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Variation in weight of stool samples prepared by the Kato–Katz method and its implications

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Summary We investigated both the extent of the variation in weight of stool samples prepared by the Kato–Katz method and its influence on egg counts and commonly used group parameters of infection derived from them. In a first study group of 795 people, the total mean weight of stool aliquots, prepared with templates designed to contain 28.3 mm^3, was 23.0 mg with 95% of the individual values lying between 12.0 and 34.0 mg. Minimum and maximum values were 2.4 and 49.5 mg, respectively. Frequency distributions of the individual weights, in series of slides prepared by different laboratory assistants, showed significant differences. In a second study group of 199 people, duplicate series of slides were prepared and variations in the weight of examined stool were related to variations in egg count. The correlation between repeated individual sample weights in this series was poor, but the correlation between egg counts was good. This was translated, at aggregate level, in very similar classifications in egg count categories. This classification was also hardly influenced by the choice of the conversion factor to transform egg counts per slide into eggs per gram. At the individual level, the variability in egg counts far outweighed the variability in sample weight and was not clearly related to it. We therefore concluded that variations in weight of examined stool are considerable, but account for only a minimal part of the important egg count fluctuations generally observed.

keywords schistosomiasis, *Schistosoma mansoni*, Kato-Katz method, stool examination, egg count variation

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Introduction

Kato and Miura (1954) introduced a new sensitive and quantitative technique, based on the examination of a relatively large and calibrated quantity of stool, for the diagnosis of intestinal helminth infections. Katz *et al.* (1972) used templates to substitute the calibrated weight of examined stool by a calibrated volume, and have in this way adapted the method to field conditions, particularly for use in *Schistosoma mansoni* infection. Despite the qualitative and quantitative advantages of this diagnostic method, there are important variations in faecal egg counts (Engels *et al.* 1996). We investigated the impact of variations in the quantity of examined stool on this egg count fluctuation.

Material and methods

The study consisted of 2 parts. The aim of the first part was to evaluate the variation in weight of stool samples prepared by the Kato–Katz method. The

second part investigated the possible influence of these variations in weight on variations in egg counts and in parasitological parameters commonly calculated at group level.

During the first part of the study, single Kato–Katz slides were examined in a study population of 795 people. To investigate the possible influence of age and diet on the studied variations in weight, 4 subgroups (adults and children from both a rural and an urban setting) of ≈200 people were considered. In order to compensate for possible systematic differences between preparers, two different laboratory assistants prepared half of the slides in each of these subgroups (Assistants 1 and 2, who prepared 397 and 398 slides, respectively).

For the second part of the study, duplicate Kato–Katz slides were examined in another study population of 199 persons (100 children, 99 adults). Both these duplicate slides were prepared from the same (small) stool specimen supplied by each individual. Each of the two slides was prepared by a different laboratory assistant. Another pair of laboratory assistants participated in this second part of the study (Assistants 1 and 3). Both slides of an individual were examined by the same microscopist, who was unaware of their origin.

For the preparation of the Kato–Katz slides, stainless steel templates 1 mm thick, with a hole 6 mm in diameter, and thus designed to contain 28.3 mm^3 of stool, were used. Microscopic slides were weighed individually, without and with their aliquot of stool. The weighing was done with an electronic scale (Sartorius type 1702, sensitivity 0.1 mg) immediately after withdrawal of the template from the slide. The slides were prepared in the same way as under operational circumstances, and examined after 45 minutes according to the technique described by Peters *et al.* (1980).

Conversion of egg counts/slide into eggs per gram (e.p.g.) was done by multiplication by 40, as under operational circumstances (presuming an average weight per sample of 25 mg). The mean egg load was calculated as the geometric mean of positive individual e.p.g. of faeces. Egg count categories were defined as: 1–100 e.p.g. (1–2 eggs/Kato–Katz slide); 101–400 e.p.g. (3–10 eggs/Kato–Katz slide); more than 400 e.p.g. (>10 eggs/Kato–Katz slide).

Statistical tests used were the χ^2 for the comparison of prevalences in independant samples, and the Mc Nemar test, the non-parametric test for the same type of comparison in paired samples. For comparison of the prevalences of the different egg count categories in paired samples, the non-parametric Wilcoxon matched pairs signed ranks test was used. Heterogeneity of variances was tested with the variance ratio test. Means and logarithmic means were compared with the *t*-test for paired and independent samples. Because of the heterogeneity of variance, a modified *t*-test accounting for unequal variances was used in independent samples. For the same reason, the non-parametric Kruskal–Wallis test was used for one-way analysis of variance. As multiple statistical comparisons of group means were performed in this procedure, the Bonferroni method was used for the adjustment of the *P*-value (Altman, 1991).

