A Comparison of quantitative cellophane-covered thick smear technic and formalin-ether concentration technic in stool examination for helminths.

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การศึกษาทดลองใช้ Katz's modified thick smear (KMTS) เพื่อตรวจหาและนับไข่พยาธิในอุจจาระของเด็กนักเรียนไทย 500 คน เปรียบเทียบกับ formalin-ether concentration (FEC) และ Stoll's egg count พบว่ามีปาราสิตทั้งหมด 150 ราย (ร้อยละ 30) ในจำนวนนี้เป็น หนอนพยาธิ 72 ราย (ร้อยละ 14)

จากการวิเคราะห์ทางสถิติ พบว่า KMTS ให้ผลดีกว่า FEC และ Stoll's egg count ในการวินิจฉัยการติดเชื้อหนอนพยาธิ นอกจากนั้นแล้วยังสิ้นเปลืองเวลาน้อยกว่า ประหยัดกว่าและทำง่ายกว่าอีกด้วย KMTS มีข้อเสียเปรียบตรงที่ไม่สามารถใช้ตรวจหาโปโตซัวในอุจจาระได้ และรูปร่างของไข่ของหนอนพยาธิที่มองเห็นโดยวิธีนี้ไม่ชัดเจนเท่าวิธีเก่า ในการศึกษาการใช้ยา มีเบนดาโซล ในการรักษาโรคติดเชื้อหนอนพยาธิ KMTS ให้ผลเป็นที่น่าพอใจกว่า formalin-ether concentration ที่ใช้อยู่เดิม จากการศึกษาครั้งนี้พบว่ามีเบนดาโซล ให้ผลดีในการรักษาโรคติดเชื้อหนอนพยาธิตัวกลมทั้งแบบติดเชื้อชนิดเดียวและหลายชนิด ส่วนผลของการรักษาโรคติดเชื้อหนอนพยาธิตัวตืดนั้นยังไม่สามารถสรุปได้ เพราะมีจำนวนผู้ป่วยน้อยเกินไป

The most popular laboratory technics employed in the diagnosis of intestinal parasitic infections nowadays are the simple smear and the formalin-ether concentration[11]. The latter has been accepted as one of the most satisfactory technics in Thailand for more than twenty years.[1,3,5,6,7,8]

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The quantitative cellophane-covered thick smear technic (Katz's modified thick smear technic) or KMTS[2], was introduced into routine stool examination for parasites in Japan seven years ago. The technic is simple, convenient, cheap, and quantitatively applicable. However, there is still no report on its use outside Japan.

This study aims to compare the merits/disadvantages of this relatively new technic with the older formalin-ether concentration by means of employing them in the parasitological stool survey and the evaluation of the therapeutic efficacy of mebendazole (methyl-5-benzoylbenzimidazole-2-carbamate, Fugacar, Janssen), a broadspectrum anthelmintic, in Thai school children in Bangkok.

The results is expected to justify more use of the drug[9-13] in prevention and control of intestinal helminthic infections in Thailand.

MATERIALS AND METHODS

1. Stool specimens were collected from 500 elementary school children in Bangkok. The schools and classes were randomly selected; all students in the selected classes were examined.
2. A portion of each specimen was examined by Katz's modified thick smear (KMTS) technic and another portion of the same specimen was examined by the formalin-ether concentration (FEC) technic.
3. The parasite eggs in the specimen as known positive by FEC and KMTS were counted by Stoll's dilution egg count technic.
4. The results of the egg count by the two technics were statistically compared.
5. The students found to have parasite eggs in their stools were treated with mebendazole (Fugacar, Janssen) according to the following regimens:

   Ascariasis, Trichuriasis and hookworm infections

   1 tablet (100 mg) 2 doses daily, 3 consecutive days

   Taeniasis and Hymenolepiasis

   1 tablet (100 mg) 2 doses daily, 5 consecutive days

   Enterobiasis

   1 tablet (100 mg) single dose
6. The post-treatment follow-up was done by repeating the stool examination at the end of 2nd and 4th weeks after the administration of the last doses. Both KMTS and FEC technics were used on the same specimens but the egg count by stoll's technic was omitted.

RESULTS

Out of 500 school children in Bangkok, 150 cases (30 percent) were found to be harboring one or more species of parasites by KMTS and FEC. Table 1 shows the details of the result.

