

INTRASPECIMEN FECAL EGG COUNT VARIATION IN *SCHISTOSOMA MANSONI* INFECTION

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Abstract. To determine the degree of intraspecimen fecal egg count variation in *Schistosoma mansoni* infection and its impact on commonly used parasitologic parameters obtained by single egg counts, 10 25-mg Kato-Katz slides were prepared from each of three stool specimens collected on different days in a study group of 20 infected people. Individual fecal egg counts in these series of examinations varied considerably and this had profound consequences for the reliability of both qualitative and quantitative diagnosis. In light infections, *S. mansoni* eggs in stools appeared to be homogeneously mixed. However, this distribution became heterogeneous as the intensity of infection increased, indicating clustering of eggs in stool. The cumulative egg counts in the 10 slides of the same 20 people examined in this study were compared with those in 14 slides prepared from seven stool samples collected on different days. This revealed significantly different mean egg counts for six people, even after such exhaustive series of examinations. Intraspecimen variation also biased considerably some operational parameters used to determine the infection status at the group level, particularly when these were determined by the examination of single 25-mg slides. The examination of duplicate or multiple slides improved the intraspecimen estimates of these parameters but did not overcome day-to-day variation. The examination of fewer samples taken on different days proved to be more adequate than examining more slides from one stool specimen for the determination of precise estimates of the real infection status.

Over the past decades, methods for the field diagnosis of *Schistosoma mansoni* and intestinal helminth infections have much improved. The Kato-Katz method¹⁻⁴ has been an especially crucial break-through in this respect. However, the accuracy of an on-the-spot diagnosis of these infections in a single stool specimen is reduced by the important variation in egg counts between different stool specimens from one person.⁵⁻¹³ Recently, we evaluated the day-to-day fluctuation in *S. mansoni* egg counts (interspecimen variation) in Burundi and their diagnostic and operational implications.¹⁴ In the current study, we compare these results with the variation of fecal egg counts within single stool specimens (intraspecimen variations).

SUBJECTS, MATERIALS, AND METHODS

A group of 20 people (10 adults and 10 children, up to 16 years old) infected with *S. mansoni* were asked to provide whole morning stools on three occasions. This group of people was a subsample of 200 subjects who participated in a study on day-to-day egg count fluctuations,¹⁴ in which duplicate 25-mg Kato slides were examined on seven occasions (days 1, 3, 5, 8, 10, 32, and 37). The whole stool samples in the present study were collected on days 28, 31, and 35. The subgroup from which these extra specimens were taken was selected on basis of the egg count results obtained from the five previous examinations. In this way, four lightly infected people (having an average of 1-100 eggs per gram [epg] of feces in the first five examinations), nine moderately infected (101-400 epg), and seven heavily infected (> 400 epg) were selected to make up the subsample. After having been duly informed, all selected people volunteered to participate in this study. For children, consent was obtained from their parents. The study was approved by the Ethical Commission within the Burundi Ministry of Health.

The whole stool specimens were collected on and covered with a cardboard tray and flattened by gentle pressure on the top tray. Ten 25-mg thick smears were prepared according

to the Kato-Katz method² from 10 randomly chosen sites in each individual stool specimen. These slides were numbered from 1 to 10 and were microscopically examined 45 min after preparation.⁴ To minimize interobserver variation bias, each of the five participating microscopists examined all slides of the same subjects throughout the study.

Final classification of individuals in egg output categories in this study was done on basis of the arithmetic mean egg count in all examined slides, which was converted into eggs per gram. Categories used were 1-100 epg (light infections), 101-400 epg (moderate infections), and > 400 epg (heavy infections). For group means, the geometric mean egg count was used and calculated as the antilog (arithmetic mean log [epg of positive individuals]).

