Comparison of methods to detect gastrointestinal parasites in llamas and alpacas

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Objective—To compare relative sensitivity and overall yields of various methods of fecal examination for gastrointestinal parasites in llamas and alpacas. 

Design—Prospective study. 

Sample Population—Fecal samples from 42 alpacas and 62 llamas. 

Procedures—Fecal samples were analyzed via direct smear, a modified McMaster technique with sucrose solution or saturated saline (approx 36% NaCl) solution, and a centrifugation-flotation procedure. McMaster flotation chambers were examined 15 and 60 minutes after loading. Centrifugation-flotation samples were examined after 10 and 60 minutes of flotation. The proportions of samples with positive results and concentrations of parasites were compared among methods. 

Results—The centrifugation-flotation technique yielded more positive results than other methods for all parasites except small coccidia. Longer flotation time increased the proportion of positive results and parasite concentrations for all parasites except Nematodirus spp. Longer time in the McMaster chamber made little difference. By use of the modified McMaster technique, sucrose solution yielded more positive results for Trichuris spp, Eimeria macusaniensis, and strongyles, whereas saline solution yielded more positive results for Nematodirus spp and small coccidia. The saline solution McMaster test yielded more positive results for small coccidia than did most other methods, and the sucrose McMaster technique yielded more positive results for Trichuris spp. 

Conclusions and Clinical Relevance—The centrifugation-flotation technique appeared to offer clear advantages in detecting infection with E. macusaniensis, Trichuris spp, Nematodirus spp, and capillarids. The saline McMaster technique appeared to offer an advantage in detecting small coccidia. (J Am Vet Med Assoc 2008;232:733–741)

Internal parasitism of camelids is one of the leading health concerns among camelid owners and veterinarians.1–4 As with other domestic livestock, gastrointestinal parasites are notable contributors to this concern. Scientific reports5–9 of morbidity and mortality rates in the North American camelid population caused by gastrointestinal parasitism are relatively few, perhaps because they are considered too mundane, but between the increasing documentation of substantial illness and rising concerns about the emergence of anthelmintic-resistant nematode populations,7 it is likely that interest in this field will increase. 

Parasite-monitoring strategies in camelids have generally followed those developed for other grazing livestock. Quantitative methods are favored over qualitative ones by many practitioners because severity of infection relates to severity of fecal shedding and because changes in egg counts during serial quantitative monitoring reflect the efficacy of control strategies. The strategy of quantitative assessment potentially fails when, despite severe disease, small numbers of eggs or oocysts are in the feces. The occurrence of severe disease in camelids with little to no fecal shedding has been documented or suggested for Nematodirus battus,7 Eimeria macusaniensis,7 and Teladorsagia or Ostertagia spp.9 Additionally, high intestinal burdens of E. macusaniensis,5 Teladorsagia spp,8 and Aonchotheca (Capillaria) spp10 have been found in camelids with low fecal egg or oocyst counts. In some cases, low fecal shedding may relate to variable egg or oocyst output, and in others, it may relate to disease occurring before or near the onset of patency. In the case of variable shedding, standard quantitative techniques useful for herd screenings may fail to detect parasites in individuals. Thus, in some cases, a highly sensitive qualitative method or quantitative method of fecal analysis could be desirable, particularly for identifying diseased individuals. 

A variety of camelid fecal analysis methods have been described.3–6,10–21 Most are adaptations of common techniques, which appears reasonable because many of the nematode parasites of camelids also infect domestic ruminants. However, camelids are also infected by several unique gastrointestinal parasites, including Camelostrostrongylus mentulatus, Trichuris tenuis, Nematodirus lamae, Lamanema chavezi, and several

**ABBREVIATIONS**

epg: Eggs per gram
opg: Oocysts per gram
species of *Eimeria*, which may present unique detection challenges. *Eimeria macusaniensis* is of particular concern because it appears to be a common pathogen in North America⁵; has been reported as a major pathogen elsewhere.¹⁹ ²⁰ ²²; and has no coccidial parasite of ruminants that is its counterpart in size, wall thickness, and density. *Eimeria leuckarti* in horses and *Eimeria cameli* in Old World camelids are similar in appearance, but comparative studies on recovery of those parasites are also rare.²³ Although reports evaluating the performance of the common fecal diagnostic techniques in various species are fairly common, such comparisons in camelids are anecdotal or extremely limited. Data comparing the performance of these tests on the unique camelid parasites, or from camelid feces, are virtually nonexistent. Additionally, the heterogeneity and poor descriptions of techniques in the existing camelid studies make cross-study comparisons difficult.

