

## COPROLOGICAL DIAGNOSIS OF SCHISTOSOMIASIS

### II — Comparative Study of Quantitative Methods

Geraldo CHAIA, Any B. Q. CHAIA, Joan MCAULLIFE, Naftale KATZ and Dorothy GASPER

#### SUMMARY

There has been performed a comparative study of Kato's, Bell's and Barbosa's methods using feces of naturally infected *S. mansoni* patients. The mean number of *S. mansoni* eggs (per g of stool) obtained by Kato's technique (545) was higher than that obtained by Barbosa's (196) and Bell's (79) methods. No great variation in the mean number of eggs was observed in the different countings performed, every 4 days, on the feces of those patients.

As Kato's technique (a method of direct stool examination) resulted in the observation of more eggs, the Authors inferred that Barbosa's and Bell's methods dispersed rather than concentrated the eggs. The slight variation in the number of eggs from the various portions of a same stool sample also led them to conclude that eggs are naturally homogenized in the feces.

Kato's technique has been found to be the method of choice, not only by providing better results but also by being easily carried out and readily prepared to be used.

#### INTRODUCTION

Innumerable are the qualitative methods proposed for the recovery of *S. mansoni* eggs in the feces. Those methods are generally based on flotation, centrifugation or spontaneous sedimentation methods.

Lately, however, a great interest in the employment of quantitative methods has been risen and investigators sponsored by the World Health Organization (WHO)<sup>8</sup> have strongly been suggesting the use of such methods even for epidemiological surveys. The evaluation of Barbosa's<sup>1</sup> and Bell's<sup>2</sup> quantitative techniques has recently been performed by KATZ & CHAIA<sup>5</sup>.

This paper presents a comparative study of Kato's<sup>4</sup>, Barbosa's<sup>1</sup> and Bell's<sup>2</sup> quantitative methods using the stool of naturally infected *S. mansoni* patients.

#### MATERIAL AND METHODS

Twenty-six patients naturally infected with *S. mansoni* were picked out for this investigation performed through coprological examination by Hoffman, Pons & Janer's<sup>3</sup> spontaneous sedimentation method. Firstly there were performed, on these patient's newly laid feces, 5 countings of *S. mansoni* eggs by Kato's<sup>4</sup>, Bell's<sup>2</sup> and Barbosa's<sup>1</sup> quantitative methods with a 3-days interval between each counting and the next one. The stool samples used in each experiment were distributed as follows:

1<sup>st</sup> counting ..... — feces from 10 patients  
2<sup>nd</sup> and 3<sup>rd</sup> countings — feces from 24 patients  
(each count)  
4<sup>th</sup> counting ..... — feces from 25 patients  
5<sup>th</sup> counting ..... — feces from 26 patients

Centro de Pesquisas René Rachou. Instituto Nacional de Endemias Rurais, Caixa Postal 1.743, Belo Horizonte, Brasil

*Bell's and Barbosa's methods* — A five-gram stool sample from each patient was put into a Borrel flask containing 10 ml water and, with the help of a glass stick, homogenized and diluted in water in the proportion of 1/10 (total volume 50 ml). This suspension was then filtered through a 500-micra-mesh nylon screen and, from the resulting filtrate, 1 ml was taken, re-filtered through a 350-micra-mesh nylon screen and collected onto a S & S filter paper sheet no. 589. The filter paper was soaked in saturate nihidrine solution and allowed to dry, overnight, in an incubator at 37°C. Afterwards it was cut into strips, which were placed, each one separately, over a slide and imbibed in water, the number of *S. mansoni* eggs already stained being then counted with a (100 X) microscope. The number of eggs detected on the strips multiplied by 10 corresponded to the number of eggs per g of stool, BELL<sup>2</sup>. The remaining 49 ml-suspension of diluted feces (1:10) was placed into a graduated 100 ml test tube and allowed to stand for spontaneous sedimentation for an hour and a half. The supernatant was drawn with the help of a vacuum pump and then discarded. A 0.05 ml-sample of the sediment was then pipetted out, placed between slide and coverslip and then, examined under microscope (100 X). The number of eggs detected on the slide multiplied by the sediment volume and divided by the product of the volume examined by the feces weight corresponded to the number of eggs per g of feces, BARBOSA<sup>1</sup>.

*Kato's method* — Stainless steel bolting cloth (105 mesh W. S. Tyler Co., Cleveland, Ohio) is placed over the patient's feces and pushed down with the help of a wooden applicator. A fecal sample (40-50 mg) is then removed by scraping the upper surface of the cloth with applicator stick, transferred to clean slide and weighed. Over this small stool sample is placed a slip of cellophane wettable (not moisture resistant, about 22X30 mm, medium thickness, 40-50 micra, previously soaked in glycerine-malachite green solution-100 ml pure glycerine, 100 ml water and 1 ml 3% aqueous malachite green). The slide is then inverted and pressed down so that the smear covers an area about 20-25 mm in diameter. The

smear is now allowed to stand at room temperature for an hour (time necessary for the feces to clear). After that the eggs are counted under microscope and, the weight of the feces examined being previously known, the number of eggs per g of stool is then evaluated (All fecal samples here used were previously weighed).

