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Comparison of filtration staining (Bell) and thick smear (Kato) for the detection and quantitation of *Schistosoma mansoni* eggs in faeces*

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Summary

We compare results of one Bell and one Kato-Katz examination performed on each of 315 stool specimens from residents in an area in north-eastern Brazil endemic for schistosomiasis mansoni. The prevalence of schistosome infection detected by the Bell technique was 76% and by the Kato-Katz technique was 63%. 81% (44/54) of the infections missed by a Kato-Katz smear were light infections (one to 50 epg range by Bell examination). Over-all, 55% (44/80) of stools in this egg count range by the Bell technique were negative on a single Kato-Katz smear. This implies that five Kato-Katz smears per stool would ensure a 95% probability ($0{\cdot}55^5 \times 100$) of detecting such light infections. However, a single Kato-Katz smear detected eggs in 97% (124/128) of stools with a Bell count >100 epg. For stools positive by both methods the egg counts per gram of stool were higher ($p<0{\cdot}001$) by Kato-Katz examination. Geometric mean egg counts for the infected population were 199 epg by the Kato-Katz and 92 epg by the Bell methods. 64% (59 v. 36) more persons were classified as heavily infected (>400epg) by the Kato-Katz method than by the Bell method. The differing measurements of schistosome infection obtained with the Bell and Kato-Katz methods must be considered when comparing data on morbidity-infection relationships.

Introduction

Faecal examination to detect and quantify schistosome infection is an integral requirement for epidemiological and clinical studies of schistosomiasis mansoni (WHO, 1980). However, few comparisons have been made between quantitative stool examination methods on populations large enough to allow statistically valid conclusions. In this study we compare the filtration-straining technique of BELL (1963) to the Katz modification (KATZ *et al.*, 1972) of the Kato thick-smear technique (KATO & MIURA, 1954), two methods which have been extensively used in the field. The two methods are compared with respect to sensitivity of detection of *Schistosoma mansoni* infection and to the results of the calculated egg counts per gram (epg) of stool. Also, by comparing the estimates of prevalence and intensity of schistosome infection obtained in a defined population by each method, we determined how the standard epidemiological indices obtained by the Bell technique related to the same indices obtained by the Kato-Katz technique. Finally, we determined what influence the choice of either faecal examination method would have on a control strategy based on selectively treating heavily infected individuals.

Methods

The study was conducted in a geographically defined rural community in North-East Brazil for which epidemiological studies of endemic schistosomiasis mansoni have been reported (LEHMAN *et al.*, 1976; SLEIGH *et al.*, 1981). A stool specimen was collected in April, 1977 from each of 315 people and examined once by each method. All age groups were included and 71·3% of the population resident in the study area in the Municipio of Castro Alves, Bahia, Brazil participated in the survey.

The original Bell filtration staining method (BELL, 1963) was modified as follows: we added merthiolate (SAPERO & LAWLESS, 1953) to the 8·0 ml of 10% formalin in which the 2 ml fresh stool sample was diluted and substituted Whatman No. 1 for Whatman No. 541 filter papers (LEHMAN *et al.*, 1976). For processing, one ml of preserved stool suspension was homogenized in 30 ml of water, then strained in equal parts under suction through 500μ mesh wire gauze on to two filter papers. The area of the filter papers used (diameter 5·5 cm) was slightly greater than one-half that of the 7 cm diameter filter employed by Bell. Thus the total amount of faeces examined per person (0·2 ml or 200 mg) and the amount strained per square cm of filter paper were similar to the amounts described by Bell. Although Whatman No. 1 filters have a smaller pore size than No. 541 filters (11μ versus 24μ), they did not clog during filtering. The egg count per gram of stool was obtained by averaging the counts of the two papers and multiplying by ten.

One Kato-Katz thick smear was prepared from each stool sample in the field using a commercial kit (Boehringer Mannheim Bioquimica S.A., Rio de Janeiro, Brazil). The method is identical to that described by KATZ *et al.* (1972) except that cardboard templates are replaced by plastic ones with a slightly smaller hole which delivers a mean weight of 41·7† mg of faeces per slide and the stainless steel screens are replaced by nylon screens. The egg count per gram was obtained by multiplying the slide count by 24.

All stool samples were preserved and thick smears prepared within four hours of collection of the specimens. The filter papers and thick smears were counted for *S. mansoni* eggs by one of the two observers (JSP and AS) between one and 30 days after

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†The manufacturer states that the egg count per smear multiplied by 24 gives the egg count per gram. Therefore, $1{,}000 \div 24 = 41{\cdot}7$.

preparation. Inter-observer concordance was established by comparing readings on 40 stools examined in duplicate.