Results

The mean weight of examined stool observed during the first part of the study (795 observations) was 23.0 mg. Observed minimum and maximum values were 2.4 mg and 49.5 mg, but 95% of the individual values lay within the range 12.0–34.0 mg. The median value was 22.4 mg. The mean weights of stool aliquots prepared by each of the two laboratory assistants, in subsamples of 397 and 398 observations, were 23.8 mg (with 95% of the values lying within 11.1–36.5 mg) and 22.1 mg (with 95% of the values lying within 13.5–30.7 mg), respectively. The difference between those means was statistically significant ($P<0.001$). The difference in frequency distribution of the individual weights is shown in Figure 1a. The mean and median weights of the stool aliquots in the different subgroups (children-adults; rural-urban residents) are summarized in Table 1. One-way analysis of variance showed an overall statistically significant difference in mean weights between the 4 subgroups ($P<0.01$). When tested 2-by-2, only 2 (urban adults *vs* urban children and rural adults *vs* urban children) of 6 possible combinations were significantly different ($P<0.05$).

Table 2 summarizes the results of the second part of the study and shows the means and some summary measures of variation for the weight of stool aliquots prepared by the two different lab assistants

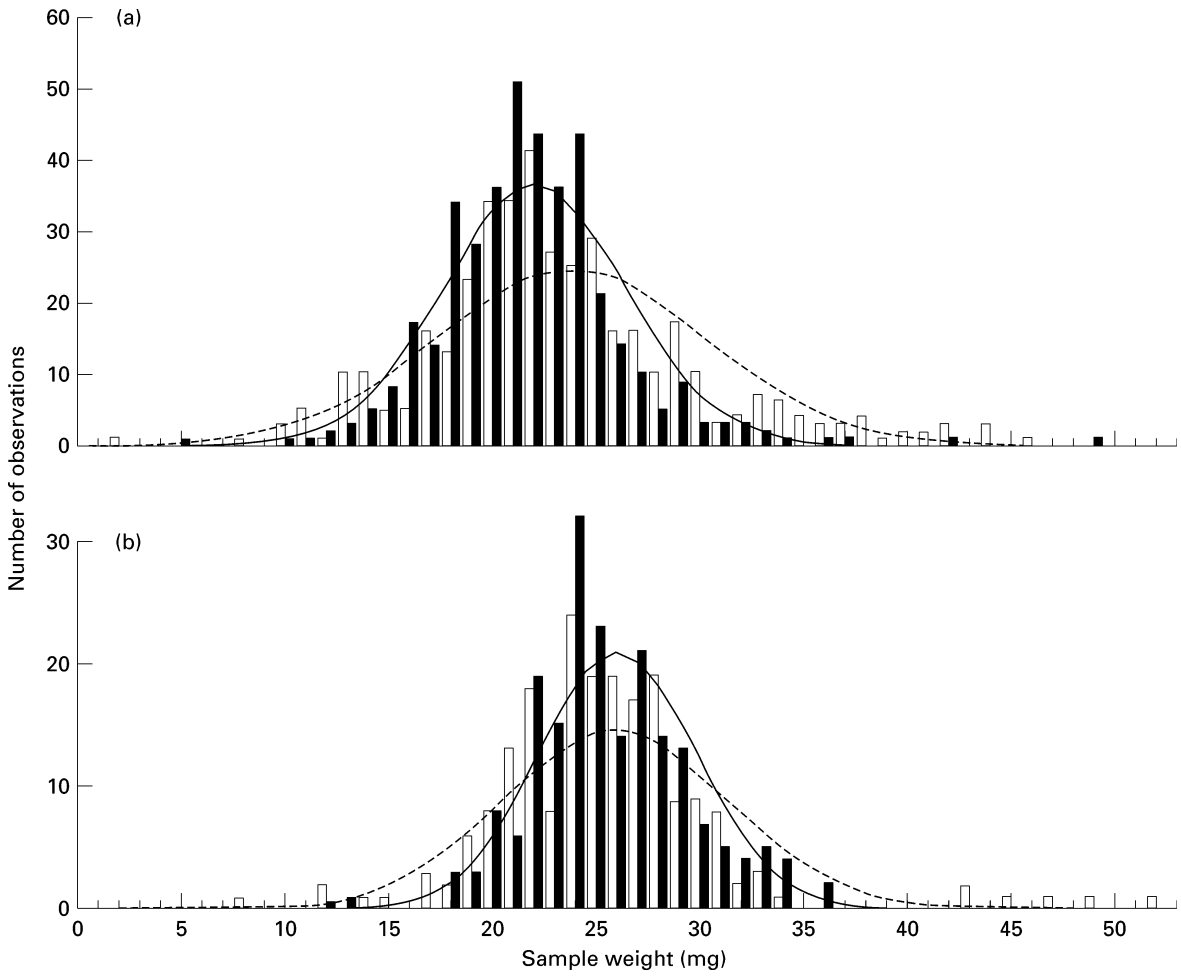

Figure 1 Frequency distributions of individual sample weights in a, two independent series of Kato–Katz slides (□, n_1=397; ■, n_2=398) prepared by different laboratory assistants (1 and 2) during the first part of the study and b, duplicate series of slides from 199 people prepared by different laboratory assistants (□, 1 and ■, 3) during the second part of the study. – – –, Fitted Gaussian curve 1. a, —, fitted Gaussian curve 2; b, —, fitted Gaussian curve 3. The figures on the *x*-axis indicate intervals:, e.g. the bars on the *x*-axis point 5 indicate the number of samples weighing 5.0–5.9 mg.

in the study group of 199 people. Although the difference in means was not statistically significant, the variance was higher for the series of slides prepared by Assistant 1 (P<0.01), as can easily be seen in Figure 1b. In this series of observations the difference in mean sample weight between children and adults was statistically significant for the samples prepared by Assistant 3 (26.8 *vs* 25.3 mg; P=0.004), but not for those prepared by Assistant 1 (25.8 *vs* 25.9 mg; P=0.897).

Table 2 also shows some commonly used group parameters of infection (prevalence, prevalences of different egg count categories, geometric mean egg load) derived from the egg counts observed in these duplicate series of observations. Total prevalences and prevalences of different egg count categories were not significantly different. Nevertheless, there is a considerable shift between the prevalences of light (1–100 e.p.g.) and moderate (101–400 e.p.g.) infections. Because some individuals were positive in one slide and negative in the other, the values of the geometric mean egg load of positives could not be statistically tested as paired observations. When, as a proxy measure, the duplicate series were considered

