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AN IMPROVED STOOL THICK-SMEAR TECHNIQUE FOR QUANTITATIVE DIAGNOSIS OF *SCHISTOSOMA MANSONI* INFECTION

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SUMMARY

The Kato's method for direct fecal smear examination was modified in order to have helminth eggs preserved and distinctly contrasted to the background. The glycerin-malachite-green solution was substituted for a preservative-clearing solution constituted by polyethylene glycol, formaline and saturated saline in the proportion of 1:1:2. Preparations with the technique described can be observed immediately. Schistosome eggs can be kept unaltered for at least 4 months at room temperature. Contrarily to what happens with the original Kato's method, hookworm eggs do not collapse.

Series of egg-counts made from the same stool specimens of 19 *Cebus* monkeys experimentally infected with *S. mansoni* were very uniform, as determined by the coefficient of variation.

The technique described is quite simple and reliable for a quantitative diagnosis of *S. mansoni* and/or other helminth infections.

INTRODUCTION

In 1954 KATO & MIURA [3] introduced a thick-smear technique for stool examination that proved very satisfactory in Japan (KATO [2]). An evaluation of the Kato's method was published by KOMIYA & KOBAYASHI [5] in 1966. More recently, CHAIA et al. [1], MARTIN & BEAVER [6], and KATZ et al. [4] determined the relative sensitivity of the method and studied the reproducibility of egg-counts made by a modified Kato's technique as compared with counts performed by other techniques.

Considering that over-clearing may cause collapse and disappearence of hookworm eggs and sometimes schistosome eggs tend to become indistinct (MARTIN & BEAVER [6]) it was found worth-while to substitute the clearing glycerin-malachite-green solution by a clearing and preservative one. This improvement in the Kato's method is here described along with the variability of repeated egg-counts and the results obtained in a long-term quantitative follow-up of a fecal examination from *Cebus* monkeys experimentally infected with *Schistosoma mansoni.*

MATERIALS AND METHODS

The materials required are: a) ordinary glass microscope slide; b) polyethylene or wettable cellophane coverslip: sheet of medium thickness, cut into rectangles of appro-

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ximately 20 x 30 mm; c) preservative-clearing (PC) solution (polyethylene glycol n.º 300, formaline, saturated sodium chloride solution in the proportion of 1:1:2); d) a small spatula or metal rods (2 mm diameter); e) 105-mesh, type 304 stainless-steel bolting cloth (W. S. Tyler Co., Cleveland, Ohio) cut into pieces of 2 x 4 cm; f) projection microscope (Visopan model, Reichert, Vienna).

The PC solution is prepared by mixing solution A (Formaline and saturated saline, 1:1) with solution B (Polyethylene glycol and saturated saline, 1:1) in equal parts.

