EGG COUNT IN URINE TO DETERMINE THE INTENSITY OF *SCHISTOSOMA HAEMATOBIUM* INFECTION

MASAAKI SHIMADA[1], MIZUKI HIRATA[2], KATSUYUKI SATO[1]
EDITH WAMBAYI[3], JOHN H. OUMA[4] AND YOSHIKI AOKI[1]

Received September 1 1986/Accepted November 10 1986

Abstract: A statistical comparison was carried out among 3 different parameters, *i.e.*, egg count/volume, total egg count/sample and egg count/hour, in regards to day-to-day variations of *S. haematobium* egg output in midday urination. Among 3 parameters, the egg count/hour showed the most stable value. In addition, the total egg count in a urine sample was not correlated with the sample volume of the urine in the same individuals. We conclude, therefore, that the adoption of the egg count/hour was best as a parameter for a quantitative unit of intensity of infection for cohort studies where the changes of intensity of infection are monitored for a long period. The existing parameter for egg output expressed in terms of the egg count/10 m*l* volume of urine seems to be a less reliable reflection of the intensity of infection.

INTRODUCTION

In order to measure the intensity of *Schistosoma haematobium* infection, the egg count/10 m*l* of urine collected at midday has long been used in socio-epidemiological studies. When we used this formulation, we frequently experienced considerable fluctuations in the count value among individual persons or even in the mean of a given group as other researchers pointed out (Wilkins, 1977; Stephenson *et al.*, 1984). This fact may be due to the urine volume, which is influenced by several factors such as volume of water consumed, air temperature or relative humidity, etc. If the density of eggs in urine varies considerably with the change of urine volume, single urine sample examinations, which we usually perform in field surveys, may lead to an erroneous interpretation of the results. The ideal parameter for expressing the intensity of infection is any count value that shows the minimal daily variations among egg output data.

In this report, we compared parameters for the intensity of infection *i.e.* an existing parameter of the egg count/10 m*l* urine and alternatives, *i.e.* egg count/urine sample and the egg

1 Department of Parasitology, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan
2 Department of Parasitology, Kurume University School of Medicine, Kurume, Japan
3 Center for Microbiology Research, Kenya Medical Research Institute
4 Division of Vector Borne Diseases, Ministry of Health, Nairobi, Kenya

This study was conducted under the Kenya-Japan Communicable Diseases Research and Control Project, supported by the Kenya Medical Research Institute (KEMRI) and the Japan International Cooperation Agency (JICA). Publication of this paper is approved by the Director of Kenya Medical Research Institute.

Correspondence address: Masaaki Shimada, M. D., D. M. Sc., Department of Parasitology, Institute of Tropical Medicine, Nagasaki University, 12–4 Sakamoto-machi, Nagasaki 852, Japan

count/hour for their range of variations, stability and reproducibility, and found that egg count/hour was superior to the other.

Materials and Methods

Twenty children from age 5 to 12 were selected systematically by a serial sampling among 203 pupils registered in a primary school in Mwachinga village in Kwale district, Coast Province, Kenya. Urine samples were collected from each of the children twice a day at about 11.30 and 12.30 hours for 5 days at the end of April, 1982. The times of the two urine samplings were recorded exactly.

The number of eggs in a known aliquot of each urine sample was counted by the nuclepore method (Peters *et al.*, 1976) using a membrane in 25 mm diameter with 12 micron porosity. If the eggs in the aliquot were too many or too few to count, we filtered another aliquot of the urine by adjusting the sample volume for examination for obtaining an appropriate egg count. From the egg count in the volume of urine filtered, the egg count in a 10 m*l* sample was calculated. The total volume of each urine sample was also measured to calculate the total egg count in the urine sample. Then, the total egg count in a sample collected at 11.30 was used for a parameter, egg count/sample, while those collected at 12.30 were expressed in terms of the egg count/sample and the egg count/hour, which was calculated by dividing by the exact time between two urinations.

Among the 20 subjects, 12 gave satisfactory data in terms of egg count/hour, but the remainder provided an insufficient number of samples or extremely low egg output. These unsatisfactory data were excluded from the analysis.

Results and Discussion

Table 1 shows the daily fluctuations in the egg counts/10 m*l* urine of individual children. Although it was impossible from our study to know how much the total daily egg output actually varies from day to day in individuals, it was possible to determine whether the egg count/10 m*l* urine is influenced by the sample volume. If the number of eggs in urine increases in proportion to the urine volume, the egg count/10 m*l* urine sample is considered not to be influenced by the sample volume, and then can be justified for practical use as a parameter of intensity of infection.

Based on this rationale, comparisons were made among correlation coefficients between the sample volumes (Tables 2 and 3) and the total egg counts per sample (Table 4) and those per hour (Table 5) in each group of samples collected at 11.30 and 12.30. The egg count data for 5 days from individual children were standardized before calculation to avoid the influence of individual differences of egg counts and sample volumes.

