Comparison of Fecal Flotation Media and Methods

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ABSTRACT: *Although many methods exist for detecting parasite eggs in feces, fecal flotation is the primary method used in small animal veterinary practice to detect intestinal parasites. It is therefore imperative that fecal flotation be easy to conduct and that it accurately diagnose parasitic infection, thereby facilitating appropriate treatment of the host to prevent the spread of potential zoonotic diseases and other parasites of domestic animals. Few studies have been conducted to determine whether use of any one flotation method is preferable in terms of providing sound diagnostic results.*

Fecal samples from 60 adult dogs and 40 puppies from Alachua County Animal Services in Gainesville, Florida, were tested for parasite ova using standing flotation and centrifugation with flotation (CF) methods. Sodium nitrate and Sheather's sugar were used as flotation media. The numbers of helminth eggs and coccidian oocysts recovered using each medium with each method were counted and compared to determine the most effective method and medium for diagnosing parasite infection. A significant difference in the number of eggs floated by each medium was found when standing flotation was used. However, no statistical difference was detected between media or methods in the number of infections diagnosed.

Veterinary technicians commonly use fecal flotation to concentrate parasite eggs and oocysts, enhance detection of parasites, and aid in parasite diagnosis. Proper diagnosis of parasites with minimum cost, effort, and unnecessary human exposure to certain zoonotic parasites is desirable. Several flotation media exist, but few studies have been conducted to test the diagnostic efficiency of different flotation methods using different media.

The Companion Animal Parasite Council (CAPC) has proposed standards for veterinary diagnostic laboratory procedures,[1] including standard fecal flotation and CF. There is currently a debate among veterinarians and parasitologists about the CAPC recommendations concerning the necessity of centrifugation as part of the flotation process. In this study, we compared the use of two different flotation media in standing flotation and CF to determine the necessity of centrifugation in fecal flotation to diagnose infection.

MATERIALS AND METHODS

In 2005, 60 adult dogs and 40 puppies from Alachua County Animal Services in Gainesville, Florida, were tested for parasites using simple standing fecal flotation and CF methods with sodium nitrate (Fecasol [Evsco Pharmaceuticals, Buena, NJ]; specific gravity = 1.20) and Sheather's sugar (specific gravity = 1.30). Only dogs with no known parasite treatment before testing were included. Fresh fecal samples were collected, placed into individual sample containers, and refrigerated until testing to ensure minimal parasite development.

Each fecal sample was mixed thoroughly, and 1 g of feces from each sample was placed in each of four 15-ml test tubes. Flotation medium was added to the feces in

Glossary

Alpha — Probability that a statistically significant result will be found where none exists; a value of .05 is standard

Antibody — An immunoglobulin protein produced in blood or tissue in response to a specific antigen that destroys, weakens, or neutralizes that antigen

Chi-square test — Statistical test of significance in which observed values are compared with expected values

Fisher exact test — Test of independence of two outcomes

Mann-Whitney rank sum test — Statistical test used when the outcome being studied does not have a normal distribution

Standard deviation — Measure of the variability of a sample

***t* test** — Statistical test used when the outcome being studied has a normal distribution

each test tube, filling the tube halfway. Two tubes were used for each medium. A wooden applicator stick was used to thoroughly break up the feces and mix them with the medium in each tube to ensure that the parasite eggs were exposed to the medium. The tubes were then filled until a slight positive meniscus formed, and a 22 × 22-mm glass coverslip was placed on top of each tube. Care was taken to avoid air bubbles under the coverslip that could have prevented eggs from reaching the coverslip.

The standing tubes were left on the laboratory bench undisturbed, while the centrifuged tubes were spun at 1,500 rpm (650 ×*g*) for 10 minutes in a floor centrifuge with swing-out heads. After centrifugation, all coverslips were removed and placed onto microscope slides to be examined under a compound microscope at a viewing magnification of 100×. Higher magnifications were used to view questionable objects. All parasite eggs and oocysts seen were identified and counted. The sodium nitrate samples were read first to avoid the crystallization that occurs over time with this medium. The same observer read the slides throughout the experiment.

Prevalence of Parasite Species Detected in 100 Dogs from Gainesville, Florida

Parasite Species	*Total Number of Dogs Infected*	*Number of Infections Detected*			
		Sodium Nitrate (standing)	*Sodium Nitrate (CF)*	*Sheather's Sugar (standing)*	*Sheather's Sugar (CF)*
Ancylostoma caninum	70	70	69	64	69
Trichuris vulpis	24	23	23	21	23
Cystoisospora spp	16	10	13	9	14
Toxocara canis	11	9	9	9	11

Average Number of Eggs (Standard Deviation) of Parasite Species Recovered from 100 Dogs[a]

Parasite Species	*Sodium Nitrate (standing)*	*Sodium Nitrate (CF)*	*Sheather's Sugar (standing)*	*Sheather's Sugar (CF)*
Ancylostoma caninum	590 (799)	901 (1,159)	123 (231)	801 (1,280)
Trichuris vulpis	19 (27)	53 (114)	11 (31)	77 (154)
Cystoisospora spp	68 (151)	273 (587)	10 (21)	110 (256)
Toxocara canis	75 (80)	274 (336)	17 (20)	340 (380)

[a]All eggs and oocysts under the entire coverslip were counted.