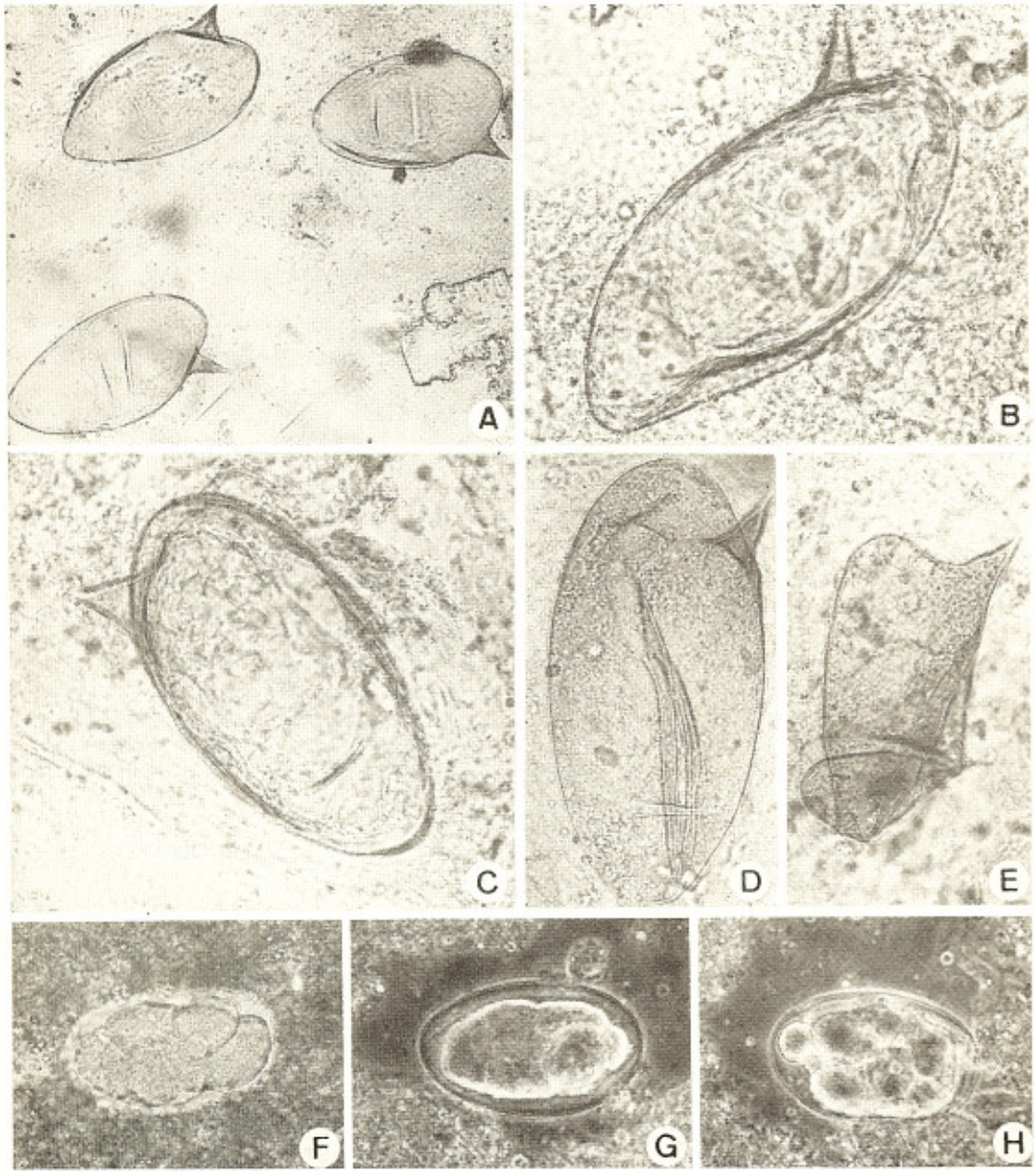

Fig. 1 — *A* to *E*: eggs of *S. mansoni* from a slide prepared one month before using PC solution and polyethylene coverslip. Note in *B* and *C* the miracidium well preserved. *E* to *H*: hookworm eggs well preserved after one month.

When cellophane was used, the rectangles were kept soaked in the preservative-clearing solution. The polyethylene coverslip were used dried without soaking in the solution.

For fecal examination 1 or 2 g of a fresh specimen was put on a disposable paper sheet, covered by a piece of the wire cloth, and a sample collected by scraping a spatula or a metal rod across the surface of the cloth.

For quantitative egg count a microscope slide was put over the pan of an analytical balance (Mettler, H-15 model) and a sample of screened feces weighed accordingly. The preparation was made by mixing the fecal sample (20 to 30 mg) with 0.02 to 0.03 ml of the PC solution, covering with a polyethylene coverslip, inverting the slide and pressing it down against a flat soft surface until the fecal mass covered an area of approximately 20 to 25 mm in diameter. When the cellophane coverslip was used the inverted slide was pressed against a sheet of an absorbent paper. Best preparations are obtained with polyethylene coverslips.

The entire film was examined under low power magnification (about 80 X) of a projection microscope and all schistosome eggs counted.

The coefficient of variation (CV) was used to evaluate the reproducibility of egg counts. For this purpose countings in 3 to 5 slides of the same fecal specimen from 19 *Cebus* experimentally infected with *S. mansoni* were performed.

The number of *S. mansoni* eggs passed by two adult *Cebus apella macrocephalus* exposed to 200 cercariae *(S. mansoni)* by the percutaneous route were determined at different periods of infection (42 up to 174 days).

RESULTS

It has been found that preparations made with PC solution can be observed immediately without waiting 1 to 1 ½ hours as in the Kato's method for the optimum clearing time. Schistosome eggs appear with the shell and internal structure well preserved (Fig. 1, *B* and *C*). Examination of human stool specimens showed that hookworm eggs do not collapse (Fig. 1, *F, G, H*) as in the Kato's method (MARTIN & BEAVER [6]) and eggs of other helminths are distinctly contrasted to the background (125 X magnification).

