



Evaluation of Sugar Flotation and Formalin-Ether Concentration Techniques in the Examination of GI Parasites of Refuge Dogs and Cats in Kanchanaburi Province, Thailand

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Abstract

This study was conducted to determine the prevalence of gastrointestinal protozoan and helminthic infections among refuge dogs and cats in Kanchanaburi Province, Thailand, using the sugar flotation technique (SF) and formalin-ether concentration technique (FECT), and to evaluate and compare the reliability and recovery efficiency of SF and FECT. Fecal samples of dogs (100) and cats (100) were collected and subjected to both SF and FECT. Each sample was examined for gastrointestinal parasites and processed in duplicate using each technique. The overall prevalence rates among the dogs by FECT were 5.0% and 5.0%, while the rates among the cats were 4.0% and 3.0% by SF, and 22.0% and 22.0% by FECT. Among the dogs, by FECT, only one protozoan species, *Giardia duodenalis*, was found; no helminths were recovered. Among the cats, SF detected only hookworm and *Toxocara cati*, while FECT detected a total of five helminth species, including *Spirometra mansoni*, *Platynosomum fastosum*, and *Dipylidium caninum*. *S. mansoni* was the most prevalent helminth among the cats (7%). The reliability of SF for detecting parasitic infections in cats was excellent ($\kappa = 0.85$, $p < 0.01$); FECT was also excellent for dogs ($\kappa = 1.0$, $p < 0.01$), and cats ($\kappa = 0.88$, $p < 0.01$). However, the overall agreement between SF and FECT for parasitic infections among cats was poor ($\kappa = 0.26$, $p < 0.01$). Although both SF and FECT were reliable, FECT was found more statistically efficient for recovering parasitic infections in the fecal specimens of dogs and cats.

Keywords: sugar flotation technique, formalin-ether concentration technique, refuge dogs and cats, gastrointestinal parasites, Thailand

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Introduction

Dogs and cats harbor several species of gastrointestinal protozoa and helminths that can cause zoonotic infections in humans. Some of these parasites reported in canine and feline in Thailand include the genera *Ancylostoma*, *Ascaris*,

Capillaria, Gnathostoma, Strongyloides, Toxocara, Trichuris, Dipylidium, Hymenolepis, Spirometra, Taenia, Fasciola, Opisthorchis, Schistosoma [1-9]; *Balantidium, Blastocystis, Cryptosporidium, Entamoeba, Giardia* and *Toxoplasma* [1,10-13].

To ensure the health and well-being of pet dogs and cats, coproscopic examinations for parasite eggs, cysts and oocysts are an important part of the daily routine for most veterinary practices. Many fecal examination techniques have been utilized and modified, each with its own advantages and limitations [14]. The techniques chosen for fecal examination depend to some degree on the suspected parasites. Fecal direct smear, to detect protozoan trophozoites, should be considered for animals presenting with diarrhea or soft-mushy stools [15,16]. The fecal flotation method is the one most frequently used to recover helminth eggs and protozoan oocysts. The centrifugal flotation method is accepted as the gold standard for screening common intestinal parasites in dogs and cats [14,16-18]. However, the technique is unreliable for the detection of nematode larvae and is not suitable for the eggs of most trematodes and large tapeworms [19-21].

The formalin-ether concentration technique (FECT), reintroduced by Ritchie [22], has been modified and refined by many others, and is widely used nowadays. It is the best of all centrifugation techniques, using no surface-active reagent. All types of worm eggs, larvae, and protozoan cysts may be recovered [19-21,23]. FECT is satisfactory for concentrating helminth eggs and moderately satisfactory for concentrating protozoan cysts [19]. It is a good choice for the examination of fecal specimens preserved in formalin [24].

In our previous study, we evaluated and compared the recovery efficiency and reliability of direct smear with FECT. The results indicated that, if the prevalence of parasitic infections is not too low (*ie*, $\geq 24\%$), the direct smear was statistically reliable and was fair-to-good in agreeing with FECT, although the recovery rate was about 50% lower [9]. The objectives of this study were to determine the prevalence of gastrointestinal parasitic infections among refuge dogs and cats in

Kanchanaburi Province, using both sugar flotation technique (SF) and FECT, and to evaluate and compare the reliability and recovery efficiency of SF and FECT.

