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A COMPARATIVE STUDY ON THE QUANTIFICATION METHODS OF *SCHISTOSOMA HAEMATOBIUM* EGGS IN THE URINE WITH SPECIAL REFERENCE TO THE POST-PRANDIAL EGG-OUTPUT

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Abstract: Four different egg count methods (egg counts per 10 m*l*, egg counts per specimen, egg counts per hour and post-prandial egg counts per hour) were applied to expression of the intensity of infection with *S. haematobium*. Egg counts per hour and post-prandial egg counts per hour revealed less variable results than egg counts per 10 m*l* and egg counts per specimen. Egg counts per hour seemed to be suitable for expression of intensity of infection with *S. haematobium*. No correlation between egg number and urine volume was shown. The post-prandial effect on egg-output of *S. haematobium* was much higher in the low egg-output group than in the high and middle egg-output groups.

INTRODUCTION

The intensity of infection with *Schistosoma haematobium* (worm burden) was assessed by egg counts in the urine specimens. For quantitative studies, 24-hour urine specimens over a number of days should be collected for examination, but it is not a practical method in large scale surveys or control programmes. Therefore, it is important to quantify schistosome eggs in the urine specimens. Recently, filtration method using nucleopore polycarbonate filter was recommended for the measurement of intensity of infection (Peters *et al.*, 1976a, b), and egg-output is usually expressed in terms of ova per 10 m*l* urine (Jordan, 1982).

In the present study, we have applied four different egg count methods to express the intensity of infection with *S. haematobium*, and evaluated statistically these methods in their applicability. We have also studied correlation between egg number and urine volume, and effect of meal on egg-output in different egg-output groups.

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Materials and Methods

We have examined 63 school children (9–14 years old) in Mtsangatamu Primary School, Kwale, Coast Province, Kenya, by four different egg count methods for 4 consecutive days. The collection procedures of urine specimens and four egg count methods applied were as follows; (1) egg counts per 10 m*l* and (2) egg counts per specimen were applied to urine specimens which was collected at 12:00 without any prior arrangement, (3) egg counts per hour were applied to urine specimens which were collected at 12:00 after urination at 11:00, and (4) post-prandial egg counts per hour was applied to urine specimens which were collected at 12:00 after urination at 11:00 and fed with "Chapati" (wheat flour cake, 250 g) and 180 m*l* soft drink at 10:45. All the specimens were transferred to the laboratory, and the whole or a portion of urine specimen was filtrated through a 25 mm-diameter Swin-Lok Holder containing nucleopore polycarbonate filter of 12 μ pore size (Nuclepore Corp.). After the filtration, the volumes of urine specimen filtrated and remained were recorded. Microscopic examination was performed under 10 $\times$ magnification to assess egg counts per unit. If urine specimens were not collected for 4 consecutive days, the data were omitted in this study.

Results

The coefficient of variations {C. V. = (standard deviation/mean) $\times$ 100} in egg numbers by four different egg count methods are plotted in Figure 1. The averages were 74.1%, 56.6%, 50.4% and 48.6% in egg counts per 10 m*l*, egg counts per specimen, egg counts per hour and post-prandial egg counts per hour, respectively. Egg counts per hour and post-prandial egg counts per hour revealed less variable results than the other two. However, there were no significant differences among these four methods.

Table 1 shows the correlation between egg number and volume of the urine which was collected without any prior arrangement. The correlation was not found and the correlation coefficients varied from 0.992 to -0.831.

In Table 2, the positive effect of meal on egg-output of *S. haematobium* is demonstrated. Urines of 34 school children were examined by one-hour collecting method (standard) and post-grandial one-hour collecting method. Post-grandial egg counts per hour was 22.8% higher than the egg count per hour. This effect was clearly found in lower egg-output groups. The increasing rate of egg number was highest in low egg-output group.

Discussion

In the epidemiological survey of schistosomiasis haematobia, the accurate egg counts is necessary to estimate the intensity of infection in the patient. A filtration method with nucleopore polycarbonate filter is gaining popularity in examination of urine specimens, as the technique is rapid, accurate, sensitive and reproducible (Peters *et al.*, 1976a, b). This procedure was used in our study.

In this study, we examined the applicabilities of four different egg counts; (1) egg counts per 10 m*l*, (2) egg counts per specimen, (3) egg counts per hour (Shimada *et al.*, 1983) and (4) post-prandial egg counts per hour. In any method, it was difficult to minimize the day-by-day