Preparations could be kept unaltered as long as 4 months (total period of observation) by placing the slides face-down at room temperature.

Table I shows the variability, as measured by the coefficient of variation, of repeated egg-counts performed in 19 *Cebus* monkeys.

Figures 2 and 3 show the results of egg-countings in 2 *Cebus* monkeys experimentally infected with *S. mansoni,* at different periods of infection.

TABLE I

Mean egg-counts per gram of feces and coefficient of variation (CV) in 19 *Cebus* monkeys experimentally infected with *S. mansoni*

Monkey	Number of slides examined	Mean egg-count per gram of feces	Coefficient of variation (C.V.)
1	5	188	46.0
2	5	372	15.0
3	5	764	2.8
4	5	763	2.8
5	5	399	17.6
6	5	322	12.6
7	4	975	5.1
8	5	220	7.3
9	4	256	19.9
10	5	242	36.5
11	5	282	17.8
12	3	187	22.3
13	5	262	9.8
14	5	216	27.7
15	5	380	2.8
16	4	145	14.3
17	5	380	2.8
18	5	224	14.6
19	5	436	16.3

DISCUSSION

The chief advantages of using the PC solution are: a) eggs can be preserved in a cleared preparation; b) there is no need of awaiting for the best period for clearing

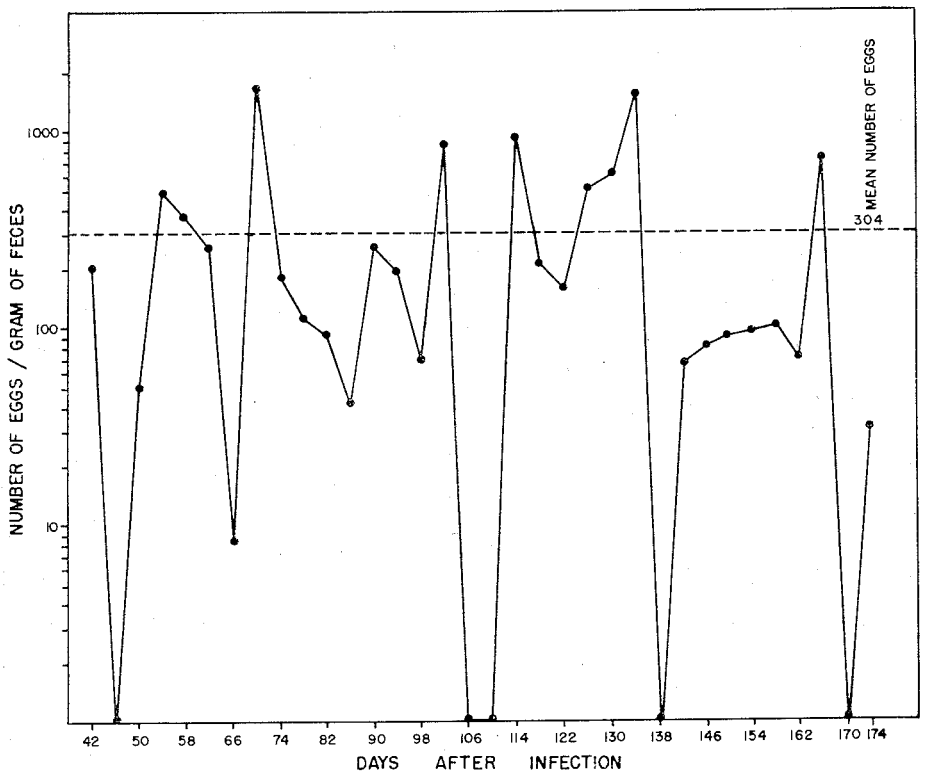

Fig. 2 — Egg-counts performed in the feces of monkey no. 5, with a low mean number of *S. mansoni* eggs (304) per gram of feces.

and c) slides can be kept unchanged for a long period. The use of the Visopan microscope greatly facilitates the scanning of the slides.

Best results were obtained using a small spatula for scraping and polyethylene coverslips. When cellophane soaked in PC solution is used, crystals of sodium chloride begin to appear after one day, rendering the examination of the slide more difficult, the eggs appearing as white spots over a dark background.

The values provided by the coefficient of variation (Table I) indicate that egg-counts for *S. mansoni* eggs are very uniform in the same fecal sample. On the other hand, variation of the number of eggs at different periods of infection is quite high (Fig. 2 and 3). It is interesting to remark that sometimes no eggs were found (5 and 3 times for monkeys 5 and 7, respectively), in spite of repeating a second slide.