As a result, we found no significant correlation between the total egg counts in urine samples and the sample volumes in both groups of samples (correlation coefficient$=0.203$ and -0.081, $n=51$ and 50 respectively). In addition, the negative correlation was significant between the egg counts/10 m*l* and the sample volumes (correlation coefficient$=-0.699$ and -0.438, $n=51$ and 50 respectively).

These results indicated that the total number of eggs in the urine sample was not correlated with the urine volume. Furthermore, the increased urine volume tended to dilute the egg density in the urine. This fact has been pointed out by Wilkins (1977) and Stephenson *et al.*

Table 1 Day-to-day variation of egg count per 10 ml of urine sample collected at 11.30

Subject No.	Day of Examination					Mean	S. D.*	C. V.**
	1	2	3	4	5			
1	1,540	940	3,300	3,280	2,730	2,360	1,070	45.3
2	100	380	60	270	60	170	150	84.0
3	440	310	630	–	–	460	160	35.0
4	710	360	380	400	280	430	170	38.7
5	–	150	1,050	730	510	610	380	61.9
6	240	120	190	–	–	180	60	33.2
7	180	120	200	190	460	230	130	58.4
8	740	1,230	820	3,320	660	1,350	1,120	82.8
9	90	–	70	990	2,330	870	1,060	122.1
10	850	960	4,000	–	3,800	2,400	1,730	72.1
11	410	60	90	–	400	240	190	80.0
12	140	310	110	–	2,940	880	1,380	157.4
						Average of C. V.**		72.6

* Standard deviation
** Coefficient of variation (%)

Table 2 Day-to-day variation of urine volume (ml) collected at 11.30

Subject No.	Day of Examination				
	1	2	3	4	5
1	54	77	22.5	12.6	23.2
2	197	70.8	190	37	226.5
3	60	85.9	100	–	–
4	58	59.2	95	34	90.5
5	–	95	15.5	4	14.3
6	21.7	76.5	18.8	–	–
7	69	52	19.5	89	21
8	22.2	29	41	8.5	28.5
9	75.5	–	57.9	12.5	9
10	46	26.5	10.5	–	11.7
11	11.7	63	90	–	71
12	76	48.8	65	–	15.1

(1984). The egg count/10 ml urine sample expresses a less reliable value of intensity of infection.

The day-to-day variation among the egg counts in each group of samples at 11.30 and 12.30 was compared in terms of the coefficient of variation for 5 days. As shown in Tables 4 and 5, the average value of the coefficient of variation in the egg counts for the 12.30 samples was significantly smaller than that of the 11.30 samples ($p<0.05$, $t=2.64$, $df=11$). This indicates

Table 3 Day-to-day variation of urine volume (m*l*) collected at 12.30, about one hour after the previous urination

Subject No.	Day of Examination				
	1	2	3	4	5
1	12.5	8.5	9.3	12.7	9.4
2	12	16.5	8.5	84	20
3	9	6.2	9.7	–	–
4	8.3	19.5	14	10	–
5	–	14	12.7	12.1	12.1
6	7.8	12.3	9.8	–	–
7	12.7	12.5	10.5	16	13.1
8	23.5	30	18.2	20.6	14
9	21	–	19	11.7	7.8
10	10.6	13.8	13.3	–	11.1
11	10.3	18	11.9	–	11.2
12	13.4	13.5	11.7	–	6.5

Table 4 Day-to-day variation of total egg count per urine sample collected at 11.30

Subject No.	Day of Examination					Mean	S. D.*	C. V.**
	1	2	3	4	5			
1	8,320	7,240	7,430	4,130	6,330	6,690	1,590	23.8
2	1,930	2,690	1,100	1,010	1,310	1,610	700	43.7
3	2,640	2,660	6,300	–	–	3,870	2,110	54.5
4	4,120	2,130	3,590	1,360	2,550	2,750	1,110	40.4
5	–	1,440	1,630	290	730	1,020	620	60.9
6	520	910	360	–	–	600	280	47.7
7	1,260	600	380	1,670	970	980	510	52.6
8	1,640	3,570	3,360	2,820	1,880	2,660	860	32.5
9	660	–	430	1,240	2,100	1,110	740	67.1
10	3,910	2,550	4,200	–	4,450	3,780	850	22.5
11	480	370	810	–	2,840	1,120	1,160	103.2
12	1,060	1,510	740	–	4,560	1,970	1,750	89.1
						Average of C. V.**		53.2

* Standard deviation

** Coefficient of variation (%)

that the egg counts in the urine samples obtained 1 hour later at 12.30 were less fluctuated than those collected at 11.30 regardless of the previous discharge of urine.