From different parts of each fecal sample of the 6 patients were taken 5 portions (40-50 mg), the number of eggs being then counted by Kato<sup>4</sup>'s method.

*Statistical Analysis* — There have been obtained the coefficients of variability from the data obtained by Kato<sup>4</sup>'s, Bell<sup>2</sup>'s and Barbosa<sup>1</sup>'s methods.

## RESULTS

Table I shows that the mean number of eggs obtained by Kato<sup>4</sup>'s method (545) was higher than that obtained by either Barbosa<sup>1</sup>'s (196) or Bell<sup>2</sup>'s (79) methods. The coefficients of variability obtained by Kato<sup>4</sup>'s, Barbosa<sup>1</sup>'s and Bell<sup>2</sup>'s methods were, respectively, 21.6%, 21.2% and 19.3%.

Table II shows, in detail, the number of eggs, per g of feces, detected by Kato<sup>4</sup>'s method in the various portions of the same fecal sample.

## DISCUSSION

Investigators sponsored by the World Health Organization (WHO<sup>8</sup>) have been showing the significance of quantitative methods in coprological examinations. The evaluation of a quantitative technique can hardly be made unless the number of eggs in the feces to be examined is previously known. Recently, KATZ & CHAIA<sup>5</sup> evaluated Barbosa<sup>1</sup>'s and Bell<sup>2</sup>'s quantitative methods by the recovery of *S. mansoni* eggs added to feces with negative tests (feces from people not infected with helminths). A comparative study of the various quantitative methods, performed on the feces of *S. mansoni* patients, provided data that could reveal the superiority of one such method. The mean number of eggs obtained by Kato<sup>4</sup>'s

TABLE I

Mean number of eggs, per g of stool, obtained by Kato's, Barbosa's and Bell's quantitative methods on the feces of patients naturally infected with *Schistosoma mansoni*

Quantitative Methods	Mean number of <i>S. mansoni</i> eggs per g of stool						
	1st* count	2nd count	3rd count	4th count	5th count	Mean number of eggs in the 5 countings	Coefficient of variability
KATO .....	600	478	662	375	612	545	21.6
BARBOSA .....	230	277	211	149	213	196	21.2
BELL .....	76	81	94	56	92	79	19.3

\* A 3-days interval between consecutive countings of eggs

TABLE II

Number of eggs per g of feces, recovered by Kato's thick smear, from fine different portions (40-50 mg weighed per sample) of each of six stool samples. The countings were performed on the feces of 6 patients naturally infected with *Schistosoma mansoni*

Patient	no. of <i>S. mansoni</i> eggs per g of stool portions examined					Coefficient of variability
	1st	2nd	3rd	4th	5th	
I	2,583	2,461	2,755	1,675	2,204	18.2
II	1,560	1,736	2,095	2,100	1,877	12.4
III	1,045	423	1,331	476	593	51.2
IV	500	488	474	604	466	11.0
V	152	199	46	46	50	60.6
VI	20	48	100	70	27	61.6

method (545/g) was observed to be higher than that detected by either Barbosa's (196/g) or Bell's (79/g) method.

Kato's method is but a direct method for stool examination and, when compared with egg-concentration methods (Barbosa's and Bell's) showed to be superior to them, thus suggesting that, possibly, concentration methods disperse rather than concentrate the eggs. Another advantage of Kato's method over the others, when coprological diagnosis of *S. mansoni* is intended, is the semi-permanency of its preparations, hardly decomposable, and which may then, be ready for use even after considerable delay.

*S. mansoni* eggs when detected by Kato's method are observed not to contain miracidium, which, however, does not render invalid the employment of this method for epidemiological surveys, since *S. mansoni* eggs are easily identified by their shell (Fig. 1).

It was interesting to observe that *S. mansoni* eggs recovered by Kato's technique

were naturally homogenized in the feces. This can be seen on Table II, which shows the coefficients of variability resulting from the examination of the various portions of a same stool sample to be relatively low (11.0 to 18.2%), with the exception of only one sample whose coefficient of variability was 51.2%. As expected, the coefficients of variability were rather high when the number of eggs in the feces was very low (patients V and VI, Table II).

MARTIN<sup>7</sup> has reported slighter variation in the number of helminth eggs when counted by the direct method than when counted by dilution methods. We believe the quantitative methods to be quite significant in studies of epidemiological nature, especially in epidemiological surveys, in the control of areas already investigated, etc., since (as can be seen by Table I) the number of eggs obtained in one counting did not vary greatly in relation to the number of eggs obtained in the succeeding countings since the