Among the options for fecal parasite detection are the direct smear, floatations, and sedimentations. Direct smear is a qualitative test, unless the amount of feces is measured, and allows no concentration of eggs or oocysts. Floatations and sedimentations allow concentration of parasite eggs or oocysts. Quantification is achieved through the use of a known weight of feces in a known amount of solution. Flotation techniques depend on the difference in specific gravity between the parasite, fecal debris, and flotation solution; viscosity of the flotation solution; permeability of the parasite for the flotation solution; and elapsed time. Counting too early may miss slow-rising parasites, and counting too late may allow parasites to become distorted, particularly those with thin walls. Various flotation solutions including sucrose, sodium chloride, and saturated saline (approx 36% NaCl) solution have been used on camelid feces. Sedimentation techniques concentrate parasite eggs or oocysts in less dense solutions, often water and often assisted by centrifugation. Techniques may be used in series, such as an initial suspension and centrifugation in water, followed by a flotation step. Quantification can then follow by use of either a standard microscope slide and coverslip or a specialized counting chamber.

The purpose of the study reported here was to compare the relative efficacies of 3 procedures and 2 flotation solutions in the detection of eggs and oocysts of common camelid parasites. Popular techniques with different theoretical detection limits were chosen for comparison. Because camelids have a variety of unique parasites, methods were altered in a variety of ways to observe the effects on success of detection. Variables included flotation solution, flotation time, method of quantification, and inclusion of a centrifugation step.

**Materials and Methods**

**Fecal samples**—Ten to 15 g of feces was collected from 104 New World camelids (42 alpacas and 62 llamas). Twenty-one samples were from specimens submitted to the Oregon State University Diagnostic Laboratory or from animals admitted to Oregon State University Large Animal Teaching hospital for treatment. The remaining 83 camels were from private farms and the university teaching herd. Samples were stored at 4°C up to 5 days until tested. All assays were run on a single sample on the same day and read by the same individual, who was unaware of specimen identities.

**Assay procedures**—All fecal samples were examined by use of 7 techniques: direct smear, a double centrifugation-sucrose flotation technique after 10 and 60 minutes of flotation, and dissolution in either saturated saline solution or sucrose solution followed by flotation in a counting chamber for 15 and 60 minutes (modified McMaster technique).

For the direct smear, approximately 0.1 g of feces was liquefied in isotonic saline solution, and approximately 25 µL was added to a microscope slide; the slide was then coverslipped. Slides were immediately examined at 10X magnification, and parasite eggs or oocysts were noted. The numbers of parasite eggs or oocysts were not quantified.

The centrifugation-sucrose flotation was performed as described,²³ with minor modifications. In brief, 2 g of feces was mixed in 98 mL of distilled water and allowed to soak overnight at 4°C to assist in separating parasite eggs and oocysts from the fecal material. The following morning, the fecal slurry was mixed, and 10 mL was immediately poured into each of 2 conical-bottom 15-mL centrifuge tubes and centrifuged at 200 X g for 5 minutes in a swinging-bucket centrifuge. The supernatant was decanted and the pellet resuspended in 2 mL of sucrose solution (Sheather solution with 3.74M; specific gravity = 1.27, checked via hydrometer). An additional 10 mL of sucrose solution was then added, and the samples were centrifuged again for 5 minutes at 200 X g. After the second centrifugation, the tubes were filled with sucrose solution until a slight convex meniscus formed, and a 22 X 22-mm coverslip was placed on top of each tube. Tubes were allowed to sit undisturbed for 10 or 60 minutes, after which the coverslips were removed and placed on microscope slides. All parasite eggs and oocysts were counted, and the total was multiplied by 5 to determine epg and opg values.

