THE POTENTIAL USEFULNESS OF THE MODIFIED KATO THICK SMEAR TECHNIQUE IN THE DETECTION OF INTESTINAL SARCOCYSTOSIS DURING FIELD SURVEYS

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Abstract. A total of 479 stool specimens were collected from rural communities of Ubon Ratchathani Province, Thailand and examined by two techniques: the modified Kato thick smear and the direct smear. The prevalence of *Opisthorchis viverrini* (14.8%), hookworm (10.2%), *Sarcocystis* spp (4.6%), *Taenia* spp (2.9%), *Strongyloides stercoralis* (2.1%), *Giardia lamblia* (1.2%), *Echinostoma* spp (0.6%), *Ascaris lumbricoides* (0.4%), *Entamoeba histolytica* (0.2%), *Chilomastix mesnili* (0.2%) and *Endolimax nana* (0.2%) were determined. The morphology of the *Sarcocystis* spp sporocysts examined by both procedures looked similar and was found to be easily recognizable. Among these specimens, 22 cases (4.6%) were positive for *Sarcocystis* infection detected by the modified Kato technique, whereas only one case (0.2%) was detected by both techniques. These differences were found to be statistically significant ($p<0.05$), indicating that the modified Kato technique was decidedly more sensitive than the direct smear procedure in identifying *Sarcocystis* infection. An epidemiological survey was conducted in Khon Kaen Province involving 1,124 stool samples using the modified Kato technique. The greatest frequency was *Opisthorchis viverrini* at 32.0% while the second highest was *Sarcocystis* spp at 8.0%. The prevalences of hookworm, *Echinostoma* spp, *Taenia* spp, *Trichuris trichiura* and *Enterobius vermicularis* were 2.7, 2.1, 1.0, 0.2 and 0.2%, respectively. Other than opisthorchiasis, northeastern Thailand may be an endemic area for sarcocystosis. This is the first report of the applicability and potential usefulness of the Kato thick smear technique for the diagnosis of *Sarcocystis* infection in a field survey.

INTRODUCTION

In 1954, Kato and Miura were the first to introduce a new method, the "cellophane thick-smear technique" which involved a principle of direct fecal sampling (Kato and Miura, 1954). It is different from the standard direct smear procedure in that a larger amount of fecal sample is employed and cellophane strips are used as cover slips instead of glass. After further refinement, the Kato thick smear technique, was adopted in control programs in Japan (Kato, 1960). At that time, it was considered to be the most reliable and practical method for the detection of even scanty helminthic infections (Komiya *et al*, 1960; Komiya and Kobayashi, 1966). Many publications have noted that the Kato technique is a suitable method in view of its sensitivity, simplicity and minimal cost, especially in epidemiological surveys (Cho *et al,* 1969; Xu *et al*,

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1995; Yu *et al*, 2003).

A quantitative study of helminthic infections using the Kato method was initially carried out by Martin and Beaver in 1968 for the detection of specific helminth eggs. When a number of *Schistosoma mansoni* eggs were added to a known amount of human feces, the "Kato-Katz" method, as it became known, yielded excellent results (Katz *et al,* 1970). It soon became evident that this method was highly sensitive, had minimal variation between samples, was simple to perform and eminently suited for field studies (Katz *et al,* 1972). Since then, this method has been adopted by the World Health Organization for quantitative and qualitative diagnosis of intestinal infections caused by helminthes, such as *A. lumbricoides*, *T. trichiura*, hookworm and *S. mansoni*, especially in control programs and chemotherapeutic studies (WHO, 1993, 2002). This was subsequently confirmed by many workers from various parts of the world (Odongo-Aginya *et al,* 1995; Ebrahim *et al,* 1997; Borel *et al,* 1999; Berhe *et al,* 2004). Nevertheless, its distinct disadvantage was its inability to detect protozoa cysts and larvae (Komiya and Kobayashi, 1966; Zamen and Cheong, 1967; Kagei and Kihata, 1976) so any survey data using the Kato-Katz method usually can not detect the presence of protozoan infections.

The present study was undertaken to compare the sensitivities of the modified Kato thick smear technique with the standard direct smear procedure in the detection of *Sarcocystis* sp, an intestinal protozoa. A secondary objective was to determine the relative frequencies of different parasitic infections in two provinces in northeastern Thailand using the modified Kato thick smear in order to evaluate the potential usefulness of this technique.