1. The two most common helminths are *Ascaris lumbricoides* (31 cases; 6 percent) and hookworms (27 cases; 5 percent). Two species of pathogenic protozoa were found namely *Giardia lamblia* (59 cases; 12 percent), and *Entamoeba histolytica* (1 case; 0.2 percent).

2. There is no significant difference of prevalences either among age-groups or sexes of examinees ($p > 0.10$).

The results of egg count by the two technics are shown in table 2.

From table 2 it can be concluded that:

1. Out of 72 cases, 25 cases were positive for helminth eggs with only KMTS but not with FEC.
2. Out of 72 cases which were positive with both KMTS and FEC, 20 cases were negative with Stool's egg count technic.
3. In 6 cases which were negative with KMTS the Stoll's egg count were also negative.
4. The efficacy of the technics can be compared as follows:
   4.1 The ratio of egg recovery rates between KMTS : FEC = 1.4 : 1
   4.2 The ratio of egg being counted by the two methods is KMTS : Stoli's = 1.43 : 1
5. The relationship between the numbers of eggs obtained by the KMTS and Stoll's technic can be statistically concluded that the numbers of eggs obtained by KMTS is 1.74 times of those by Stoll's technic.
6. From 4.2 and 5, the relative efficiency of FEC + Stoll's technics is computed to be approximately 57.50 - 69.70 % of KMTS.
7. Number of *Asacris* egg per gram of stool recovered by KMTS is more than by Stoll's technic in 23 cases out of 25 cases (92 percent); in the case of hookworms egg, *Trichuris* egg, and *Enterobius* egg, the superiority of the KMTS over Stoll's technic are 100 percent (24 out of 24), 87 percent (7 out of 8) and 100 percent (6 out of 6) respectively.

   Taenia egg and *Hymenolepis* egg were found in only two cases each; therefore the number cannot be statistically interpreted.

After treatment with mebendazole, the follow-up stool examinations were done at the end of 1st, 2nd, 3rd, and 4th week. The efficacy of mebendazole in elimination of eggs from stool is shown in Table 3. The cure rates for ascariasis and hookworms infection are 96 percent and 86 percent respectively. Due to small number of cases of other helminthic infections under trial, the results are not statistically interpretable.

The study of egg reduction after treatment in some selected cases of single helminthic infection shows mark reduction in all cases (Table 4).

DISCUSSION

KMTS proves to be more effective in the demonstration of helminth eggs in stool than FEC (1.40 times) and in egg counting than Stoll's technic (1.43 times). However KMTS cannot be used for the diagnosis of protozoa infections while FEC can recover both eggs and cysts and to tell the species of helminthic eggs with KMTS the microscopist must be fairly well-trained (Fig. 1).

KMTS is also more time-saving, economical, and simpler than the combination of FEC and Stoll's technic; therefore it was very satisfactory when used in the evaluation of therapeutic efficacy of a new anthelmintic.

The results show that mebendazole is very effective against ascariasis and enterobiasis (cure rate of 96 percent and 100 percent respectively). Hookworms and *Trichuris trichiura* are somewhat more resistant (cure rate of 86 percent and 89 percent respectively). The numbers of trial cases for *Taenia* spp. and *Hymenolepis nana* are too small to be conclusive.

