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Stability of faecal egg excretion in *Schistosoma mansoni* infection*

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Summary

Stability of *Schistosoma mansoni* egg excretion was studied in 23 residents of a rural endemic area in North-east Brazil where the over-all prevalence rate was 87% and the peak geometric mean egg excretion was 308 eggs/ml (Bell method) in the 10 to 14-year-old age group. Stool examinations by the Kato method were performed for three to four consecutive days each month for three consecutive months. Both raw and transformed data showed significant stability of *S. mansoni* egg excretion from day to day and month to month in this population. A single Kato examination detected 68% of individuals who were excreting more than 400 eggs per gramme. Although *S. mansoni* egg excretion is stable over time, identifying individuals with high egg excretion in endemic populations requires sensitive quantitative methods.

Introduction

In studies on Manson's schistosomiasis, quantitative egg counts are now widely used, and levels of egg excretion are utilized as an index of the intensity of infection. CHEEVER'S (1968) post-mortem study in Brazil demonstrated unequivocally that the worm load was a factor which correlated directly with the severity of the disease due to infection with *Schistosoma mansoni*.

Subsequent investigations performed in various endemic areas have repeatedly shown a relationship between faecal egg counts and the morbid consequences of the infection. KLOETZEL (1974) has proposed that a minimum egg count be a criterion to determine the use of "selective and preventive chemotherapy" in ages at maximal risk. This thesis has been elaborated by WARREN & MAHMOUD (1976) and they suggested that rapid quantitative techniques be deployed in the field to identify those heavily infected individuals at risk of developing severe disease, thus qualifying for "targeted mass treatment".

Confidence in the use of such strategies assumes that faecal egg excretion is reasonably stable and that heavily and lightly infected persons can be identified and segregated on the basis of one or two parasitological examinations. MCCULLOUGH & BRADLEY (1973) elegantly demonstrated such stability in *S. haematobium* infections in Tanzania, but few data on longitudinal egg excretion in infections with *S. mansoni* are available. The present investigation employed an egg-counting technique appropriate for use in the field and examined short term variation in *S. mansoni* egg excretion by individuals living in an endemic area.

Population and methods

Study Area and Population

The individuals studied came from a rural population in north-east Brazil being followed longitudinally for morbidity due to schistosomiasis mansoni and Chagas's disease. The study area is located in the municipio (county) of Castro Alves, State of Bahia, and is highly endemic for schistosomiasis and Chagas's disease. The area and the epidemiological methods employed have been described (LEHMAN *et al.*, 1976; MOTT *et al.*, 1976).

A sub-population was chosen in an area which in 1974 contained 120 individuals with an over-all prevalence of *S. mansoni* infection of 87%; the 10 to 14-year-old age group had a peak geometric mean egg excretion of 308 eggs/ml as calculated by the Bell method (BELL, 1963) of faeces. In 1976, 23 infected individuals were selected on the basis of previous egg counts using a table of random numbers with the objective of studying persons with high, medium and low levels of egg excretion. Each individual entering the study agreed to provide stool specimens on three consecutive days for each of three consecutive months (August, September and October of 1976). In instances where fewer than three stools were provided in a three-day period, a fourth day was added to obtain

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Table I—Individual characteristics of 23 individuals according to age, sex, number of Kato examinations performed over 3 months and analysis of *S. mansoni* egg count data for the entire period. Individuals are listed according to increasing mean counts.

Individual No.	Age	Sex	No. of Kato slides	Mean egg count	Standard deviation	Coef. of variation	Median count	Minimum count	Maximum count
1	67	F	16	5	13·1	290	0	0	48
2	47	M	18	32	31·9	100	24	0	96
3	69	F	16	57	42·8	75	48	0	168
4	51	M	18	65	47·1	72	48	0	168
5	41	F	18	180	103·9	58	168	48	504
6	8	F	16	194	172·9	89	168	0	576
7	25	F	18	277	155·8	56	264	72	720
8	13	F	18	367	225·7	62	324	24	960
9	23	M	18	372	277·0	75	324	96	1272
10	58	F	18	385	165·6	43	408	48	624
11	30	M	18	400	249·6	62	300	96	984
12	35	F	18	416	100·5	24	432	264	648
13	18	F	18	432	179·8	41	432	96	720
14	16	F	10	698	512·9	73	696	144	1560
15	29	M	18	704	428·4	61	564	168	1512
16	19	F	16	834	738·5	89	540	0	2400
17	31	F	18	927	666·6	72	780	96	2428
18	15	M	18	976	554·8	57	948	0	2304
19	78	F	14	977	363·2	37	996	360	1608
20	36	F	18	1153	335·9	29	1116	648	1800
21	11	F	18	1348	767·0	57	1248	432	3264
22	13	M	16	1505	633·9	42	1440	648	3168
23	13	F	14	2103	1699·2	81	1704	432	5232

additional samples. In general, compliance was excellent; two of us (M.L.B. and J.T.F.S.) supervized stool collections in the field.