In 15 patients with *S. mansoni* infection, as demonstrated by the conventional Kato's technique, schistosome eggs were found in all cases using the technique here described.

The results of the present study show that the thick-smear technique is easy to perform and provides a reliable method for the quantitative diagnosis of schistosome or other helminth infections.

RESUMO

Modificação do método de Kato de esfregaço coprológico espêsso, para o diagnóstico quantitativo da infestação pelo Schistosoma mansoni.

O método de Kato para o exame direto de fezes foi modificado com o intuito de se obter ovos de helmintos preservados e fàcilmente visíveis em preparações clarificadas. A solução de glicerina-verde de malaquita foi substituída por uma solução con-

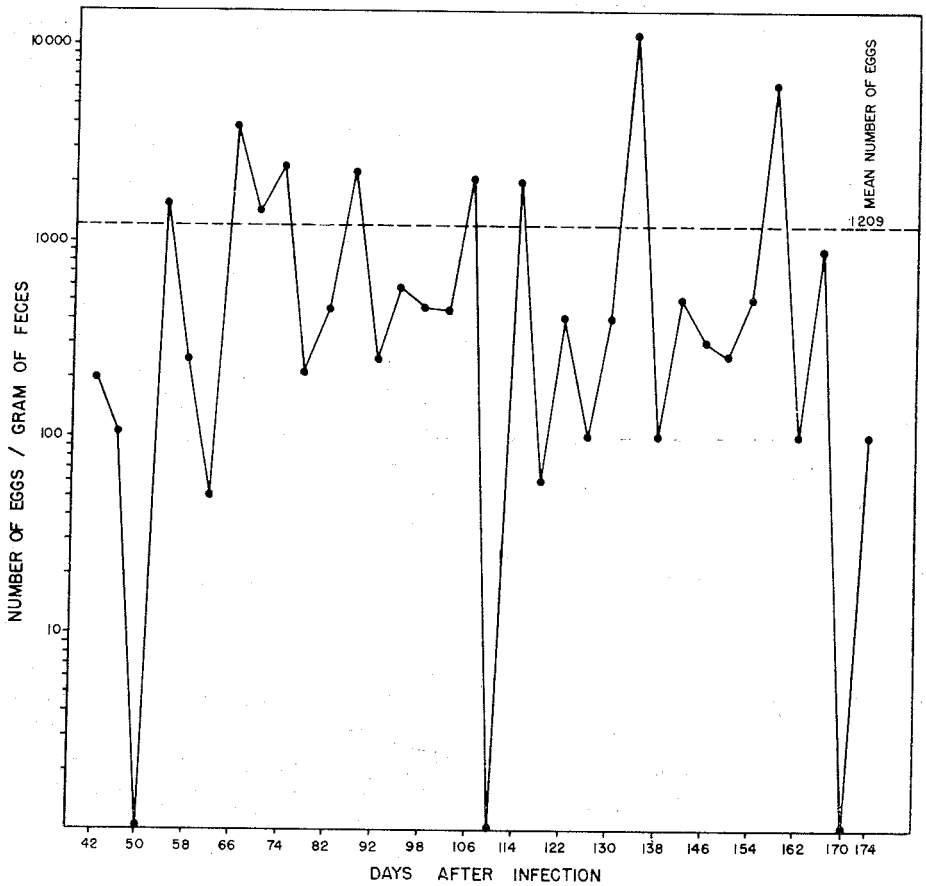

Fig. 3 — Egg-counts performed in the feces of monkey no. 7, with a high mean number of *S. mansoni* eggs (1209) per gram of feces.

tendo polietileno glicol, formol e salina saturada na proporção de 1:1:2. Com a técnica descrita, as lâminas podem ser observadas imediatamente. Ovos de *S. mansoni* ficam inalterados pelo menos por 4 meses (período total de observação), à temperatura ambiente. Ao contrário do que acontece com o método de Kato convencional, os ovos de ancilostomídeos são conservados.

Contagens seriadas feitas nas mesmas amostras de fezes de 19 macacos *Cebus* infetados com *S. mansoni* foram bem uniformes quando avaliadas pelos coeficientes de variação.

A técnica descrita é muito simples e pode ser usada para determinações quantitativas de ovos em infecções por *S. mansoni* e outros helmintos.

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