Materials and methods

Specimen collection and examination

Fecal samples were collected from dogs and cats in an animal refuge in Kanchanaburi Province, about 130 km west of Bangkok. A total of 200 samples, 100 from dogs and 100 from cats, were collected. The specimens were transported to the laboratory at the Faculty of Tropical Medicine, Mahidol University, in Bangkok. All fecal samples were processed in duplicate by both SF and FECT, and examined for gastrointestinal protozoa (trophozoites, cysts, and oocysts) and helminths (eggs and larvae).

Sugar flotation technique (SF)

The sugar flotation solution (modified Sheather's solution) used for SF was prepared by boiling 454 g of sugar in 355 ml of distilled water. When cool, 2 ml of 37% formaldehyde were added as a preservative. The specific gravity of the flotation solution was measured using a hydrometer to be about 1.27 [14,18,25-27].

Two grams of feces were mixed thoroughly with 10 ml of sugar flotation solution in a cup and strained through two layers of gauze into a conical 15-ml centrifuge tube. The liquid remaining in the gauze strainer was squeezed from the feces by tongue depressor. After centrifugation at 1,000xg for 5 minutes, the tube was removed, placed in a test-tube rack, and filled to the top with sugar flotation solution. A 22 x 22 mm coverslip was placed on the tube, left for 10 minutes, removed and placed on a glass slide. The entire coverslip was then examined under a light microscope [14,18,25-27].

Formalin-ether concentration technique (FECT)

One gram of feces was mixed well with 10 ml water and strained through two layers of wet gauze into a conical 15-ml centrifuge tube. After

centrifugation at 1,000xg for two minutes, the supernatant was discarded. Seven milliliters of 10% formalin were added, followed by 3 ml of ether. The stoppered tube was shaken vigorously for 1 minute, then centrifuged for two minutes. After centrifugation, all supernatant and fecal scum were decanted. One drop of the recovered sediment, using a Pasteur pipette, was placed on a glass slide and a 22 x 22 mm coverslip was applied. The entire coverslip was then examined under a light microscope [19,20].

Statistical analysis

The two techniques, SF and FECT, were compared using Chi square and Fisher’s exact tests for differences, and Kappa test for agreements [Kappa value (κ): $\kappa > 0.75$ = excellent agreement, $\kappa = 0.40-0.75$ = fair to good agreement, $\kappa < 0.40$ = poor agreement] [28].

Results

The prevalences of gastrointestinal parasite infections among the sample dogs and cats are shown in Tables 1 and 2, respectively. The degree of agreement between SF-1 and SF-2, FECT-1 and FECT-2, and SF-1 and FECT-1 are also shown in Tables 1 and 2. The association between positive fecal samples from the dogs and cats examined by FECT-1 is tabulated in Table 3.

Table 1 (dog fecal samples): the overall

prevalence among the dogs, by SF-1 and SF-2 were both 0% (0/100), and by FECT-1 and FECT-2 both 5.0% (5/100). Only one species of protozoa, *Giardia duodenalis*, was found; no helminths were recovered. The degree of agreement between FECT-1 and FECT-2 for overall parasitic infections among the dogs did not occur by chance ($p < 0.01$); there was excellent agreement beyond chance ($\kappa = 1.0$).

Table 2 (cat fecal samples): the overall prevalence among the cats, by SF-1 and SF-2 were 4.0% (4/100) and 3.0% (3/100), respectively; and by FECT-1 and FECT-2 22.0% (22/100) and 22.0% (22/100), respectively. Two species of protozoa, *G. duodenalis* and *Cystoisospora* sp, were detected only by FECT (Fig 1). SF could only detect hookworm and *Toxocara cati* among the five helminth species detected by FECT–*Spirometra mansoni*, *Platynosomum fastosum*, and *Dipylidium caninum* (Fig 1). *S. mansoni* was the most prevalent helminth among the cats. The degree of agreement between SF-1 and SF-2 for overall parasitic infections in cats was excellent ($\kappa = 0.85, p < 0.01$). The agreements between FECT-1 and FECT-2 for protozoan, helminthic, and overall parasitic infections in cats were all excellent ($\kappa = 1.0, 0.84$ and 0.88 , respectively). However, the degree of agreement between SF-1 and FECT-1 did not occur by chance ($p < 0.01$), with poor agreement by chance ($\kappa = 0.26$).

Table 1 Number and percentage of dog fecal samples (n = 100) positive for protozoa and helminths by SF and FECT (Kappa test).