The modified McMaster technique was performed as described,²⁶ with minor modifications. Briefly, a fecal solution of 4 g of feces and 26 mL of either a saturated sodium chloride solution (specific gravity = 1.20, checked via hydrometer) or sucrose solution (as described) was prepared with a mortar and pestle, and the slurry was filtered through gauze into a 50-mL tube. The filtrate was mixed and used to fill both chambers of a 2-chamber McMaster counting slide. Slides were allowed to sit undisturbed for 15 minutes, after which both chambers were read at 100X magnification. Slides were allowed to sit for an additional 45 minutes and read a second time. The number of parasite eggs and oocysts was quantified by adding together the counts from both chambers for each type present and multiplying the total number of each by 25 to determine concentrations of eggs and oocysts. Eggs and oocysts were classified as strongyle type, capillarid, *Nematodirus* spp, *Trichuris* spp, tapeworm, *E macusaniensis*, and small coccidia on the basis of physical characteristics.
Statistical analysis—The proportions of the unknown samples that yielded positive results for each parasite were compared for heterogeneity among the 7 procedures by use of the Cochrane Q test for non-parametric matched data with dichotomous outcomes. These comparisons were repeated after eliminating samples in which the parasite was not detected by any method. To investigate differences in values of epg or oog of feces, positive results were compared among methods by use of the Friedman repeated-measures ANOVA on ranks. The Tukey test was used for pairwise comparisons if a significant difference was found. Methods yielding <10 positive results were excluded from this analysis. To specifically investigate the effects of longer flotation time on values of epg or oog of feces, paired counts from samples with positive results for the 2 time points for each of the 3 types of quantitative procedure (all but the direct smear) were compared by use of the Wilcoxon signed rank test. All comparisons were considered significant at values of \( P < 0.05 \).

To evaluate the performance of the various detection methods at different egg or oocyst concentrations, results were stratified on the basis of count and depicted graphically. The lowest group contained samples within the lowest detection threshold for all quantitative methods. Higher groups were determined on the basis of arbitrary thresholds.

Results

Of the 104 fecal samples, small coccidia and strongyle-type eggs were each found in 91 samples, Nematodirus spp in 57 samples, Aonchotheca (Capillaria) spp in 40 samples, E macusaniensis in 35 samples, and Trichuris spp in 33 samples. Additionally, tapeworm eggs were found in 3 samples, and large coccidial oocysts resembling Eimeria tiviensis were found in 3 samples. Egg or oocyst counts were as high as 12,250 epg for small coccidia, 2,225 epg for strongyle-type eggs, 215 epg for Nematodirus spp, 155 epg for Capillaria spp, 54,350 oog for E macusaniensis, and 100 epg for Trichuris spp.

Small coccidia were detected in a significantly higher proportion of the 104 fecal samples via centrifugation-flotation at 60 minutes, either saline McMaster technique, and the sucrose McMaster technique at 60 minutes than by centrifugation-flotation at 10 minutes or the sucrose McMaster technique at 15 minutes (Table 1). The same tests detected small coccidia in a significantly higher proportion of the 91 samples with positive results (Table 2). Small coccidia were not evaluated by use of direct smear because they often were obscured by background debris. The saline McMaster technique appeared to offer a detection advantage over a wide range of fecal oocyst counts (Figure 1). Whereas the saline McMaster technique at either time point detected small coccidia at all concentrations >250 oog and sucrose flotation detected all known samples with positive results >500 oog, centrifugation-flotation at either time point did not detect occasional samples with positive results until counts exceeded 2,000 oog.

Eimeria macusaniensis was detected in a significantly higher proportion of the 104 fecal samples via centrifugation-flotation at 60 minutes than by use of any other technique (Table 1). This organism was also detected in a significantly higher proportion of samples via centrifugation-flotation at 10 minutes, direct smear, and saline McMaster technique after 15 minutes (Table 2).

Table 1—Number of positive results for eggs or oocysts of various parasites among 104 fecal samples from llamas and alpacas tested by use of various techniques.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Technique</th>
<th>Direct</th>
<th>CFSuc10</th>
<th>CFSuc60</th>
<th>NaCl15</th>
<th>NaCl60</th>
<th>Suc15</th>
<th>Suc60</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small coccidia</td>
<td>ND</td>
<td>64 a</td>
<td>75 b</td>
<td>78 b</td>
<td>76 b</td>
<td>65 a</td>
<td>73 a</td>
<td>73 a</td>
<td>91</td>
</tr>
<tr>
<td>Eimeria macusaniensis</td>
<td>16 c</td>
<td>21 a</td>
<td>27 a</td>
<td>10 c</td>
<td>10 c</td>
<td>18 a</td>
<td>18 a</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Trichuris spp</td>
<td>10 c</td>
<td>21 a</td>
<td>26 b</td>
<td>1 c</td>
<td>0 a</td>
<td>11 b</td>
<td>13 a</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Nematodirus spp</td>
<td>16 b</td>
<td>53 a</td>
<td>48 b</td>
<td>15 a</td>
<td>15 a</td>
<td>6 c</td>
<td>6 c</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>Capillarids</td>
<td>5 a</td>
<td>17 b</td>
<td>31 c</td>
<td>8 a</td>
<td>8 a</td>
<td>8 a</td>
<td>8 a</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Strongyle type</td>
<td>24 a</td>
<td>81 b</td>
<td>65 a</td>
<td>36 c</td>
<td>36 a</td>
<td>40 b</td>
<td>40 b</td>
<td>91</td>
<td></td>
</tr>
</tbody>
</table>