Method of Stool Examination

The Kato egg count method (MARTIN & BEAVER, 1968) as modified by KATZ *et al.* (1972), for which a kit is available commercially (KIT-AK, AK Industria e Comercio Ltda., Belo Horizonte, Minas Gerais, Brazil), was utilized. In this method, faeces are strained through nylon gauze to remove debris and then placed in a plastic template which delivers a fixed stool volume (about 40 mg) on to a glass slide. The sample is covered with a cellophane coverslip (22 × 33 mm) impregnated with an aqueous solution of glycerol and malachite green. Slides are inverted and pressed on to a bed of absorbent paper, turned face up and allowed to clear for at least six hours. Slides are examined microscopically using a magnification of 100×. The number of *S. mansoni* eggs per slide is multiplied by a factor of 24 to obtain the number of eggs per gramme of faeces. All slides were read within 30 days of collection. Two slides were prepared from each faecal specimen and read independently. All slides were examined by one observer (M.L.B.) who randomly re-read a subsample to examine concordance with the first reading.

Statistical Methods

All data were tabulated, and the means and variances of egg counts for individuals were calculated on the basis of the number of slides read. In order to render the variances independent of the means and to achieve normal distributions, Taylor's power law (TAYLOR, 1961) was used and the appropriate transformation performed. Subsequently, standard statistical methods, such as correlation coefficient and Student's *t* test, were used to compare grouped data. In general, the treatment of data followed that of MCCULLOUGH & BRADLEY (1973), as the objectives of our work on *S. mansoni* egg excretion and their investigations on *S. haematobium* egg excretion were similar.

Results

Table I presents a summary of the grouped data accumulated for each individual during the study, based on the number of slides counted. Within this population there was a spectrum of mean egg counts, ranging from four to 2,103 eggs per gramme. The individual mean and median egg counts coincided reasonably well, indicating that the distribution of egg counts for each individual was nearly symmetrical around the mean. The coefficients of variation (standard deviation/mean × 100) within the sample demonstrated considerable individual variation in egg counts throughout the study period, and the average coefficient of variation was around 70%. For many persons, there was a considerable spread between minimum and maximum egg counts.

The mean, standard deviation and coefficient of variation for each individual by month are shown

Table II—Monthly *S. mansoni* mean egg counts in 23 individuals. Standard deviation and coefficient of variation of the egg counts are given. The order is the same as in Table I.

	AUGUST				SEPTEMBER				OCTOBER			
Individual No.	No. of slides	Mean egg count	Stand dev.	Coef. of var.	No. of slides	Mean egg count	Stand. dev.	Coef. of var.	No. of slides	Mean egg count	Stand. dev.	Coef. of var.
1	6	8	19·6	245	4	4	9·8	245	6	4	9·8	245
2	6	12	20·1	167	6	60	25·2	42	6	24	30·4	127
3	6	56	62·0	111	6	48	33·9	71	4	72	19·6	27
4	6	76	49·0	64	6	78	51·3	68	6	44	41·3	94
5	6	248	135·5	55	6	164	70·2	43	6	128	65·6	51
6	6	220	204·4	93	6	116	100·0	86	4	270	205·0	76
7	6	180	77·0	43	6	360	218·9	84	6	292	94·1	32
8	6	504	254·9	51	6	296	251·1	85	6	300	106·0	35
9	6	444	133·0	30	6	496	410·9	83	6	176	60·1	34
10	6	496	99·1	20	6	248	165·4	67	6	412	133·6	32
11	6	320	124·9	39	6	456	394·4	77	6	424	248·8	59
12	6	440	119·2	27	6	360	94·8	26	6	448	73·8	16
13	6	388	71·9	19	6	384	232·2	61	6	524	151·4	29
14	4	192	65·0	34	6	1036	359·3	35	—	—	—	—
15	6	876	421·9	48	6	860	435·6	51	6	376	249·7	66
16	6	480	452·6	94	4	1836	492·8	27	6	520	462·1	89
17	6	736	538·7	73	6	1088	967·0	89	6	956	455·2	48
18	6	548	507·4	93	6	1324	576·4	44	6	1048	276·0	26
19	6	748	250·1	33	2	672	135·8	20	6	1308	212·4	16
20	6	1456	283·0	19	6	948	236·5	25	6	1056	273·6	26
21	6	1108	471·4	43	6	1820	1127·0	62	6	1116	326·5	29
22	6	1332	408·1	31	4	1326	592·9	54	6	1796	815·4	45
23	6	1012	833·1	82	4	1650	1434·7	87	4	4194	901·7	22

