Am. J. Trop. Med. Hyg., 54(4), 1996, pp. 319–324

DAY-TO-DAY EGG COUNT FLUCTUATION IN *SCHISTOSOMA MANSONI* INFECTION AND ITS OPERATIONAL IMPLICATIONS

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Abstract. In a study group of 183 people in a *Schistosoma mansoni*–endemic area in Burundi, stool examinations were performed with duplicate 25-mg Kato-Katz slides on seven occasions (days 1, 3, 5, 8, 10, 32, and 37). Point prevalences detected by single examinations of 25 mg and 50 mg of stool varied from 41.0% to 57.9% and from 55.7% to 63.9%, respectively. The cumulative prevalence for all seven measurements was 82.0%. The individual day-to-day variation in egg output was important. The majority of infections missed by the examination of single slides and specimens were light ones. The Kato-Katz method applied on a single stool specimen is more suitable for morbidity control, but less suitable for control of infection. When a precise quantitative diagnosis on the individual level is required, several measurements on different days are necessary. The data presented validate recently developed statistical models and charts predicting true prevalences.

The Kato-Katz method is the current standard method for the field diagnosis of intestinal schistosomiasis. However, use of a single stool examination can severely underestimate true *Schistosoma mansoni* prevalences.[1] As a quantitative measure of infection, helminth egg output can also show important variation.[2–8] The present study evaluates in detail the day-to-day variability in egg counts of *S. mansoni* and its consequences for the interpretation of individual and population-based results of fecal screening. The study was conducted in Burundi, a country where the majority of schistosomal infections are mild to moderate.[9]

MATERIALS AND METHODS

One hundred adults (20 or more years of age) and 100 children and adolescents (less than 20 years of age) were asked to produce stool specimens on seven occasions: five times within a period of two weeks (days 1-3-5-8-10), and another two times three weeks later (days 32 and 37). From each specimen, two 25 mg–thick smears were prepared according to a modified Kato-Katz method.[5,9,10] The two slides (A and B) were examined after 45 min[11] by different microscopists. A total of six microscopists participated in the study: three of them examined all A-slides and the other examined all B-slides. The same pair of microscopists examined the same slides of the same people throughout the study.

Classification in egg output categories was done on the basis of the total egg output detected in all examined slides of that individual, and output was converted into eggs per gram (epg) according to the amount of stool examined. Categories used were 1–100 epg (light infections), 101-400 epg (moderate infections), and > 400 epg (heavy infections). Where relevant, the latter two categories were combined (moderate and heavy infections, i.e., > 100 epg). Geometric mean egg loads were calculated in two commonly applied ways: 1) the antilog {arithmetic mean log (egg count/g of positive individuals)} and 2) the antilog {arithmetic mean log (egg count/g + 1 of all individuals)} − 1.

RESULTS

To allow strict comparison of the results on different days, all individuals with missing values were excluded from the analysis, leaving a study group of 183 people.

Figure 1 shows the operational performance of single and duplicate slides with regard to the detection of infections of gradually increasing intensity. In this figure, three non-mutually exclusive categories were used: all infections (> 0 epg), moderate and heavy infections (all infections > 100 epg), and heavy infections (all infections > 400 epg). The relationship between point prevalences of these categories and the cumulative values at each of the seven measurements shows that the examination of a single stool specimen, whether it be a single slide or duplicate 25-mg Kato-Katz slides, seriously underestimated the final cumulative prevalence. For moderate and heavy infections (> 100 epg) and heavy infections (> 400 epg), the advantages of repeated stool examinations were less marked.

Detailed figures of the different parameters more classically used to determine infection in a population are shown in Table 1. The point prevalence of infection detected by a single 25-mg slide ranged from 41.0% to 57.9%. The group of three microscopists examining the B-slides tended to detect fewer eggs than the ones examining the A-slides, resulting in generally lower values of the B-parameters, except for the cumulative prevalence of light infections (1–100 epg). These findings illustrate the possible influence of interobserver variation on group parameters of infection. The percentage of people with at least one positive egg count in all examined duplicate 25-mg slides increased from 63.9% on day 1 to 82.0% on day 37 (seventh count). Previously undiagnosed infections were detected up to the seventh (and last) egg count. Thirty subjects who were still negative on day 37 were selected for further study; four (13%) of them were found to be positive after three additional stool examinations with duplicate 25-mg slides (Engels D, unpublished data). Table 1 also shows that the geometric mean egg load of infected people calculated from single 25-mg slides was higher than when calculated from duplicate slides (50 mg of stool). Its cumulative value consistently decreased to 40–60% of the initial value on day 37. This is due to a mathematical bias as more light infections are diagnosed with duplicate slides, decreasing the mean when it is calculated for positive subjects only. The geometric mean egg load of all individuals (based on eggs counts + 1) showed an inverse and less paradoxical pattern, but with much lower figures.