Table 1 Weights of examined stool in different subgroups of the study population of 795 people

Subgroup	Number	Weight (mg)		
		Mean	Standard deviation	Median
Children				
Rural	196	22.4	4.6	22.1
Urban	200	21.9	5.3	21.8
Adults				
Rural	200	24.1	6.3	22.6
Urban	199	23.4	5.9	22.8

as independent samples, these values were not significantly different. The same conclusion remained valid when the arithmetic means or the geometric means of the egg count (+1) of all individuals were considered and tested as paired observations.

Table 3 shows the influence of the conversion factor, used to transform the number of eggs detected in duplicate Kato–Katz slides into eggs per gram, on group parameters of infection in the second part of the study. Five different conversion factors were used. The first factor (19.3) was based on the detected mean sample weights of each single slide (25.9 and 26.0 mg, respectively). The second (20) was the commonly used factor based on a presumed weight of 25 mg per single slide. The third (21.7) is based on a presumed weight of 23 mg per single slide. The fourth (17.7), theoretically the most correct, is based on a volume of 28.3 mm^3 per single slide. Because of the volumetric approach here, the conversion thus obtained should be expressed in cm^3. To constitute the last column, each egg count was converted according to the individual sample weights in each of the duplicate slides and the conversion factor was therefore different for each individual observation. The use of different conversion factors had obviously no impact on the total prevalence and had only a minimal impact on the prevalences of the different egg count categories. The most directly affected parameter was the geometric mean egg load.

Pearson's coefficient of correlation (r) between the sample weights of the individual duplicate slides in the group of 199 people was 0.130 (P=0.067). When only those individuals who were positive in any of the 2 duplicate slides (n=88) were considered, the correlation coefficient between sample weights was −0.046 (P=0.673), whereas the one between the corresponding egg counts was 0.778 (P<0.001).

Figure 2 quantifies the discrepancies in egg counts and sample weights and shows the direct relation between them, in the same series of 88 duplicate slides. The ratio of the egg counts detected in these duplicate slides varied from 0.12–4.00, whereas the ratio of the weights of the same slides varied only from 0.52–1.59. Because of the simple fact that their possible range of values is more restricted, the egg count ratios in light infections are more concentrated at the values 0.5, 1 and 1.5. But no substantial differences in degree of egg count variation appeared to exist between light and moderate to heavy infections. Pearson's correlation coefficient corresponding to this scattergram, using a $\log_{10}$ transformation to make distributions approximately normal, is −0.126 (P=0.241). The value for r^2, indicating the percentage of variation in egg counts which is explained by the variation in sample weight, is 0.016 or 1.6%. Spearman's rank correlation coefficient (r_s) is −0.054 (P=0.617).

