

COMPARATIVE STUDY OF KATO'S THICK-SMEAR TECHNIQUE WITH CONCENTRATION FORMALIN-ETHER AND FLOTATION METHODS FOR QUANTITATIVE AND QUALITATIVE DIAGNOSIS OF INTESTINAL HELMINTH INFECTIONS

J. Massoud
F. Arfaa
H. Jalali
M. Reza

ABSTRACT

This investigation was performed on 1056 stool samples collected from Isfahan area (with high intensity of infection) and Dasht-Mishan area of Khuzestan. As indicated in Tables 1, 2 and 3 the results of Kato thick-smear technique is comparable to those conventional methods of Formalin-ether and Flotation methods, plus low cost, simplicity and practicability of Kato's thick-smear. For *Ascaris* eggs the Kato method is ideal for epidemiological studies: For Hookworm and *Trichostrongylus* infections, Kato method was less sensitive but still it was at an acceptable level for epidemiological surveys. Also, Kato technique was more sensitive in areas where the intensity of infection is high. The conclusion is that the Kato Thick-Smear technique is cheap, fast, convenient and ideal for mass-diagnosis in epidemiological surveys of intestinal helminthiasis.

INTRODUCTION

A cellophane thick-smear technique was introduced by Kato and Miura (1954)¹ and later on improved and evaluated by Kato (1960)², Komiya and Kobayashi (1966)³, Martin and Beaver (1968)⁴, Chaia et al (1968)⁵ and Kato et al (1970)⁶. This method has now been widely

This investigation has been supported by the School of Public Health and Institute of Public Health Research, Teheran University.

used in epidemiological surveys for helminthiasis.

The thick-smear technique is simple and highly effective for detecting eggs of helminths particularly *Ascaris* and *Schistosomiasis* in the faeces.

Large scale control of intestinal helminthiasis recently started by the Ministry of Health and Welfare in Iran needs a simple, quick and reliable method of stool examination for mass diagnosis of these infections before, and the evaluation of the effect of control measures after mass-chemotherapy.

The present study has been conducted to compare the reliability of conventional and rather complicated methods of stool examination with the newly introduced Kato thick-smear method. Both qualitative and quantitative methods were compared during this study.

MATERIALS AND METHOD

Five hundred and fifty six stool specimens from the inhabitants of three villages around the central part of Isfahan and 500 specimens from five villages in the Dasht-Mishan area of Khuzestan, South-Western Iran were collected. Each sample was examined with three methods, Formalin-Ether concentration, Flotation and Kato thick-smear. In Kato thick-smear technique about 50 mg of fresh stool was put on a slide glass covering with an impregnated malachite green cellophane sleeper, and slightly pressed with a rubber stopper to produce a uniform spread of specimen on the glass slide. Examination of each slide was within 30-40 minutes after preparation to ensure enough transparency of the slides.

For quantitative egg count study a total of 100 stool specimens from Khuzestan were tested by Kato-Katz technique and compared with Stoll's standard technique for the same specimens. From each sample one slide was prepared by a faecal measuring device which was constructed out of rectangular cardboard (3cm x 1cm x 1.37mm) by making a hole of 6mm in diameter in its centre. Faeces previously strained through 105 mesh stainless steel cloth (I.W.S. Tyler Co., Cleveland, Ohio), are placed in the hole by the toothpick transfer. The figure was multiplied by 1000 and divided by the faecal weight (50 mg) to obtain the number of eggs per gramme of faeces. Mostly this study was based on the *Ascaris* egg counts in both Isfahan and Khuzestan areas where this infection is prevalent.

RESULTS

As can be seen in Tables 1 and 2, the Kato thick-smear technique in an area like Isfahan with a high prevalence and intensity of Ascariasis infection is well matched compared with Formalin-Ether and flotation methods. In the Isfahan area, 63% of 556 samples examined were positive for *Ascaris* infection while Flotation and Formalin-Ether methods showed 59% and 49% respectively. But in the Dasht-Mishan area, with a rather lower prevalence and intensity of *Ascaris*, Kato thick-smear still matched well with the two other methods, with 45% for Kato technique, 29% for flotation and 42% for Formalin-Ether. In the case of *Trichostrongylus* infection, Kato technique showed a lower prevalence than flotation and concentration methods. The result, as shown in Tables 1 and 2, in the Isfahan area was 48% for concentration, 31% for flotation and 27% for Kato technique and in Dasht-Mishan area was 28% for concentration, 21% for flotation and 17% for Kato technique. In the Isfahan area the rate of *Trichostrongylus* and hookworm infections were negligible, but in Dasht-Mishan area, where the percentage of hookworm infection was high, Kato technique showed a lower percentage than the two other techniques (53% for Kato, 70% for flotation and 72% for concentration techniques). In the case of *Trichostrongylus* infection the Kato technique was much poorer and the prevalence rate of infection obtained by the three methods are significantly different, as shown in Table 2 (Kato technique with 10%, flotation with 39% and concentration with 36%). Also, in the case of *Hymenolepis* infection, the Kato technique is poor with 6% compared with 16% and 13% respectively for flotation and concentration techniques. The intensity of infection is given in Tables 1 and 2. *Ascaris* in Isfahan showed a higher egg count with 31500 egg/gram of faeces, than the Dasht-Mishan area, with a mean egg count of 10700 egg/gram of faeces (Table 1 and 2).