Table 1

Prevalence of parasitic infections in Thai school children, Bangkok, Thailand

July 1978 - January 1979

Parasites \ Age (years)	5 - 9		10 - 14		Total
	male	female	male	female	
Total examination	130	120	117	133	500
Total positive	31 (23.84 %)	44 (36.66 %)	36 (30.76 %)	39 (26.00 %)	150 (30.00 %)
Single Protozoa	14 (10.76 %)	20 (16.66 %)	18 (15.37 %)	13 (9.77 %)	65 (31.00 %)
Multiple Protozoa	5 (3.84 %)	4 (3.33 %)	1 (0.85 %)	3 (2.25 %)	13 (2.60 %)
Single Helminth	13 (10.00 %)	18 (15.00 %)	12 (10.25 %)	17 (12.78 %)	60 (12.00 %)
Multiple Helminth	1 (0.76 %)	1 (0.83 %)	2 (1.71 %)	1 (0.75 %)	5 (1.00 %)
Mixed infection	1 (0.76 %)	4 (3.33 %)	-	2 (1.51 %)	7 (1.40 %)
Entamoeba histolytica	-	1 (0.83 %)	-	-	1 (0.20 %)
Entamoeba coli	7 (5.38 %)	6 (5.00 %)	2 (1.71 %)	4 (3.01 %)	19 (3.80 %)
Iodamoeba butschlii	1 (0.76 %)	2 (1.66 %)	-	1 (0.75 %)	4 (0.80 %)
Endolimax nana	6 (4.61 %)	2 (1.66 %)	3 (2.58 %)	5 (3.76 %)	16 (3.20 %)
Giardia lamblia	9 (6.92 %)	20 (16.66 %)	17 (14.52 %)	13 (9.77 %)	59 (11.80 %)
Trichomonas hominis	-	-	-	1 (0.75 %)	1 (0.20 %)
Ascaris lumbricoides	8 (6.15 %)	7 (5.83 %)	3 (2.58 %)	13 (9.77 %)	31 (6.20 %)
Trichuris trichiura	1 (0.76 %)	3 (2.50 %)	3 (2.58 %)	2 (1.51 %)	9 (1.80 %)
Enterobius vermicularis	1 (0.76 %)	-	-	1 (0.75 %)	2 (0.40 %)
Hook-worms	5 (3.84 %)	9 (7.50 %)	9 (7.69 %)	4 (3.01 %)	27 (5.40 %)
Strongyloides stercoralis	-	1 (0.83 %)	1 (0.85 %)	-	2 (0.40 %)
Opisthorchis viverrini	-	2 (1.66 %)	1 (0.85 %)	4 (3.01 %)	7 (1.40 %)
Taenia spp.	-	1 (0.83 %)	-	-	1 (0.20 %)
Hymenolepis nana	-	-	1 (0.85 %)	1 (0.75 %)	2 (0.40 %)

Table 2

Results of egg count per one gram of stool specimens from the application of Katz's modified thick smear and formalin-ether concentration technics; Bangkok primary school children, July 1978–February 1979.

	Case No.	Katz's modified thick smear EPG	Stoll's dilution egg count EPG
Ascaris lumbricoides	1	179,080	16,750
	*2	39,683	26,800
	3	331,187	263,000
	4	19,518	13,200
	*5	28,509	22,200
	*6	38,832	33,800
	7	14,245	10,400
	8	64,454	33,000
	9	0#	0#
	*10	66,896	35,200
	11	0#	0#
	12	20,313	17,200
	13	3,571	1,200
	*14	31,225	12,600
	15	18,124	11,100
	16	19,378	17,000
	*17	35,236	7,900
	*18	19,320	7,200
	*19	1,104	1,400
	20	95,546	20,000
	21	38,157	30,400
	22	4,784	160
	23	60,053	38,400
	24	39,514	29,600
	25	345	0
	26	4,899	5,200
	27	61,847	56,400

Table 2 (continued)

	Case No.	Katz's EPG	Stoll's EPG
Trichuris trichiura	1	276**	0
	2	259**	0
	*3	148**	0
	4	0#	0#
	*5	297	200
	6	56**	0
	7	241**	0
	*8	184	200
	9	161**	0
Enterobius vermicularis	*1	74**	0
	2	0#	0#
	*3	19**	0
	*4	19**	0
	*5	777**	0
	6	115**	0
Hookworm	1	888	600
	*2	907	800
	3	759	600
	4	0#	0#
	5	0#	0#
	6	3,793	3,600
	7	6,845	5,200
	*8	981	800
	*9	2,017	1,500
	10	1,866	200
	*11	315**	0
	12	1,499	600
	13	2,886	2,700
	14	204	200
	15	315**	0
	16	370**	0

Table 2 (continued)

	Case No.	Katz's EPG	Stoll's EPG
	*17	290	200
	*18	46**	0
	19	1,127	400
	*20	460	100
	*21	3,105	1,400
	22	184**	0
	23	1,035	220
	24	414	400
	25	1,725	1,200
	26	368**	0
Taenia spp	1	851	800
	*2	19**	0
Hymenolepis nana	1	76**	0
	*2	11,040	23,400

* 25 cases positive with Katz's technic only

** 20 cases positive with Katz's technic and F-E concentration but Stoll's egg count=0

6 cases positive only with F-E concentration and Stoll's egg count=0

Table 3

Efficacy of mebendazole (Fugacar, Janssen) against Ascariasis, Trichuriasis, Enterobiasis, hookworm infections, Taeniasis and Hymenolepiasis in Thai school children, Bangkok, Thailand.
(July 1978-February 1979)