Direct = Direct smear. CFSuc10 = Centrifugation-sucrose flotation after 10 minutes. CFSuc60 = Centrifugation-sucrose flotation after 60 minutes. NaCl15 = Saturated saline McMaster technique after 15 minutes. NaCl60 = Saturated saline McMaster technique after 60 minutes. Suc15 = Sucrose McMaster technique after 15 minutes. Suc60 = Sucrose McMaster technique after 60 minutes. Total = Number of samples positive by any technique. ND = Not done.

Table 2—Percentages of llama and alpaca fecal samples with positive results for each parasite in which eggs or oocysts from that parasite were detected by each specific technique.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Technique</th>
<th>Direct</th>
<th>CFSuc10</th>
<th>CFSuc60</th>
<th>NaCl15</th>
<th>NaCl60</th>
<th>Suc15</th>
<th>Suc60</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small coccidia</td>
<td>ND</td>
<td>70 a</td>
<td>82 b</td>
<td>86 b</td>
<td>84 b</td>
<td>71 a</td>
<td>80 b</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>E macusaniensis</td>
<td>47 c</td>
<td>60 a</td>
<td>77 b</td>
<td>29 c</td>
<td>29 a</td>
<td>51 a</td>
<td>51 a</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Trichuris spp</td>
<td>30 a</td>
<td>64 a</td>
<td>88 f</td>
<td>3 a</td>
<td>0 a</td>
<td>35 a</td>
<td>35 a</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Nematodirus spp</td>
<td>28 a</td>
<td>93 b</td>
<td>84 b</td>
<td>26 a</td>
<td>26 a</td>
<td>11 a</td>
<td>11 a</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>Capillarids</td>
<td>13 a</td>
<td>43 a</td>
<td>78 b</td>
<td>20 a</td>
<td>20 a</td>
<td>20 a</td>
<td>20 a</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Strongyle type</td>
<td>26 a</td>
<td>89 a</td>
<td>83 a</td>
<td>40 a</td>
<td>40 a</td>
<td>53 a</td>
<td>53 a</td>
<td>91</td>
<td></td>
</tr>
</tbody>
</table>

Total positive = No. of samples with a positive result obtained by use of any of the techniques. See Table 1 for key.
or either sucrose McMaster technique than by either saline McMaster technique. The same relationships were true for detection of the 35 samples with positive results (Table 2). Centrifugation-flotation appeared to offer the greatest detection advantage at counts up to 25 opg (Figure 2; the McMaster technique yielded identical results at either time point). Centrifugation-flotation at 60 minutes and the sucrose McMaster technique at either time point detected all known samples with positive results with egg concentrations > 25 opg. Centrifugation-flotation at 10 minutes detected all samples with positive results > 100 opg, direct smear detected all samples with positive results > 250 opg, and the saline McMaster technique detected all samples with positive results at either time point > 500 opg.

*Trichuris* spp were detected in a significantly higher proportion of the 104 fecal samples via centrifugation-flotation at either time point than via any other technique (Table 1). This organism was also detected in a significantly higher proportion of samples via direct smear and via either sucrose McMaster technique than by use of either saline McMaster technique. The same detection relationships were found among the 33 samples with positive results, except that use of the centrifugation-flotation at 60 minutes detected *Trichuris* spp in a significantly higher proportion of samples than did use of the same technique at 10 minutes (Table 2). Centrifugation-flotation, particularly at 60 minutes, appeared to offer a detection advantage at counts up to 25 epg (Figure 3; the McMaster technique yielded similar results at either time point). At higher concentrations, use of sucrose McMaster techniques appeared to give similar results. Use of saline McMaster techniques rarely detected *Trichuris* spp at any concentration.

