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# COPROLOGICAL DIAGNOSIS OF SCHISTOSOMIASIS

## I — Evaluation of quantitative techniques

Naftale KATZ and Geraldo CHAIA

SUMMARY

This paper presents an evaluation of Bell's and Barbosa's quantitative techniques through the recovery of *S. mansoni* eggs added to human faeces. The mean percentage of eggs recovered by Barbosa's technique (45 to 84.7%) was higher than that obtained by Bell's method (18.6 to 67.5%). Both techniques, however, showed great sensitivity as with 100 eggs per gram of stool. It was also demonstrated that the number of eggs found in the different portions of a same sample did not vary greatly.

## INTRODUCTION

There have been developed recently, some quantitative methods for the determination of the number of *S. mansoni* eggs in the faeces, and studies to evaluate the sensitivity of these methods have been performed on the faeces of infected individuals. However, a reliable evaluation of a quantitative method can hardly be obtained, unless the actual number of eggs in the patients, stools be previously known.

This paper deals with the evaluation of BARBOSA's [1] and BELL's [2] quantitative methods through the recovery of *S. mansoni* eggs previously added to human faeces.

## MATERIAL AND METHODS

*Recovery of eggs added to human faeces* — To each 5 g sample of human faeces were added 100 to 10,000 *S. mansoni* eggs per gram of stool. The faeces were homogeneized by means of an electric stirrer and the previously counted *S. mansoni* eggs were simultaneously added. They were then diluted in water in the proportion of 1 to 10 (total volume — 50 ml) and afterwards filtered through a nylon screen with meshes of 500 micra. From this suspension, 4 samples of 1 ml each were taken and separately filtered through a nylon screen with meshes of 350 micra onto S & S filter paper n.° 589 and then submerged into a ninidryn saturated solution.

The sheets of filter paper were allowed to dry overnight in an incubator at 37°C and, afterwards, cut into slips which were placed over a slide and the imbibed with water. The *S. mansoni* eggs, already stained, were counted and multiplied by 10, the number of eggs per gram of faeces being then obtained, BELL [2]. The remaining 46 ml from each suspension of diluted faeces (1:10) were poured into a graduated test tube where it was left 1 hour and a half for sedimentation. The supernatant was removed by means of a vacuum pump and discarded. From the sediment, 4 samples of 0.05 ml were then placed between slide and coverslip and examined under microscope. The number of eggs on the slide multiplied by the volume of sediment and then divided by the product resulting from the volume of faeces examined and their weight, corresponded to the number of eggs per gram of faeces, BARBOSA [1].

*Procurement and counting of* S. mansoni *eggs* — The eggs were recovered from the

Centro de Pesquisas René Rachou, Instituto Nacional de Endemias Rurais. Caixa Postal 1743, Belo Horizonte, Brasil

intestine of mice experimentally infected with *S. mansoni*, PELLEGRINO [3], placed into a 10% formol solution and then kept in a refrigerator at 4°C. When needed, this suspension was homogeneized and 5 samples of 0.1 ml were taken for egg countings. The arithmetical mean of the number of eggs found in the 5 samples examined represented the number of eggs in the 0.1 ml suspension.

*Human faeces* — The human faeces employed were from 2 individuals with several negative parasitological and immunological examinations (complement fixation and intradermal tests) for *S. mansoni*. The faeces were used on the same day they were collected, no preservative being used.

*Statistical analysis* — The coefficients of variability from the data obtained by Barbosa's and Bell's methods have been calculated.

## RESULTS

As regards the fecal samples to which 10,000, 5,000 and 3,000 eggs per gram were added, the mean percentages of egg recovery were, respectively, 64.0, 78.8, and 72.0% (Barbosa's method) and 22.0, 19.0, and 18.6% (Bell's method). Concerning the other samples to which 1,000, 700, 400, 200, and 100 eggs were added, the mean percentages of egg recovery were, respectively, 79.6, 84.7, 70.0, 45.0, and 50.0% (Barbosa) and 34.5, 20.7, 33.7, 43.7, and 67.5% (Bell).

The coefficient of variability from the number of eggs found in the various examinations of a same sample of faeces varied from 7.1 to 28.6% (Barbosa's method) and from 6.7 to 52.1% (Bell's method). Further data are shown on Table I.