	No. cases Treated	Cured	% Cured
Ascaris lumbricoides	27	26	96
Trichuris trichiura	9	8	89
Enterobius vermicularis	6	6	100
Hookworm	26	24	86
Taenia spp.	2	2	100
Hymenolepis nana	2	2	100
Total	72	68	94.4

Table 4

Results of egg counts after treatment with mebendazole (Fugacar, Janssen) in 4 Thai school children, Bangkok, Thailand
(July 1978-February 1979)

	Case No.	EPG Pre-treatment	EPG Post-treatment	Percent of reduction
Ascaris lumbricoides	3	331,187	1,850	99.4
Trichuris trichiura	2	259	18	93.0
Hookworm	6	3,793	370	90.2
	7	6,845	462	93.3

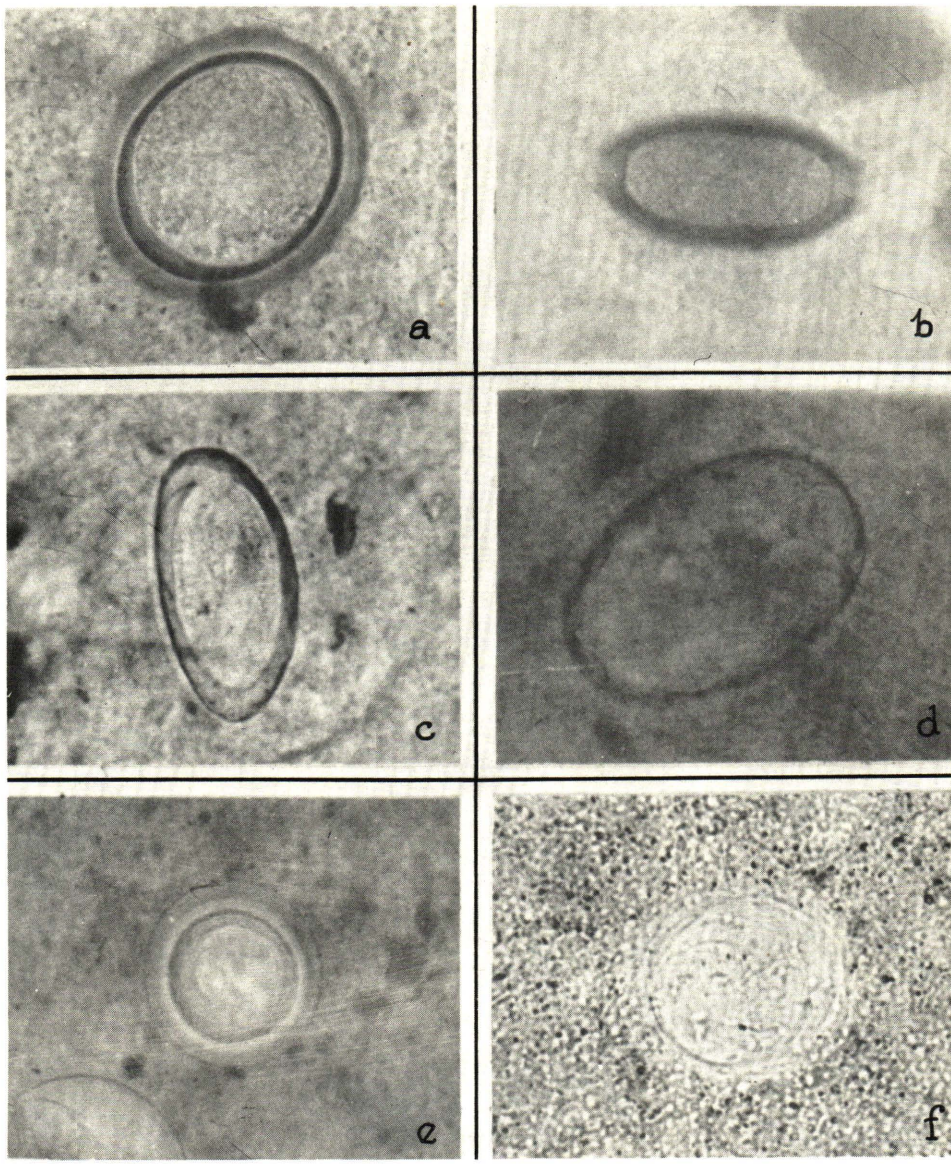

Fig. 1 Helminthic eggs as appeared in Katz's modified thick smear technic. (X450)

a. *Ascaris lumbricoides*
b. *Trichuris trichiura*
c. *Enterobius vermicularis*
d. Hookworm
e. *Taenia* spp.
f. *Hymenolepis nana*