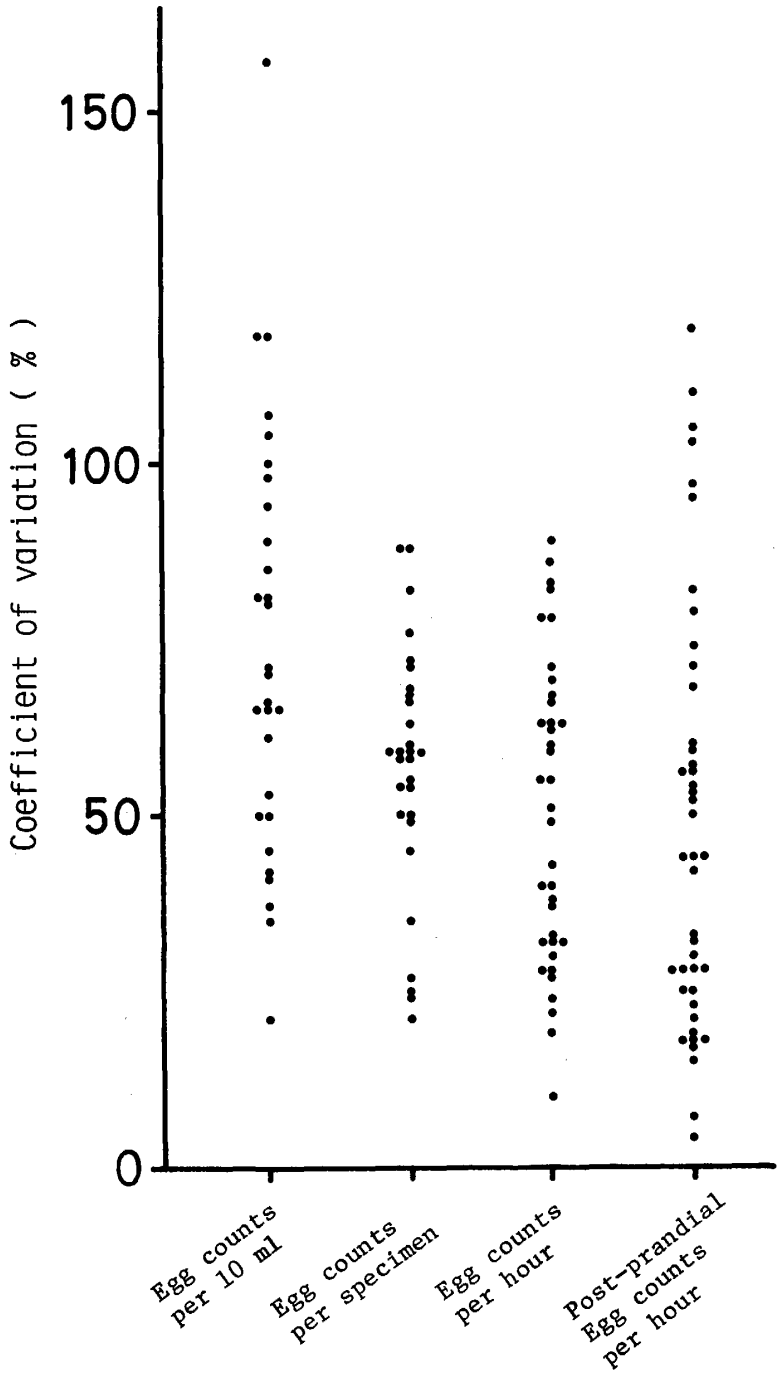

Figure 1 The coefficient of variation of egg number by four different egg-count methods applied on urine specimens for 4 consecutive days each.

variation in egg counts. The high coefficient of variation in urine specimens was recorded by Stimmel and Scott (1956). However, as shown in Table 1 and Figure 1, it seemed that both of the one-hour collecting methods were more excellent than the other two, because the averages of coefficients of variation in egg number were slightly smaller than that of egg counts per specimen, and much smaller than that of egg counts per 10 m*l*. The one-hour collecting method

Table 1 The correlation between egg number and urine volume in individual specimens collected from 29 subjects for 4 consecutive days at 12 : 00

