Evaluation of the Importance of Centrifugation as a Component of Zinc Sulfate Fecal Flotation Examinations

Fifty canine fecal samples were evaluated by five flotation procedures to compare the sensitivity of the zinc sulfate (ZnSO₄) centrifugation flotation test with ZnSO₄ flotation tests using benchtop incubation during the flotation period. One or more parasite species were detected in 40 samples. Results showed that centrifugation with ZnSO₄ solution was significantly more likely to detect a positive sample than benchtop procedures. The difference in procedures was due primarily to increased detection of *Trichuris* eggs and *Giardia* cysts by centrifugal flotation. No significant difference was seen in the ability of benchtop procedures to detect positive samples when tests sat either for 5 or 10 minutes before examination.


Anne M. Zajac, DVM, PhD
Jamil Johnson, BS
Susan E. King, MT (ASCP)

Introduction

The increased awareness of canine and feline giardiasis in recent years has contributed to practitioner interest in the use of 33% zinc sulfate (ZnSO₄) fecal flotation solution when performing fecal examination for parasites. This solution has been recommended as an effective flotation solution for recovery of *Giardia*, because it produces less distortion of cysts than other flotation solutions of higher specific gravity. Generally, the ZnSO₄ flotation test is described as a centrifugation procedure. However, based on reports from practitioners, the ZnSO₄ flotation procedure is also performed as a standard benchtop test, allowing the mixture of feces and ZnSO₄ solution to sit in a pill vial or other container for a period of approximately 5 or 10 minutes before collecting the floated material for examination. In one commercial flotation kit that provides ZnSO₄ as the flotation medium, the package instructions direct the user to mix feces with flotation solution in the provided container and allow the mixture to sit for 5 or 10 minutes before inspection. The purpose of this study was to determine if the benchtop ZnSO₄ technique provides comparable results to the ZnSO₄ centrifugation procedure. Additionally, a comparison was made between results obtained by the ZnSO₄ procedures and the Sheather’s sugar centrifugal flotation technique to investigate whether 33% ZnSO₄ flotation solution is as effective in recovering common helminth eggs as a solution of higher specific gravity.

Materials and Methods

Fecal Samples

Fifty fecal samples were used in the study. The samples were collected from dogs at the local animal shelter and from newly acquired research dogs (also originating from animal shelters) at the Virginia-Maryland Regional College of Veterinary Medicine. An effort was made to collect samples from young dogs or dogs with diarrhea, with the expectation...
that they were more likely to be fecal positive for parasitism than clinically normal adult dogs. In some cases, fecal-positive dogs were sampled more than once on different days.

**Flotation Procedures**

Fecal samples were examined for parasites within 24 hours of collection. Each sample was thoroughly mixed, and five 5-gram subsamples were taken. The subsamples were allocated to the following five groups: 1) 33% ZnSO₄ centrifugal fecal flotation following the procedure used in the diagnostic parasitology laboratory of the Virginia-Maryland Regional College of Veterinary Medicine Teaching Hospital (ZnSO₄ VA Tech); 2) ZnSO₄ flotation centrifugation using ZnSO₄ provided in the Ovassay kit; 3) ZnSO₄ flotation as described in the Ovassay kit with a 5-minute incubation; 4) same as Group 3, but with 10-minute incubation; 5) Sheather’s sugar centrifugation flotation procedure. All the procedures were carried out by a single individual.

**Group 1, ZnSO₄ VA Tech procedure:** The ZnSO₄ solution used in the diagnostic laboratory of the veterinary college is produced by mixing water, 33 grams of heptahydrate ZnSO₄, and 20% sulfuric acid to a total volume of 100 mL. The specific gravity of the final solution is checked with a hydrometer, and water or ZnSO₄ is added as needed to achieve a specific gravity of 1.18. The test is performed by mixing approximately 20 mL flotation solution to a 5-gram sample and straining the mixture through a double layer of cheesecloth into a 15-mL conical centrifuge tube. Additional flotation solution is added to the tube to create a reverse meniscus, and a coverslip is placed on the top of the tube in contact with the flotation solution. The tube and coverslip are placed in a swinging bucket benchtop centrifuge and spun for 5 minutes at 400 G. After the centrifuge comes to a complete stop, the coverslip is removed and microscopically examined.