The numbers of samples diagnosed positive or negative for each parasite were compared statistically using a chi-square test or a Fisher exact test, depending on the data. Alpha was set at 0.05. A *t* test was used to compare the egg counts by parasite and method except when the egg count data were not normally distributed, in which case a Mann-Whitney rank sum test was used. Only dogs in which infection with a specific parasite was diagnosed by at least one method were included in the chi-square and *t* tests. The results for *Dipylidium caninum* were not analyzed because only two of 100 dogs were positive for this parasite.

RESULTS

The prevalence of *Ancylostoma caninum* was the highest of any parasite detected (70%). The other parasites detected were *Trichuris vulpis* (24%), *Cystoisopora* spp (16%), *Toxocara canis* (11%), and *D. caninum* (2%). Sodium nitrate standing flotation detected significantly more *A. caninum* infections than did Sheather's sugar standing flotation ($P = .037$). There were no other significant differences in number of infections diagnosed for either medium or method used.

However, the *t* tests revealed significant differences between the number of eggs detected by Sheather's sugar standing flotation and the numbers detected using the other methods. For all parasite species detected except *Cystoisospora* spp, significantly more eggs were seen when sodium nitrate was used in standing flotation and CF and when Sheather's sugar was used in CF than when Sheather's sugar was used in standing flotation.

DISCUSSION

When fecal samples are examined for parasites, a reliable medium is required for flotation, and a rapid test is optimal only if it yields correct results. The specific gravity of the medium should not be high enough to damage the eggs or cause feces to float along with the eggs. Sodium nitrate is a good all-purpose solution because it has an intermediate

Preparing Sodium Nitrate

Sodium nitrate solution is prepared by mixing 400 g of sodium nitrate ($NaNO_3$) with 1,000 ml of hot water until dissolved and allowing the solution to cool. The optimum specific gravity of sodium nitrate is 1.20 to 1.25. The specific gravity of the solution used in this study was 1.20. Specific gravity can be altered by varying the ratio of sodium nitrate to water. An alternative is to purchase Fecasol.

Preparing Sheather's Sugar

Sheather's sugar solution is prepared by melting 6.5 g of phenol crystals in a 5-L beaker over low heat. Once the phenol crystals are melted, 320 ml of distilled water is added while stirring, and 500 g of table sugar (sucrose) is added. The solution is kept over low heat and stirred until the sugar is completely dissolved. After the solution has cooled, the specific gravity is measured with a hydrometer. The specific gravity of Sheather's sugar should be between 1.27 and 1.30.

specific gravity, mixes well with feces, and floats only the lightest particles of feces. However, sodium nitrate often crystallizes on the slide, creating time constraints for the technician performing the examination. Furthermore, eggs tend to lose water in the presence of sodium nitrate, resulting in the embryos shrinking away from the egg shell, which may make identification more difficult. Sheather's sugar solution is known for its effectiveness at floating coccidian oocysts as well as other, heavier eggs. Slides made with the more viscous sugar solution are readable for longer periods than slides made with sodium nitrate. Problems inherent in using sugar solution are that it regularly gets onto countertops and attracts ants and it is more difficult to clean up than salt solution.

The main objective of this study was to determine the prevalence of infection with various parasite species as diagnosed by using standing flotation and CF with different media and to determine the necessity of centrifugation in this process. The chi-square analysis showed no significant differences in the number of dogs that tested positive or negative for any parasite species except *A. caninum*, for which sodium nitrate standing flotation was more efficient than Sheather's sugar standing flotation. No differences were found between the flotation techniques for any parasite, only between the media. Therefore, the difference in the number of parasite infections diagnosed using standing flotation compared with CF was not significant enough to change current practice methods of detecting parasite eggs by using standing flotation.

Results from the *t* tests suggest that parasite detection may be less accurate when standing flotation with Sheather's sugar is used. This may be a result of the short amount of time allowed for the flotation (10 minutes). Because Sheather's sugar is very viscous, eggs may need more time to float to the surface. Additionally, the feces were not strained before the eggs were floated, and large fecal particles tended to float during standing flotations with Sheather's sugar. Because no significant difference was found between methods when sodium nitrate was used, lack of centrifugation is probably not the sole reason for the inaccuracy of the standing Sheather's sugar test. Rather, it is likely the combination of a viscous solution with no centrifugation and a short flotation period.

It is important to note that little information about worm intensity can be gained from an egg count because eggs are rarely produced by female parasites at a constant rate. Worm age, host immunity, host nutrition, and other factors apparently influence helminth egg production.[2] A false-negative parasite diagnosis is of more concern than a low egg count because even a low count indicates that worms are shedding eggs and the dog is infected.

Dryden et al[3] tested standing flotation, CF, and direct smear techniques to detect parasites, using Sheather's sugar, sodium nitrate, and zinc sulfate media. They concluded that differences in egg counts were the result of the method used, not the flotation solution. Therefore, they concluded that centrifugation was an important technique in fecal flotation. Zajac et al[4] tested the efficacy of centrifugation with zinc sulfate as a flotation medium. In their study, they concluded that centrifugation maximizes the accuracy of fecal flotation. In our study, we found no significant diagnostic difference between CF and standing flotation.

Overall, the most important aspect of fecal flotation is its ability to aid the small animal veterinarian in the diagnosis of internal parasite infections in dogs and cats. Our results showed that diagnostic accuracy was not compromised by the flotation method used and that centrifugation did not increase parasite egg and oocyst detection. Thus, centrifugation was not needed to diagnose canine intestinal parasites.

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