*Nematodirus* spp were detected in a significantly higher proportion of the 104 fecal samples by use of centrifugation-flotation at either time point than by use of any other technique (Table 1). This organism was also found in a significantly higher proportion of samples via direct smear or either saline McMaster technique than by use of either sucrose McMaster technique. The same detection relationships were found among the 57 samples with positive results (Table 2). Centrifugation-flotation appeared to offer the greatest detection advantage at counts up to 100 epg (Figure 4; the McMaster technique yielded identical results at either time point). Detection via direct smear and saline McMaster techniques improved at the higher concentrations. Sucrose McMaster techniques appeared to be unreliable at the concentrations of *Nematodirus* spp detected.

Capillarid eggs were detected in a significantly higher proportion of the 104 fecal samples by use of centrifugation-flotation at 60 minutes than by any other technique (Table 1). Capillarid eggs were also found by use of centrifugation-flotation at 10 minutes in a significantly higher proportion of samples than by use of direct smear and either sucrose or saline McMaster technique. The same detection relationships were found among the 40 samples with positive results (Table 2). Centrifugation-flotation, particularly at 60 minutes, appeared to offer a detection advantage at counts up to 25 epg (Figure 5; the McMaster technique yielded identical results at either time point). Too few samples were found with higher egg counts to draw conclusions.

Strongyle-type eggs were detected in a significantly higher proportion of the 104 fecal samples by use of ei-
ther centrifugation-flotation technique than via all other techniques (Table 1). These eggs were also found in a significantly higher proportion of samples by use of either sucrose McMaster technique than by use of saline McMaster techniques or direct smear and by saline McMaster techniques, compared with direct smear. The same detection relationships were found among the 91 samples with positive results (Table 2). Centrifugation-flotation appeared to offer the greatest detection advantage at counts up to 100 epg (Figure 6; the McMaster technique yielded identical results at either time point). These methods and sucrose McMaster techniques detected all known samples with positive results with egg concentrations > 250 epg. Direct smear and the saline McMaster techniques detected all known samples with positive results with egg concentrations > 500 epg.

Comparison of counts from samples with positive results via all methods, excluding those with < 10 positive results, revealed that use of the saline McMaster test at either time point and the sucrose McMaster test at 60 minutes yielded significantly more positive results for small coccidial oocysts than either centrifugation-flotation method or the sucrose McMaster test at 15 minutes (Table 3). Median and interquartile boundaries were 2 to 6 times as high for these McMaster tests as for the centrifugation-flotation methods. The centrifugation-flotation test at 10 minutes further yielded significantly fewer positive results for small coccidia than did the same test at 60 minutes or the sucrose McMaster test at 15 minutes.

The centrifugation-flotation method at 60 minutes and the sucrose McMaster test at either time point yielded significantly (\(P = 0.032\)) higher concentrations of *E. maysa*
usaniensis than did centrifugation-flotation method at 10 minutes and the saline McMaster technique at either time point (Table 3). The saline and interquartile boundaries were approximately 4 times as high for the saline McMaster tests. The centrifugation-flotation technique at 10 minutes yielded intermediate values.

The sucrose McMaster test at either time point yielded approximately 3 times as many *Trichuris* spp eggs as the centrifugation-flotation technique at 10 minutes (Table 3). The saline McMaster technique yielded too few *Trichuris*-positive results for analysis. The centrifugation-flotation technique at 60 minutes yielded significantly more *Nematodirus* spp eggs than either saline McMaster test. The sucrose McMaster yielded too few *Nematodirus*-positive results for analysis. Both the saline and sucrose McMaster techniques yielded too few *Capillaria*-positive samples for comparison.
Figure 6—Number of fecal samples from llamas and alpacas found to contain strongyle-type eggs by various detection methods at various egg concentrations. To classify samples by egg concentration, the highest egg count determined by any detection method was used. See Figures 1 and 2 for key.