**Group 2, ZnSO₄ centrifugation procedure using ZnSO₄ provided in the Ovassay kit:** The test was performed as described for Group 1 (ZnSO₄ VA Tech), except that the flotation solution was prepared following the kit instructions. Water was added to a measured amount of ZnSO₄ up to the line indicated on the bottle. The specific gravity of the resulting solution was approximately 1.2. No adjustment to the specific gravity was made.

**Group 3, Ovassay ZnSO₄ procedure:** Feces and ZnSO₄ solution were mixed in a collection vial provided in the kit. A reverse meniscus was formed with a coverslip placed on top, and the flotation mixture was allowed to sit for 5 minutes in the vial before collection and examination.

**Group 4, Ovassay ZnSO₄ procedure:** This procedure was performed as for Group 3, except the flotation mixture was allowed to sit for 10 minutes before examination.

**Group 5, Sheather’s sugar centrifugation procedure:** This centrifugation procedure was performed as for Group 1, except Sheather’s sugar solution (specific gravity, 1.25) was used as the flotation solution.

At the end of each flotation procedure, the coverslip was collected, placed “wet” side down on a microscope slide, and systematically scanned using the 10× objective of a compound microscope (eyepiece magnification was also 10× for a total magnification of 100×). The 40× objective lens was used for confirmation of *Giardia* cysts. *Toxocara* eggs, *Trichurus*, *Ancylostoma*, and *Giardia* cysts were counted up to a maximum of 300 per species per slide. The same student technician who performed the procedures also read each slide.

**Statistical Analysis**

For the positive samples for each parasitic species, the GLIMMIX macro of the SAS system was used to fit a generalized linear mixed model. Dogs were considered a random effect, and treatment solutions were considered a fixed effect. For egg counts of the positive samples, the MIXED procedure of SAS was used to perform a mixed effects analysis of variance on the log counts. Single degree of freedom contrasts were used to test hypotheses. For analysis of differences between centrifugal and noncentrifugal ZnSO₄ techniques, results of the two centrifugation procedures were combined and compared to results of the combined benchtop procedures. Results were considered significant at *P* ≤ 0.05.

**Results**

A total of 40 samples tested positive for one or more of the following parasites: *Toxocara canis*, *Trichurus vulpis*, *Ancylostoma spp.*, and *Giardia spp*. *Isospora oocysts* were found in only one sample and so were not included in the analysis of results. No other parasite species was detected.

The Table shows the number of samples that tested positive for each parasite by the five flotation procedures. The ZnSO₄ VA Tech procedure detected parasites in the greatest number of samples (n=39), followed by the Ovassay centrifugation procedure (n=33 positive samples), the Sheather’s sugar flotation technique (n=31 positive samples), Ovassay flotation for 5 minutes (n=25 positive samples), and Ovassay flotation for 10 minutes (n=24 positive samples). Centrifugation with either ZnSO₄ or sugar solution was significantly more likely to produce a positive result than the benchtop procedures (*P* ≤ 0.0001). No significant difference in positive results was seen between the 5-minute benchtop flotation and the 10-minute flotation. Centrifugation with ZnSO₄ (Groups 1 and 2 combined) was significantly more likely to produce a positive result than Sheather’s sugar flotation (*P* < 0.001).

