



# Prevalence of Gastrointestinal Parasitic Infections in Refuge Dogs and Cats and Evaluation of Two Conventional Examination Techniques

Wichit Rojekittikhun<sup>1</sup>, Aongart Mahittikorn<sup>2</sup>, Samrerng Prummongkol<sup>3</sup>,  
Supalarp Puangsa-art<sup>4</sup>, Kittipong Chaisiri<sup>1</sup>, Teera Kusolsuk<sup>1</sup>

<sup>1</sup> Department of Helminthology, <sup>2</sup> Department of Protozoology, <sup>3</sup> Bangkok School of Tropical Medicine,  
<sup>4</sup> Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University,  
420/6 Ratchawithi Road, Bangkok 10400, Thailand

## Abstract

The purposes of the study were to determine the prevalence of gastrointestinal protozoan and helminthic infections among “refuge” dogs and cats in Pathum Thani and Nakhon Pathom provinces, using direct/simple smear (SS) and formalin-ether concentration techniques (FECT), and to evaluate the recovery efficiency and reliability of SS compared with FECT. Fifty samples each from dogs and cats in Pathum Thani Province, and 100 samples each from dogs and cats in Nakhon Pathom Province, were collected. All fecal samples were subjected to the two techniques, processed in duplicate, and examined for gastrointestinal parasites. In Pathum Thani refuge, the overall prevalence rates among the dogs by SS were 8.0% and 4.0%, and by FECT 10.0% and 8.0%; among the cats, the rates by SS were 34.0% and 34.0%, and by FECT 82.0% and 78.0%. In Nakhon Pathom refuge, the rates among the dogs by SS were 7.0% and 6.0%, and by FECT 28.0% and 20.0%; among the cats by SS the rates were 24.0% and 22.0%, and by FECT 48.0% and 46.0%. *Toxocara cati* was the most prevalent helminth among cats in Pathum Thani refuge, whereas hookworm infections were highest in both dogs and cats in Nakhon Pathom refuge. The infection intensity among all dogs and cats in both refuges was considered light. All positive fecal samples detected by SS were also detected by FECT. The recovery rate for SS was 25.0% (7/28) to 80.0% (4/5), compared with FECT. In the Pathum Thani observation, the reliability of SS for detecting parasitic infections in dogs and cats was statistically poor ( $\kappa = -0.5$  and  $0.38$ ), while the reliability of FECT for dogs was excellent ( $\kappa = 0.88$ ) and cats fair to good ( $\kappa = 0.50$ ). The agreement between SS and FECT for overall parasitic infections was poor ( $\kappa = 0.31$  and  $0.20$ ). In the Nakhon Pathom observation, the reliability of SS for detecting parasitic infections among dogs and cats was excellent ( $\kappa = 0.92$  and  $0.94$ ); FECT was also excellent ( $\kappa = 0.62$  and  $0.84$ ). The agreement between SS and FECT for overall parasitic infections among dogs was poor ( $\kappa = 0.32$ ), whereas among cats it was fair to good ( $\kappa = 0.51$ ). The results indicate that if the sample size of the animals is large enough ( $n = 100$ ) and the prevalence of parasitic infections is not too low ( $\geq 24\%$ ), SS was statistically reliable and was fair-to-good in agreeing with FECT, although the recovery rate was about 50% lower.

## Correspondence:

Wichit Rojekittikhun; E-mail: [wichit.roj@mahidol.ac.th](mailto:wichit.roj@mahidol.ac.th); Office Tel: 02-6435600; Phone: 089-8244925

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## Introduction

As reservoir hosts, dogs and cats frequently harbor several species of gastrointestinal protozoa and helminths causing zoonotic infections in humans. Parasites of dogs and cats commonly found in Thailand and tropical countries include the genera *Toxocara*, *Ancylostoma*, *Trichuris*, *Strongyloides*, *Gnathostoma*, *Ascaris*, *Dipylidium*, *Taenia*, *Spirometra*, *Hymenolepis*, *Opisthorchis*, *Giardia*, *Entamoeba*, *Blastocystis*, and *Toxoplasma* [1-8].

Many fecal examination techniques have been described and modified in the medico-veterinary scientific literature. Medically, the choice of technique(s) for use in a survey/control program is a major consideration, but two other matters are of greater importance. First, a chosen technique need not be the most accurate, but its level of reliability should be known and determined by the persons using it. Second, the choice of technique can best be made by the laboratory personnel where it is to be used [9]. There is no all-purpose technique, nor is there any best technique for any one purpose. Therefore, for the reliable diagnosis of intestinal parasites, a combination of several techniques is required [10].