Differences between individual (arithmetic) mean egg counts were tested with a *t*-test accounting for unequal variances and sample sizes.¹⁵ The Pearson's coefficient of correlation was used to compare egg counts between the two 25-mg components of duplicate slides. To test the operational value of different variants of the Kato-Katz method, the accuracy of their estimations of the number of people classified in the different egg count categories was compared with the overall results obtained after all 30 examinations. The Kappa value (K), which measured the degree of non-random agreement, was used for this purpose. This agreement measure between two sets of observations is defined as the ratio of the agreement effectively obtained (i.e., the observed percentage of identical classification in groups minus the percentage of agreement expected purely by chance) over the maximum obtainable agreement (i.e., 100 minus the percentage of agreement expected purely by chance). It has a maximum value of 1.00 when agreement is perfect, while zero indicates no agreement better than chance. The different variants of the Kato-Katz method tested in this way were single slide examinations (1 × 25 mg) from one stool specimen, duplicate examinations (2 × 25 mg) from one stool specimen, six examinations (6 × 25 mg) from one stool specimen, single examinations from three stool specimens col-

TABLE 1

Individual variation in egg counts in each of the three series of ten 25-mg Kato-Katz slides from one stool specimen, total mean values of all three series and of a series of seven stool examinations evaluating the between-days egg count variation

Patient			Day 28			Day 31			Day 35			Total		Mean* (eggs/g) after seven double slides on different days
			Mean* (eggs/slide)	Min-max† (eggs/slide)	D‡	Mean* (eggs/slide)	Min-max† (eggs/slide)	D‡	Mean* (eggs/slide)	Min-max† (eggs/slide)	D‡	Mean* (eggs/slide)	(eggs/g)	
1	25	F	0.5	0-2	1.44	0.4	0-3	2.33	0.9	0-3	1.35	0.6	24.0	20.0
2	12	F	0.4	0-1	0.67	0.9	0-2	0.60	0.5	0-3	1.89	0.6	24.0	1,031.4§
3	60	F	0.8	0-3	1.33	0.3	0-2	1.52	1.1	0-4	1.91	0.7	29.3	34.3
4	11	F	1.0	0-4	1.78	0.3	0-2	1.52	3.4	1-10	2.17	1.6	62.7	85.7
5	10	M	0.5	0-2	1.00	2.3	0-5	1.07	2.0	1-5	0.89	1.6	64.0	14.3§
6	30	M	0.7	0-2	0.97	0.8	0-3	1.33	3.9	0-10	1.85	1.8	72.0	485.7§
7	25	M	1.8	0-4	1.09	2.8	0-9	2.92	2.2	0-4	0.59	2.3	90.7	208.6
8	52	M	3.4	0-7	1.78	2.9	1-6	0.88	1.5	0-4	0.93	2.6	104.0	94.3
9	12	M	0.8	0-2	0.78	9.3	3-16	2.01	0.5	0-2	1.00	3.5	141.3	414.3§
10	25	F	1.5	0-4	1.07	6.3	1-12	1.94	3.9	1-5	0.48	3.9	156.0	145.7
11	18	M	3.7	0-16	5.65	5.9	0-16	5.74	2.4	0-5	1.22	4.0	160.0	171.4
12	30	F	1.2	0-6	2.74	8.9	1-21	3.86	3.2	0-7	1.24	4.4	177.3	222.9
13	10	F	2.5	0-6	1.80	7.8	2-14	2.05	4.6	1-11	2.18	5.0	198.7	348.6
14	8	F	4.5	1-15	3.67	6.7	2-14	2.12	8.5	3-14	1.18	6.6	262.7	151.4¶
15	30	F	11.1	5-17	1.19	4.8	1-7	0.64	7.7	5-11	0.49	7.9	314.7	140.0§
16	36	F	7.0	1-13	1.78	-	-	-	12.7	7-23	2.66	9.9	394.0	271.4
17	10	F	15.1	5-29	2.73	2.9	0-9	4.25	17.5	7-26	2.05	11.8	473.3	897.1
18	13	M	16.8	7-31	3.86	27.3	19-41	2.14	15.8	17-37	1.58	23.3	932.0	1,494.3
19	6	M	7.2	1-21	4.44	53.4	16-86	12.39	56.8	10-124	36.17	39.1	1,565.3	1,325.7
20	9	M	33.3	6-56	7.17	29.9	15-52	3.67	83.1	33-156	11.89	48.8	1,950.7	1,708.6

* Arithmetic mean.

† Minimum-maximum.

‡ Index of dispersion = variance/mean.