When the four parasite species were considered independently, the proportion of positive samples detected by combined ZnSO₄ centrifugal flotation tests was significantly greater for eggs of the helminths compared to the benchtop procedures (*P* < 0.001). Additionally, the geometric mean number of eggs detected in positive samples was sig-
nificantly greater for each parasite in the combined ZnSO₄ centrifugation procedures when compared to both ZnSO₄ benchtop procedures (P<0.001). Significantly greater numbers of Giardia cysts were also detected by ZnSO₄ centrifugation when compared to the benchtop procedures (P<0.05). Significantly fewer positive Giardia samples were detected by centrifugation with the Ovassay ZnSO₄ flotation solution when compared to the diagnostic laboratory solution (P<0.05).

**Discussion**

As early as 1941, a study comparing sodium nitrate flotation procedures found that centrifugation provided superior results compared to benchtop incubation.³ Results of this study showed that centrifugation utilizing ZnSO₄ fecal flotation solution can significantly improve the recovery of common parasite species. Even increasing the time period from 5 to 10 minutes for the benchtop procedure did not significantly improve detection.

The difference between centrifugation and bench incubation in the study was due, in large part, to the variation in detection of Giardia cysts. For many years, Giardia infection was considered of little importance in small animals. The use of flotation solutions that quickly distort and destroy cysts only served to reinforce the general impression that Giardia was unusual or insignificant. However, more recent information shows that Giardia is, in fact, a common parasitic infection that can produce clinical disease in dogs and cats.⁴ Equally important is evidence indicating that at least some small animal isolates of Giardia are probably infective for humans.⁵ Given the importance of the parasite, optimal techniques for flotation procedures in veterinary practices should include the ability to detect Giardia cysts.

Zinc sulfate was initially proposed as a general-purpose centrifugal flotation solution for protozoan cysts and helminth eggs in human feces in 1939.⁶ Because of the success of ZnSO₄ in floating Giardia cysts, its use in veterinary practices appears to be increasing. However, some practitioners and technicians have adopted the flotation solution as a benchtop procedure following the introduction of commercial kits that provide ZnSO₄ solution for use in a standard benchtop test. The importance of the test procedure was evident in one comparison from 1942 between ZnSO₄ centrifugation flotation and benchtop flotation, which found that the results of the two tests were similar when the benchtop technique employed a 1-hour “sit” time period.⁷ Not only is this time period much longer than practical in most veterinary practices, but the authors have observed that prolonged exposure to 33% ZnSO₄ also causes collapse of Giardia cysts.

A significant difference was also found in numbers of Giardia-positive samples between the ZnSO₄ solution routinely prepared by the authors’ diagnostic laboratory and the ZnSO₄ provided in the commercial kit. This difference may have occurred because the resulting specific gravity of

<table>
<thead>
<tr>
<th>Flotation Procedure</th>
<th>Toxocara canis</th>
<th>Trichuris vulpis</th>
<th>Ancylostoma spp.</th>
<th>Giardia spp.</th>
<th>Total Positive Samples†</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZnSO₄ VA Tech</td>
<td>10</td>
<td>22</td>
<td>12</td>
<td>16</td>
<td>39</td>
</tr>
<tr>
<td>Ovassay ZnSO₄</td>
<td>10</td>
<td>21</td>
<td>10</td>
<td>11</td>
<td>33</td>
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<td>centrifugation</td>
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<td>(56)</td>
<td>(20)</td>
<td>(70)</td>
<td>(80)</td>
</tr>
<tr>
<td>Ovassay, 5 min</td>
<td>10</td>
<td>11</td>
<td>11</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>(24)</td>
<td>(4)</td>
<td>(14)</td>
<td>(4)</td>
<td></td>
</tr>
<tr>
<td>Ovassay, 10 min</td>
<td>7</td>
<td>13</td>
<td>11</td>
<td>1</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>(41)</td>
<td>(4)</td>
<td>(22)</td>
<td>(2)</td>
<td></td>
</tr>
<tr>
<td>Sheather’s sugar flotation</td>
<td>10</td>
<td>20</td>
<td>12</td>
<td>1</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>(94)</td>
<td>(30)</td>
<td>(114)</td>
<td>(67)</td>
<td></td>
</tr>
</tbody>
</table>

* Number provided in parentheses
† Some samples were positive for more than one parasite

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the solution prepared from the kit was always higher than the intended level of 1.18. This higher specific gravity may have distorted cysts in samples with lower numbers, making their detection difficult. These results demonstrate the value of checking the specific gravity of flotation solutions. A hydrometer can be inexpensively purchased from a scientific supply company and is easy to use.