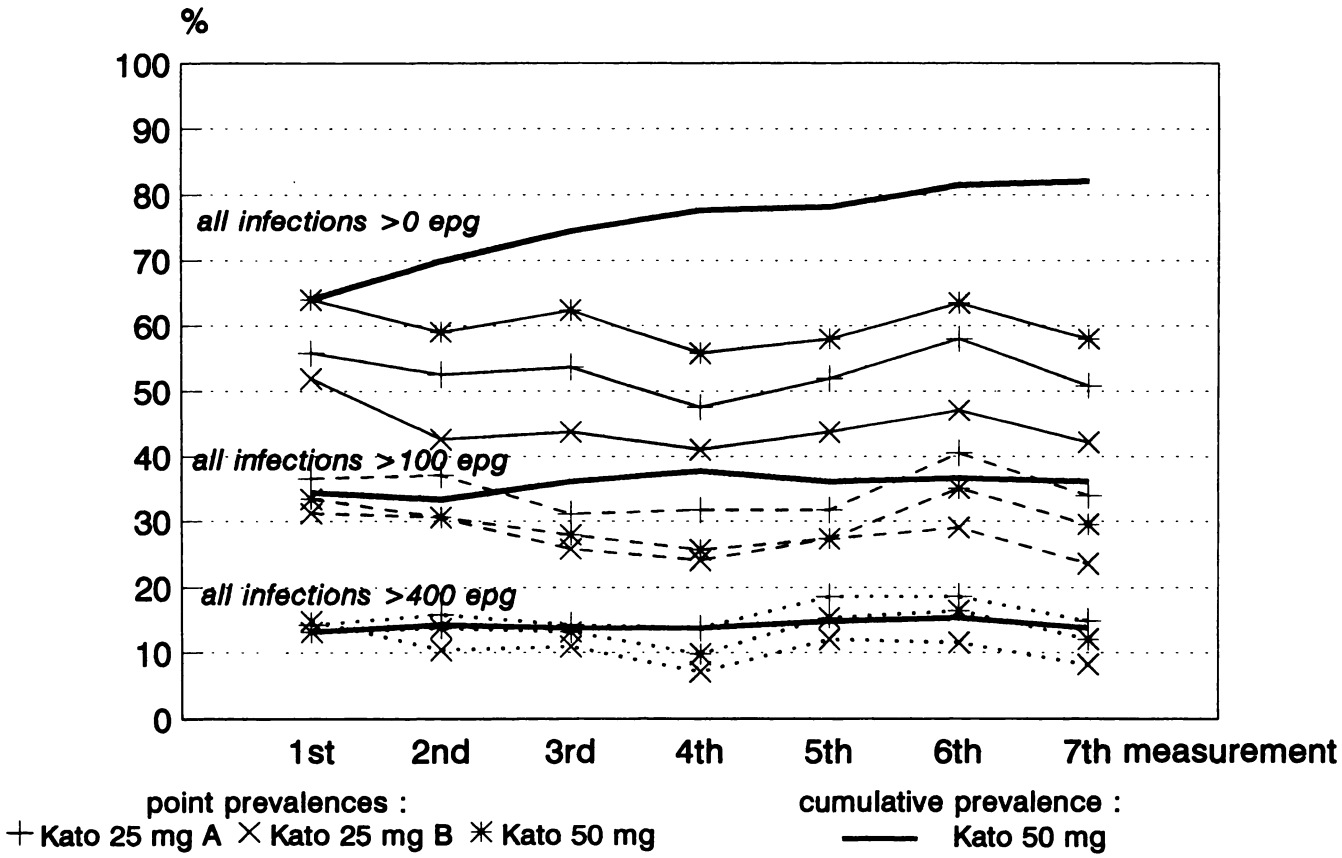

FIGURE 1. Point and cumulative prevalences of all infections (> 0 epg), moderate and heavy infections (> 100 epg), and heavy infections (> 400 epg) detected after each of seven repeated stool examinations using the Kato-Katz method in a study group of 183 people. epg = eggs per gram.

Day-to-day fluctuation of egg counts in individuals is partly due to the low concentration and uneven distribution of eggs in stools. It can thus be expected that such variation is less when a larger amount of stool is examined. Table 2, however, shows that the individual day-to-day variation in egg counts found in 50 mg of stool was only slightly less than in 25 mg.

