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Evaluation of Two Fecal Examination Techniques for Detection of *Trypanoxyuris* spp. Infection in Owl Monkeys (*Aotus nancymae*)

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Infections of *Trypanoxyuris* spp. pinworms in *Aotus nancymae* and other New World primates are typically subclinical, but infection during experimental use could confound interpretation of experimental data. Further, *Trypanoxyuris* species are highly infective, and rapid diagnosis is important to prevent an outbreak in the animal colony. This study sought to determine whether a fecal flotation technique was sensitive enough to replace the perianal tape test for diagnosis of *Trypanoxyuris* spp., thereby reducing stress to the animal and sample collection time. On days 0 and 3, we collected fecal samples from 45 animals confirmed to be infected with *Trypanoxyuris* spp. by perianal tape testing. Fecal samples were evaluated by both a commercial analysis system and by sucrose flotation with centrifugation. For both detection methods, no significant difference in sensitivity was detected between tests conducted on day 0 versus day 3. The sensitivity of repeated commercial tests was 80%, significantly higher than the 60% for sucrose flotation. The commercial test was significantly more sensitive than sucrose flotation, indicating that the commercial system was a better method for detecting *Trypanoxyuris* spp. However, sensitivity of only 80% confers a considerable risk of false negatives, thereby potentially delaying treatment and further contributing to environmental contamination. In our opinion, neither method of fecal analysis was a suitable replacement for the perianal tape test to diagnose *Trypanoxyuris* spp. in owl monkeys.

Abbreviations: PBS, phosphate buffered saline

Aotus monkeys can be infected by the common pinworm, *Trypanoxyuris*, in their natural habitat and while in captivity.[17] A prevalence study conducted previously with 128 *Aotus nancymae* monkeys showed 54.7% of the animals were naturally infected with this pinworm.[13] This genus of nematode is classified in the family Oxyuridae, and like other genera of pinworms, has a direct life cycle.[7,17] Infection occurs through the ingestion of larval eggs, with colonization of the cecum with adults followed by the migration of gravid females to the perianal skin, where eggs are deposited.[3,5,17] The deposition of eggs on the perianal skin is the basis for the tape test and its effectiveness at detecting pinworm infections, although Felt and colleagues[5] describe the lack of circadian-based egg deposition with *Trypanoxyuris microon*, unlike the situation with human pinworm (*Enterobius vermicularis*) infection.

Trypanoxyuris infections produce minimal pathologic change in the intestinal tracts of infected nonhuman primates. The vast majority of infections with *Trypanoxyuris* spp. are asymptomatic.[17] Similar to pinworm infection in other nonhuman primates, irritability associated with anal pruritus and irritation may present clinically.[19] However, the death of a spider monkey with an overwhelming infestation, presumably in the cecum or colon, has been reported.[3] Although pinworm infection in nonhuman primates usually lacks clinical sequelae, it can potentially confound research data through undetermined physiologic, immunologic, or behavioral effects, as has been documented for other laboratory animals.[1,10,12,16,18]

Routine parasitologic examination of fecal material from the *Aotus* colony at the Naval Medical Research Center Detachment's laboratory animal facility indicated the presence of *Trypanoxyuris* spp. infection.[2] Examinations were conducted as part of the preventive medicine program to detect the presence of various intestinal parasites, not solely *Trypanoxyuris*. The detection of *Trypanoxyuris* eggs from the routine screen raised the possibility of fecal collection and examination as an alternative to perianal tape testing. In human medicine, cellophane tape preparations are preferred for pinworm detection, and submission of fecal samples is not recommended, because pinworms are only occasionally recovered from those specimens.[6] The purpose of the present study was to determine whether fecal flotation could replace perianal tape testing for *Aotus*, because feces can be collected directly from the cage pan, thereby decreasing handling and stress to the monkeys.

Materials and Methods

Experimental design. Fresh feces were collected from a total of 45 *Trypanoxyuris*-infected animals, confirmed by perianal tape testing, on the day of the tape test (day 0) and 3 d later. Collected feces were examined by both a commercial analysis system (Fecalyzer, Evsco Pharmaceuticals, Buena, NJ; n = 90) and standard sucrose flotation[9] (n = 90) for the presence of *Trypanoxyuris* eggs. If 1 or more eggs were detected during microscopic examination, the test was considered positive for *Trypanoxyuris*.