For all the helminth species detected, the number of eggs recovered in positive samples was significantly greater with the ZnSO₄ centrifugation procedure than with the ZnSO₄ benchtop procedure. It is likely that where parasite egg numbers are low, the benchtop procedure may detect no eggs or only very few, which could be missed during a rapid scan of the coverslip. In this study, inaccuracies in data collected from samples with low egg numbers could have arisen from two sources. If low numbers of eggs were not evenly distributed throughout the sample, some subsamples may not have contained any eggs. Also, because this experiment was not carried out as a blind study, the possibility of unconscious bias cannot be excluded.

The difference between benchtop and centrifugation ZnSO₄ techniques in recovery of Trichuris eggs was particularly marked. The eggs of whipworms have a higher specific gravity (1.14) than other helminth eggs detected in these tests (Toxocara, 1.09; Ancylostoma, 1.06) and are less likely to complete their flotation in the short time permitted in the benchtop procedure. However, results of this study did not show that the Sheather’s sugar flotation technique (specific gravity, 1.25) was more effective in identifying whipworm-positive samples than ZnSO₄ centrifugation, although the geometric mean number of eggs/sample was higher with the sugar flotation solution. The results add support to the recommendation that ZnSO₄ is an effective routine flotation solution when the centrifugation procedure is used.

The most desirable solution to use for routine screening flotation tests is one that is readily available and will allow the flotation of the greatest number of parasite species without distortion. Unfortunately, those solutions with the highest specific gravities effectively float eggs of many helminth species but also distort and destroy parasite eggs and cysts more rapidly than solutions of lower specific gravity. Clearly, no single flotation solution currently in use is best for recovering every parasite found in small animal fecal samples. The 33% ZnSO₄ solution seems to offer the best balance for a simple screening test between effective flotation of common helminth eggs and protozoan cysts and oocysts. It is clear, however, that centrifugation of a flotation test produces significantly better results than benchtop incubation. This situation applies regardless of the flotation solution used, and every effort should be made in veterinary practices to perform this test as a centrifugation procedure.

The procedures in this study used a swinging bucket centrifuge. However, fixed-angle centrifuges are probably more common in veterinary practices. These fixed-angle centrifuges can also be used for flotation procedures with a slight modification of the technique. Centrifuge tubes should be filled as full as possible without spilling when placed in the angled rotor. After centrifuging the tube, the top layer of fluid can be gathered in two ways. A microbiological loop or the rounded bottom of a small glass tube (such as an empty blood-collection tube) can be touched to the surface of the liquid in the tube after centrifugation. The loop or tube is then touched to a microscope slide, and a drop or two of fluid is deposited. A coverslip should be added and the slide examined. Alternatively, the tube can be removed from the centrifuge after spinning and then placed in a test-tube rack. Additional flotation solution is added until a reverse meniscus forms, and then a coverslip is placed on top. The tube is allowed to sit for an additional 5 minutes to allow parasite eggs to float the rest of the distance to the coverslip. The coverslip is then removed and placed on a microscope slide as described for this study. Centrifuges commonly found in veterinary practices also provide adequate force for centrifugal flotations. As a general guideline, centrifuging a tube at the same speed used for spinning blood or urine should be sufficient for flotation procedures.²

Practitioners and technicians may argue that centrifugation adds inconvenience to the simple flotation test, but maximizing the sensitivity of this common laboratory procedure will improve detection and treatment of parasitic infections in companion animals.

References


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