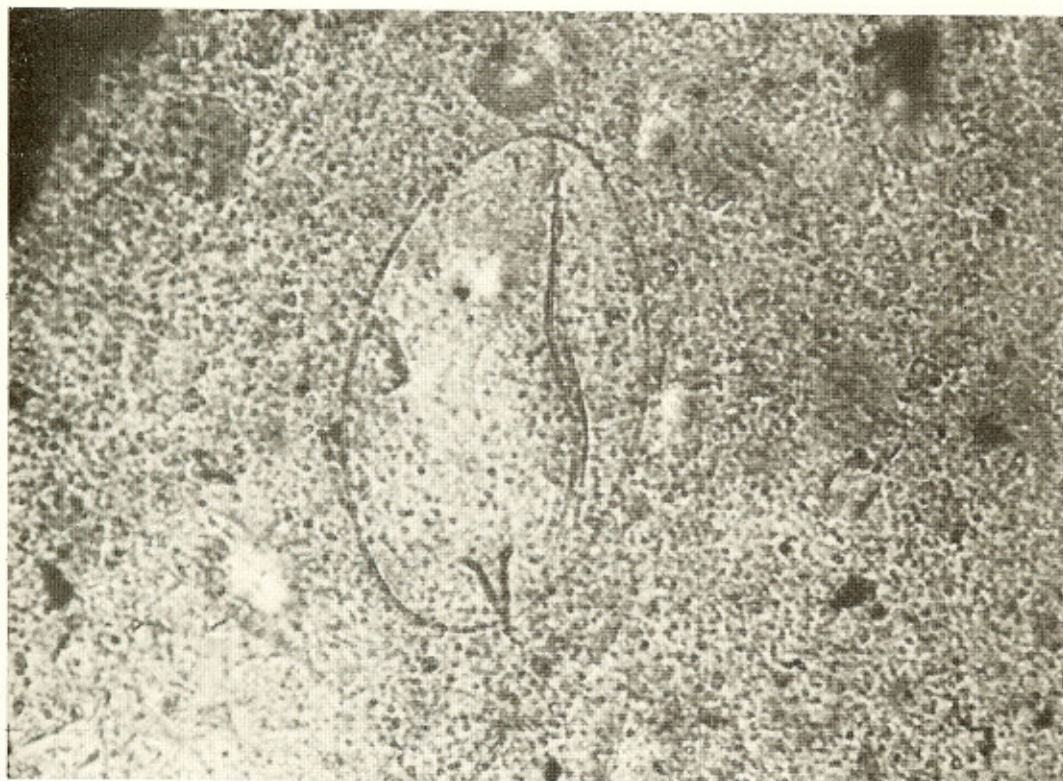


Fig. 1 — Aspect of *Schistosoma mansoni* egg observed after Kato's method (60 X)

coefficients of variability were relatively low (19.2% to 21.6%). MARTIN & BEAVER<sup>6</sup> found that the Kato<sup>4</sup>'s technic secures a high sensitivity for detecting all common types of helminth eggs from stools. The conclusion was then drawn that Kato<sup>4</sup>'s technique is indeed the method of choice, not only because of its easy performance, but also because of the steady nature of its preparations (which may be kept ready made for a long time), not escaping mentioning again it was the method that revealed the largest number of eggs per g of feces.

Although Kato<sup>4</sup>'s method suggested the employment of stainless steel bolting cloth for filtration of laboratory animal's stool, we were led to use it on the feces of human patients as well, since they were observed to contain a large number of fibers, which may have been caused by the type of food ingested by the people in the region where the investigation was carried out. The great amount of fibers and coarse elements usually found in the feces of those patients must probably have been responsible for the small number of eggs detected by Bell<sup>2</sup>'s method. A lot of eggs may have remained under such fibers, having thus escaped counting. This may be one of the reasons accounting for the low number of eggs in the feces.

#### RESUMO

#### Diagnóstico coprológico da esquistossomose. II — Estudo comparativo dos métodos quantitativos

Os Autores fizeram estudo comparativo dos métodos quantitativos de Kato, Bell e Barbosa nas fezes de pacientes infetados naturalmente pelo *Schistosoma mansoni*. O número médio de ovos de *S. mansoni*, por g de fezes, obtido pelo método de Kato (545 ovos) foi superior aos números obtidos pelo método de Barbosa (196 ovos) e pelo método de Bell (79 ovos). Não houve grande variação do número médio de ovos obtidos nas diferentes contagens, realizadas com intervalos de 3 dias, nas fezes destes pacientes.

Sendo o método de Kato um método de exame direto das fezes e tendo sido superior aos métodos de concentração de Barbosa e Bell, os Autores puderam deduzir que os

métodos de concentração diluíram mais do que concentraram os ovos. Pela pequena variação obtida na contagem de ovos de diferentes porções de uma mesma amostra de fezes, os Autores concluíram que os ovos já vêm naturalmente homogeneizados nas fezes. Os Autores concluíram pela preferência do método de Kato, pois além de ter sido superior aos demais, é um método de fácil execução e suas preparações podem ser quase que permanentes.

#### ACKNOWLEDGEMENTS

We are indebted to the Department of Parasitology, Tulane University (U.S.A.), supervised by Prof. Paul C. Beaver, for having through Captain Larry K. Martin, provided the facilities for the performance of Kato's method. Thanks are also due to Heitor de Souza Moraes and Gersy de Souza Moraes, laboratory technicians.

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Recebido para publicação em 10/5/1968.