The ordinary direct/simple smear technique (SS) is the oldest, simplest, and easiest technique for fecal examination. It is used mainly to demonstrate helminth eggs, larvae, protozoan trophozoites, and cysts [11]. Although SS is characterized by no egg-loss during the course of examination, light infections escape detection by the technique because of the limited (2-3 mg) fecal sample used [12]. However, for the detection of clinically significant infections, SS, if properly prepared and skillfully examined, is adequate in most cases [10]. SS is recommended as a routine procedure in the hospital or clinic, and all laboratories at the peripheral level [10,11].

The formalin-ether concentration technique (FECT) was reintroduced by Ritchie, in 1948 [13]. It has been modified and refined by many others,

and is widely used nowadays. FECT is the best of all centrifugation techniques using no surface-active reagent. All types of worm eggs, larvae, and protozoan cysts may be recovered [10-12,14]. The technique is satisfactory for concentrating helminth eggs and moderately satisfactory for concentrating protozoan cysts [10]. FECT is a good choice for checking on the thick smear (or the technician using it) and also for the examination of fecal specimens preserved in formalin [9]. It is recommended for all samples at Health Center Laboratories and District hospitals [11].

For veterinary science, the procedure(s) chosen for fecal examination will depend to some extent on which parasites are suspected. The 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 [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 [10,12,14].

It has been recently reported that FLOTAC techniques are sensitive, accurate and precise methods for qualitative and quantitative copromicroscopic analysis. When compared with more widely used diagnostic methods for parasite detection in animals (eg, McMaster and Wisconsin egg-counting techniques) and humans (eg, Kato-Katz and ether-based concentration techniques), the FLOTAC techniques showed higher sensitivity and accuracy [19]. In a report comparing the diagnostic accuracy of Kato-Katz, Koga agar plate, ether-concentration, and FLOTAC for *S. mansoni* and soil-transmitted helminths, FLOTAC showed the highest sensitivity for *S. mansoni* diagnosis, but revealed lower egg counts [20]. In another report on the comparison of Flotac-400 dual technique and FECT for the diagnosis of human intestinal

protozoan infection, while the Flotac-400 (results from two flotation solutions combined) found higher prevalence of *E. coli*, *B. hominis*, and *G. duodenalis*, the FECT detected higher prevalence of *E. histolytica*/*E. dispar* and four species of nonpathogenic intestinal protozoa. It should also be considered that *E. coli* cysts, as well as *S. mansoni* eggs, were somewhat deformed when subjected to the FLOTAC technique. Despite these constraints, the diagnostic performance of the Flotac-400 dual technique for detection of intestinal protozoa is promising, at least when compared with the widely used FECT [21].

A study comparing the performance of different European reference laboratories in diagnosing helminths and intestinal protozoa, using the ether-concentration method, concluded that although common helminths were reliably diagnosed by the study laboratories, there was only moderate agreement between Centers for pathogenic intestinal protozoa. It should be noted that there was no method equally suitable for all parasites; a standard technique has to be chosen if no specific parasite infection is suspected for which a particular technique could be employed. A reliable diagnosis of helminths and pathogenic protozoa was more important than the correct identification of non-pathogenic species [22].

The objectives of the study were to determine the prevalence of gastrointestinal parasitic infections in dogs and cats in two refuges in Pathum Thani and Nakhon Pathom provinces, using both SS and FECT, and to evaluate the recovery efficiency and reliability of SS compared with FECT.

## Materials and methods

Animal fecal samples were collected from two animal refuges, one in Pathum Thani Province and the other in Nakhon Pathom Province. A total of 100 samples, 50 from dogs and 50 from cats were collected from Grandma Mani's refuge in Pathum Thani Province. Two hundred samples, 100 each from dogs and cats from Aunt Nida's refuge in Nakhon Pathom Province, were also collected. The specimens were transported to the

laboratory in the Faculty of Tropical Medicine, Mahidol University, Bangkok. All fecal sample were subjected to both SS and FECT, each of which was processed in duplicate, and examined for gastrointestinal protozoa (trophozoites, cysts and oocysts) and helminths (eggs and larvae).

For SS, about 2 mg of feces, taken from various parts of the sample by means of a wooden applicator stick, were mixed with a drop of 0.85% sodium chloride (normal saline) solution on a slide. A 22 x 22 mm coverslip was placed on the mixture and the sample was examined under a light microscope [12].

For FECT, about 0.5 g of feces were mixed with 10 ml of 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 decanted, 7 ml of 10% formalin was added, followed by 3 ml of ether. The mixture was shaken vigorously for 10 minutes and centrifuged again [12]. The recovered sediment was adjusted to 0.3 ml (300  $\mu$ l), and one drop (about 75  $\mu$ l) was then cover-slipped on a slide and examined under a light microscope. Under these conditions, FECT could be used to estimate infection intensity.

Fecal samples were recorded as positive when a single protozoan cell or helminth egg of interest was found in the examined slide. Statistical comparisons were performed using Chi square and Fisher's exact tests for differences, and Kappa test for agreements (Kappa value:  $\kappa > 0.75$  = excellent agreement beyond chance,  $\kappa = 0.40-0.75$  = fair to good agreement beyond chance,  $\kappa < 0.40$  = poor agreement beyond chance) [23].