in Table II. There was month to month variation in egg counts, but the average coefficients of variation for each month were around 70%, indicating that the counts, grouped by month, varied to a similar degree. When the mean egg counts for each month were plotted on a logarithmic scale (Fig. 1), there

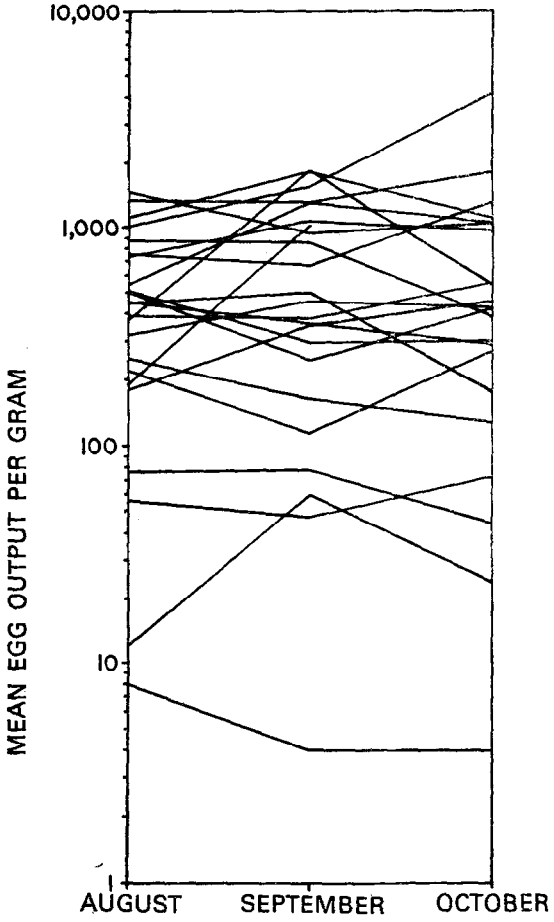

Fig. 1. Changes in mean *S. mansoni* egg output of 23 individuals over 3 months, plotted on logarithmic scales.

was apparent stability of counts for most individuals. However, further analysis of these raw data was not possible because of the strong correlation of the individual variances to the means, as demonstrated in Fig. 2. The linear relation suggested that a transformation of the data was necessary in order to render the variances independent of the means. Thus, Taylor's power law (TAYLOR, 1961) has been used to suggest a precise transformation of the raw data. In the general case, the equation,

$$\log s^2 = \log a + b \log \bar{x},$$

when solved for b, indicates a specific type of transformation. In our data, b was approximately 1·4 (Table III), and as these values of b were close to 1·33 according to Taylor's power law, a cube-root transformation was appropriate. The second part of Table III shows that when this transformation was made the correlation of variances to means disappeared, which was distinctly different from the highest significant correlations of the untransformed data.

The cube-root transformed data by month are presented in Table IV. There were few statistically significant (at the 5% level) changes in egg counts, based on month-to-month comparisons of the transformed data. From August to September, three persons showed increased and two persons showed decreased mean counts; from September to October, only one person increased and one decreased.