§ $P < 0.01$.

¶ $P < 0.05$.

lected on different days ($3 \times 1 \times 25$ mg), and duplicate examinations also from three stool specimens collected on different days ($3 \times 2 \times 25$ mg). For each variant, randomly drawn combinations of slides were used to determine series of 30 classifications of the study group in egg count categories. For each of these classifications, the degree of agreement with the reference test was calculated.

Homogeneity of *S. mansoni* eggs in stools would correspond to a Poisson distribution whereby the variance = the mean. The index of dispersion ($D = \text{variance}/\text{mean}$) should therefore be equal to 1. Variance exceeding the mean is more indicative of a heterogeneous distribution of eggs (e.g., in case of a negative binomial distribution). To test a general relationship between the variance and the mean, regression analysis was performed for all 3×20 series of observations. For this regression analysis, a $\log_{10}$ transformation was first performed on both variance and mean to obtain approximately normal distributions. Since measurement variation has to be considered in both the dependent (log variance) and independent variables (log mean), Deming regression was used.¹⁶ We chose to minimize the perpendicular distance of each point to the regression line. In this way an equal weight was given to measurement error of both x- and y-values. For the statistical comparison of different regression models (linear and stepwise linear), the likelihood ratio test was used.¹⁷

RESULTS

Apart from one person on the second occasion, a full stool specimen was obtained on all three occasions from all 20 people participating in the study. The individual variation in

egg counts in each of the three series of 10 repeated measurements is summarized in Table 1 and compared with the total cumulative (arithmetic) mean egg output. The number of eggs found in a single 25-mg slide varied considerably. Also, 120 (20%) of the 590 examined slides were negative. This percentage was 43% among the lightly infected, 10% among the moderately infected, and 33% among the heavily infected people. In patient no. 1, who was the least infected, 20 of the 30 slides were negative. In this study group of 20 infected people, 14 subjects (70%) had at least one negative slide among the 30 examined. This figure was 100% (7 of 7) for the lightly infected (1-100 epg), 67% (6 of 9) for the moderately infected (101-400 epg), and 25% (1 of 4) for the heavily infected (> 400 epg) people.

Table 1 also shows for the same people the (arithmetic) mean egg output obtained after duplicate 25-mg Kato-Katz slides from seven other stools examined on different days in the larger fluctuation study.¹⁴ For six of them, the mean egg counts in the two studies were significantly different. The detailed individual egg counts of these people obtained in both studies are shown in Table 2. The variation in egg counts was particularly important in cases no. 2, 6, and 9.

Table 1 also shows the index of dispersion ($D = \text{variance}/\text{mean}$) for each of the 59 series of 10 egg counts. In 48 (81.4%) of them, it was greater than 1. Each of these series is graphically represented by its variance and mean in Figure 1. From the scattergram it is clear that the variance increases with increasing intensity of infection. The relative increase in variance also becomes more important at higher levels of the mean egg count. This is confirmed by the fact that to fit these data, stepwise linear Deming regression appeared to provide a better model than linear Deming regression. When

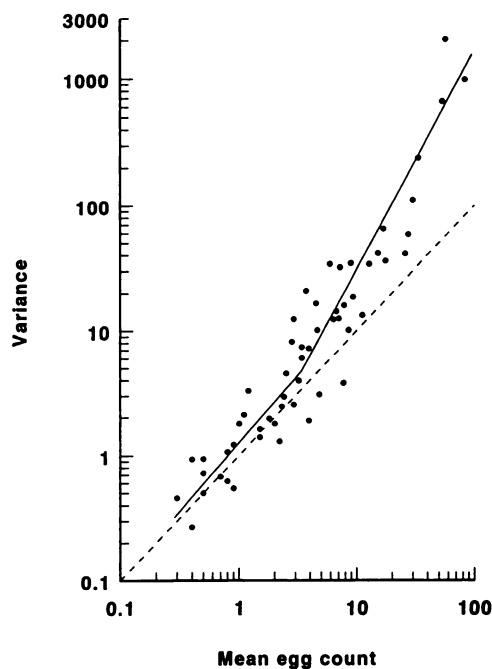


FIGURE 1. Scattergram of the variances and mean egg counts in the 59 series of 10 25-mg slide examinations. The dashed line indicates a variance = mean, which would correspond to a homogeneous distribution of eggs in stool. The solid line represents the combination of regression lines ($\log \text{variance} = a + b \times \log \text{mean}$) based on stepwise linear Deming regression with a breakpoint at $x = 3.4$ (median value of the mean egg counts) and with the following characteristics: intercept = 0.078; slope₁ = 1.09; slope₂ = 1.73. The regression line based on non-stepwise linear Deming regression (not shown) had the following characteristics: intercept = 0.034; slope = 1.45.