Results

Sensitivity

Significantly more stools were positive by the Bell than by Kato-Katz examination (Table I). Because of the superior sensitivity of the Bell technique, the examined prevalence of infection and the predictive value of a negative examination were greater than with the Kato-Katz technique (Table II). The sensitivity of the Kato-Katz method was directly proportional to the intensity of infection; Kato-Katz examinations were positive in 45% (36/80), 80% (24/30) and 97% (89/92) respectively of stools with a Bell count range of one to 50, 51 to 100, and 101 to 400 epg (Table III). As the probability of missing an infection in the Bell count range of one to 50 epg by a single Kato-Katz smear was 0·55 (44/80) or 55%, the probability of missing such light infections should be reduced to $0{\cdot}55^5$ or 5% by examining up to four more smears on persons initially negative.

Egg Counts

The arithmetic and geometric mean egg counts for Kato-Katz positive individuals are more than double the means for Bell positive individuals (Table IV). Comparison of the logarithms of the geometric means shows this difference to be highly significant ($t = 5{\cdot}409$, d.f. 434, $p<0{\cdot}001$).

Table I—Comparative sensitivity of Bell and Kato-Katz methods

		Kato-Katz (eggs detected) +	−	Total
Bell (eggs detected)	+	184	54	238
	—	14	63	77
	Total	198	117	315

χ^2, 1 df, (paired analysis) = 22·37
$p<0{\cdot}001$

Table II—Efficiency of Bell and Kato-Katz methods for detecting schistosome eggs

Method	No. of examinations*			Indicators of test efficiency		
	observed positive (a)	observed negative (b)	known missed positive (c)	prevalence estimated $(\frac{a}{a+b} \times 100)$	Predictive value of a negative result $(\frac{b-c}{b} \times 100)$	over-all sensitivity $(\frac{a}{a+c} \times 100)$
Bell	238	77	14	76	82	94
Kato-Katz	198	117	54	63	54	79
Combined Result	252	63	—	80	—	—

*Total = a + b = 315

Table III—Comparison of schistosome egg counts for Bell and Kato-Katz stool examinations

Bell (eggs per gram)	Kato-Katz (eggs per gram) 0	1-50	51-100	101-400	401-800	>800	Total
0	63	7	6	1	0	0	77
1-50	44	12	13	10	1	0	80
51-100	6	6	5	11	1	1	30
101-400	3	12	7	38	21	11	92
401-800	1	1	1	7	7	7	24
>800	0	0	0	2	0	10	12
Total	117	38	32	69	30	29	315

Table IV—Estimates of mean intensity of *S. mansoni* infection obtained by the Bell and Kato-Katz methods

Mean egg count*	Method used Bell	Kato-Katz
Arithmetic ± SEM (epg)	216 ± 20·4	506 ± 75·8
Geometric (epg) ± SEM ($\log_{10}$ values)	92 ± 0·0451	199 ± 0·0409

*Infected persons only. N = 238 and 198 for Bell and Kato-Katz means respectively

Table V—Comparison of proportion of individuals identified with high egg counts by Bell and Kato-Katz methods

		Kato-Katz (eggs per gram) ≤400	>400	Total
Bell (eggs per gram)	≤400	244	35	279
	>400	12	24	36
	Total	256	59	315

χ^2 (paired analysis) = 10·30
$p<0·01$
1df.

However, the lower Bell mean in the population could be the result of weighting by the 44 individuals whose Bell counts were low (one to 50 epg) and whose Kato-Katz counts were zero. These individuals were excluded from the calculations of the Kato-Katz mean (Table III). To avoid this bias we log-transformed the egg counts of the 184 individuals positive by both tests and then subtracted each transformed Bell count from the paired transformed Kato-Katz count. The mean difference showed that egg counts were still significantly higher for the Kato-Katz method ($t = 5·337$, d.f. 183, $p<0·001$). This difference is most evident when the Kato-Katz counts are over 400 (Table III). When individuals are classified into those passing ≤400 and >400 epg of stool the two tests differed significantly ($p<0·01$) and classified 15% (47/315) of the persons examined inconsistently (Table V). With the Kato-Katz method 64% more individuals (59 v. 36) were classified as passing more than 400 epg of stool than were identified by the Bell method.