## Results

The prevalence of gastrointestinal parasitic infections in dogs and cats from Grandma Mani's refuge is shown in Tables 1a and 1b, and from Aunt Nida's refuge in Tables 2a and 2b. The degree of agreement between SS-1 and SS-2, FECT-1 and FECT-2, and SS-1 and FECT-1 are also shown. Tables 3 and 4 show the association between positive fecal samples from the dogs and cats from the two refuges examined by FECT-1.

Table 1a shows that the overall prevalence among the dogs from Grandma Mani's refuge, by SS-1 and SS-2, were 8.0% (4/50) and 4.0% (2/50), and by FECT-1 and FECT-2, 10.0% (5/50) and 8.0% (4/50), respectively. One species of protozoa, *Giardia duodenalis*, was found among the dogs. One case of *Ascaris lumbricoides* was detected by both SS-1 and FECT-1; however, one case of *Dipylidium caninum* was detected only by FECT. The agreement between SS-1 and SS-2 for overall parasitic infections in dogs (occurred by chance,  $p > 0.05$ ) was poor ( $\kappa = -0.05$ ); that of FECT-1 and FECT-2 (occurred not by chance,  $p < 0.01$ ) was excellent ( $\kappa = 0.88$ ); and that of SS-1 and FECT-1 (occurred not by chance,  $p < 0.01$ ) was poor ( $\kappa = 0.31$ ).

In Table 1b, the overall prevalence among the cats from Grandma Mani's refuge by SS were 34.0% (17/50) and 34.0% (17/50), and by FECT 82.0% (41/50) and 78.0% (39/50). *Isospora* sp was the only protozoan parasite found in the cats. Two cases of cat liver-fluke, *Platynosomum fastosum*, were detected only by FECT. *Toxocara cati* was found to be the most prevalent helminth among the cats. Agreements between the techniques show similar results to Table 1a, except that the agreement between FECT-1 and FECT-2 for overall parasitic infections in cats was fair to good ( $\kappa =$

0.50,  $p < 0.01$ ).

Table 2a reveals that the overall prevalence among the dogs from Aunt Nida's refuge by SS were 7.0% (7/100) and 6.0% (6/100), and by FECT 28.0% (28/100) and 20.0% (20/100). *G. duodenalis* was found in the dogs by both SS and FECT, whereas *Isospora* sp was detected only by FECT. One case of *T. canis*, 2 cases of *T. vulpis*, and one case of *H. diminuta*, were also detected only by FECT. The agreement between SS-1 and SS-2 for overall parasitic infections in the dogs was excellent ( $\kappa = 0.92$ ,  $p < 0.01$ ); that of FECT-1 and FECT-2 was fair to good ( $\kappa = 0.62$ ,  $p < 0.01$ ); and that of SS-1 and FECT-1 was poor ( $\kappa = 0.32$ ,  $p < 0.01$ ).

In Table 2b, the overall infection rates among the cats from Aunt Nida's refuge by SS were 24.0% (24/100) and 22.0% (22/100), and by FECT 48.0% (48/100) and 46.0% (46/100). As in the dogs, *Isospora* sp was only found in the cats by FECT. One case of *Opisthorchis viverrini*-like egg was detected only by FECT. The agreement between SS-1 and SS-2 for overall parasitic infections in cats was excellent ( $\kappa = 0.94$ ,  $p < 0.01$ ); that of FECT-1 and FECT-2 was excellent ( $\kappa = 0.84$ ,  $p < 0.01$ ); and that of SS-1 and FECT-1 was fair to good ( $\kappa = 0.51$ ,  $p < 0.01$ ).

**Table 1a Number and percentage of dog fecal samples (n = 50) positive for protozoa and helminths by simple smear and formalin-ether concentration techniques, Grandma Mani's refuge, Pathum Thani Province (Kappa test).**

Parasite species (Dogs)	No. positive (% positive)							
	SS-1	SS-2	Kappa value	P-value	FECT-1	FECT-2	Kappa value	P-value
Protozoa	3 (6.0)	2 (4.0)	0.79	<0.0001**	3 (6.0)	3 (6.0)	1.00	<0.0001**
<i>Giardia duodenalis</i>	3 (6.0)	2 (4.0)			3 (6.0)	3 (6.0)		
Helminths	2 (4.0)	2 (4.0)	-0.04	0.6259 <sup>ns</sup>	4 (8.0)	3 (6.0)	0.85	<0.0001**
Hookworm	0	1 (2.0)			2 (4.0)	2 (4.0)		
<i>Toxocara canis</i>	1 (2.0)	1 (2.0)			2 (4.0)	2 (4.0)		
<i>Dipylidium caninum</i>	0	0			1 (2.0)	1 (2.0)		
<i>Ascaris lumbricoides</i>	1 (2.0)	0			1 (2.0)	0		
Overall parasitic infections	4 (8.0)	2 (4.0)	-0.05	0.6648 <sup>ns</sup>	5 (10.0)	4 (8.0)	0.88	<0.0001**
	4 (8.0)				5 (10.0)		0.31	0.0012**