Table 3—Median and interquartile range of parasite egg and oocyst concentrations (egp or opg) from camelid fecal samples with positive results as analyzed by use of various techniques. Only samples with positive results from all techniques were included. Techniques yielding < 10 positive results were excluded.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Technique</th>
<th>CFSuc10</th>
<th>CFSuc60</th>
<th>NaCl15</th>
<th>NaCl60</th>
<th>Suc15</th>
<th>Suc60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small coccidia</td>
<td>60</td>
<td>100</td>
<td>325</td>
<td>350</td>
<td>200</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>(n = 45 samples)</td>
<td>(25–245)</td>
<td>(65–488)</td>
<td>(150–1,244)</td>
<td>(189–1,300)</td>
<td>(75–513)</td>
<td>(175–1,169)</td>
<td></td>
</tr>
<tr>
<td>E. macusaniensis</td>
<td>103</td>
<td>248</td>
<td>63</td>
<td>75</td>
<td>250</td>
<td>283</td>
<td></td>
</tr>
<tr>
<td>Trichuris spp</td>
<td>15</td>
<td>40</td>
<td>ND</td>
<td>ND</td>
<td>50</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>(n = 9)</td>
<td>(5–25)</td>
<td>(19–63)</td>
<td>ND</td>
<td>ND</td>
<td>50</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Nematodirus spp</td>
<td>50</td>
<td>65</td>
<td>25</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Capillarids</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Strongyle type</td>
<td>128</td>
<td>168</td>
<td>88</td>
<td>75</td>
<td>63</td>
<td>83</td>
<td></td>
</tr>
</tbody>
</table>

*a,b,c* Within a row, median values with different superscript letters are significantly (P ≤ 0.05) different. See Table 1 for remainder of key.

Table 4—Median and interquartile range of parasite egg and oocyst concentrations from camelid fecal samples with positive results as analyzed by use of various techniques with various flotation times.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Technique</th>
<th>Centrifugation-flotation</th>
<th>Saline McMaster</th>
<th>Sucrose McMaster</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>10 min</td>
<td>60 min</td>
</tr>
<tr>
<td>Small coccidia</td>
<td>60</td>
<td>45</td>
<td>(15–105)</td>
<td>(33–188)</td>
</tr>
<tr>
<td>E. macusaniensis</td>
<td>16</td>
<td>38</td>
<td>(13–273)</td>
<td>(15–523)</td>
</tr>
<tr>
<td>Trichuris spp</td>
<td>19</td>
<td>10</td>
<td>(5–19)</td>
<td>(10–38)</td>
</tr>
<tr>
<td>Strongyle type</td>
<td>76</td>
<td>25</td>
<td>(10–80)</td>
<td>(15–123)</td>
</tr>
</tbody>
</table>

*a* Significant (P < 0.05) difference between time points for a given method. See Table 1 for remainder of key.
The centrifugation-flotation technique at 60 minutes yielded significantly more strongylo-type eggs than the sucrose McMaster technique at either time point or the saline McMaster technique at 60 minutes (Table 3). The centrifugation-flotation technique yields were approximately 50% greater than the McMaster technique yields.

Comparison of the values of epg or opg at different time points for the same method revealed that the longer flotation time for the centrifugation method significantly increased the counts of all parasite eggs and oocysts except for Nematodirus spp (Table 4). The differences were most profound for small coccidia and E. macusaniensis, where median and interquartile boundaries were 2 to 3 times as high at 60 minutes as at 10 minutes. The longer time had little effect on the McMaster technique, slightly decreasing the yield of strongylo parasites with saline solution and more than doubling the yield of small coccidia with sucrose solution.

Discussion

The major finding of this investigation was that no single method of fecal analysis proved superior to all others for the detection of all parasites at both low and high concentrations. The centrifugation-sucrose flotation technique yielded the highest proportion of positive results for all parasites except small coccidia. This advantage was most marked at low egg counts, and by concentrations of approximately 250 epg for strongyles and 100 epg for Capillaria spp, saline and sucrose flotation detected a similar proportion of samples with positive results as the centrifugation-sucrose flotation methods. Saline flotation detected a similar proportion of samples with > 40 epg of Nematodirus spp or > 500 epg of E. macusaniensis as the centrifugation-sucrose flotation techniques, and sucrose flotation detected a similar proportion with > 40 epg of Trichuris spp or > 25 opg of E. macusaniensis as the centrifugation-sucrose flotation techniques. For some of these parasites, the ability to detect small numbers of eggs may be clinically unimportant, but given that E. macusaniensis and possibly Trichuris spp and other parasites7,8 are associated with clinical disease at low fecal egg or oocyst counts, the higher detection proportion of the centrifugation-flotation procedure could have an important positive impact on the ability to diagnose and treat these infections in a timely fashion.