MATERIALS AND METHODS

Data collection was divided into two parts. The first part was conducted in rural communities of Ubon Ratchathani Province located in northeastern Thailand where a total of 479 stool specimens were collected and examined by both the direct wet smear and modified Kato thick smear procedures. The standard direct smear was prepared by emulsifying a small amount of fresh feces in normal saline and mounting under a 22x22 mm-cover glass, while the modified Kato thick smear was processed according to the method of Kato and Miura (1954). The materials used were prepared in accordance with standard laboratory in-house procedures. Thus, the glycerin-malachite green solution was mixed with 1 ml of 3% malachite green, 100 ml of 6% phenol and 100 ml of pure glycerin. The cellophane strips, each 22x40 mm, were soaked in this solution for at least 24 hours before use. Additionally, in order to eliminate fibers or seed, the technique was modified by pressing a 105-mesh stainless steel grid onto the sample which was then filtered, transferred to slides covered by the cellophane-soaked cover slips and allowed to stand for 30 minutes. All preparations were initially screened with a low-power (10x) objective lens. Suspected parasitic objects were subsequently examined under a high-power (40x) objective. The stool samples were preserved in 10% formalin for later confirmation, if needed. Every positive case of *Sarcocystis* infection identified by the modified Kato method was confirmed by formalin-ether concentration before a definitive diagnosis was established.

The second part consisted of an epidemiological survey in the field of 1,124 stool samples from 10 villages in Khon Kaen Province (approximately 400 km northeast of Bangkok) using the modified Kato thick smear technique only. The subjects were of both genders with an age range of 4-79 years. As in the first part, all positive cases of intestinal sarcocystosis were confirmed in the central laboratory with the formalin-ether technique. This was the application phase of this study based on the outcomes of the initial compara-

tive investigation.

Statistical analysis was done using SPSS version 8, statistical software (Chicago, IL, USA). Statistical analysis of the difference between the prevalences observed by the different techniques was done by means of the McNemar test for paired proportions. When fewer than 10 cases had different outcomes (positive or negative) with the 2 techniques, the binomial distribution was used to calculate the exact value of P. The standard normal deviate, z, was used to analyze the differences between the independent proportions of the prevalence of the parasitic infections in the two provinces.

RESULTS

The relative frequencies and their respective percentages of 11 different parasitic infections found in 479 stool samples from the Ubon Ratchathani Province in northeastern Thailand using the Kato thick smear and the direct smear techniques are shown in Table 1. The most prevalent infections were *O. viverrini* (14.8%), followed by hookworm (10.2%) and *Sarcocystis* spp (4.6%). The remainder of the identified parasites was <3.0%.

Table 2 presents a further analysis of the contents of Table 1 and compares the number of helminth or protozoan cases classified as positive for a specific infection by the direct smear method, the modified Kato procedure or both. The number of cases detected by the direct smear procedure or the modified Kato method did not include cases detected by both methods. With the exception of *Strongyloides* and *A. lumbricoides*, the detection rates for the helminthic infections using only the direct smear procedure were quite low ranging from 1.4% to 33.3%. The modified Kato method for the same infectious group was considerably higher with percentages ranging from 50.0% to 74.6%. The z scores comparing the proportions between the direct smear and the Kato technique for *Opisthorchis viverrini* and hookworm were 8.95 and 6.80, respectively. Both were significant (p=0.001). The helminthic detection rate for both methods varied from 23.9% to 66.7%.

For protozoan infections, of 22 *Sarcocystis* infections, 21 (95.4%) were identified by modified Kato method only, while only 1 (4.5%) case was detected by both methods, meaning the direct smear technique was able to

Table 1
Prevalence of parasitic infections in Ubon Ratchathani Province, examined by both the modified Kato thick smear and direct smear.

Parasitic infections[a]	Number of positive/total number examined	Percentage of total
Opisthorchis viverrini	71/479	14.8
Hookworm	49/479	10.2
Sarcocystis spp	22/479	4.6
Taenia spp	14/479	2.9
Strongyloides stercoralis	10/479	2.1
Giardia lamblia	6/479	1.2
Echinostoma spp	3/479	0.6
Ascaris lumbricoides	2/479	0.4
Entamoeba histolytica	1/479	0.2
Chilomastix mesnili	1/479	0.2
Endolimax nana	1/479	0.2

[a]Many cases had multiple parasitic infections

Table 2
Comparison of the numbers and percentages of cases detected by the direct smear technique, the modified Kato method and by both procedures in 479 stool specimens.