The approximate weight of the faeces used in the Katz et al (1973)⁷ cardboard technique for egg count was 50 mg. The total number of *Ascaris* eggs per gram of faeces found in 100 patients was 3975 by the Stoll technique and 2258 by the cardboard technique and for hookworm was 792 for Stoll and only 96 for the cardboard technique. Comparing the results of these two methods for quantitative estimation of helminth eggs, the conventional Stoll technique showed a marked superiority (Table 3).

DISCUSSION

The amount of faeces examined in Kato thick-smear is 50 to 100 mg, but in flotation and Formalin-Ether methods is about one gram. The convenience, speed, simplicity and practicability of the Kato technique has been found to be superior to other techniques used. For *Ascaris* eggs the Kato method is ideal for epidemiological studies as shown in Tables 1 and 2. The differences in the percent positive in Kato technique was in the same range as in the two other methods.

In hookworm, *Trichostrongylus* and *Trichuris* infections, Kato technique was less sensitive but still it was at an acceptable level for epidemiological surveys. The result of this study shows Kato technique is sensitive in areas where the intensity of infection is high, particularly in the case of helminths where the egg laying capacity is also considerable.

The conclusion is that the Kato thick-smear technique is cheap, fast, convenient and ideal for mass-diagnosis in epidemiological surveys. In Iran where the mass-chemotherapy campaign against intestinal helminthic parasites has been started, Kato thick-smear will be the most practical method for mass-diagnosis and evaluation of the campaign when used by experienced field technicians.

Table 1
Comparison of the sensitivity of three methods for the recovery of intestinal helminth eggs from cases in Isfahan area, 1976

Technique used	No. exam.	% Positive with:				
		<i>Ascaris</i>	Hook-worm	<i>Tricho-strongylus</i>	<i>Trich-uris</i>	<i>Hymeno-lepis</i>
Formalin-ether	556	49	0	0	48	7
Flotation	556	59	0	7	31	9
Kato thick-smear	556	63	0	0	27	4

Table 2
Comparison of the sensitivity of three methods for the
recovery of intestinal helminth eggs from cases in Dasht-Mishan area
of Khuzestan, 1976

Technique used	No. exam.	% positive				
		A.	H.	Tri.	T.T.	H.n.
Formalin-ether	500	42	72	36	28	13
Flotation	500	29	70	39	31	16
Kato thick-smear	500	45	53	10	17	6

Table 3
Comparison of Stoll and Kato thick-smear egg count technique

Tech- nique used	No. examined	Mean Ascaris eggs/grm	Mean Hookworm eggs/grm	Mean Trichostron- gylus eggs/grm	Mean Trichuris eggs/grm
Stoll	100	3975	792	261	444
Kato thick- smear	100	2258	96	47	48

REFERENCES

1. Kato, K. and Miura, M. (1954). Comparative examinations. Jap. J. Parasit. 3, 35.
2. Kato, K. (1960). Introduction of the thick-smear technique with a cellophane cover for helminth eggs in faeces. A pamphlet, 5pp.
3. Komiya, Y. and Kobayashi, A. (1966). Evaluation of Kato's thick-smear technique with a cellophane cover for helminth eggs in faeces. Jap. J. Parasit. 19, 59-64.
4. Martin, L.K. and Beaver, P.C. (1968). Evaluation of Kato thick-smear technique for quantitative diagnosis of helminth infections. Am. J. Trop. Med. Hyg. 17, 382-391.
5. Chaia, G., Any, B.G., McAullife, J., Katz, N. and Gasper, D. (1968). Coprological diagnosis of Schistosomiasis. II. Comparative study of quantitative methods. Rev. Inst. Med. Trop. S. Paulo, 10, 349-353.
6. Kato, N., Coelho, P.M.Z., and Pellegrin, J. (1970). Evaluation of Kato's Quantitative method through recovery of *Schistosoma mansoni* eggs added to human faeces. J. Parasit. 56, 1032-1033.
7. Katz, N., Chavea, A. and Pellegrin, J. (1973). A simple device for quantitative determination of *Schistosoma mansoni* eggs in faeces examined by the thick-smear technique. WHO/Schisto/73.26.