Animals. We selected 45 (18 male, 27 female) captive-bred *Aotus nancymae* monkeys (age, 1 to 7 y) from the facility animal colony for assignment to this protocol. The experimental protocol (NMRCD06-1) was approved by the institutional animal care and use committee. The laboratory animal facility is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International; therefore all husbandry and

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Figure 1. Components and set up of the commercial fecal analysis system.

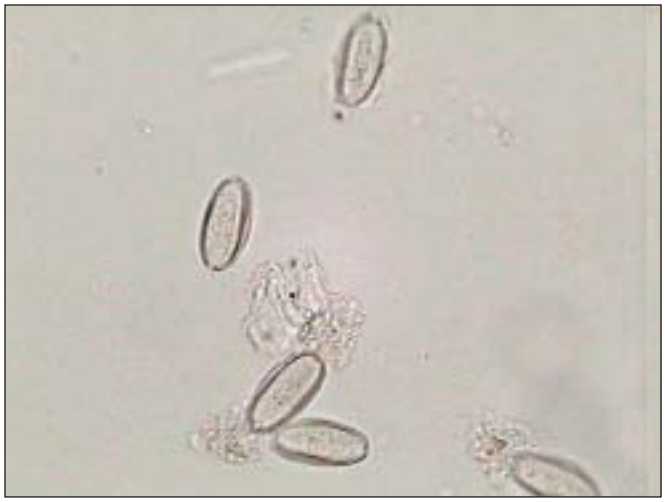

Figure 2. Appearance of *Trypanoxyuris* eggs recovered from the commercial fecal analysis system. Magnification, ×400.

experimental procedures were performed in compliance with the *Guide for the Care and Use of Laboratory Animals*.[14] Animals were single-housed in standard metal cages with nest boxes and perches. Cage pans were cleaned daily, and nest boxes and cages were sanitized every 2 wk on an alternating schedule to allow continuous scent marking. Each monkey daily received 10 pellets of a commercially formulated monkey diet (New World Primate Diet 8794N, Harlan Teklad, Madsion, WI), which was supplemented with a variety of fresh fruits and monkey biscuits purchased from the regional primate center (Instituto Veterinario de Investigacones Tropicales y de Altura, Iquitos, Peru). Distilled water was provided ad libitum. Animals were provided a reverse 12:12-h light:dark cycle that is offset from the normal day so that monkeys can be observed during their active time. Animals were caught in a net while in their cages and manually restrained for perianal tape testing.

Perianal tape test. Unsedated animals were positioned with tail extended and perineum exposed. The perineum was checked for the presence of adult pinworms, urine, and feces. If the perineum was urine-soaked or covered in feces, the area was blotted gently (not wiped) with a 2 × 2 in. gauze pad.[5] When the perineum was dry, an approximately 3-cm piece of ¾-in., clear cellophane tape was applied directly to the anus. The tape was pressed against the anus 3 times before the tape was transferred, sticky side down, to a glass microscope slide. The slide was examined for the presence of *Trypanoxyuris* eggs by using a light microscope with a 10× to 40× objective . If 1 or more eggs were observed, the animal was considered positive for *Trypanoxyuris* infection.

Commercial fecal analysis system. Fecal samples were collected directly from the cage pan with the commercial analysis system (Figure 1) by lifting the cap and removing the green insert. The small end of the green insert was pushed into the fecal sample and then replaced in the container and the cap closed. In the laboratory the cap was opened, and sodium nitrate solution (specific gravity, 1.2; Fecasol, Evsco Pharmaceuticals) was added to fill approximately half the cylinder. The green insert was agitated back and forth to separate ova from the fecal sample. The green insert was seated firmly with thumb pressure. Afterward, the remainder of the cylinder was filled with sodium nitrate solution to form a meniscus. A glass cover slip was floated on the meniscus for 15 to 20 min. The cover slip was transferred to a glass microscope slide and examined for the presence of *Trypanoxyuris* eggs by use of a light microscope with a 10× to 40× objective.