the median mean egg count (3.4 eggs/slide) was taken as a break point, the likelihood ratio test statistic, being the difference between $2 \times \log$ (maximum likelihood) of the large model (stepwise linear) and the one of the small model (linear) was 11.1 (degrees of freedom = 1, $P < 0.001$). This indicated a statistically significant difference in favor of the former model. In this model, the slope of the regression line for the D values less than the median mean egg count was 1.09 whereas it was 1.73 for the regression line above the breakpoint. The first part of the regression line is situated very closely to the 1/1 line. These individual values of the variance/mean ratio can therefore be considered compatible with a Poisson or homogeneous distribution of eggs in stool. The second part of the regression line deviates from the 1/1 line, which is more consistent with a heterogeneous distribution of eggs in stool. In reality, as can be seen from the scattergram, the relative increase in variance with increasing mean is a progressive phenomenon, indicating that the distribution of eggs in stool becomes more and more heterogeneous as the intensity of infection increases.

Figure 2 shows how the intraspecimen variation in egg counts can influence the percentage of people classified in each of the different egg count categories as well as the (group) geometric mean egg output. To allow comparison of these data in the three specimens, the individual who did not submit a stool specimen on the second occasion was ex-

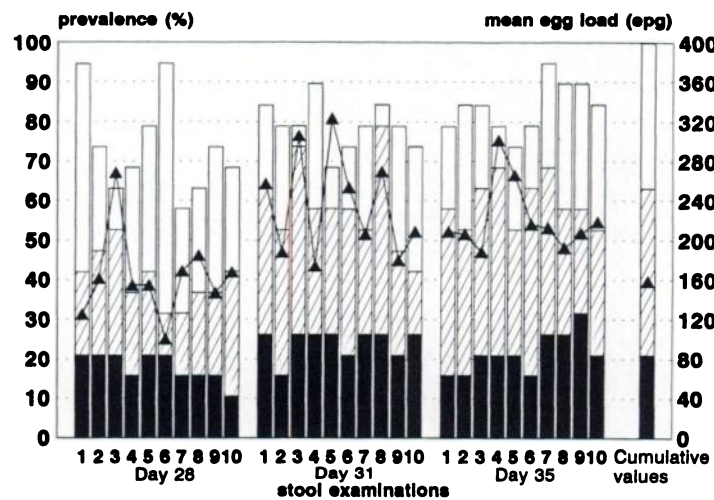
TABLE 2
Individual egg count values for the six people whose between-days and within-specimen (arithmetic) mean egg counts were significantly different

Patient			Egg count in each 25 mg slide on										
No.	Age (years)	Sex	Day 1*	Day 3*	Day 5*	Day 8*	Day 10*	Day 28†	Day 31†	Day 32*	Day 35†	Day 37*	
2	12	F	23-35	46-53	24-31	19-23	24-30	1-1-0-0-0-1-0-0-0-1	1-1-0-1-0-0-2-1-1-2	2-4	1-0-0-0-0-0-3-1-0	35-12	
5	10	M	1-1	1-0	0-0	0-0	1-0	1-0-0-0-1-0-1-2-0-0	4-3-3-3-0-1-2-5-1-1	0-0	1-1-3-5-3-1-2-1-1-2	1-0	
6	30	M	2-4	28-5	8-2	9-8	23-18	2-1-0-0-0-1-0-0-1-2	0-0-2-1-0-1-1-3-0-0	15-4	6-10-0-5-3-4-3-2-3	23-21	
9	12	M	10-18	5-3	12-13	2-5	17-17	1-2-0-0-1-2-1-0-0-1	13-10-12-3-16-5-4-12-7-11	20-18	0-0-1-2-0-0-1-0-1-0	3-2	
14	8	F	7-7	6-0	3-0	2-0	2-0	2-4-6-2-15-1-2-3-6-4	6-2-7-3-4-5-7-7-12-14	5-10	10-8-3-9-8-9-12-7-14-5	5-6	
15	30	F	5-9	3-2	3-1	2-0	11-3	14-12-5-6-12-14-11-10-17-10	7-5-6-4-3-6-5-5-6-1	10-0	8-9-5-9-7-5-6-8-11-9	0-0	