Discussion

The paradox of lower sensitivity and higher mean egg counts by the Kato-Katz method could be explained as follows. The Bell method examines more faeces (200 mg v. 41·7 mg) and the eggs are easier to distinguish than on a Kato-Katz smear, critical factors when egg counts are low. On the other hand, at higher egg counts the small concentration factor of 3 to 38% (KATZ *et al.*, 1970; TEESDALE & AMIN, 1976a, b; KNIGHT *et al.*, 1976; JORDAN *et al.*, 1981) introduced by forcing the faeces through the nylon screen could contribute to higher Kato-Katz counts. The lower Bell counts could result from egg loss during processing, but this would have to be selectively operative when eggs were numerous in order to also fit our findings of superior Bell sensitivity at low counts. BELL (1963) originally concluded that eggs were not retained by the sieve through which the diluted stools were strained on to the filter paper. However, KATZ & CHAIA (1968), using a modification of the Bell technique, recovered only 19·6 to 22·0% of schistosome eggs added in concentrations of 3,000 to 10,000 epg of uninfected stool, although these findings were based on very few observations.

MARTIN & BEAVER (1968) and TEESDALE & AMIN (1976a), using other modifications of the Kato technique, reported that Kato counts tended to be higher than Bell counts. CHAIA *et al.* (1968) observed much higher counts with Martin's quantitative Kato method than with the Bell method; however, the Bell counts reported by them may have been low because of failure to include a fixative in the stool diluent before subsampling and examination. For the Kato-Katz smears a relatively small faecal sample, often obtained from the stool surface, was strained and subsampled for direct examination. These considerations would lead one to expect higher Kato-Katz counts if eggs are more numerous on the stool surface. On the other hand, if the egg distribution is non-random, but not with more surface eggs, the homogenization step would favour egg detection by the Bell method. Unfortunately, as the actual distribution of schistosome eggs in stools remains controversial after 50 years of study (KATZ & CHAIA, 1968; MARTIN & BEAVER, 1968; TEESDALE & AMIN, 1976a; CHAIA *et al.*, 1968; KHALIL & SALEN EL DIN, 1930; MARTIN, 1965; BLAIRE *et al.*, 1968; SIONGOK *et al.*, 1976; WOODSTOCK *et al.*, 1971) we cannot determine how sampling procedures may have influenced the detection and quantitation of *S. mansoni* eggs.

We and others (JORDAN *et al.*, 1981) have shown that the Bell technique identifies many more light infections than the Kato-Katz technique. This would be an important consideration for investigations such as incidence and post-treatment follow-up studies which require individual accuracy. However, for prevalence estimation there is an alternative approach. The mean intensity of *S. mansoni* infection in an endemic area tends to increase as the prevalence rises (KNIGHT *et al.*, 1976) implying that the sensitivity of the Kato-Katz test, which we showed to vary with the intensity of infection, also varies with the prevalence. JORDAN *et al.* (1975) used multiple qualitative stool examinations to detect *S. mansoni* infection in eight communities with prevalences ranging from 16 to 79% and demonstrated that the sensitivity of the method did vary with the prevalence of infection. Using polynomial regression they could relate the prevalence rates based on one stool examination per person ("one stool prevalence") to the rates based on multiple examinations ("true prevalence"). A similar approach applied to Kato-Katz data should eliminate the need for multiple examinations when attempting to estimate prevalence accurately. Then, because of its simplicity, lower cost and rapidity the Kato-Katz technique would be particularly appropriate for large scale surveys and if used consistently in repeated surveys would allow reliable comparison of results.

The greater number of individuals observed to be heavily infected (> 400 epg) by the Kato-Katz method as compared to the Bell method is important when considering a control strategy of selective chemotherapy of heavily infected persons. To selectively detect heavily infected individuals (e.g. ≥ 400 e.p.g.) the volume of stool examined by the Kato-Katz method might be decreased so that the results would be positive for all individuals at or above this infection level and negative for most other persons. The smears would clear faster (PETERS *et al.*, 1980) and counting would become unnecessary.

A mathematical formula relating Kato-Katz egg counts to Bell egg counts would be helpful when correlating morbidity and infection as assessed by the two methods of stool examination. However, neither linear nor polynomial regression of the raw or log-transformed paired counts could satisfactorily relate the counts obtained by the two methods. We conclude that the Bell and Kato-Katz methods give quite different assessments of the prevalence and the intensity of *S. mansoni* infection and this could confuse attempts to compare epidemiological data when such dissimilar methods of faecal examination have been used.

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