Parasites	Number of positive cases (n= 479)	Number of detected cases (detection rates,%)		
		Positive by direct smear method only (%)	Positive detection by modified Kato method only (%)	Positive detection by both methods direct and methods Kato methods (%)
Helminths, eggs/larvae				
Opisthorchis viverrini, egg	71	1 (1.4)	53 (74.6)	17 (23.9)
Hookworm, egg	49	1 (2.0)	26 (53.1)	22 (44.9)
Taenia spp, egg	14	0	8 (57.1)	6 (42.9)
Strongyloides stercoralis, rhabditiform larva	10	10 (100)	0	0
Echinostoma spp, egg	3	1 (33.3)	0	2 (66.7)
Ascaris lumbricoides, egg	2	0	1 (50)	1 (50)
Protozoa, cysts/sporocyst				
Sarcocystis spp, sporocyst	22	0	21 (95.4)	1 (4.5)[a]
Giardia intestinalis, cyst	6	6 (100)	0	0
Entamoeba histolytica, cyst	1	1 (100)	0	0
Chilomastix mesnili, cyst	1	1 (100)	0	0
Endolimax nana, cyst	1	1 (100)	0	0

[a]Sensitivity of direct smear when compared with the modified Kato method = 1/22 * 100 = 4.54%
Relative risk ratio = 22.76 (14.98-34.58) Fisher exact' test p=0.045

Table 3
Prevalence of parasitic infections in Khon Kaen Province, examined (only) with the modified Kato thick smear.

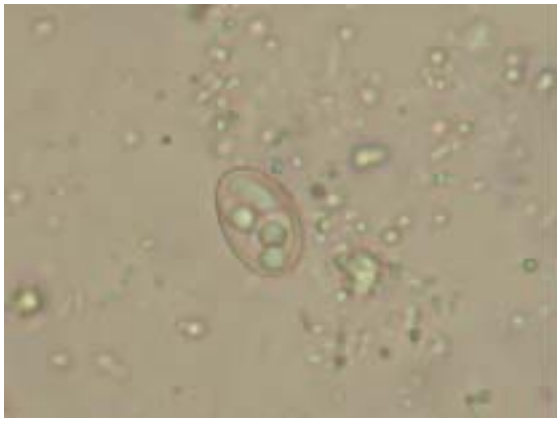
Parasitic infections[a]	Number positive	Percentage of total
Opisthorchis viverrini	360/1,124	32.0
Sarcocystis spp	90/1,124	8.0
Hookworm	30/1,124	2.7
Echinostoma spp	24/1,124	2.1
Taenia spp	11/1,124	1.0
Trichuris trichiura (Tt)	2/1,124	0.2
Enterobius vermicularis (Ev)	2/1,124	0.2

[a]Many cases had multiple parasitic infections

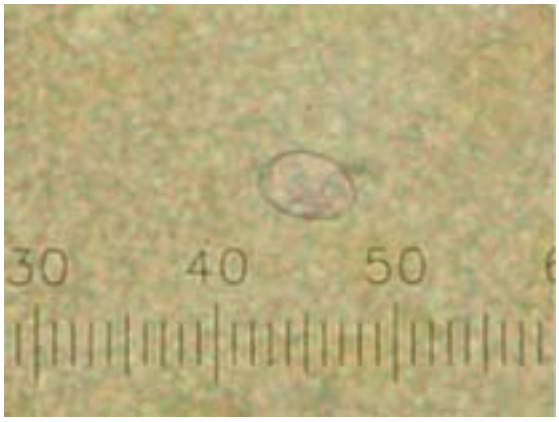
identify only 1 of 22 cases of *Sarcocystis* spp. Independent verification of the above 22 cases was confirmed at our central laboratory by formalin-ether technique. Four hundred fifty-seven were negative by both procedures. These differences are statistically significant (p = 0.045, Fisher exact test). All 4 of the remaining protozoan infections were identified by direct smear only.

Table 3 presents frequencies of parasitic infections in Khon Kaen Province, northeastern Thailand obtained by examining 1,124 stools using the modified Kato thick smear technique. The most common infection was *O. viverrini,* (32%) followed distantly by *Sarcocystis* spp at 8.0%, the latter of which was independently confirmed by formalin-ether technique. All 5 remaining infections ocurred in less than <3%.

The two photomicrographs in Fig 1 show the morphology of the sporocysts of *Sarcocystis* spp using a light microscope with a magnification of 400. The direct smear procedure was employed in panel 1A which revealed similar features to that displayed by the



1A



1B

Fig 1–Photomicrographs at 400 magnification showing the morphology of *Sarcocystis* sp sporocysts in normal saline (1A) compared with the appearance of sporocysts after processing using the Kato thick smear technique (1B).

formalin-ether technique (data not presented) while the modified Kato technique was used in panel 1B. Both procedures clearly distinguished the sporocysts from their background matrix.