Patient No.	Egg counts per specimen (Urine volume, m*l*)				Correlation coefficient
	day 1	day 2	day 3	day 4	
2	381 (37)	588 (84)	337 (91)	873 (71)	0.112
3	1,894 (31)	5,708 (67)	2,600 (50)	2,130 (31)	0.934
7	791 (17)	1,176 (2)	2,867 (47)	1,404 (31)	0.817
8	9,360 (24)	11,050 (25)	9,135 (35)	5,513 (37)	−0.766
9	5,650 (66)	3,357 (44)	2,960 (400)	980 (99)	−0.177
14	1,414 (36)	13,366 (82)	12,052 (131)	15,563 (79)	0.653
17	7,138 (43)	5,904 (123)	8,153 (104)	4,961 (41)	0.289
19	34,335 (63)	41,902 (41)	30,645 (67)	52,746 (59)	−0.394
20	26 (130)	148 (106)	350 (140)	134 (167)	0.110
21	81 (162)	132 (30)	192 (120)	21 (210)	−0.662
23	1,964 (35)	1,100 (50)	763 (35)	2,724 (106)	0.772
24	1,804 (55)	722 (86)	2,944 (115)	1,438 (12)	0.857
25	464 (232)	261 (145)	1,152 (134)	649 (94)	−0.319
26	6,916 (26)	9,747 (19)	4,785 (87)	11,132 (22)	−0.823
27	27,690 (39)	8,586 (81)	18,715 (95)	6,831 (253)	−0.716
30	84 (168)	200 (50)	418 (110)	125 (249)	−0.477
34	1,572 (79)	3,654 (126)	2,879 (101)	10,858 (215)	0.992
35	16 (80)	80 (134)	72 (120)	68 (75)	0.631
36	1,312 (68)	312 (195)	1,051 (51)	1,974 (210)	0.054
38	17,952 (68)	36,334 (37)	32,745 (37)	10,578 (41)	−0.467
39	12,750 (51)	20,623 (41)	10,989 (27)	2,036 (40)	0.131
40	6,674 (71)	12,436 (141)	4,259 (51)	1,418 (225)	−0.235
41	473 (21)	731 (84)	422 (62)	140 (40)	0.591
42	412 (20)	735 (75)	710 (5)	1,585 (121)	0.851
44	1,344 (24)	2,999 (84)	3,626 (153)	5,415 (150)	0.891
47	874 (52)	1,617 (37)	1,418 (105)	1,240 (248)	−0.080
50	1,076 (53)	175 (175)	1,789 (43)	891 (165)	−0.831
52	21,504 (48)	25,555 (95)	14,706 (171)	493 (170)	−0.741
54	1,462 (85)	689 (53)	261 (45)	208 (65)	0.781

is somewhat complicate in the process of urine collection in the field condition, but it is not such a serious matter. To know the applicability of this method for epidemiological survey, a study is ongoing at Mwachinga, Kwale District, Kenya. In conclusion, we recommend the one-hour collecting method at egg-output peak and egg counts per hour for expression of intensity of infection of *S. haematobium*. If it is impossible to perform it, egg counts per specimen should be used instead of egg counts per 10 m*l*.

In general, the reproduction of parasite egg is of synchronized nature in both parasite and host. Egg-output of *S. haematobium* in the urine is of circadian diurnal nature only in host; McMahon (1976) observed that a partial shift to a nocturnal pattern occured when day shift workers changed to night shift. In fact, Nojima *et al.* (1984) reported the enhancement of egg

Table 2 Comparison of egg numbers in urine specimens between one-hour collecting method as the standard and post-prandial one-hour collecting method

Change of egg counts	Number of patients			
	Degree of egg-output*			Total
	Low (1–999)	Middle (1,000–9,999)	High (10,000–)	
Increase†	9	10	3	22
Decrease‡	3	6	3	12
Total	12	16	6	34
Increase of§ egg counts (%)	+47.2	+17.2	−11.1	+22.8

* Egg counts per hour

† Egg counts per hour < Post-prandial egg counts per hour

‡ Egg counts per hour > Post-prandial egg counts per hour

§ $|(\text{Post-prandial egg counts per hour}/\text{Egg counts per hour}) - 1| \times 100$

excretion into bladder by midday meal, and suggested that the functional congestion of blood in the abdominal organs may have promoted the excretion of eggs after the ingestion of lunch meal. Strenuous exercise before micturition did not increase the number of eggs (Jordan, 1962; Weber *et al.*, 1967; Dukes and Davidson, 1968). These findings suggest that the egg-output depends on human life, such as midday activity and meal. Therefore, further study to know factors of various human behaviours which influence on egg-output and to improve egg count method suitable in the field is necessary.

Stimmel and Scott (1956) and Nojima *et al.* (1984) observed no correlation between the volume of voided urine and the number of eggs included. In this study, we also confirmed absence of statistical correlation between them.

In the present study, we showed that egg counts by post-prandial one-hour collecting method were higher than those by one-hour collecting method. The post-prandial effect was much stronger in a low egg-output group than in high and middle egg-output groups. The present study suggests that post-prandial collecting method may be applicable in the survey in area with low prevalence or in the examination of patients with low intensity of infection.

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ビルハルツ住血吸虫症患者の検尿方法の比較および虫卵排泄に及ぼす昼食の影響

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ビルハルツ住血吸虫症の疫学調査で必要な排泄虫卵数をより正確に表わす方法を見つける目的で，ケニア国においてビルハルツ住血吸虫症患者から4日間連続して採尿し，排泄虫卵数を4通りの方法，すなわち (1) egg counts/10 ml, (2) egg counts/specimen, (3) egg counts/hour および (4) 食事後の egg counts/hour で表わし，その変動（日差）の大きさを比較した。方法毎の排泄虫卵数の変動は (1)(2)(3)(4) の順に小さくなり，排泄虫卵数を表わす方法としては egg counts/hour が適当であると考えられた。また排泄虫卵数と患者尿量の間に相関は認められなかった。患者に食事を与えた後に採尿した場合には，与えない場合に比べて，排泄虫卵数が増加した。その増加の程度は排泄虫卵数が少ない患者において大きかった。

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