SS = Direct/simple smear technique, FECT = Formalin-ether concentration technique, \*\* = highly significant difference, ns = no significant difference, Kappa value ( $\kappa$ ):  $\kappa > 0.75$  = excellent agreement beyond chance,  $\kappa = 0.40$ -0.75 = fair to good agreement beyond chance,  $\kappa < 0.40$  = poor agreement beyond chance

**Table 1b Number and percentage of cat fecal samples (n = 50) positive for protozoa and helminths by simple smear and formalin-ether concentration techniques, Grandma Mani's refuge, Pathum Thani Province (Kappa test).**

Parasite species (Dogs)	No. positive (% positive)							
	SS-1	SS-2	Kappa value	P-value	FECT-1	FECT-2	Kappa value	P-value
Protozoa	1 (2.0)	0	0.00	>0.05	3 (6.0)	3 (6.0)	1.00	<0.0001**
<i>Isospora</i> sp	1 (2.0)	0			3 (6.0)	3 (6.0)		
Helminths	16 (32.0)	17 (34.0)	0.41	0.0018**	40 (80.0)	38 (76.0)	0.54	0.0001**
Hookworm	4 (8.0)	3 (6.0)			20 (40.0)	22 (44.0)		
<i>Toxocara cati</i>	13 (26.0)	15 (30.0)			28 (56.0)	25 (50.0)		
<i>Platynosomum fastosum</i>	0	0			1 (2.0)	2 (4.0)		
Overall parasitic infections	17 (34.0)	17 (34.0)	0.38	0.0039**	41 (82.0)	39 (78.0)	0.50	0.0002**
	17 (34.0)				41 (82.0)		0.20	0.0087**

SS = Direct/simple smear technique, FECT = Formalin-ether concentration technique, \*\* = highly significant difference, ns = no significant difference, Kappa value ( $\kappa$ ):  $\kappa > 0.75$  = excellent agreement beyond chance,  $\kappa = 0.40-0.75$  = fair to good agreement beyond chance,  $\kappa < 0.40$  = poor agreement beyond chance

**Table 2a Number and percentage of dog fecal samples (n = 100) positive for protozoa and helminths by simple smear and formalin-ether concentration techniques, Aunt Nida's refuge, Nakhon Pathom Province (Kappa test).**

Parasite species (Dogs)	No. positive (% positive)							
	SS-1	SS-2	Kappa value	P-value	FECT-1	FECT-2	Kappa value	P-value
Protozoa	1 (1.0)	1 (1.0)	1.00	<0.0001**	12 (12.0)	4 (4.0)	0.20	0.0085**
<i>Giardia duodenalis</i>	1 (1.0)	1 (1.0)			10 (10.0)	3 (3.0)		
<i>Isospora</i> sp	0	0			3 (3.0)	1 (1.0)		
Helminths	6 (6.0)	5 (5.0)	0.90	<0.0001**	19 (19.0)	17 (17.0)	0.87	<0.0001**
Hookworm	6 (6.0)	5 (5.0)			18 (18.0)	17 (17.0)		
<i>Toxocara canis</i>	0	0			1 (1.0)	1 (1.0)		
<i>Trichuris vulpis</i>	0	0			1 (1.0)	2 (2.0)		
<i>Hymenolepis diminuta</i>	0	0			1 (1.0)	0		
Overall parasitic infections	7 (7.0)	6 (6.0)	0.92	<0.0001**	28 (28.0)	20 (20.0)	0.62	<0.0001**
	7 (7.0)				28 (28.0)		0.32	<0.0001**

SS = Direct/simple smear technique, FECT = Formalin-ether concentration technique, \*\* = highly significant difference, ns = no significant difference, Kappa value ( $\kappa$ ):  $\kappa > 0.75$  = excellent agreement beyond chance,  $\kappa = 0.40-0.75$  = fair to good agreement beyond chance,  $\kappa < 0.40$  = poor agreement beyond chance

Surprisingly, the overall parasitic infection rates in the cats were about eight-fold those of the dogs ( $p < 0.01$ ) from Grandma Mani's refuge (Table 3), and about two-fold those of cats compared with dogs ( $p < 0.01$ ) from Aunt Nida's refuge (Table 4).