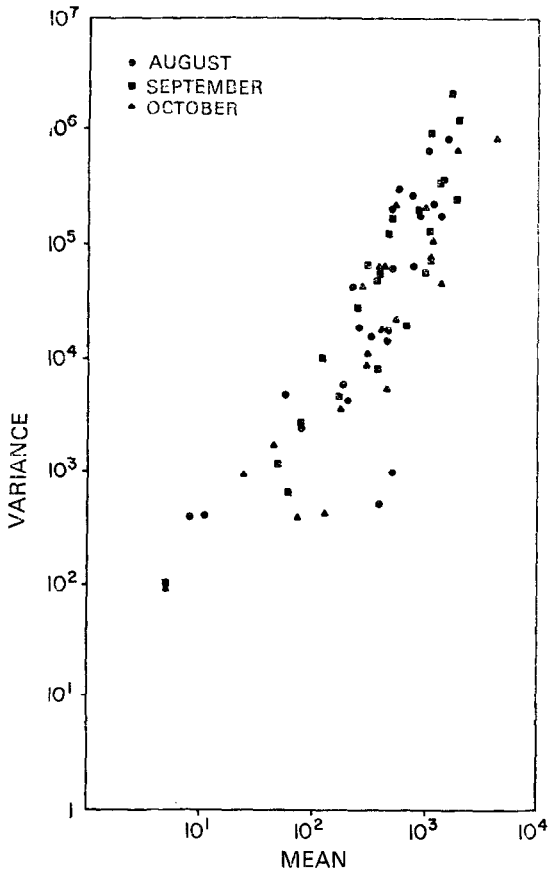

Fig. 2. The relationship between the mean and variance of monthly individual *S. mansoni* egg counts plotted on logarithmic scale. Each point represents the egg counts of one individual in one month.

In order to test for any important variations in the day-to-day egg excretions for the populations as a whole, the cube-root transformed data for three consecutive days in August were further transformed to a standardized mean of 15 for each individual for each day. This weighted mean was used to avoid the effects of very high or very variable egg counts on the over-all egg output. The mean transformed and weighted egg count was

Table III—The relationship between the *S. mansoni* mean egg count and variance for each monthly series of examinations. The effect of cube root transformation on the data is shown

Month	Mean of Means	Mean of Variances	Gradient (b)*	Correlation Coefficient	Degrees of Freedom	Significance Level
Logarithms of statistics from untransformed data						
August	2·483	4·395	1·333	0·873	22	0·1%
September	2·557	4·614	1·521	0·918	22	0·1%
October	2·508	4·288	1·373	0·917	21	0·1%
Statistics of cube root transformed data						
August	6·9	3·1	—0·080	0·056	22	NS
September	7·5	3·1	0·302	0·283	22	NS
October	7·4	1·5	—0·050	0·138	21	NS

* as calculated according to Taylor's power law

Table IV—The mean and standard deviation of cube root transformed *S. mansoni* egg counts for individuals represented in Table I

	AUGUST		SEPTEMBER		OCTOBER	
Individual No.	Mean egg count	Stand. dev.	Mean egg count	Stand. dev.	Mean egg count	Stand. dev.
1	0·6	1·5	0·5	1·2	0·6	1·2
2	1·1	1·7	3·8	0·6	1·8	2·0
3	2·8	2·3	3·1	1·6	4·1	0·4
4	4·1	0·9	3·7	1·9	6·0	1·7
5	6·1	1·0	5·3	1·0	4·9	0·8
6	5·0	2·9	4·5	1·4	6·2	1·4
7	5·5	0·9	6·8	1·6	6·6	0·7
8	7·8	1·3	6·1	2·2	6·6	0·8
9	7·6	0·7	7·5	2·0	5·5	0·8
10	7·9	0·5	6·0	1·5	7·4	0·8
11	6·7	0·9	7·2	2·1	7·3	1·4
12	7·6	0·7	7·1	0·6	7·6	0·4
13	7·3	0·5	6·9	1·8	8·0	0·8
14	5·7	0·6	10·0	1·3	—	—
15	9·3	1·7	9·3	1·6	7·0	1·5
16	7·3	2·1	12·2	1·1	6·1	4·7
17	8·4	2·7	9·5	3·0	9·6	1·6
18	6·6	4·2	10·8	1·6	10·1	0·9
19	9·0	1·1	8·7	0·6	10·9	0·6
20	11·3	0·8	9·8	0·8	10·1	0·9
21	10·2	1·5	11·7	2·8	10·4	1·1
22	10·9	1·3	10·8	1·6	11·9	1·8
23	9·9	2·6	11·1	3·4	16·1	1·2

calculated for each of the three days (Table V), and compared (Student's *t* test) with the expected mean of 15. The calculated values were very close to 15, indicating little over-all variation in the day-to-day egg excretion of the population.

In terms of the total egg excretion of the 23 study individuals, the transformed data by month showed a total increase of 6·1% between August and September and a decrease of 0·4% between September and October. Additionally, the transformed individual mean counts of August and October were highly correlated (r = 0·94), These observations provide further evidence of relative stability of egg excretion.