It is important to note that the advantage of the centrifugation-flotation procedures may have resulted from any of a number of factors, including the addition of an overnight soak and sedimentation in water, centrifugation, and the final flotation in sucrose solution. Pelleted camelid feces are relatively dry and compact. Parasite eggs are released more readily from sandy soil than from clay,27 and it is logical to presume that they would also be released slowly from compact feces. The overnight soak appeared to allow complete dissolution of the feces. Shorter soak times were not evaluated.

Centrifugation may also have played a role in the higher detection proportion. A previous comparison of methods used for canine fecal analysis revealed that the inclusion of a centrifugation step appeared to be more important for increasing yields than which flotation solution was used afterward.28 Given the large differences between the centrifugation-flotation procedure and the sucrose McMaster technique, it is likely the effects of full dissolution and centrifugation were greater than that of the choice of flotation solution for camelid parasites.

Allowing the centrifugation-flotation samples to float for 60 minutes led to even greater recovery for all parasites except Nematodirus. This advantage in detection was marked for small coccidia, E. macusaniensis, Trichuris spp, and Capillaria spp at low concentrations. The advantage in yield was most marked for small coccidia and E. macusaniensis. Flotation time is a balance between the time required for the eggs or oocysts to rise and the time before they become saturated with the solution. Given how rarely, and at what low concentrations, the shorter flotation times offered advantages in sensitivity, saturation appeared not to be a problem within 60 minutes. Some of the advantages seen with the 60-minute centrifugation-flotation procedure might have been the result of picking too short a time, 10 minutes, for the early analysis. Twenty minutes is recommended by 1 source, but 10 minutes is considered adequate by others.29 Considering that, except for Nematodirus spp, there was little disadvantage in allowing the samples to float longer, a 60-minute flotation time should be strongly considered when analyzing camelid feces by use of the centrifugation-sucrose flotation technique.

The effect of a longer flotation time on the McMaster procedure was most evident with small coccidia in sucrose solution. This may have been a viscosity issue. Small coccidia, with deformable walls, may have had delayed rising in the more viscous sucrose solution.

The advantage of the sucrose solution over the saline solution flotation was apparent with strongylo-type eggs; E. macusaniensis; and, most markedly, Trichuris spp. However, saline solution appeared to offer the advantage with small coccidia and Nematodirus spp. The ability of a parasite egg or oocyst to float in a particular flotation solution varies with the difference in specific gravity, viscosity of the solution, and elapsed time. The specific gravities of camelid parasites have not been reported. Trichuris spp and strongyles from other species are reported to have specific gravities less than that of either saturated saline solution or sucrose solution.30 The strongyle infections missed by the saline McMaster method generally had low fecal egg counts, were beyond the detection threshold of this method in some samples, and may not have been clinically relevant in some samples. The failure of the saline McMaster test to detect these strongyle infections may have been related to the fact that camelids are parasitized by several unique strongyles that may have denser eggs than their ruminant counterparts. Additionally, flotation solutions with higher specific gravities yield higher strongyle egg counts in sheep.31 The near inability of the saline McMaster technique to detect Trichuris spp suggests camelid whipworm eggs might have higher specific gravity than other Trichuris spp. Given the clinical importance of this parasite, saline solution flotation appeared to be unacceptable for its detection.

The sucrose McMaster technique also detected almost twice as many samples with E. macusaniensis as did...
the saline solution version. One previous report also suggests poor flotation of *E. macusaniensis* in solutions with specific gravity ≤ 1.2 and suggests use of solutions with specific gravity ≥ 1.28, and another report suggests poor flotation of *E. macusaniensis* even in sucrose solution, but still others report apparent success in detecting either *E. macusaniensis* or the closely related and perhaps identical *E. cameli* in saturated saline solution. Further complicating matters, a recent report and our findings suggest some heterogeneity in populations of large coccidia in camels. The importance and prevalence of *Eimeria ivitaenensis* in North America have not been established, but this organism’s role as a copathogen in South America suggests it plays a similar role to *E. macusaniensis*. Because of the possible importance of even modest fecal shedding of *E. macusaniensis*, choosing the flotation solution and method for optimal recovery could have a positive effect on health care. On the basis of our findings, the centrifugation-sucrose flotation with the 60-minute flotation time appeared to offer the greatest likelihood of finding the parasite.