In summary, *T. cati* was the most prevalent helminth found among the cats from Grandma Mani's refuge. The rate was significantly higher

than *T. canis* infection in the dogs from the same refuge (Table 3). Hookworm infections were found to be the highest in both dogs and cats from Aunt Nida's refuge. Similar to Grandma Mani's refuge, *T. cati* infection in cats was also significantly higher than *T. canis* infection in dogs (Table 4). The levels of infection intensity in all dogs and cats in both refuges were estimated to be light. All positive fecal

samples detected by SS could also be detected by FECT. No sample found positive by SS was missed by FECT. The SS recovery rate was 25.0% (7/28) (Table 2a) to 80.0% (4/5) (Table 1a), compared with FECT. It is interesting to note that when the sample size of the animals examined was large enough ( $n = 100$ ), and the prevalence of overall parasitic infections was

not too low ( $\geq 24\%$ ), SS was statistically reliable and was fair to good in agreement with FECT although the recovery rate was about 50% lower (Table 2b).

## Discussion

*G. duodenalis* was found only among the dogs, from both Grandma Mani's and Aunt

**Table 2b Number and percentage of cat fecal samples ( $n = 100$ ) positive for protozoa and helminths by simple smear and formalin-ether concentration techniques, Aunt Nida's refuge, Nakhon Pathom Province (Kappa test).**

Parasite species (Dogs)	No. positive (% positive)							
	SS-1	SS-2	Kappa value	P-value	FECT-1	FECT-2	Kappa value	P-value
Protozoa	0	0	-	-	2 (2.0)	1 (1.0)	-0.01	0.5571 <sup>ns</sup>
<i>Isospora</i> sp	0	0			2 (2.0)	1 (1.0)		
Helminths	24 (24.0)	22 (22.0)	0.94	<0.0001**	48 (48.0)	46 (46.0)	0.84	<0.0001**
Hookworm	15 (15.0)	14 (14.0)			38 (38.0)	36 (36.0)		
<i>Toxocara cati</i>	10 (10.0)	10 (10.0)			13 (13.0)	13 (13.0)		
<i>Taenia</i> sp	1 (1.0)	0			2 (2.0)	4 (4.0)		
<i>Platynosomum fastosum</i>	1 (1.0)	1 (1.0)			4 (4.0)	2 (2.0)		
<i>Opisthorchis viverrini</i>	0	0			1 (1.0)	0		
Overall parasitic infections	24 (24.0)	22 (22.0)	0.94	<0.0001**	48 (48.0)	46 (46.0)	0.84	<0.0001**
	24 (24.0)				48 (48.0)		0.51	<0.0001**

SS = Direct/simple smear technique, FECT = Formalin-ether concentration technique, \*\* = highly significant difference, ns = no significant difference, Kappa value ( $\kappa$ ):  $\kappa > 0.75$  = excellent agreement beyond chance,  $\kappa = 0.40$ -0.75 = fair to good agreement beyond chance,  $\kappa < 0.40$  = poor agreement beyond chance

**Table 3 The association between number (and percentage) of dog and cat fecal samples ( $n = 50$ ) positive for protozoa and helminths by formalin-ether concentration technique (FECT-1), Grandma Mani's refuge, Pathum Thani Province.**

Parasite species	No. positive (% positive)		
	Dogs	Cats	P-value
Protozoa	3 (6.0)	3 (6.0) <sup>a</sup>	1.00 <sup>ns</sup>
<i>Giardia duodenalis</i>	3 (6.0) <sup>a</sup>	-	0.242 <sup>ns</sup>
<i>Isospora</i> sp	-	3 (6.0) <sup>a</sup>	0.242 <sup>ns</sup>
Helminths	4 (8.0)	40 (80.0) <sup>b</sup>	<0.0001**
Hookworm	2 (4.0)	20 (40.0) <sup>b</sup>	<0.0001**
<i>Toxocara canis</i> ( <i>T. cati</i> in cat)	2 (4.0)	28 (56.0) <sup>b</sup>	<0.0001**
<i>Dipylidium caninum</i>	1 (2.0)	-	-
<i>Ascaris lumbricoides</i>	1 (2.0)	-	-
<i>Platynosomum fastosum</i>	-	1 (2.0)	-
Overall parasitic infections	5 (10.0)	41 (82.0) <sup>b</sup>	<0.0001**

SS = Direct/simple smear technique, FECT = Formalin-ether concentration technique, a = Fisher's exact test, b = Chi square test, ns = no significant difference, \*\* = highly significant difference



**Table 4 The association between number (and percentage) of dog and cat fecal samples (n = 100) positive for protozoa and helminths by formalin-ether concentration techniques (FECT-1), Aunt Nida's refuge, Nakhon Pathom Province.**