Discussion

These figures show that the variation in weight of individual stool samples prepared by the Kato–Katz method under operational conditions is considerable. The frequency distribution of individual sample weights and their means can indeed be substantially different in different series of stool samples. This was the case, in the first part of the study, for the two series of samples prepared by different laboratory assistants. Three elements can be assumed to play a role in this variation in sample weight: varying consistency of stool and random and systematic inter-assistant variations in volume. All three elements contribute to the sample weight variation observed in this first part of the study.

In the second part of the study, duplicate slides were prepared from the same individual stool specimens and so had the same consistency. Although the mean weights were not significantly different in these two series, variance was higher for samples prepared

Table 2 Group prevalences and intensities of infection in relation to the mean weight of stool samples prepared by two different laboratory assistants in the study group of 199 people

Parameter		Assistant 1		Assistant 3
Weight (mg)	Mean	25.9	(P=0.766)	26.0
	Standard deviation	5.5		3.8
	Minimum value	8.4		13.9
	Maximum vgalue	52.5		36.5
	Median	25.5		25.5
Parasitological	Total prevalence (%)	36.7	(P=0.324)	33.2
	Prevalence of uninfected	63.3		66.8
	Prevalence of infections 1–100 epg (%)	22.1	(P=0.827)	14.6
	Prevalence of infections 101–400 epg (%)	9.5		15.1
	Prevalence of infections >400 epg (%)	5.0		3.5
	Geometric mean egg load	102.3		123.1

Table 3 Influence of the conversion factor (used to convert the number of eggs detected in duplicate Kato–Katz slides into eggs per gram) on group parameters of infection in a group of 199 people

	Conversion factor				
Parameter	× 19.3[1]	× 20[2]	× 21.7[3]	× 17.7[4]	individual[5]
Prevalence of infections 1–100 epg (%)	26.1	26.1	25.1	26.1	26.1
Prevalence of infections 101–400 epg (%)	14.1	14.1	14.1	14.6	14.6
Prevalence of infections >400 epg (%)	4.0	4.0	5.0	3.5	3.5
Geometric mean egg load (epg)	70.7	73.4	79.8	64.8	70.1

[1] Based on detected mean sample weights: 1000/(25.9+26.0)
[2] Based on presumed mean weight of 25 mg: 1000/(2 × 25)
[3] Based on presumed mean weight of 23 mg: 1000/(2 × 23)
[4] Based on volume of 28.3 mm^3: 1000/(2 × 28.3)–the unit here is then eggs/cc
[5] Based on individual sample weights: $1000/(\text{weight}_{\text{sample 1, individual } i} + \text{weight}_{\text{sample 2, individual } i})$ (i=1, . . . , 199).

by Laboratory Assistant 1. As this was also the case in the first part of the study, it illustrates the fact that systematic inter-assistant variations in volume can occur. Assistant 1 was indeed the least experienced of the 3 laboratory assistants participating in the study. This probably accounted for the wider fluctuations in volume (and thus weight) of the prepared samples. But despite this difference in variance, both frequency distributions in Figure 1b are similar. This suggests, at first sight, that repeated measurements on the same individuals might correlate quite well, which was not the case. In fact, the correlation between the sample weights of each of the individual duplicate slides was very poor. This illustrates the extent of random inter-assistant variations in volume, which are not reflected in the frequency distributions and apparently constitute the most important factor accounting for sample weight variation in Kato–Katz slides.

The results of the first part of this study also suggest a possible difference in the mean weight of stool samples between adults and children. This could be due to a systematic difference in consistency of stool due to functional reasons or diet. The second part of the study could only partly confirm this finding. More appropriately designed studies would therefore be necessary to obtain further evidence or disproof such a difference.