## DISCUSSION

Investigations sponsored by the World Health Organization (W.H.O.[4]) have shown the need for quantitative methods for coprological examination. The quantitative techniques so far studied for *S. mansoni* eggs were based on the data provided by faeces from infected individuals. However, a reliable evaluation of a quantitative technique could hardly be obtained unless the actual number of eggs existing in the patient's faeces were previously known. An attempt at solving such problem has been made by adding a known number of eggs to faeces of non infected people. This method, however, may undergo some misleading influences such as the presence, in the suspension, of a large number of immature eggs resulting from the scraping of the mice's intestinal mucosa and from the manipulation necessary for mixing the eggs with the faeces.

Notwithstanding it made possible the comparison of the two quantitative techniques, since both methods were assessed under the same conditions. From the data obtained, Barbosa's quantitative technique has been proved superior to that of Bell's. The mean percentages of egg recovered by Barbosa's technique were higher (45 to 84.7%) than those obtained by Bell's method (18.6 to 67.5%). It must be pointed out that the mean percentage of egg recovery was not proportional to the number of eggs added to the faeces. When 10,000 eggs were added per gram of stool, only 64.0% were recovered whereas when 700 eggs were added per gram of stool, the percentage was 84.7% (Table I). This fact was also observed with regard to Bell's technique (with 10,000 eggs the percentage of recovery was 22.0% and with 100 eggs the percentage was 67.5%). There has not been observed great variation in the number of eggs recovered from different portions of a same sample, except in one single sample, whose coefficient of variability was 52.1% (Table I).

## RESUMO

*Diagnóstico coprológico da esquistossomose. I — Avaliação de técnicas quantitativas*

Os Autores fizeram avaliação das técnicas quantitativas de BELL e BARBOSA, por intermédio da recuperação de ovos de *Schistosoma mansoni* adicionados a fezes humanas. Os percentuais médios de ovos recuperados pela técnica de BARBOSA (45 a 84,7%) foram superiores aos percentuais obtidos pela técnica de BELL (18,6 a 67,5%). Ambas as técnicas demonstraram sensibilidade de

TABLE I

Evaluation of Barbosa's and Bell's techniques for the coprological diagnosis of Schistosomiasis. Study concerning the recovery of eggs added to 5-gram samples of human faeces

| no. of eggs added per gram of faeces | Sedimentation method (Barbosa) | | | | | | | Method of Bell | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Volume of sediment (ml) | no. of eggs per gram of stool Portions examined (0.05 ml) | | | | Mean percentage of egg recovered | C.V. | no. of eggs per gram of faeces Examinations performed | | | | Mean percentage of egg recovered | C.V. |
| | | 1st | 2nd | 3rd | 4th | | | 1st | 2nd | 3rd | 4th | | |
| 10,000 | 5 | 7,320 (366) | 5,480 (274) | — | — | 64.0 | — | 2,200 (220) | — | — | — | 22.0 | — |
| 5,000 | 9 | 3,960 (110) | 3,924 (109) | — | — | 78.8 | — | 970 (97) | — | — | — | 19.0 | — |
| 3,000 | 9 | 2,016 (56) | 2,304 (64) | — | — | 72.0 | — | 560 (56) | — | — | — | 18.6 | — |
| 1,000 | 4 | 640 (40) | 896 (56) | 960 (60) | 688 (43) | 79.6 | 19.5% | 420 (42) | 300 (30) | 340 (34) | 320 (32) | 34.5 | 15.2% |
| 700 | 3 | 548 (49) | 576 (48) | 600 (50) | 648 (54) | 84.7 | 7.1% | 170 (17) | 140 (14) | 110 (11) | 160 (16) | 20.7 | 18.2% |
| 400 | 2 | 336 (42) | 304 (38) | 232 (29) | 248 (31) | 70.0 | 17.2% | 110 (11) | 90 (9) | 240 (24) | 100 (10) | 33.7 | 52.1% |
| 200 | 5 | 60 (3) | 100 (5) | 120 (6) | 80 (4) | 45.0 | 28.6% | 30 (3) | 50 (5) | 170 (17) | 100 (10) | 43.7 | 6.8% |
| 100 | 5 | 40 (2) | 60 (3) | 40 (2) | 60 (3) | 50.0 | 23.0% | 120 (12) | 90 (9) | 20 (2) | 40 (4) | 67.5 | 6.7% |

C.V. = Coefficient of variability

Note — The figures in brackets correspond to the number of eggs on each slide

recuperação de ovos relativamente alta, pois com 100 ovos por grama de fezes os percentuais médios de recuperação foram bem elevados. Ficou também demonstrado não haver grande variação entre os números de ovos encontrados em diferentes porções examinadas de uma mesma amostra de fezes.

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