Parasite species	No. positive (% positive)		P-value
	Dogs	Cats	
Protozoa	12 (12.0)	2 (20.0) <sup>b</sup>	0.006**
<i>Giardia duodenalis</i>	10 (10.0) <sup>a</sup>	-	0.002**
<i>Isospora</i> sp	3 (3.0)	2 (2.0) <sup>a</sup>	1.00 <sup>ns</sup>
Helminths	19 (19.0)	48 (48.0) <sup>b</sup>	<0.0001**
Hookworm	18 (18.0)	38 (38.0) <sup>b</sup>	0.002**
<i>Toxocara canis</i> ( <i>T. cati</i> in cat)	1 (1.0)	13 (13.0) <sup>b</sup>	0.001**
<i>Trichuris vulpis</i>	1 (1.0) <sup>a</sup>	-	1.00 <sup>ns</sup>
<i>Hymenolepis diminuta</i>	1 (1.0) <sup>a</sup>	-	1.00 <sup>ns</sup>
<i>Taenia</i> sp	-	2 (2.0) <sup>a</sup>	0.497 <sup>ns</sup>
<i>Platynosomum fastosum</i>	-	4 (4.0) <sup>a</sup>	0.121 <sup>ns</sup>
<i>Opisthorchis viverrini</i>	-	1 (1.0) <sup>a</sup>	1.00 <sup>ns</sup>
Overall parasitic infections	28 (28.0)	48 (48.0) <sup>b</sup>	0.004**

SS = Direct/simple smear technique, FECT = Formalin-ether concentration technique, a = Fisher's exact test, b = Chi square test, ns = no significant difference, \*\* = highly significant difference

Nida's refuges, while *Isospora* sp was found only among the cats from Grandma Mani's refuge. The results were similar to our previous recent study in a refuge in Nakhon Nayok Province; we have discussed the species complex of *G. duodenalis* in relation to the host specificity of different assemblages implying animal infection. *Giardia* assemblage B has been found to infect humans and dogs, but not cats. Assemblages C and D are specific genotypes commonly infecting dogs, and assemblage F only infects cats [24,25]. In *Isospora* infection, the reason for this may be the rigid host specificity of the coccidian. Canine *Isospora* will not infect felines and the reverse is true for feline *Isospora* [26,27].

In this study, although *T. cati* was the most prevalent helminth found among the cats from Grandma Mani's refuge (56.0% *T. cati* vs 44.0% hookworms), hookworm infections were found to be the highest in both dogs and cats from Aunt Nida's refuge. This is consistent with previous studies, conducted in 2001-2002, of stray dogs and cats in Bangkok temples, where the prevalence of hookworm was 70.3-88.3% in dogs and 55.4-76.6% in cats [unpublished

data], and that conducted in 2012, of refuge dogs and cats in Nakhon Nayok Province, where the prevalence was 30.6% in dogs and 34.7% in cats [8]. Hookworms have been found to be the most common intestinal parasites of canines and felines in most reports from Thailand [1,5,7,28].

One case of *A. lumbricoides* was found in a dog in Grandma Mani's refuge. This is not common, although there has been a report of *Ascaris* infection, 0.4% (4/941) in dogs and 0.3% (1/296) in cats in northeastern Thailand [1]. It has been demonstrated that in communities where promiscuous defecation patterns exist, dogs play a major role in broadening the range of dissemination and environmental contamination of infective stages of *A. lumbricoides* [29]. A report from Egypt has suggested that dogs could act as reservoir hosts of *A. lumbricoides* and environmental contaminants that increase the risk of human infection [30].

The levels of infection intensity in all dogs and cats from both refuges were estimated to be light. Only a few helminth eggs or protozoan cysts/oocysts were observed in each mount. The lowest prevalence and lightest infection intensity were found in dogs from Grandma Mani's refuge. The overall parasitic infections in these dogs

were about eight times lower than cats from the same refuge. This is surprising, since most dogs and cats are housed in close proximity to each other. Some cats are caged individually, while most dogs can roam freely in a confined area of the refuge. The low prevalence of gastrointestinal parasites in canines and felines have been reported recently in several articles from Thailand and other countries [5,31-33]. Low prevalence rates in refuge dogs and cats may be due to good healthcare and appropriate nutrition provided by the animal keepers. It may also be related to the light intensity of parasitic infections, which may hamper the recovery efficiency of the techniques used [34].

It has been reported that the recovery rate (sensitivity) of SS for hookworms was 18.3% (false negative rate was 81.7%), for *A. lumbricoides* 26.5-32.0%, and *T. trichiura* 14.0% [35,36]. A comparative evaluation of SS and FECT for the identification of *Cryptosporidium* reported *Cryptosporidium* oocysts in 18.1% of HIV-seropositive patients using SS, and in 18.7% using FECT; there was no statistically significant difference between the two methods [37]. On the contrary, a comparison of the two techniques for diagnosing intestinal parasites showed that FECT detected 65.3% of positive specimens for one or more intestinal parasites, while SS was 34.7% effective. A significant number of the infected population was missed by SS [38]. Uga *et al*, in 2010 [34], re-evaluated the recovery efficiency of the original FECT and modified it. They reported that the highest sensitivity among the five techniques used was the modified FECT (95%), followed by the commercially available kit (90%), original FECT (76%), Kato-Katz (57%) and SS (50%).