Saturated saline solution appeared to offer a detection advantage for *Nematodirus* spp and small coccidia. In both instances, this may have reflected the greater viscosity of the sucrose solution. The small coccidia are thin-walled and presumably more pliant than the other eggs and oocysts that were evaluated. They may deform and develop greater resistance to rising. It is also possible that the greater specific gravity of the sucrose solution caused more debris to float and obscure the coccidial oocysts. The larger *Nematodirus* ova may have also been inhibited from rising. Although not evaluated in the present study, our findings suggested that using saturated saline solution as the flotation solution after centrifugation may reveal the most *Nematodirus* ova. The same may be true for small coccidia, although the value of the centrifugation step was not established.

The direct smear technique, although not quantitative, detected *E. macusaniensis* and *Trichuris* spp in more samples than did the saline McMaster test and detected more *Nematodirus* spp than either McMaster method. Direct smears also often detected low concentrations of ova or oocysts missed by other methods. Thus, the direct smear technique may be a useful adjunct to other methods, especially when looking for dense parasite eggs and oocysts via a flotation method or for parasites that are pathogenic even with low fecal egg counts. Direct smears were used in this capacity in 1 clinical report and appeared to have been helpful.

In addition to differences in number of samples that yielded positive results, the different techniques yielded different egg or oopg values. Compared with the saline McMaster test, centrifugation-flotation yielded fewer small coccidia and more *E. macusaniensis* and nematode eggs. The differences between the sucrose McMaster and centrifugation-flotation were most marked for small coccidia and strongyles. The sucrose and saline McMaster tests differed mainly in the higher counts associated with *E. macusaniensis* and *Trichuris* spp, the parasites with denser eggs or oocysts, by use of sucrose solution. Flotation time played a major role in the centrifugation-flotation yields as well. Finding more eggs or oocysts by use of a particular technique did not necessarily indicate that those infections were more important. In fact, some reports or anecdotes suggesting parasitic disease concurrent with low fecal egg or oocysts counts may be the result of inefficient parasite egg or oocyst recovery with the method used. Additionally, it must be remembered that fecal egg counts may inaccurately correlate with the degree of host infection. More data are needed to interpret these findings. For now, we must be aware that if a different analytic method is used and a higher or lower yield obtained, it may be necessary to also change the set points for antiparasitic interventions established by use of another method.

### References


Selected abstract for JAVMA readers from the American Journal of Veterinary Research

Sedative and cardiopulmonary effects of medetomidine hydrochloride and xylazine hydrochloride and their reversal with atipamezole hydrochloride in calves

**Objective**—To assess the sedative and cardiopulmonary effects of medetomidine and xylazine and their reversal with atipamezole in calves.

**Animals**—25 calves.

**Procedures**—A 2-phase (7-day interval) study was performed. Sedative characteristics (phase I) and cardiopulmonary effects (phase II) of medetomidine hydrochloride and xylazine hydrochloride administration followed by atipamezole hydrochloride administration were evaluated. In both phases, calves were randomly allocated to receive 1 of 4 treatments IV: medetomidine (0.03 mg/kg; n = 6), xylazine (0.3 mg/kg) followed by atipamezole (0.04 mg/kg; 7), medetomidine (0.03 mg/kg; 6) followed by saline solution (10 mL), and xylazine (0.3 mg/kg; 6) followed by saline solution (10 mL). Atipamezole solution was administered 20 minutes after the first injection. Cardiopulmonary variables were recorded at intervals for 35 minutes after medetomidine or xylazine administration.

**Results**—At these doses, xylazine and medetomidine induced a similar degree of sedation in calves; however, the duration of medetomidine-associated sedation was longer. Compared with pretreatment values, heart rate, cardiac index, and PaO2 decreased and central venous pressure increased with medetomidine or xylazine. Systemic arterial blood pressures and vascular resistance increased with medetomidine and decreased with xylazine. Atipamezole reversed the sedative and most of the cardiopulmonary effects of both drugs.

**Conclusions and Clinical Relevance**—At these doses, xylazine and medetomidine induced similar degrees of sedation and cardiopulmonary depression in calves, although medetomidine administration resulted in increases in systemic arterial blood pressures. Atipamezole effectively reversed medetomidine- and xylazine-associated sedative and cardiopulmonary effects in calves. (Am J Vet Res 2008;69:319–326)

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