* Duplicate samples from a single stool specimen.

† Each number represents a subsample randomly selected from a single stool specimen (intra-stool specimen).

(a) Kato-Katz 25 mg



(b) Kato-Katz 2x25 mg

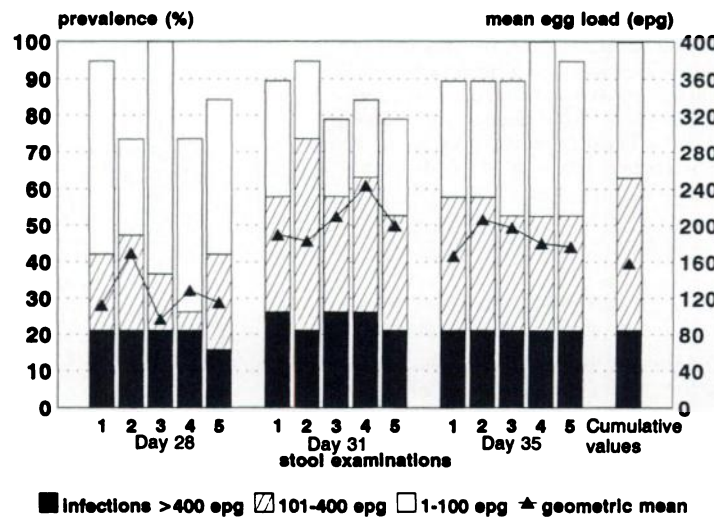


FIGURE 2. Ranges in a group of 19 people of prevalences of different egg output categories and geometric mean egg output determined by the examination of **a**, 10 25-mg Kato-Katz slides or **b**, five duplicate Kato-Katz slides (50 mg) prepared from each of three stool specimens collected on different days. epg = eggs per gram (of feces).

cluded from this analysis. This left the study group with 19 subjects.

Figure 2a shows these parameters as they were detected by single 25-mg slides. The total prevalence of infection varied from 58% (11 of 19) to 95% (18 of 19). The prevalence of light, moderate, and heavy infections varied from 5% (1 of 19) to 63% (12 of 19), from 11% (2 of 19) to 53% (10 of 19), and from 11% (2 of 19) to 32% (6 of 19), respectively. The geometric mean egg count varied from 101 epg to 323 epg. The cumulative values for all 30 examinations were 100% for the total prevalence; 37%, 42%, and 21% for, respectively, the prevalence of light, moderate, and heavy infections and 159 epg for the geometric mean egg count.

Figure 2b shows the same parameters, but determined by duplicate slides. Since the results of each two consecutive single slides in Figure 2a were combined for this purpose, this figure visualizes directly the effect on the diagnostic performance brought about by the examination of a greater quantity of stool. The prevalences of infection now varied from 74% (14 of 19) to 100% (19 of 19). The prevalence of light, moderate, and heavy infections varied, respectively, from 21% (4 of 19) to 63% (12 of 19), from 5% (1 of 19) to 53% (10 of 19), and from 16% (3 of 19) to 26% (5 of 19). The geometric mean egg count measured by duplicate slides varied from 97 epg to 244 epg. These latter figures are lower than those determined by single slides, which is due to a mathematical bias since more light infections are

