trophozoites may have been dead at time of examination. Students recorded that the SNAP® Giardia fecal antigen test identified 55 of 116 samples as Giardia positive and ZnSO<sub>4</sub> centrifugation technique recorded 45 of 116 samples as positive.

At a wet lab conducted at the Central Veterinary Conference in 2005 twenty-seven (27) participants returned completed fecal data forms. When a centrifugation fecal flotation technique was compared to passive flotation technique the data demonstrated that centrifugation with either 1.18 sp. gr. ZNSO<sub>4</sub> or 1.27 sp. gr. Sheather's sugar solution routinely recovers more eggs and oocysts than the passive Ovassay technique. Not only did the centrifugation technique recover more eggs and oocysts in addition the participants recorded many more samples as positive with the centrifugation technique. Strikingly only once (*T. canis* – Ovassay - ZNSO<sub>4</sub>) did the Ovassay technique recover all parasites in all samples, while only once did the centrifugation technique fail to recover all parasites in all samples. In the group that used 1.18 sp. gr. ZNSO<sub>4</sub> solution only 2 of 14 participants recovered *Taenia* sp. eggs. While in the group using 1.27 sp. gr. Sheather's sugar solution all 13 participants recovered *Taenia* sp. eggs using.

Even though the participants knew the samples were positive for Giardia recovery and identification of Giardia sp. oocysts was problematic for the 27 participants irregardless of technique. Only 6 of the 27 participants were able to recover and identify Giardia sp. oocysts from a known positive sample. One participant each using the Centrifugation with ZNSO<sub>4</sub>, Ovassay with ZNSO<sub>4</sub> and Ovassay with Sugar was able to recover and identify *Giardia* sp. cysts. Three participants using the Centrifugation with Sugar were able to recover and identify *Giardia* sp. cysts. All 27 participants had a positive SNAP® Giardia fecal antigen test on the mixed sample.

As part of a weeklong clinical Parasitology training program, veterinarians participated in a wet-lab evaluating fecal examination techniques.<sup>9</sup> Three classes were offered during 2010, 2011 and 2012, for a total of 9 classes that included 56 participants. Fecal samples were collected from dogs at the local animal shelter, verified as positive for various parasite diagnostic stages and mixed to form composite samples. While species of parasites in fecal samples varied, all 9 classes evaluated samples that contained *A. caninum*, *T. canis* and *T. vulpis* eggs. Each participant conducted a direct smear, an Ovassay using a 1.18 sp. gr. ZnSO<sub>4</sub> solution, a centrifugation procedure using 1.18 sp. gr. ZnSO<sub>4</sub> solution and a centrifugation procedure using 1.24 sp. gr. sugar solution. Using the direct smear technique, participants recovered *T. canis*, *T. vulpis* and *A. caninum* eggs 30.4% (17/56), 26.8% (15/56) and 30.4% (17/56) of the time, respectively. The Ovassay recovered *T. canis*, *T. vulpis* and *A. caninum* eggs 57.1% (32/56), 41.1% (23/56) and 87.5% (49/56) of the time, respectively. The centrifugation procedure with ZnSO<sub>4</sub> recovered *T. canis*, *T. vulpis* and *A. caninum* eggs 94.6% (53/56), 85.7% (48/56) and 100% (56/56) of the time, respectively. The centrifugation procedure with the sugar solution recovered *T. canis*, *T. vulpis* and *A. caninum* eggs 96.4% (54/56), 100% (56/56) and 100% (56/56) of the time, respectively. When the Ovassay technique was used, only 33.3%, 11.1% and 44.4% of the time did every participant recover *T. canis*, *T. vulpis* and *A. caninum* eggs, respectively. When the participants used the centrifugation procedure with sugar solution, every participant in every class recovered eggs of *T. vulpis* and *A. caninum* and 77.8% of the time every participant recovered eggs of *T. canis*.

## Conclusions

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 me 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, these data 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 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 method, it consistently recovered fewer parasite eggs than did NaNO<sub>3</sub> 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 test, to be sure their flotation solutions are formulated to attain a SG heavy enough to allow *T. vulpis* eggs to float. Spirurid (e.g. *Physaloptera* sp; SG 1.2376<sup>1</sup>) and tapeworm (e.g., *Taenia* sp; SG 1.2251) eggs are even heavier and require a SG of 1.22 or greater to effectively recover eggs from fecal samples.

ZnSO<sub>4</sub> has been shown to be the most efficient flotation solution for recovery of *Giardia* cysts and is often used in veterinary practices. The wet lab conducted at the CVC highlighted a potential problem in using 1.18 sp. gr. ZnSO<sub>4</sub> even in a centrifugation procedure. Only 2 of 14 (14.29%) participants that used the 1.18 sp. gr. ZnSO<sub>4</sub> centrifugation procedure correctly recorded that sample as positive for *Taenia* sp. eggs. While 100% of the participants using the 1.27 sp. gr. sugar solution recovered *Taenia* sp. eggs from the same sample. This result was not completely unexpected since *Taenia* sp. eggs have an average sp. gr. of 1.2251. This indicates that veterinary practices using 1.18 sp. gr. ZnSO<sub>4</sub> as their flotation solution are likely failing to identify some dogs infected with *Taenia* sp. tapeworms and possibly other parasites that shed heavy eggs such as *Physaloptera* sp. which has eggs with an average sp. gr. of 1.2376.

If Giardiasis is on the differential list of a dog (or cat) with diarrhea the data suggests that conducting both ZnSO<sub>4</sub> centrifugation fecal examination and a SNAP *Giardia* sp. fecal antigen test 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.

The major question is what procedure or procedures should be conducted for routine fecal examinations. Data from this current study and another study previously published by this author would suggest that swing-head centrifugation technique using 1.27 sp. gr. Sheather's sugar solution is the most efficient in recovering many commonly encountered parasite eggs and oocysts. While the sugar solution is effective for many eggs and oocysts it will distort and/or destroy most *Giardia* sp. cysts making them often unrecognizable to most veterinarians and technicians. Many practices therefore use ZnSO<sub>4</sub> as their flotation solution. However, as demonstrated in this investigation a ZnSO<sub>4</sub> flotation solution may not be able to float some heavy parasite eggs. In addition, there are the previously mentioned problems in many practices of correctly identifying *Giardia* sp. cysts even using ZnSO<sub>4</sub>. In this investigation registered veterinary technicians and veterinarians had great difficulty in identifying cysts even when informed the samples were positive. While proper training of veterinarians and clinical staff in correctly identifying *Giardia* sp. cysts is important and would likely greatly improve correct diagnoses, it may be difficult on a large scale basis.

Due to the inability of 1.18 sp gr. ZnSO<sub>4</sub> flotation solution to consistently recover heavier parasite eggs these authors recommend that for routine fecal examinations 1.27 sp. gr. Sheather's sugar solution should be used in a swing-head centrifugation technique. In addition, if Giardiasis is encountered in your practice area then the fecal examination should be accompanied by an efficient *Giardia* sp. fecal antigen snap test. Due to the difficulty of identifying *Giardia* sp. cysts, the in-clinic soluble *Giardia* spp. fecal antigen snap test likely will improve a clinic's ability to arrive at a correct diagnosis.

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