Parasite species (Dogs)	No. positive (% positive)							
	SF-1	SF-2	Kappa value	P-value	FECT-1	FECT-2	Kappa value	P-value
Protozoa								
<i>Giardia duodenalis</i>	0	0	-	-	5 (5.0)	5 (5.0)	1.0	<0.0001**
Helminths								
Helminth sp	0	0	-	-	0	0	-	-
Overall parasitic infections	0	0	-	-	5 (5.0)	5 (5.0)	1.0	<0.0001**
	0 (0.0)				5 (5.0)		0.0	-

SF = Sugar flotation technique, FECT = Formalin-ether concentration technique, ** = highly significant difference, Kappa value (κ): $\kappa > 0.75$ = excellent agreement, $\kappa = 0.40-0.75$ = fair to good agreement, $\kappa < 0.40$ = poor agreement

Table 2 Number and percentage of cat fecal samples (n = 100) positive for protozoa and helminths by SF and FECT (Kappa test).

Parasite species (Cats)	No. positive (% positive)							
	SF-1	SF-2	Kappa value	P-value	FECT-1	FECT-2	Kappa value	P-value
Protozoa	0	0	-	-	8 (8.0)	8 (8.0)	1.0	<0.0001**
<i>Giardia duodenalis</i>	0	0	-	-	3 (3.0)	3 (3.0)	1.0	<0.0001**
<i>Cystoisospora</i> sp	0	0	-	-	5 (5.0)	5 (5.0)	1.0	<0.0001**
Helminths	4 (4.0)	3 (3.0)	0.85	<0.0001**	15 (15.0)	15 (15.0)	0.84	<0.0001**
Hookworm	3 (3.0)	2 (2.0)	0.80	<0.0001**	3 (3.0)	3 (3.0)	1.0	<0.0001**
<i>Toxocara cati</i>	1 (1.0)	1 (1.0)	1.0	<0.0001**	1 (1.0)	2 (2.0)	0.66	<0.0001**
<i>Spirometra mansoni</i>	0	0	-	-	7 (7.0)	6 (6.0)	0.75	<0.0001**
<i>Platynosomum fastosum</i>	0	0	-	-	4 (4.0)	4 (4.0)	1.0	<0.0001**
<i>Dipylidium caninum</i>	0	0	-	-	4 (4.0)	2 (2.0)	0.32	0.0004**
Overall parasitic infections	4 (4.0)	3 (3.0)	0.85	<0.0001**	22 (22.0)	22 (22.0)	0.88	<0.0001**
	4 (4.0)				22 (22.0)		0.26	0.0001**

SF = Sugar flotation technique, FECT = Formalin-ether concentration technique, ** = highly significant difference, Kappa value (κ): $\kappa > 0.75$ = excellent agreement, $\kappa = 0.40-0.75$ = fair to good agreement, $\kappa < 0.40$ = poor agreement



Fig 1 (A) Egg packet of *Dipylidium caninum* (each egg = 35-60 μ m) and (B) *Cystoisospora* sp oocyst (38-51 x 27-39 μ m) found in feces of cats.

Table 3 shows that the prevalence of the protozoan parasite (*G. duodenalis*) among the dogs and cats examined by FECT-1 was not significantly different (5% vs 3%, $p > 0.05$), while that of the helminths was quite different (0% vs 15%, $p < 0.01$). Although the infection rates of each helminth species among the dogs and cats showed no significant difference, and only *S. mansoni* infection in the cats (7%) was significantly higher than in the dogs ($p < 0.05$), there was a highly significant difference in overall parasitic infections between cats and dogs ($p < 0.01$).

In summary, the degree of agreement between SF-1 and SF-2 for overall parasitic infections in cats was excellent. The agreements between FECT-1 and FECT-2 for overall infection rates in dogs and in cats were both excellent. In contrast, the degree of agreement between SF-1 and FECT-1 for overall infections in cats was poor. This implies that although both SF and FECT were reliable, FECT was more statistically efficient in the recovery of parasitic infections in dog and cat fecal samples (*i.e.*, the overall parasitic infection rates among the cats by FECT-1 were about five-fold those by FS-1).

Discussion

G. duodenalis was found among the refuge dogs and cats, while *Cystoisospora* sp was found only among the cats. The result was similar to our previous study in Nakhon Nayok Province [8]. *Cystoisospora* infection tends to have rigid host specificity, *ie*, canine *Cystoisospora* will not infect felines and the reverse is true for feline *Cystoisospora* [29].