Table V—The mean and standard deviation of the standardized daily *S. mansoni* egg output in the study group during the month of August

Day	No. of stools	Mean	Stand. dev.
1	19	15·2	1·5
2	21	14·8	1·5
3	22	14·9	1·4

Because interest has been shown in identification and treatment of persons with high egg counts, an analysis of the efficiency of detection of such individuals was made. 19 of the 23 persons had an egg count over 400 during the study (Table I). The first examination (in August) detected 13 of the 19 (68%), the first three examinations detected 16 of the 19 (84%), and the first five detected 18 of the 19 (95%). One individual with a high count was detected on the eighth examination in September.

Discussion

Investigations of variation in faecal egg excretion in *S. mansoni* infection have been few in number. In the original and only detailed study, SCOTT (1938) followed three Egyptian farmers for one month and demonstrated coefficients of variation for *S. mansoni* egg output similar to those for egg excretion in *Ancylostoma duodenale* infection. SCOTT suggested that quantitation of schistosome egg output might correlate with morbidity due to the infection and would have value as a direct measure of the contamination of the environment by infected individuals. He concluded that the degree of variation of *S. mansoni* egg excretion permitted

analysis of the data especially if egg counts were expressed in terms of eggs per unit volume of stool rather than eggs per unit of time.

WARREN *et al.* (1974), in a study of 218 Yemeni immigrants in the USA, found no significant difference in egg counts between two stools taken three days apart. SIONGOK *et al.* (1976) evaluated stability of egg counts within a Kenyan population of 416 individuals by re-examining a random 10% of persons three weeks after the initial stool collection; 68% of the individuals remained within the original egg excretion class.

Stool egg count results in 142 St. Lucian children over a two to four year period were examined by COOK *et al.* (1974). Using designations of heavy (>400 eggs/gramme), moderate (100 to 300) and light (10 to 75) egg counts, relatively good agreement between the initial and final grouping was shown; over two to four years, only four persons changed by more than one class.

With respect to *S. haematobium* infection MCCULLOUGH & BRADLEY (1973) demonstrated that, despite considerable short-term fluctuations in egg output by Tanzanian children, the trend over three years was toward stabilization of counts. In particular, high egg excretors tended to remain high and low egg excretors low. They attributed this steady state to the existence of concomitant immunity, but this conclusion has been disputed (JORDAN *et al.*, 1974).

In this study, we followed a group of individuals infected with *S. mansoni* for an intermediate time period, and based our analysis on that of MCCULLOUGH & BRADLEY (1973). Our objective was to test the hypothesis that egg excretion in schistosomiasis mansoni is sufficiently stable to allow conclusions to be drawn from one or two egg counts.

The data show a fairly large variation over a short period of time (i.e. three consecutive days in each of three months) as demonstrated by coefficients of variation of around 70%. (It is interesting that MCCULLOUGH & BRADLEY, 1973, showed similar coefficients of variation within their collection periods.) Much of this variation is undoubtedly contributed by persons with low egg counts, particularly those who had counts of zero. However, when the counts are analysed for the entire three-month period, using either the raw data or the transformed data, it is clear that egg excretion is relatively stable.

The results presented here confirm the utility of mathematical transformation of egg count data to render them more tractable to analysis. Although we used a specific transformation in the present analysis, i.e. Tayler's power law, the general use of logarithmic transformation and of geometric mean egg counts when describing age-intensity relationship for populations seems desirable.

Improved egg-counting techniques and the wide use of a standard methodology in various endemic areas will help define the natural history of schistosomiasis and how preventive or curative procedures should be deployed and evaluated. For example, if selective chemotherapy is to be used as recommended by WARREN & MARMOUD (1976), it is necessary to know the efficiency of detection of high egg excretors by whatever counting technique is utilized.

In the present study, mean *S. mansoni* egg excretion and variance was stable over the three-month period of observation. However, the variation between consecutive quantitative stool examinations must be emphasized. Only two-thirds of individuals demonstrated during the study to have high egg counts (>400) were identified on the first examination using the modified Kato method. Each quantitative counting method must be evaluated in large populations in terms of efficiency and sensitivity, before use in selective mass treatment programmes. In spite of considerable variation between the results of consecutive examinations, over time there is considerable stability of egg excretion.

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