In the present study, no sample positive by SS was missed by FECT. The recovery rate of SS was 25.0% to as high as 80.0%, when compared with FECT. The reliability of SS was found to be fair to excellent. It was poor only in the detection of helminths in dog samples and detection of protozoa in cat samples, when the parasite-infection rates were very low (<4.0% in dogs, and <2.0% in cats). The reliability of

FECT was excellent in all experiments, except for the detection of protozoa in dog samples, where double infections were detected and with low prevalence (<3.0% and <10.0%). The results indicate the good performance and usefulness of the two techniques. Although SS cannot substitute for FECT in many circumstances, the two combined are very valuable. We believe that the recovery efficiency of the techniques is not a major problem in routine medical or veterinary parasitology. Shortages of skilled and experienced technicians in this field pose a real problem in the future.

In conclusion, SS and FECT, for the detection of gastrointestinal parasitic infections in dogs and cats from two refuges in Pathum Thani and Nakhon Pathom provinces, were very reliable, although the diagnostic agreement between the two techniques for overall parasitic infections was generally poor to fair.

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## References

1. Impand P, Thirachandra S, Bunnag T. Helminth faunas of rats and domestic animals and their zoonotic potential role in north and northeast Thailand. *J Parasit Trop Med Assoc Thailand*. 1983;6:105-16. [in Thai].
2. Ballweber LR. *Veterinary parasitology: the practical veterinarian*. Boston: Butterworth-Heinemann; 2001.
3. Kaewthamasorn M, Niwetpathomwat A, Assarasakorn S, Wongsamee S, Tiawsirisup S. A surveillance of canine gastrointestinal parasites in fecal samples from public areas of Bangkok, Thailand. *J Anim Vet Adv*. 2006;5(12):1209-13.
4. Inpankaew T, Traub R, Thompson RC, S. Canine parasitic zoonoses in Bangkok



- temples. Southeast Asian J Trop Med Public Health. 2007;38(2):247-55.
5. Jittapalapong S, Inparnkaew T, Pinyopanuwat N, Kengradomkij C, Sangvaranond A, Wongnakphet S. Gastrointestinal parasites of stray cats in Bangkok metropolitan areas, Thailand. Kasetsart J (Nat Sci). 2007;41:69-73.
6. Holyoake CS. A national study of gastrointestinal parasites infecting dogs and cats in Australia [Ph.D. Thesis]. Western Australia: Murdoch University; 2008.
7. Enes JE, Wages AJ, Malone JB, Tesana S. Prevalence of *Opisthorchis viverrini* infection in the canine and feline hosts in three villages, Khon Kaen Province, Northeastern Thailand. Southeast Asian J Trop Med Public Health. 2010;41:36-42.
8. Rojekittikhun W, Chaisiri K, Mahittikorn A, Pubampen S, Sa-nguankiat S, Kusolsuk T, *et al*. Gastrointestinal parasites of dogs and cats in a refuge in Nakhon Nayok, Thailand. Southeast Asian J Trop Med Public Health. In press 2013.
9. Beaver PC, Yokogawa M. Diagnostic techniques and training. In: Yokogawa M, Hayashi S, Kobayashi A, *et al*. Collected paper on the control of soil-transmitted helminthiasis Vol I. Tokyo: The Asian Parasite Control Organization; 1980. p. 35-40.
10. Beaver PC, Jung RC, Cupp EW. Clinical parasitology. 9<sup>th</sup> ed. Philadelphia: Lea & Febiger; 1984.
11. World Health Organization. Basic laboratory methods in medical parasitology. Geneva: WHO; 1991.
12. Suzuki N. Examination technics for helminth eggs. In: Color atlas of human helminth eggs. 3<sup>rd</sup> ed. Tokyo: JAPC & JOICFP; 1981.
13. Ritchie LS. An ether sedimentation technique for routine stool examinations. Bull US Army Med Dep. 1948;8(4):326.
14. Truant Al, Elliott SH, Kelly MT, Smith JH. Comparison of formalin-ethyl ether sedimentation, formalin-ethyl acetate sedimentation, and zinc sulfate flotation techniques for detection of intestinal parasites. J Clin Microbiol. 1981;13(5):882-4.
15. Diagnostic Parasitology Service Laboratory, University of Tennessee College of Veterinary Medicine. Detection of parasitic infections by fecal examination, 2009. [cited 2013 Oct 15]. Available from: <http://www.vet.utk.edu-diagnostic-parasitology-Detections of Parasitic Infections by Fecal Exam.pdf>
16. Bowman DD, Lucio-Forster A. The importance of routine fecal exams protecting pets and their owners from parasitic infections. DX Consult. 2010;3(1):10-11.
17. Dryden MW, Payne PA, Ridley R, Smith V. Comparison of common fecal flotation techniques for the recovery of parasite eggs and oocysts. Vet Ther. 2005;6(1):15-28.
18. Robertson J. Routine parasitic screening and identifying infectious causes of diarrhea. DX Consult. 2010;3(1):11.
19. Cringoli G, Rinaldi L, Maurelli MP, Utzinger J. FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. Nat Protoc. 2010;5(3):503-15.
20. Glinz D, Silue KD, Knopp S, Lohourignon LK, Yao KP, Steinmann P, *et al*. Comparing diagnostic accuracy of Kato-Katz, Koga agar plate, Ether-concentration, and FLOTAC for *Schistosoma mansoni* and soil-transmitted helminths. PLoS Negl Trop Dis. 2010;4(7):e754. doi:10.1371/ journal.pntd.0000754
21. Becker SL, Lohourignon LK, Speich B, Rinaldi L, Knopp S, N'Goran EK, *et al*. Comparison of the Flotac-400 dual technique and the formalin-ether concentration technique for diagnosis of human intestinal protozoon infection. J Clin Microbiol. 2011;49(6):2183-90.
22. Utzinger J, Botero-Kleiven S, Castelli F, Chiodini PL, Edwards H, Kohler N, *et al*. Microscopic diagnosis of sodium acetate-acetic acid-formalin-fixed stool samples for helminths and intestinal protozoa: a comparison among European reference laboratories. Clin Microbiol Infect. 2010;16:267-73.
23. Fleiss JL. Statistical methods for rates and proportions. 2<sup>nd</sup> ed. New York: John Wiley and Sons; 1981.