Sucrose fecal flotation. Feces were collected directly from the cage pan by use of a wooden tongue depressor and placed in a plastic urine specimen cup for transportation to the parasitology laboratory. Feces (4 to 5 g) were homogenized with 20 to 25 ml of phosphate-buffered saline. Fecal suspensions were filtered through gauze and rinsed with 10 ml phosphate-buffered saline. The suspension was centrifuged at 1000 × *g* for 5 min. The resulting supernatant was decanted and the sediment emulsified with 4 to 5 ml of PBS to form a slurry. The sediment slurry was added to a 50-ml plastic tube containing 25 ml of sucrose solution (700 g/l; specific gravity, 1.18) and centrifuged at 3000 × *g* for 10 min. The uppermost 5 ml of supernatant was transferred with a pipette to a clean 50-ml plastic tube. Phosphate-buffered saline was added to fill the 50-ml tube. The tube was centrifuged at 3000 × *g* for 10 min. The supernatant was decanted and the sediment collected and examined for the presence of *Trypanoxyuris* eggs by use of a light microscope with a 10× to 40× objective.

Statistical analysis. Sensitivity, the percentage of infected animals that tested positive, for the first and second tests was calculated separately for each fecal diagnostic test in addition to the combined sensitivity for each repeated test. The combined tests for both the commercial assay and sucrose flotation were considered positive if at least 1 of the 2 samples were positive. Confidence intervals at the 95% level were calculated by using the exact binomial distribution. Comparison of diagnostic test sensitivities was evaluated by using the Exact McNemar Chi Square test for paired dichotomous data. The concordance between the results of the first and second sample in each monkey was evaluated with kappa statistics and calculated separately for the results from both the commercial and sucrose flotation tests. Kappa statistics usually range between 0 and 1, and high values represent concordance exceeding that expected due to chance, although negative values to –1 indicate extreme discordance between the 2 measures compared. All statistical analyses were performed by use of Stata 9.2 software (StataCorp, College Station, TX).

Results

All animals enrolled in the study tested positive (100%) for *Trypanoxyuris* eggs by the tape test. After the first fecal examination, 30 of 45 animals (sensitivity, 67%) tested positive by the commercial system compared with 18 of 45 monkeys (sensitiv-

Table 1. Comparison of results from *Trypanoxyuris*-infected owl monkeys by use of commercial and sucrose floatation test systems on days 0 and 3 after perianal tape testing

Test	Day 0	Day 3	No. positive/no. tested (%)
Commercial	positive	positive	22/45 (49)
	positive	negative	8/45 (18)
	negative	positive	6/45 (13)
	negative	negative	9/45 (20)
Sucrose	positive	positive	8/45 (18)
	positive	negative	10/45 (22)
	negative	positive	9/45 (20)
	negative	negative	18/45 (40)

ity, 40%) by sucrose flotation ($P = 0.012$). Figure 2 depicts the microscopic appearance of *Trypanoxyuris* eggs recovered from the commercial system. After a second fecal examination by each method 3 d after the first, 28 of 45 animals (62%) were positive by the commercial test, compared with 17 of 45 animals (38%) by sucrose flotation. Again, the commercial test was significantly ($P = 0.019$) more sensitive than was the sucrose flotation test, but the reduction in sensitivity between the first and second fecal examinations was not statistically significant for either of the tests ($P = 0.790$ and $P = 1.000$, respectively).

Despite the similar overall sensitivity between the 2 examination time points for both test methods, the concordance of the test results for each animal was fairly low, and both the commercial assay and sucrose flotation test were associated with kappa values of –1. Table 1 depicts the variation in test results between days 0 and 3 for each of the fecal flotation methods. Therefore, the combined sensitivity of the commercial test over the 2 examination time points was higher than that for a single fecal examination ($P = 0.012$), reaching 80% sensitivity (95% confidence interval, 65% to 90%). The combined sensitivity of 2 sucrose flotation tests also was improved compared with that of a single 1 test ($P = 0.004$), reaching a 60% sensitivity (95% confidence interval, 44% to 74%). The combined sensitivity of the 2 commercial tests remained higher than that of the 2 sucrose flotation tests ($P = 0.049$). Figure 3 depicts the mean sensitivities of both tests after the first and second sampling and the combination of the 2 samplings.