The point prevalences and intensities of infection, detected with more elaborate quantitative variants of the Kato-Katz method, are shown in Table 3. The difference between the results of three and five duplicate slides seems to lie essentially in the figures of total prevalence and geometric mean egg load of all individuals.

A summary of the diagnostic performance of all discussed variant methods, as compared with the gold standard of the cumulative results is given in Table 4.

DISCUSSION

In this study, an on-the-spot diagnosis with a single 25-mg Kato-Katz slide on any of the seven examination days would have revealed only 50–71% of all the infections detected with our reference test (cumulative value of duplicate 25-mg Kato-Katz slides on the seven different days). With a double smear (50 mg), this would have been 68–78% (Table 4). Also, at the group level, the detected prevalence of infection varied considerably from day to day. Under operational circumstances, where the control for bias due to different microscopists is less systematic, these variations are probably much more important.

The statistical model described by De Vlas and Gryseels[1] and De Vlas and others[12, 13] and the pocket chart worked out by De Vlas and others,[14] which allows one to estimate a true prevalence in a population from figures obtained from a single specimen survey, predicts for our group a prevalence between 85% and 90%. We detected a prevalence of 82% for seven repeated measurements, but it was later proven that a few light infections were missed despite these repeated measurements. Our study can thus be interpreted as a positive validation of that statistical model and the ensuing pocket chart. Similar validation exercises in other areas should be encouraged because a universally applicable chart to estimate true prevalences from a single specimen survey would clearly be very useful.

Despite the relatively poor sensitivity of a single Kato-Katz slide, most of the undiagnosed infections were light ones (Table 4). Also, from a more operational point of view, Figure 1 confirms the limited advantage of repeated measurements to estimate prevalences of moderate and heavy (> 100 epg) and heavy (> 400 epg) infections. This confirms the adequacy of its use in large-scale operational screening programs aimed at the control of morbidity,[15, 16] and as the

TABLE 1

Day-to-day (point) and cumulative values of parameters currently used to determine infection in a population, detected after each of seven repeated stool examinations with single and duplicate 25-mg Kato-Katz slides in a study group of 183 people*

		Day 1		Day 3		Day 5		Day 8		Day 10		Day 32		Day 37	
		Slide A	Slide B	Slide A	Slide B	Slide A	Slide B	Slide A	Slide B	Slide A	Slide B	Slide A	Slide B	Slide A	Slide B
Single 25-mg slide															
Total prevalence (%)	Point	55.7	51.9	52.5	42.6	53.6	43.7	47.5	41.0	51.9	43.7	57.9	47.0	50.8	42.1
	Cumulative	55.7	51.9	64.5	59.6	71.6	63.4	73.2	67.8	75.4	69.9	80.3	72.1	81.4	73.2
Prevalence of infections 1–100 epg (%)	Point	19.1	20.8	15.3	12.0	22.4	18.0	15.8	16.9	20.2	16.4	17.5	18.0	16.9	18.6
	Cumulative	19.1	20.8	28.4	29.5	33.3	33.3	34.4	37.2	37.7	41.0	39.9	42.1	40.4	41.5
Prevalence of infections 101–400 epg (%)	Point	22.4	16.4	21.3	20.2	16.9	14.8	18.0	16.9	13.1	15.3	21.9	17.5	19.1	15.3
	Cumulative	22.4	16.4	20.8	15.8	23.0	16.4	23.5	18.6	22.4	17.5	24.0	18.0	25.1	20.2
Prevalence of infections >400 epg (%)	Point	14.2	14.8	15.8	10.4	14.2	10.9	13.7	7.1	18.6	12.0	18.6	11.5	14.8	8.2
	Cumulative	14.2	14.8	15.3	14.2	15.3	13.7	15.3	12.0	15.3	11.6	16.4	12.0	15.8	11.5
Geometric mean egg load (epg) of positive individuals†	Point	188.7	190.6	225.8	202.3	169.0	170.7	200.3	145.5	210.6	174.2	237.5	186.8	223.6	165.7
	Cumulative	188.7	190.6	152.9	139.8	119.1	117.9	108.8	95.6	103.5	88.2	98.5	84.8	98.5	80.6
Geometric mean egg load (epg) of all individuals‡	Point	17.7	14.3	16.3	8.7	14.6	8.5	11.5	6.7	15.1	8.6	22.8	10.7	14.8	7.6
	Cumulative	17.7	14.3	24.8	18.1	30.2	19.9	30.5	21.2	32.8	22.3	39.8	24.3	41.9	24.5
Duplicate 25-mg slides (50 mg)															
Total prevalence (%)	Point	63.9		59.0		62.3		55.7		57.9		63.4		57.9	
	Cumulative	63.9		69.9		74.3		77.6		78.1		81.4		82.0	
Prevalence of infections 1–100 epg (%)	Point	29.5		28.4		34.4		30.1		30.6		28.4		28.4	
	Cumulative	29.5		36.6		38.3		39.9		42.1		44.8		45.9	
Prevalence of infections 101–400 epg (%)	Point	21.3		16.9		14.8		15.8		12.0		18.6		17.5	
	Cumulative	21.3		19.1		22.4		24.0		21.3		21.3		22.4	
Prevalence of infections >400 epg (%)	Point	13.1		13.7		13.1		9.8		15.3		16.4		12.0	
	Cumulative	13.1		14.2		13.7		13.7		14.8		15.3		13.7	
Geometric mean egg load (epg) of positive individuals†	Point	137.0		141.2		111.0		117.9		137.0		149.9		130.3	
	Cumulative	137.0		114.4		96.5		81.5		83.1		80.6		79.8	
Geometric mean egg load (epg) of all individuals‡	Point	22.6		17.7		18.1		13.4		16.5		23.3		15.9	
	Cumulative	22.6		26.9		29.3		30.2		31.5		36.0		36.3	