DISCUSSION

While the modified Kato method has commonly been employed in the diagnosis of helminthiasis, it is rarely used to detect protozoan cysts. Several kinds of eggs (*A. lumbricoides, T. trichiura,* hookworm, *S. mansoni*) found by Kato method are similar but recognizable by the standard method. Apart from its inability to detect protozoa and larvae, it has been suggested that the Kato-Katz method has a low sensitivity for identifying hookworm eggs. In the course of processing, hookworm eggs may readily collapse, or become altered and distorted from their original characteristics, leading to easily missed diagnoses (Komiya and Kobayashi, 1966; Kagei and Kihata, 1976). Additionally, when the thick smear has been cleared by glycerin, it causes the disappearance of hookworm eggs, resulting in an underestimation of their actual number (Zamen and Cheong, 1967). This implies that the Kato method may not be suitable for the detection of helminth eggs with thin egg shells. Hence, it may be suggested that all protozoa cysts might be similarly cleared and therefore, quite difficult to detect.

In this study comparison of the sensitivities between the simple smear and the modified Kato method for *Sarcocystis* determination is shown in Table 2. These differences are statistically significant and indicate greater sensitivity for the Kato procedure (100%) over the direct smear technique (4.5%). Approximately 20 to 30 times more stool is employed in the Kato thick smear procedure than in the standard direct smear technique. This may have contributed to the higher sensitivity in detecting *Sarcocystis* infection than using the direct smear technique. Since the wall of the *Sarcocystis* sporocyst is thicker than the cyst wall of other protozoa, (Morrissette and Sibley, 2002; Yang *et al*, 2005) the clearing agent (glycerin) in the Kato method may not have been able to destroy this protozoa as well as *Isospora*. As a result, the morphology of the *Sarcocystis* spp sporocyst examined by both procedures (simple smear and Kato method) looked similar and was found to be easily recognizable (Fig 1).

In the two conducted surveys, the three most frequent parasitic infections were remarkably similar. In both provinces, *O. viverrini* was the most prevalent while the hookworm and *Sarcocystis* spp traded places as second and third most common in one survey and vice versa in the other. The high prevalence of *Opisthorchis*, *Sarcocystis* and hookworm infections in these two provinces implies that northeast Thailand may be endemic for these three parasites. Other epidemiological surveys in these areas are necessary to confirm our findings. In this study it was observed that the degree of infection for most of the cases in both provinces was graded as small which is relevant to the results in Table 3. The direct smear procedure is not sensitive enough to identify these mild infections so the detection rates are quite low. On the other hand, the modified Kato technique gives a higher yield as its sensitivity is greater. For those parasitic infections which could be identified by both methods, their degree of infection was high enough for detection by the direct smear procedure.

Bunyaratvej and Unpunyo (1992) reported that between 1981 and 1990, twenty-two cases of sarcosporidiosis (in humans) were found after surgical resection necessitated by a condition of segmental enterocolitis. The subsequent microscopic examinations suggested that the parasitic structures were at an excystation stage of *Sarcosystis hominis.* In 1982 six cases of human intestinal sarcosporidiosis were observed in patients age 3 to 70 years, after the diagnosis of either segmental eosinophilic enteritis or segmental necrotizing enteritis. Histopathological sectioning of the small intestine revealed sexual forms of sarcosporidia which were contained in raw or undercooked beef consumed by the patients. The authors suggested that the identified species was an oxman parasite similar to *Sarcocystis hominis* (Bunyaratvej *et al*, 1982). In a random sample of 362 Thai laborers, a total of 13 intestinal parasites were found in stool samples (Wilairatana *et al,* 1996). The three most common parasites were *Opisthorchis viverini* (40.3%), *Sarcocystis* spp (23.2%) and hookworm (21.5%). With the exception of lower percentages, our study replicates to a high degree these earlier findings. A major difference between the two studies was in the ages of the subjects surveyed. Our target population ranged in age from 4 to 79 years while the age of the Thai laborers ranged from 20 to 50 years. Interestingly, a subset of 278 Thai laborers from northeastern Thailand had a significantly higher incidence (26.6%) of *Sarcocystis* infection than the larger sample. All had a history of consuming raw beef and pork, coupled with poor hygienic habits. We agree with the authors' contention that "*Sarcocystosis* could be a significant food-borne zoonotic infection in Thailand" (Wilairatana *et al,* 1996). We suggest further epidemiological studies may uncover other identifiable parasitic infestations.

Within the limitations of this experiment, it may be concluded that in addition to opisthorchiasis and hookworm infection, northeastern Thailand is an endemic area for intestinal sarcocystosis. Modified Kato thick smear is more sensitive than the direct smear procedure in detecting intestinal sarcocystosis. This is the first report on the applicability and potential usefulness of the Kato thick smear technique for the diagnosis of *Sarcocystis* spp infection in a field survey.

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