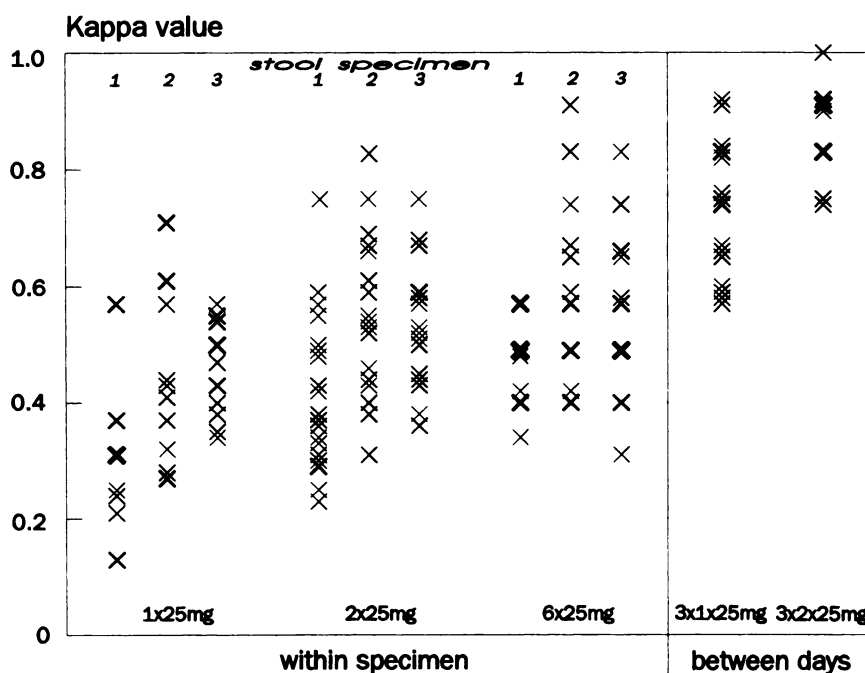


FIGURE 3. Variation in the degree of nonrandom agreement (Kappa) between the number of people classified in the different egg count categories by each of five quantitative variants of the Kato-Katz method and the cumulative outcome of all 30 25-mg slides. Each mark corresponds to a Kappa value relative to one classification of the study group in egg count categories, determined on basis of the egg count in a randomly drawn combination of slides. For each variant (and for each stool specimen in case of intraspecimen variants) a series of 30 such classifications was compared with the reference test. The thickness of the marks is related to the number of observations having resulted in that same Kappa value (graded from 1 to 11).

diagnosed with duplicate slides, decreasing the mean when it is calculated for positive subjects only. The intraspecimen variation in parameters was reduced by the examination of 50 mg of stool but important differences between specimens remained. The prevalences of light and moderate infections, for example, differed considerably on day 28 and on day 31. This is also reflected in the geometric mean egg count, which fluctuated within a different range in those two specimens. As a consequence, the ranges of values observed over the three specimens were not much smaller than those observed with single slides.

Figure 3 shows, again for the same group of 19 people, the variation in degree of nonrandom agreement (Kappa) between the number of people classified in the different egg count categories by the five quantitative variants of the Kato-Katz method and the reference test (cumulative outcome of all 30 slides). The degree of agreement was generally higher for duplicate slides than for single ones. Six slides on one stool specimen again performed slightly better, although the advantage was less marked. The Kappa values obtained by three single slides collected from specimens on different days were more precise. The examination of three duplicate slides collected from different specimens resulted in the highest Kappa values and the most narrow ranges.

The method that was used here to combine single slides into duplicate ones is different from what is done in routine surveys in which both duplicate slides are collected from one small (sub)specimen of stool. This is reflected in the coefficients of correlation between egg counts in the two 25-mg components, which varied from 0.48 to 0.99 in our 15 series of 19 duplicate slides. This is a wider range than

the 0.68–0.95 found in similar series of slides collected in a routine manner (Engels D, unpublished data). However, the degree of correlation in the two components of a duplicate slide did not influence the correlation between their combined egg count and the one in the reference test. It can therefore be assumed that the results presented here are also valid under routine circumstances.