* epg = eggs per gram (of feces).
† Antilog {arithmetic mean log (egg count/g)}.
‡ Antilog {arithmetic mean log (egg count/g + 1)} − 1.

TABLE 2

Measures of dispersion of the individual coefficients of variation in the number of eggs detected in each of seven repeated stool examinations with single and duplicate 25-mg Kato-Katz slides, among positive individuals in a study group of 183 people

		No. of positive individuals	Coefficient of variation (%)					Percentage of people with a coefficient of variation			
			Mean	Standard deviation	Minimum	Maximum	Median	0–60	61–120	121–180	>180
Single 25-mg slide	A*	149	121	64	33	245	105	17%	41%	24%	17%
	B*	134	128	60	28	245	116	9%	46%	26%	19%
Duplicate 25-mg slides (50 mg)		150	108	54	29	245	94	17%	50%	21%	12%

* A and B refer to two different 25-mg slides prepared from the same stool sample on each of the seven days, but examined by different microscopists.

diagnostic method of choice for basic health services.[17] In these services, a highly sensitive method for the diagnosis of early intestinal morbidity due to schistosomiasis is not required or possibly not even preferable. The detection of many light infections would increase the tendency of health personnel to attribute the presented symptoms to schistosomiasis when in fact they are more likely due to another etiology. In a primary health care context, the single 25-mg Kato-Katz slide, which can be examined within 1 hr after preparation, also provides a sensitive diagnosis for hookworm, a common and pathogenic intestinal helminth in many areas endemic for schistosomiasis.

For most epidemiologic surveys, we prefer to use duplicate slides, not only because of higher sensitivity, but also for the inherent control of interobserver errors. The further validation of recently developed mathematical models and predictive charts allowing investigators to infer true parasitologic parameters from single specimen surveys[12–14] will greatly enhance the value of such data.

In our study group, the individual variation in egg output was higher than that described in various other studies.[3, 18–20] A larger study group, more repeated examinations, and a methodology closer to that applicable to operational conditions are undoubtedly factors influencing these higher figures. Also, the day-to-day variation was almost as important in duplicate slides as in single ones. Therefore, in small-scale surveys conducted for specific research purposes, in which a more precise quantitative diagnosis on the individual level is required, repeated stool examinations are clearly necessary. This is not always easy for logistic and sociocultural reasons. Based on our data, the examination of three stool specimens (with duplicate 25 mg slides) at intervals of a few days appears to be a reasonable compromise between accuracy and practicability (77–88% correct classification in egg output categories). The examination of duplicate slides on five different days, which requires twice as long, only scored slightly better as far as the overall sensitivity is concerned (95% versus 90–91%, Table 4), and this advantage was obtained only by a more sensitive diagnosis of light infections.