S. mansoni was the most prevalent helminth among the cats. By contrast, our previous studies found hookworm infections (*T. cati* in one instance) were almost always the highest in both dogs and cats [8,9]. Hookworms have been found to be the most common intestinal parasites of canines and felines in most reports from Thailand [1,6,7,30].

The overall parasitic infections among the dogs were about four times lower than the cats. Moreover, only 5% of dogs were infected with only one species of protozoa, *G. duodenalis*. This is surprising, since most dogs and cats are housed in close proximity to each other. Some cats are even caged individually, while most dogs can roam freely in a confined area of the refuge. The

Table 3 The association between number (and %) of dog and cat fecal samples (n = 100) positive for protozoa and helminths by FECT-1.

Parasite species	No. positive (% positive)		
	Dogs	Cats	P-value
Protozoa	5 (5.0)	8 (8.0) ^b	0.390 ^{ns}
<i>Giardia duodenalis</i>	5 (5.0)	3 (3.0) ^a	0.721 ^{ns}
<i>Cystoisospora</i> sp	0	5 (5.0) ^a	0.059 ^{ns}
Helminths	0	15 (15.0) ^a	<0.0001 ^{**}
Hookworm	0	3 (3.0) ^a	0.246 ^{ns}
<i>Toxocara cati</i> (<i>T. canis</i> in dogs)	0	1 (1.0) ^a	1.00 ^{ns}
<i>Spirometra mansoni</i>	0	7 (7.0) ^a	0.014 [*]
<i>Platynosomum fastosum</i>	0	4 (4.0) ^a	0.121 ^{ns}
<i>Dipylidium caninum</i>	0	4 (4.0) ^a	0.121 ^{ns}
Overall parasitic infections	5 (5.0)	22 (22.0) ^b	0.001 ^{**}

FECT-1 = Formalin-ether concentration technique (trial 1), a = Fisher's exact test, b = Chi square test, ns = no significant difference, * = significant difference, ** = highly significant difference

lower prevalence among the dogs may be due to better healthcare and more appropriate nutrition provided by both the former owners and the latter refuge keepers. It may also be related to the light intensity of parasitic infections, which may reduce the recovery efficiency of the techniques used [31].

Dryden *et al* reported the evaluation of each group of fecal samples known to contain either hookworm (*A. caninum*) eggs, ascarid (*T. canis* or *T. cati*) eggs, or whipworm (*T. vulpis*) eggs: the direct-smear technique failed to detect hookworm eggs, ascarid eggs and whipworm eggs, 72.82%, 85.38%, and 92.61% of the time, respectively. The sugar flotation centrifugation technique yielded false-negative results 0.97%, 10.53%, and 4.93% of the time, respectively, and recovered >50 eggs/slide 74.76%, 1.18%, and 23.65% of the time, respectively. For fecal samples known to contain either tapeworm (*Taenia* sp) eggs or coccidia (*Cystoisospora* sp) oocysts, the direct smear technique failed to detect tapeworm eggs and coccidia oocysts 96.15% and 94.34% of the time, respectively. The sugar flotation centrifugation technique yielded false-negative results 11.54% and 5.66% of the time, respectively [14].

Akujobi *et al* found *Cryptosporidium* oocysts in 35 (18.1%) of HIV-seropositive patients using direct stool smear method and in 36 (18.7%) using FECT [32]. FECT was also found to detect 65.3% of positive specimens for one or more intestinal parasites, while the direct smear technique was 34.7% effective [33]. Uga *et al* compared five fecal examination techniques for three parameters: recovery efficiency, sensitivity and mean number of eggs detected. They reported the highest sensitivity among the five techniques was modified FECT (95%), followed by the commercially available kit (90%), original FECT (76%), Kato-Katz (57%), and direct smear (50%). The mean numbers of *Ascaris lumbricoides* eggs recovered by the techniques were 148, 97, 41, 11, and 6, respectively. The modified FECT is superior in the above-mentioned three parameters and also due to its ease of microscopic observation [34].

In the present study, no SF-positive sample was missed by FECT. The reliability of SF was

excellent, although only two species of helminths were detected. The reliability of FECT was excellent in all protozoan, helminthic and overall parasitic infections. The recovery rate using SF was only 13.6-18.2% (about 5-fold) lower than FECT, leading to poor agreement between the two techniques.

The results indicate that FECT performs better than SF. The poor recovery rate of SF may be due to at least two steps: preparation of the sugar flotation solution, which requires the precise specific gravity, and the difficulty in straining through two layers of gauze caused by the high viscosity of the sugar flotation solution.

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