24. Monis PT, Caccio SM, Thompson RCA. Variation in *Giardia*: towards a taxonomic revision of the genus. *Trends Parasitol.* 2009;25:93-100.
25. Tangtrongsup S, Scorza V. Update on the diagnosis and management of *Giardia* spp infections in dogs and cats. *Top Companion Anim Med.* 2010;25:155-62.
26. Dubey JP, Lindsay DS, L. Toxoplasmosis and other intestinal coccidial infections in cats and dogs. *Vet Clin North Am Small Anim Pract.* 2009;39:1009-34.
27. Companion Animal Parasite Council (CAPC). Intestinal parasites: coccidia. [updated 2013 Apr; cited 2013 Sep 15]. Available from: <http://www.capcvet.org/capc-recommendations/coccidia/>
28. Rojekittikhun W, Chaayasith T, Yaemput S. Dog gnathostomosis in Nakhon Nayok Province. *J Trop Med Parasitol.* 2000;23:43-52.
29. Traub RJ, Robertson ID, Irwin P, Mencke N, Thompson RCA. The role of dogs in transmission of gastrointestinal parasites in a remote tea-growing community in northeastern India. *Am J Trop Med Hyg.* 2002;67:539-45.
30. Shalaby HA, Abdel-Shafy S, Derbala AA. The role of dogs in transmission of *Ascaris lumbricoides* for humans. *Parasitol Res.* 2010;106:1021-6.
31. Gates MC, Nolan TJ. Endoparasite prevalence and recurrence across different age groups of dogs and cats. *Vet Parasitol.* 2009;166(1-2):153-8.doi:10.1016/j.vetpar.2009.07.041.
32. Asano K, Suzuki K, Asano R. Prevalence of intestinal parasites in dogs in the national capital region of Japan. *J Anim Vet Adv.* 2011;10(20):2666-8.
33. Gaunt MC, Carr AP. A survey of intestinal parasites in dogs from Saskatoon, Saskatchewan. *Can Vet J.* 2011;52:497-500.
34. Uga S, Tanaka K, Iwamoto N. Evaluation and modification of the formalin-ether sedimentation technique. *Trop Biomed.* 2010;27(2):177-84.
35. Kobayashi A. Tests on the efficacy of anthelmintics. In: Yokogawa M, Hayashi S, Kobayashi A, *et al.* Collected paper on the control of soil-transmitted helminthiasis Vol I. Tokyo: The Asian Parasite Control Organization; 1980. p. 41-5.
36. Komiya Y, Kobayashi A. Evaluation of Kato's thick smear technic with a cellophane cover for helminth eggs in feces. In: Yokogawa M, Hayashi S, Kobayashi A, *et al.* Collected paper on the control of soil-transmitted helminthiasis Vol I. Tokyo: The Asian Parasite Control Organization; 1980. p. 57-62.
37. Akujobi CN, Ogunsola FT, Iregbu KC, Odugbemi TO. Comparative evaluation of direct stool smear and formol-ether concentration methods in the identification of *Cryptosporidium* species. *Nigerian J Health Biomed Sci.* 2005;4(1):5-7.
38. Oguoma VM, Ekwunife CA. The need for a better method: comparison of direct smear and formol-ether concentration techniques in diagnosing intestinal parasites. *Internet J Trop Med.* 2007;3(2).doi:10.5580/17.