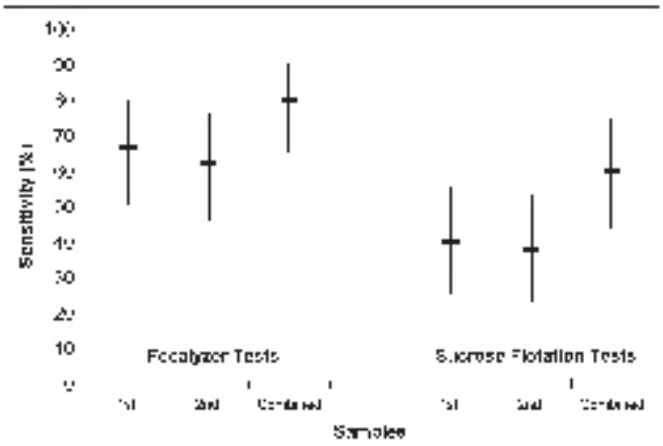

Figure 3. Individual and combined mean sensitivity for the first and second commercial tests and sucrose flotation with centrifugation tests. The second sample was collected 3 d after the first sample.

Discussion

We demonstrated that simple fecal flotation with neither sodium nitrate nor sucrose flotation with centrifugation was a suitable replacement for the detection of *Trypanoxyuris* eggs by perianal tape test in owl monkeys. Our results demonstrated that 2 fecal tests by either method were more sensitive than 1 test. The number of positive animals detected by each method was remarkably similar between the 2 different test days, and there was no significant difference in sensitivity between the first and second test for either flotation method.

Both flotation methods occasionally provided conflicting results for individual animals between the 2 sample days. This finding explains the significant increase in sensitivity between the first test and the combination of the first and second test for either flotation method ($P = 0.031$ for the commercial system and $P = 0.004$ for sucrose flotation) despite the similar sensitivity of each test independently. By performing 2 tests, there is a greater opportunity to find positives and reduce the number of false negatives. The sensitivity of each individual test and the combination of the 2 tests was significantly (first test, $P = 0.012$; second test, $P = 0.019$; combination, $P = 0.049$) greater for the commercial system than sucrose flotation, indicating that the commercial system we used is the better choice for detecting *Trypanoxyuris* eggs. Our findings contradict recommendations to always use centrifugation flotation techniques, because flotation methods that do not incorporate centrifugation often are not sufficiently sensitive to recover small numbers of organisms in the feces.[11] Further, Dryden and colleagues recommended centrifugation flotation rather than simple flotation because centrifugation floatation consistently yielded higher fecal egg counts.[4] However, we did not evaluate egg counts in our study, only the presence or absence of eggs.

In addition to the improved sensitivity with repeat tests (80% versus 60%), the advantages of the commercial system include that it is relatively inexpensive, commercially available and technically simple and provides rapid results (15 to 20 min).[4,15] Although sucrose flotation can detect the most common helminth ova and coccidian oocysts, this method requires a trained laboratory technician to perform numerous steps for sample preparation, a centrifuge and other supplies, and a considerable amount of time (approximately 2 h).

Collecting feces directly from the cage pan for fecal examination would be preferable to handling and manual restraint required for the perianal tape test because of decreased stress to the animal and sample collection time. Although perianal tape tests can be conducted while the animal is anesthetized (for example, semiannual physical exams), the recommendation from human medicine is to have 5 consecutive negative tests before considering a patient truly negative, in light of intermittent shedding of ova,[8] therefore reducing stress associated with repeated handling remains desirable. However, with a combined sensitivity of 80% after 2 tests, the commercial

system is not a suitable replacement for perianal tape testing, in our opinion. With only 80% sensitivity, there is too great a risk that false negatives will occur. Because *Trypanoxyuris* is highly infective[5] and because its eggs can persist in the environment, detection of positive animals is imperative, so that treatment of the colony with effective anthelmintics[2] can be initiated and environmental contamination eliminated.

Because combining repeated tests led to increased sensitivity, perhaps additional tests may further improve the sensitivity of these 2 fecal flotation methods, thereby making replacement of the perianal tape test a possibility. If this improvement in sensitivity were to occur, whether the reduction in stress from handling outweighs the need for timely results that may be delayed by conducting multiple fecal sample collections must be considered. Future studies evaluating more than 2 fecal samples and different time intervals between collections may reveal whether the sensitivity of fecal flotation methods can be improved.

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