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ANALYSIS OF COMPONENTS OF VARIATION IN SCHISTOSOMA MANSONI EGG COUNTING (*)

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SUMMARY

Analysis of variance was applied to data collected from counting of **S. mansoni** eggs obtained from fecal specimens of seventeen patients. The technique used (Bell method) was unable to show a statistically significant difference among individuals, although these patients had varying degrees of severity of **S. mansoni** infections. The highest variation was due to sample to sample variation. The smallest variation was due to interobserver variation. The variation among single counting was very high therefore influencing the results found in the other components. Regarding the conditions under which this experiment was carried out, one must be careful in considering classifiying individual patients, according to the severity of **S. mansoni** infections, based on a single egg counting.

INTRODUCTION

The use of egg counting in **S. mansoni** infections had been suggested as a measure of worm load [2], as a means of classifying clinical severity of Schistosomiasis [4] and as a technique preferable for morbidity surveys and other more precise studies [7]. A technique using filtration followed by ninhydrin staining of the eggs was developed by BELL [1] as a more sensitive method for evaluating **S. mansoni** egg output. Nonetheless, there are several criticisms of these quantitative techniques on the grounds of a daily variation of egg output [1,3,5], as well as other sources of error which could seriously influence the reliability of these methods [5]. Therefore, this investigation was designed to ascertain the extent of the interobserver, intraobserver and other types of variation that might influence the accuracy of the Bell method for **Schistosoma mansoni** egg counting. With identification of main sources of errors it may be possible to reduce them in order to strengthen the reliability of the method studied.

MATERIAL AND METHODS

Seventeen patients were selected on the basis of a positive stool examination for **S. mansoni** eggs using a simple qualitative sedimentation technique. There were ten males and seven females with an age range from 19 to 32 years. Six of the patients had the intestinal form of the disease while eleven had liver and spleen enlargement.

The technique used to prepare the specimens for examination followed the method described by BELL [1] and was always performed by the same person. Three observers previously trainned to recognize **S. mansoni** eggs were selected. One was a laboratory technician and the other two were physicians. Stool specimens were collected between 6 and 8 a.m. and prepared for examination on the same day. Within a period of three to five days a new specimen was collected from each patient. Each stool samples was divided into two sub-samples after having been homogenized.

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Each observer counted the total number of eggs in one coded plate and recorded it separately. The next day the code was changed by a person not involved in the experiment and a second counting was performed by the three observers. Thus one observer performed a total of eight counting for each patient and the same patient had twenty four determinations of egg excretion.

The method of analysis used was one way analysis of variance with five classifications in a hierarchal or nested model [6].

RESULTS AND COMMENTS

The collected data fit a one way analysis of variance and are presented in Tables I and II.

The method of analysis shows the following results: it was not possible to detect statistically significant differences between individuals ($p > 0.05$) although its contribution to the total variation was 27.84%. This fact can be explained by the high variation found from sample, taken from the same individual.

T A B L E I

Analysis of variance of in **Schistosoma mansoni** egg counting using the Bell's method

Source of variation	df	SSQ	MSQ	F	Level of significance
Among individuals	16	5,209,882.5	325,617.65	$F_{16.17} = \frac{325.617}{175,682,33} = 2.065$	$p > 0.05$
Between sample of the individual	17	2,680,600.0	157,682.35		
Between sub-samples of the samples	34	369,316.7	10.862.25	$F_{17.34} = \frac{157.682.35}{10.862.25} = 14.516$	$p < 0.01$
Among observers of the sub-samples	136	607,633.3	4,467.89	$F_{34.136} = \frac{10,862,25}{4,467.89} = 2.431$	$p < 0.01$
Among determinations by the observers	204	561,500.0	2,752.45	$F_{136,204} = \frac{4,467.89}{2,752.45} = 1.623$	$p < 0.01$
Total	407	9,428,932.5	—		

T A B L E II

Estimates of the contribution of different components to the total variation in **S. mansoni** egg counting (Bell technique)

Source of variation	Estimates of variation	%
Determinations	2.752.45	11.74
Observers	871.22	3.71
Sub-sample	1.065.73	4.54
Sample	12.235.01	52.17
Individuals	6.528.51	27.84
Total	23.452.92	100.00

These results show that individual egg counting can not be either a safe parameter for estimation of **S. mansoni** egg output, or used as a reliable means of classifying degree of severity of **S. mansoni** infections. The time which elapsed between the collection of the samples may have played some role in the variation from sample to sample. Statistically significant differences were found between observers of the same sub-sample, $p < 0.01$, and between sub-sample of the same sample. The contribution of these components was 3.71% and 4.54%, respectively. This variation may be reduced to nonsignificant levels if a very strict methodology is followed in the preparation of the specimens and precise standardized criteria is followed. The variation from determination to determination is mostly due to experimental error. Its contribution to the total variation is 11.74% which is considered a very high variation and may be responsible for the differences found in the other components studied.

In regard to this experiment, the Bell method for **S. mansoni** egg counting has shown a high variation from sample to sample of stool specimens of the same individual. This variation was due to a high experimental error that jeopardized the reliability of the method for individual egg counting, although the possibility of using this technique as a tool for assessing **S. mansoni** prevalence or level endemicity of a community or region is not excluded. Ne-

vertheless, under the conditions in which this experiment was carried out, it would be unwise to classify individuals according to the severity of the disease, based on a single egg counting.

RESUMO

Análise dos componentes de variação na contagem de ovos do Schistosoma mansoni

Análise de variância foi aplicada em dados coletados sobre contagem de ovos de **Schistosoma mansoni** obtidos em amostra de material fecal de 17 pacientes. O método utilizado foi o de filtração e coloração pela nihidrina (Método de Bell). Os resultados não mostraram diferenças estatisticamente significantes entre indivíduos, embora entre estes houvessem portadores de diferentes formas clínicas da doença. A análise dos componentes de variação mostrou uma maior variação entre amostras e a menor variação foi verificada entre observadores. O erro entre as determinações foi considerado muito grande, influenciando deste modo o resultado dos outros componentes. Tendo em vista estes resultados deve-se ter cautela em classificar pacientes portadores de esquistossomose mansônica quanto ao grau de severidade, baseado em apenas uma contagem de ovos.

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