ALL ABOUT FECAL DIAGNOSTICS: CLASSIC AND CUTTING EDGE

Michael W. Dryden DVM, MS, PhD, DACVM (parasitology)
University Distinguished Professor of Veterinary Parasitology
College of Veterinary Medicine
Kansas State University
Manhattan KS 66502

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.[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 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 ($NaNO_3$; SG 1.18 to 1.2), magnesium sulfate ($MgSO_4$; SG 1.2), and zinc sulfate ($ZnSO_4$; 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^2 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 $ZnSO_4$ solution readily floats, hookworm (*A. caninum*) eggs (SG 1.0559[1]); 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.[2] 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 ($ZnSO_4$) 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 $ZnSO_4$ 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 $NaNO_3$ solution results in significantly higher fecal egg counts than the simple flotation method, which is allowed to stand for 5 or 10 minutes.[2] 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.[2-9]

From 2000 to 2004, students at KSU evaluated 206 fecal samples known to contain hookworm (*A. caninum*) eggs.[2] 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.[2]

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.[2]

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.[2]

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.[2]

Evaluations of centrifugation fecal techniques and IDEXX SNAP® Giardia fecal antigen test kits of puppy fecal samples by 2nd 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 $ZnSO_4$ 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. $ZNSO_4$ 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 - $ZNSO_4$) 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. $ZNSO_4$ 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 regardless 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 $ZNSO_4$, Ovassay with $ZNSO_4$ 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.[9] 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. ZnSO4 solution, a centrifugation procedure using 1.18 sp. gr. ZnSO4 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 ZnSO4 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.

Addition of Fecal Antigen Testing to your Standard Diagnostic Procedures. New methodology for detecting protein biomarkers secreted or excreted by nematodes in the intestinal lumen. Unique biomarkers now available for ascarid, hookworm, and whipworm. These biomarkers are produced by the worms and not the eggs. Beneficial because they can overcome issues with misidentification, spurious eggs from coprophagy and even prepatent periods.[10]

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