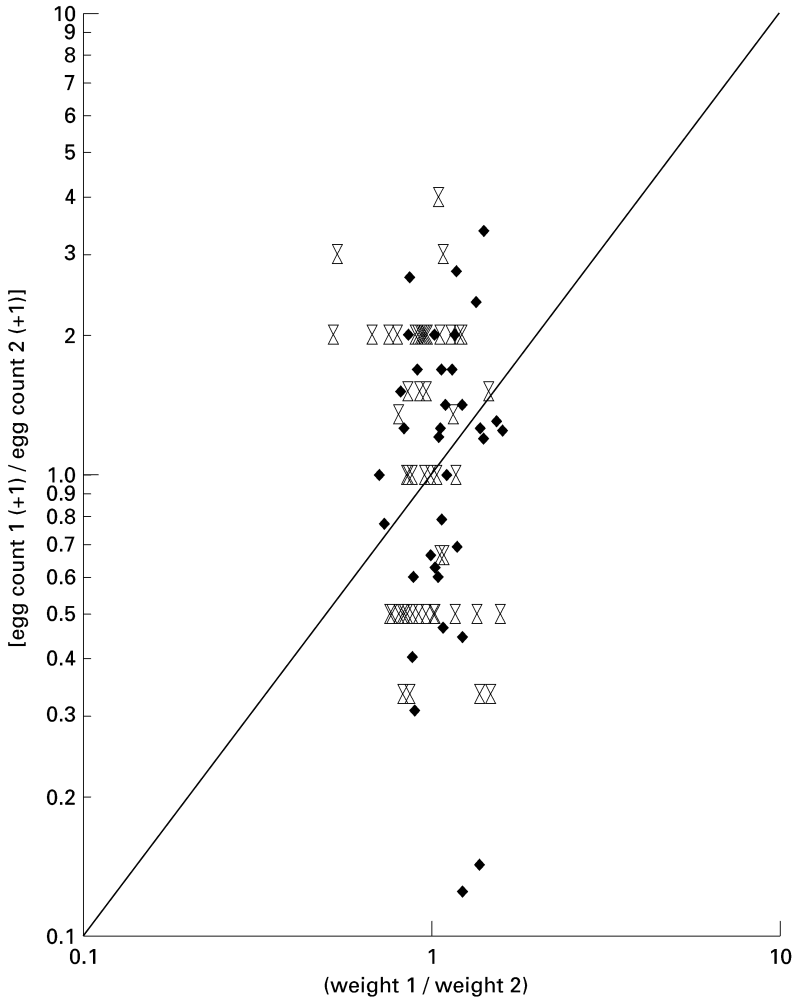

Figure 2 Scattergram of the relation between the ratio of (egg counts +1) and the ratio of sample weights in a series of duplicate slides prepared by the Kato–Katz method by 2 different laboratory assistants in a group of 88 people, known to be infected with *S. mansoni*. Open marks indicate light infections (up to 5 eggs detected in both slides); closed marks indicate moderate to heavy infections (6 or more eggs detected in both slides).

Although the correlation between sample weights in duplicate slides was poor ($r = -0.046$), the correlation between repeated egg counts was high ($r = 0.778$). This merely indicates that, regardless of the variation in weight of duplicate slides, the number of eggs counted in them tends to be systematically high or low depending on whether the individual is heavily or lightly infected. This is translated at group level in very similar classifications of people in egg count categories on the basis of each of the two series of duplicate slides. The shifts between the prevalences of the different egg count categories, especially between light and moderate infections, are directly caused by variations in egg count around the delicate cut-off point of 2–3 eggs/slide and are probably due to observer rather than assistant variation. The geometric mean egg load is also subject to a mathematical bias which enhances small variations as more or less light infections are detected (Engels *et al.* 1996).

The same parasitological parameters at group level are also directly influenced by the choice of the factor used to convert the egg count per slide into eggs per gram. And given the important variation in sample weight, the factors which are commonly used to make this conversion ($\times$ 40 for single 25 mg slides, $\times$ 20 for duplicate slides) may appear rather arbitrary. But our results demonstrate that, for operational purposes, these factors are appropriate and convenient. Other, strictly speaking more correct,

factors indeed complicate calculations and have a direct effect only on the geometric mean egg load (Table 3), a parameter which is also subject to other biases (Engels *et al.* 1996; Fulford 1994). For community diagnosis, the differences induced by the use of different conversion factors are unlikely to have a major influence on operational decisions.

Apart from the influence of sample weight on group parameters of infection, we also investigated whether the variation in egg count at the individual level was directly related to the variation in weight of the examined stool sample. If this were true, one would expect similar ratios for both in the duplicate series of slides prepared by different laboratory assistants and the individual points in Figure 2 would be distributed along the 1/1 line. This was clearly not the case. No clear relation could be demonstrated between the ratios of the egg counts and the ratios of the sample weights; the correlation, both linear and non-parametric, was very poor. The same figure further shows that the variability in egg counts far outweighs the variability in sample weight. We therefore conclude that sample weight fluctuations when using the Kato–Katz method can be considerable, but account for only a minimal part of the important egg count fluctuations which are generally observed.

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