As more light infections are diagnosed with increasingly sensitive diagnostic methods, the geometric mean egg load of positive individuals decreases (Table 1). This makes the interpretation of this parameter difficult as a measure of intensity of infection in a population. In our study population, the mean egg load of positive subjects based on seven egg counts was 79.8 epg, with 17% having heavy infections (> 400 epg) and 44% having moderate and heavy infections (> 100 epg). The calculation of the geometric mean egg load of all individuals increased as more examinations were performed, but remained unrealistically low and appears even less suitable for the estimation of the intensity of infection in a population. Problems with interpretation of geometric mean egg loads is further complicated after treatment, when most infected persons become negative or have low mean egg loads. Also, statistical comparison of differences in geometric means, already beyond standard methodology,[21] becomes a difficult matter in such circumstances. Therefore, we believe that the percentage of people classified in each of the different egg output categories is a more accurate and pragmatic way of assessing and monitoring the intensity of infection in a population.

The Kato-Katz method, because of its many practical advantages and good performance in diagnosing moderate and heavy infections, will remain the standard for the field diagnosis of intestinal schistosomiasis and a cornerstone in

TABLE 3

Point prevalences and intensities of infection detected during the study with more elaborate quantitative variants of the Kato-Katz method*

	Three duplicate 25-mg slides (150 mg)			Duplicate 25-mg slides	Duplicate 25-mg slides
	Days 1-3-5	Days 3-5-8	Days 5-8-10	Days 1-3-5-8-10 (250 mg)	Days 1-3-5-8-10-32-37 (350 mg)
Prevalence (%)	74.3	73.8	74.3	78.1	82.0
Prevalence of infections 1–100 epg (%)	38.3	39.3	43.2	42.1	45.9
Prevalence of infections 101–400 epg (%)	22.4	21.9	16.4	21.3	22.4
Prevalence of infections >400 epg (%)	13.7	12.6	14.8	14.8	13.7
Geometric mean egg load (epg) of positive individuals†	96.1	85.4	82.1	83.1	79.8
Geometric mean egg load (epg) of all individuals‡	29.4	26.2	26.1	31.5	36.4

* epg = eggs per gram (of feces).
† Antilog {arithmetic mean log (egg count/g)}.
‡ Antilog {arithmetic mean log (egg count/g + 1)} − 1.

TABLE 4

Performance of different quantitative variants of the Kato-Katz method, compared with the reference test of 350 mg (duplicate 25-mg slides on seven different days)*

	Single 25-mg slide	Double 25-mg slide (50 mg)	Double 25-mg slide on three different days† (150 mg)	Double 25-mg slide on five different days‡ (250 mg)
Sensitivity	50–71%	68–78%	90–91%	95%
Percentage of undiagnosed infections				
Light infections (1–100 epg)	79–98%	91–100%	100%	100%
Moderate infections (101–400 epg)	2–19%	0–9%	0%	0%
Heavy infections (>400 epg)	0–3%	0%	0%	0%
Percentage of correct classification in egg output categories§ (uninfected, light, moderate and heavy infections)	44–57%	55–69%	77–88%	87%
Percentage of misclassifieds classified in a category immediately adjacent to the correct one	81–96%	92–100%	100%	100%

* epg = eggs per gram (of feces).
† On days 1-3-5, 3-5-8, and 5-8-10, respectively.
‡ On days 1-3-5-8-10.
§ Determined by the total egg output in all seven duplicate 25-mg slides.

control efforts. However, it must be realized that this method lacks sensitivity for the detection of light infections. Therefore, it should be modulated and its use adapted to particular research or control purposes. Especially in hypoendemic or mesoendemic areas, selective treatment based on screening with the Kato-Katz method is in fact a kind of targeted control of morbidity due to heavy infections. Thus, the choice between selective population chemotherapy or indiscriminate mass chemotherapy is not one between two different approaches of morbidity control, but between morbidity control and infection control. In countries such as Burundi and probably many others where resources are limited, morbidity control remains the prime objective, and can be satisfactorily delivered by basic health services.[17] In such circumstances, active selective population chemotherapy, which is labor-intensive and difficult to sustain, should not be encouraged as a national strategy. On the other hand, where infection control is the major objective, mass chemotherapy should be preferred. Depending on local epidemiologic conditions, health priorities, and cost-efficiency considerations, the main strategic choices in schistosomiasis control are between primary health care–based case detection and blanket mass chemotherapy, not between selective and mass treatment.

Acknowledgments: We thank the microscopists A. Gakeme, J. Kanurwe, P. Ndarwarukanye, A. Njangwa, J. Muhutu, and S. Vyumvuhore and their team leader L. Nkulikiyinka for diligence during this study. Our thanks also go to the population of Gihungwe for collaboration and compliance in repeatedly supplying stool specimens.

Financial support: The Schistosomiasis Research and Control Program in Burundi receives financial support from the National Ministry of Health, the Belgian Technical Cooperation, the European Development Fund, and the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases (TDR).

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