The ideal count value to express the intensity of infection is the one which can give the least daily variation in egg count. In the present study, it was shown that the egg count/hour, which can be calculated from the 2nd sample of 2 consecutive urine samples collected at an hour interval, is a more stable parameter giving the least day-to-day fluctuation compared with other

Table 5 Day-to-day variation of egg count per hour, *i.e.*, egg count per urine sample collected at 12.30, about one hour after the previous urination

Subject No.	Day of Examination					Mean	S. D.*	C. V.**
	1	2	3	4	5			
1	5,090	6,990	4,380	3,640	5,510	5,120	1,260	24.7
2	2,150	1,790	1,680	1,300	2,550	1,890	480	25.2
3	1,710	670	1,490	–	–	1,290	550	42.7
4	2,050	1,150	1,040	1,720	–	1,490	480	32.0
5	–	1,150	490	990	760	850	290	33.9
6	380	280	180	–	–	280	100	36.1
7	1,140	530	900	1,680	910	1,030	420	41.1
8	2,500	1,720	1,760	3,150	3,880	2,600	920	35.5
9	670	–	610	380	730	600	150	25.2
10	2,890	3,090	5,950	–	8,040	4,990	2,470	49.4
11	300	210	380	–	1,170	520	440	85.7
12	4,160	2,650	4,240	–	2,740	3,450	870	25.2
						Average of C. V.**		38.1

* Standard deviation
** Coefficient of variation (%)

parameters. Our results support the opinion of Clarke (1966) that intensity of infection should be expressed as the egg count excreted for a certain fixed time.

In conclusion, we considered that egg count/hour should be adopted especially for cohort studies where changes of the intensity of infection are monitored repeatedly for a long period. The disadvantage of this formulation is that the examinees are requested to bring urine samples twice within about one hour.

Acknowledgements

We express appreciation to Professor M. Mugambi (Director of KEMRI), Dr. T. K. Arap Siongok (Director, Division of Communicable Diseases Control), Dr. J. N. Kaviti (Director, National Public Health Laboratory Services), Dr. D. K. Koech (Director, Division of Vector Borne Diseases) and Mr. K. Onoda (JICA) for their encouragement. We are also grateful to the late Mr. P. Bebora and his staff, Mr. Saidi (Chief field worker), the headmaster of Mwachinga Primary School and the children's parents. We wish to express special appreciation to Drs. T. Yoshimura, M. Ikeda and K. Makiya for their helpful criticism and suggestions in the preparation of the manuscript.

References

1) Clarke, V. de V. (1966): The influence of acquired resistance in the epidemiology of bilharziassis, Cent. Afr. J. Med., 12 (6, suppl.), 1–30
2) Peters, P. A. S., Mahmoud, A. A. F., Warren, K. S., Ouma, J. H. and Arap Siongok, T. K. (1976):

Field studies of a rapid, accurate means of quantifying *Schistosoma haematobium* eggs in urine samples, Bull. WHO, 54, 159–162
3) Stephenson, L. S., Latham, L. C., Kinoti, S. N. and Oduori, M. L. (1984): Sensitivity and specificity of reagent strips in screening of Kenyan children for *Schistosoma haematobium* infection, Am. J. Trop. Med. Hyg., 33 (5), 862–871
4) Wilkins, H. A. (1977): Variation in urinary creatinine concentration and *Schistosoma haematobium* egg count, Trans. Roy. Soc. Trop. Med. Hyg., 71 (5), 411–415

感染強度の指標としての尿中ビルハルツ住血吸虫卵数の表現法

嶋田　雅暁[1]・平田　瑞城[2]・佐藤　克之[1]・E. WAMBAYI[3]
J. H. OUMA[4]・青木　克己[1]

昭和61年9月1日　受付／昭和61年11月10日　受理

ビルハルツ住血吸虫症では，感染の強度を表す指標として，単位尿量中の排泄虫卵数が一般的に用いられている。しかし，検査毎の虫卵数が異なるのは当然とはいえ，同じ患者から得られた検体でも，時にその日々の変動の大きさは無視できないことがある。我々は，検査毎の虫卵数が出来るだけ一定で変動の少ない方法を見いだすため，この研究を行った。

ケニア人小学生12名から，5日間午前11時30分と午後12時30分の2回採尿した。するとどちらの採尿時間のものでも，その尿量と含まれる虫卵数との間には相関はなく，むしろ単位尿量中の虫卵数は，尿量と逆相関していた。また，先立つ排尿の時間を無視して午前11時30分に採尿したものと、その1時間後の午後12時30分に採尿したものの検体中の虫卵数を比較すると，午後12時30分に採尿したものの方が日々の変動が少ないことが明らかになった。

以上の結果から，特にコホート研究においては，ビルハルツ住血吸虫症患者の感染の強度を尿中の排泄虫卵数で表す場合，単位尿量中の排泄虫卵数よりも単位時間当たりの排泄数で表現することが望ましいと考えられる。

1 長崎大学熱帯医学研究所寄生虫学部門　2 久留米大学医学部寄生虫学教室
3 Center for Microbiology Research, Kenya Medical Research Institute
4 Division of Vector Borne Diseases, Ministry of Health, Kenya