

## Short Report

### Implications of faecal egg count variation when using the Kato-Katz method to assess *Schistosoma mansoni* infections

M. L. Barreto<sup>1</sup>, D. H. Smith<sup>2</sup> and A. C. Sleigh<sup>3</sup> <sup>1</sup>Departamento de Medicina Preventiva, UFBA, Rua Padre Feijó 29, 4º Andar, Canela, 40.140 Salvador, Bahia, Brazil; <sup>2</sup>Departamento de Zoologia, Instituto de Biologia, Universidade Federal da Bahia, 40.000 Salvador, Bahia, Brazil; <sup>3</sup>Tropical Health Program, Medical School, University of Queensland, Herston, Queensland, 4006, Australia and Department of Tropical Public Health, Harvard School of Public Health, 665 Huntington Avenue, Boston, Massachusetts, 02115, USA

Faecal egg counts from Brazilians with chronic *Schistosoma mansoni* infections, as assessed by the Kato-Katz (KK) technique (KATZ *et al.*, 1972), tend to remain stable over a 3-month period (BARRETO *et al.*, 1978). However, when counts are low, short-term variation is considerable. In another Brazilian study, single KK smears were often negative from patients with light schistosome infections (SLEIGH *et al.*, 1982). Evidently, multiple KK smears are needed to characterize infection status accurately.

The KK method is now widely used to diagnose and grade *S. mansoni* and *S. japonicum* infections (MOTT & CLINE, 1980; MOTT, 1982). Therefore, we re-analysed our original data to compare the diagnostic efficiency of repeating smears on the same stool sample or on stools collected on consecutive days or successive months.

Stool specimens were collected on 3 consecutive days during each of 3 successive months from 23 Brazilians known to be infected with *S. mansoni* (BARRETO *et al.*, 1978). For each specimen, 2 KK smears were prepared from sub-samples obtained from different parts of the stool. All smears were examined by the same observer (M.L.B.).

One person (subject no. 1), from whom 14 of 16 smears were negative, was excluded from the analysis. For each of the remaining 22 individuals, the egg count variance noted between smears obtained on the same day, on different days, and on different months was analysed as follows. First, for each person, the correlation between the daily mean egg count and the respective daily variance was calculated separately for untransformed, log-transformed and Taylor's power law-transformed egg counts (BARRETO *et al.*, 1978; TAYLOR, 1961). The data, expressed in the form that had shown the least correlation between mean and variance (SOUTHWOOD, 1978), were then used for an individual analysis of egg count variance using one-way hierarchical ANOVA formulas (SNEDECOR & COCHRAN, 1967). Subsequently, the relative contributions of within-day, between-day and between-

month variation were calculated for each subject as a percentage of the total variance observed (SNEDECOR & COCHRAN, 1967; SOKAL & ROHLF, 1969). Finally, the individual percentage contributions were averaged for the 22 persons.

The correlation between the egg count means and variances was minimum for untransformed counts in 4 subjects, for log-transformed counts in 7 subjects, and for Taylor's law-transformed counts in the remaining 11 subjects. In all instances, these minimum correlations between the means and the variances were not statistically significant ( $P > 0.05$ ). Thus, after transforming the data for 18 subjects, the analysis of egg count variance was unhindered by correlation between means and variances.

Most of the egg count variation occurred between stool samples obtained on consecutive days (Table). Overall, of the total variation observed, the average percentage contributions of between-day, within-day and between-month differences in egg counts were 46.1%, 31.5% and 22.4%, respectively.

Individual diagnostic accuracy is important when proof of infection or cure is required in connection with experimental schistosomicidal treatment, for evaluation of the sensitivity and specificity of new diagnostic methods, or for incidence studies (WHO, 1980). In contrast, individual accuracy of this degree is not necessary when measuring the impact of control

Table. Relative contribution of sources of *S. mansoni* egg count variance over 3 months in 22 chronically infected Brazilians