DISCUSSION

This study shows that the intraspecimen variation of egg counts in *S. mansoni* infection is high, confirming the findings of Barreto and others.⁵ Also, the sensitivity of a qualitative diagnosis can be quite low, especially in lightly infected people. Indeed, despite the fact that all people in our study group were infected, a considerable proportion of all examined slides were negative, especially in this latter group of people. However, single slide examinations were able to detect most heavy infections. Since it is generally assumed that morbidity is related to intensity of infection, they may therefore be appropriate for interventions in which control of morbidity is the aim and only qualitative diagnosis is required. This is the case for the passive detection of cases in basic health services or for active screen-and-treat programs at the population level.

This study contradicts the findings previously described by other investigators,^{3, 18, 19} i.e., a homogeneous distribution of *S. mansoni* eggs in stools. According to our findings, this distribution, which can be considered homogeneous in the case of light infections, appears to become more and more heterogeneous as the intensity of infection increases. Al-

though our methodology was not specifically designed for this purpose, we can assume that our sampling was aleatoric enough for this conclusion to be of value. Such heterogeneity, suggesting clustering of eggs in stool, is likely to have profound consequences in the quantitative diagnosis of *S. mansoni* by means of a stool examination. Such clustering makes it necessary to examine multiple slides from one specimen to get a reliable idea of the individual infection status.

However, even the examination of 10 slides per specimen did not necessarily result in consistent mean egg counts in each of the specimens from three different days (Table 1). This further illustrates the relativity of such a quantitative diagnosis and points out the important day-to-day variation that has been discussed more in detail elsewhere.¹⁴ What is most remarkable is the fact that two fairly exhaustive approaches to determine accurate individual egg counts can still result in very different values. Indeed, 10 25-mg Kato-Katz slides on three different days and duplicate 25-mg slides on seven different days still resulted in significantly different mean values for about 30% of the people. Some of these people were even manifestly classified in a different egg output category according to each of the two approaches. The figures of case no. 2 in Table 2 are particularly remarkable in this respect. Because of the special attention that was paid during the study to avoid interchanges of people, we have every reason to believe the specimens came from one and the indicated person.

This suggests that the individual gold standard of intensity of infection is not even reached after seven or 10 repeated examinations and that a very exhaustive series of repeated examinations may be required to get a precise idea of a patient's true infection status. Such exhaustive series are not feasible in daily practice. At the group or population level statistical modeling and the development of predictive charts^{12, 13} could provide a solution to this problem. At the individual level, these findings plead in favor of the development of new diagnostic techniques more directly and consistently related to worm load, such as those based on antigen detection.²⁰

This study also demonstrates that intraspecimen variation can considerably bias operational parameters used to determine the infection status at group level, especially when they are obtained by single egg counts. Point infection, obtained by the examination of a single, 25-mg Kato-Katz slide, varied strongly, especially for light and moderate infections. Total prevalence was considerably underestimated. The use of duplicate slides made these figures more stable and provided better estimates of the total prevalence, but did not overcome day-to-day variation. This is probably the main reason why duplicate slides restricted to one specimen did not perform much better than single ones (Figure 3). Neither did the examination of six slides per specimen consistently improve the agreement with the reference test. The examination of three single slides from different specimens did overcome day-to-day variation and gave better and more precise estimates. These findings confirm in a more empirical way what has been stated by Barreto and others,⁵ i.e., that day-to-day egg count variation is more important than intraspecimen variation, but conclude beyond this that fewer examinations on different days should be preferred to more

examinations on the same specimen. These considerations have to be taken into account when precision is weighed against cost or operational advantages of multiple examinations of the same stool. The examination of duplicate slides from several specimens collected on different days gave the best estimates and appears to be the best compromise between accuracy and practical feasibility, as already stated elsewhere.¹⁴

As a general conclusion it can be stated that due to important intraspecimen and interspecimen variation, a parasitologic diagnosis made by a single stool examination is often not an accurate reflection of the real individual infection status and can also considerably bias parameters at the group level. However, single specimen examinations are able to detect most heavy infections and may therefore be acceptable for interventions aimed at the control of morbidity. Accurate classification of the real infection status requires the examination of exhaustive series of samples, which is beyond feasible limits in current practice. Therefore, additional statistical tools and/or new diagnostic methods need to be developed, if only for research purposes. When multiple stool samples are required, it must be taken into consideration that the examination of a few samples taken on different days provides better estimates of the individual infection status than the examination of many samples from one stool specimen.

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