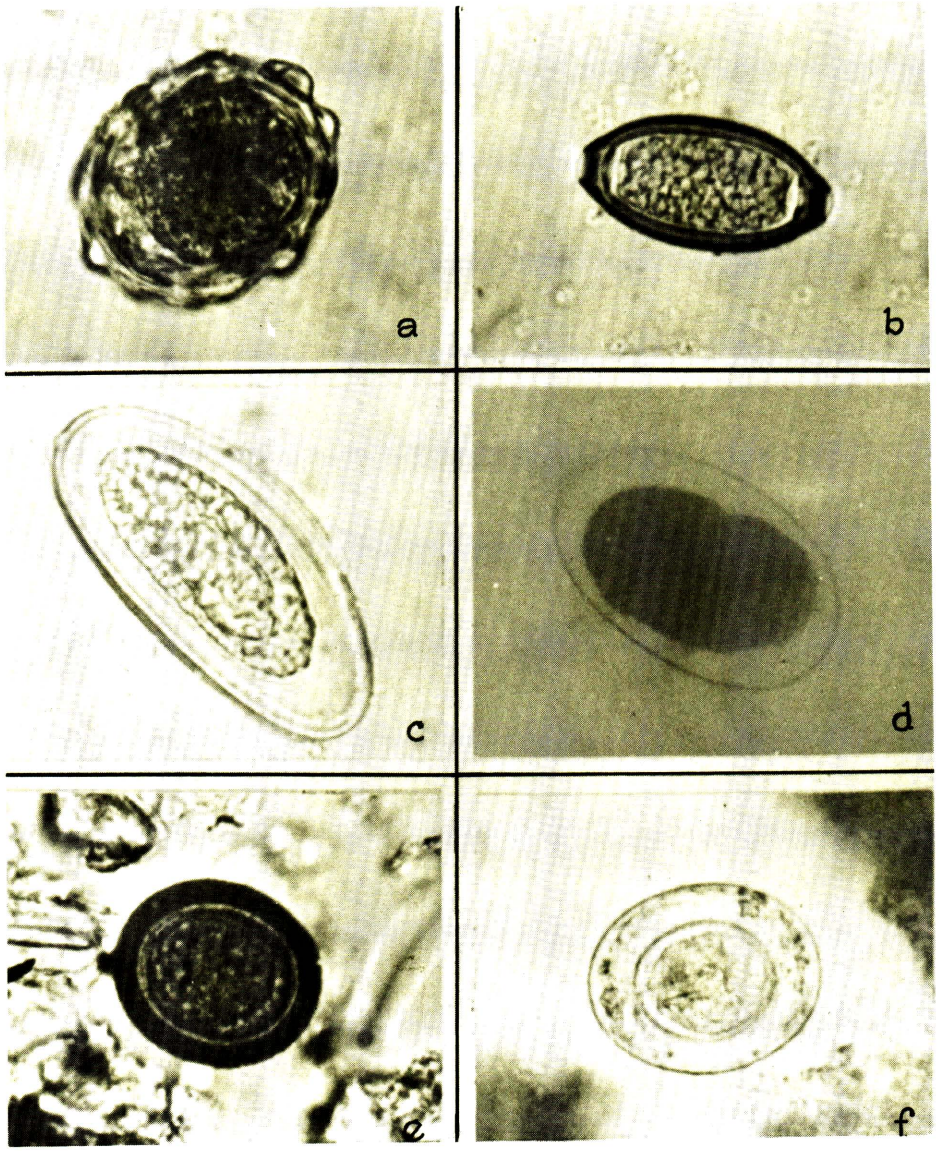

Fig. 2 Helminthic eggs from Formalin-ether concentration technic. (X450)

a. *Ascaris lumbricoides*
b. *Trichuris trichiura*
c. *Enterobius vermicularis*
d. Hookworm
e. *Taenia* spp
f. *Hymenolepis nana*

ACKNOWLEDGEMENT

The authors wish to express their appreciation for the kind assistance of Dr. Anek Hirunraks of the Department of Biostatistics, in the statistical analysis of the data.

They are also most grateful to Col. Pisit Chularerk, Chief, Devision of Pathology, Army Institute of Pathology, for his advice and comments throughout the study.

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