Subject no. <sup>a</sup>	No. of counts	Eggs/smear <sup>b</sup>	Percentage of total variance		
			Within days	Between days	Between months
2	18	1.0	36	29	36
3	16	1.9	53	35	13
4	18	2.2	71	24	6
5	18	6.6	30	61	9
6	16	5.6	45	46	10
7	18	10.1	57	33	10
8	18	12.5	18	78	4
9	18	12.8	52	17	31
10	18	14.2	11	60	29
11	18	14.2	48	48	4
12	18	16.9	49	49	1
13	18	16.4	15	80	5
14	10	21.3	12	4	84
15	18	24.2	16	49	35
16	16	18.9	8	79	13
17	18	29.2	23	59	19
18	18	29.1	11	59	30
19	14	37.9	39	8	62
20	18	46.2	43	13	43
21	18	49.2	15	84	2
22	16	44.4	46	42	12
23	14	60.2	5	58	38
Group mean	17	21.6 <sup>c</sup>	31.5	46.1	22.4

<sup>a</sup>Subjects were ranked by arithmetic mean egg count (BARRETO *et al.*, 1978). Subject no. 1 was excluded from analysis (see text).

<sup>b</sup>Geometric mean number of eggs per smear:  $[\text{antilog}\{\log(x+1) \div n\} - 1]$ , where  $x$  is the egg count of a smear and  $n$  is the total number of smears.

<sup>c</sup>Arithmetic mean for the whole group of the individual geometric mean egg counts/smear. Group mean egg count per gram of faeces was  $24 \times 21.6 = 518.4$ .

measures on the average worm burden of a large group of people (MOTT & CLINE, 1980). Examination of a single KK smear for each person should be sufficient to calculate the geometric mean egg count of an infected population; individuals in whom infection was misgraded by the single examination would be equally likely to have had counts that were higher or lower than the true count. However, for estimating the prevalence of infection, the need for multiple stool examinations depends on the average intensity of schistosome infections (SLEIGH *et al.*, 1982). In lightly infected populations, a single KK examination would fail to detect many infections.

When accurate classification of individual infection status is required, multiple KK smears should be obtained in a way that incorporates as much of the short-term egg count variation as possible. Our data reveal that accuracy is maximized by examining stool samples obtained on consecutive days. Multiple examinations from the same stool sample yield information that is a little less accurate but is much less expensive for community surveys. Thus the best faecal sampling strategy depends on both the degree of accuracy needed and on the resources available.

#### References

- Barreto, M. L., França Silva, J. T., Mott, K. E. & Lehman, J. S. (1978). Stability of faecal egg excretion in *Schistosoma mansoni* infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **72**, 181–187.
- Katz, N., Chaves, A. & Pellegrino, K. (1972). A simple device for quantitative determination of *Schistosoma mansoni* eggs in faeces examined by the thick smear technique. *Revista do Instituto de Medicina Tropical de São Paulo*, **14**, 397–400.
- Mott, K. E. (1982). Control of schistosomiasis: morbidity-reduction and chemotherapy. *Acta Leidensia*, **49**, 101–111.
- Mott, K. E. & Cline, B. L. (1980). Advances in epidemiology survey methodology and techniques in schistosomiasis. *Bulletin of the World Health Organization*, **58**, 639–647.
- Sleigh, A., Hoff, R., Mott, K., Barreto, M., Mais de Paiva, T., de Souza Pedrosa, J. & Sherlock, I. (1982). Comparison of filtration staining (Bell) and thick smear (Kato) for the detection and quantitation of *Schistosoma mansoni* eggs in faeces. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **76**, 403–406.
- Sokal, R. R. & Rohlf, F. J. (1969). *Biometry: The Principles and Practice of Statistics in Biological Research*. San Francisco: W. H. Freeman.
- Southwood, T. R. E. (1978). *Ecological Methods with Particular Reference to the Study of Insect Populations*. New York: John Wiley.
- Snedecor, G. W. & Cochran, W. G. (1967). *Statistical Methods*. Ames, Iowa: Iowa State University Press.
- Taylor, L. R. (1961). Aggregation, mean and variance. *Nature*, **169**, 732–735.
- WHO (1980). *Epidemiology and Control of Schistosomiasis: Report of an Expert Committee*. Geneva: World Health Organization, Technical Report series, no. 643.

Received 6 April 1989; revised 2 January 1990; accepted for publication 6 February 1990