TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (1997) 91, 222-225

Daily fluctuation of levels of circulating cathodic antigen in urine of children infected with *Schistosoma mansoni* in Brazil

J. Disch[2], M. M. A. Garcia[2], G. W. Krijger[1], M. N. Amorim[2], N. Katz[1], A. M. Deelder[1], B. Gryseels[3] and A. Rabello[2] *[1]Laboratory of Parasitology, Medical Faculty, University of Leiden, P.O. Box 9605, 2300 RC Leiden, The Netherlands; [2]Laboratório de Esquistossomose, Centro de Pesquisas 'René Rachou', Fundação Oswaldo Cruz–FIOCRUZ, Avenida Augusto de Lima 1715, CEP 3019-002, Caixa Postal 1743, Belo Horizonte (MG), Brazil; [3]Institute for Tropical Medicine 'Prins Leopold', Nationalestraat 155, 2000 B Antwerpen, Belgium*

Abstract

The fluctuation of circulating cathodic antigen (CCA) levels in urine was studied in 69 Brazilian schoolchildren infected with *Schistosoma mansoni* and compared to egg counts. Faeces and urine samples were simultaneously collected at 7 times during a period of 2 weeks. CCA was determined by enzyme-linked immunosorbent assay and could be detected in 96% of the urine samples; the individual mean CCA level ranged from 609 to 350700 pg/mL. 90% of the faecal samples contained *S. mansoni* eggs and the individual mean egg output ranged from 9 to 5510 eggs/g. The Spearman rank correlation ceofficient between these individual means was 0·69. Kendall's coefficient of concordance (*W*) was 0·88 for CCA levels and 0·80 for egg counts.

Keywords: schistosomiasis, *Schistosoma mansoni*, diagnosis, circulating cathodic antigen

Introduction

As eradication of schistosomiasis is still beyond human and financial resources, the aim of most control programmes remains the reduction of morbidity by treating the infected people on an individual or population basis (WHO, 1993). In this context, the value of reliable diagnostic procedures is evident.

Parasitological methods, based on the demonstration of schistosome eggs in excreta, are widely used in clinical practice and control programmes. Several studies based on schistosomiasis in Africa have shown that the egg output fluctuates, which complicates effective screening (POLDERMAN *et al.*, 1985; TEESDALE *et al.*, 1985), and repeated samples must be examined if high sensitivity and reliability are required (DE VLAS & GRYSEELS, 1992). Other studies, in Brazil, found that no significant difference could be observed in the egg counts of consecutive faecal samples (BARRETO *et al.*, 1978; LAMBERTUCCI *et al.*, 1983; LIMA E COSTA *et al.*, 1984). Conclusions on fluctuation of egg output are, however, strongly influenced by the method of examination chosen. Low, fluctuating egg output may yield unreliable results in epidemiological studies, especially in areas of low endemicity.

Various immunodiagnostic methods have been developed as an alternative to parasitological methods. Highly sensitive assays are available to detect antibodies directed against schistosome worms or eggs, and some of these are useful for individual diagnosis (FELDMEIER *et al.*, 1986). However, in general, antibody levels do not correlate strongly with egg counts, and often remain positive for a long period after loss of the infection.

New serological methods for the diagnosis and quantification of schistosome infections are based on the detection of metabolites of the parasites in body fluids. These 'circulating antigens' are thought to give a more direct quantitative reflection of worm burdens and to allow differentiation between active and past infection. The most thoroughly investigated circulating antigens with diagnostic potential are the circulating anodic antigen (CAA) and the circulating cathodic antigen (CCA) (DEELDER *et al.*, 1994). These circulating antigens, emanating from the worm gut, have been demonstrated in serum and urine of infected animals and humans.

Highly sensitive enzyme-linked immunosorbent assays (ELISAs) based on monoclonal antibodies have been developed to assess CAA and CCA levels in serum and urine (DEELDER *et al.*, 1989; DE JONGE *et al.*, 1990). The advantages of testing urine instead of serum samples are obvious, for urine is easy to collect even if large or repeated samples are required. Recent field trials of antigen detection in urine have confirmed that this is a feasible and valuable approach (DE JONGE *et al.*, 1989, 1990; VAN LIESHOUT *et al.*, 1992; DEELDER *et al.*, 1994; KREMSNER *et al.*, 1994). Several authors have reported higher levels of CCA in urine than of CAA, whereas the reverse is often found in serum. Lower renal filtration of the CAA than CCA, due to its relatively larger size and its polarity, may explain this phenomenon (KESTENS *et al.*, 1988a, 1988b). Therefore, CCA seems to be the most promising candidate for urine-based antigen detection assays.

In epidemiological surveys it is highly preferable to be able to rely on evidence derived from a single specimen per subject. Therefore, many studies have been performed to estimate the fluctuation of faecal egg counts. In order to evaluate further the potential of circulating antigen detection in urine, we aimed to assess the fluctuation of CCA levels in urine by comparing them with the fluctuation of faecal egg counts in Brazilian children infected with *Schistosoma mansoni*. A similar study on individuals infected with *S. mansoni* in Burundi has recently been reported (VAN ETTEN *et al.*, 1996).

Patients, Materials and Methods

Among an initial group of all 134 schoolchildren in the first to fourth classes of a primary school, 69 with proven *S. mansoni* infection were selected after screening by examination of 2 thick smears made from one stool sample (KATZ *et al.*, 1972). The study group consisted of 25 females and 44 males, with a median age of 12 years (range 7–16). None of the children had previously been treated for schistosomiasis. During a period of 2 weeks, 7 faecal and urine samples were collected simultaneously from these children. This period started on the day of collecting the first sample. The second sample was collected on the first day of the second week, and during the following 5 consecutive days samples 3, 4, 5 and 6 were collected. The last sample (no. 7) was collected on the first day of the third week. For the collection of faeces, small cups were distributed; urine was collected in plastic tubes of 25 mL capacity. Cups and tubes were labelled with the name, class, and a registration number, and distributed the day before collection. The samples were immediately transported to the laboratory, 30 min drive from the school: faecal samples were kept at 4°C and urine samples at –20°C until use. Faecal samples were examined within 3 d after collection; urine samples were examined within 3 months.

The number of eggs in the faeces was counted on du-

Address for correspondence: J. Disch, Laboratório de Esquistossomose, Centro de Pesquisas 'René Rachou', Fundação Oswaldo Cruz–FIOCRUZ, Avenida Augusto de Lima 1715, CEP 3019-002, Caixa Postal 1743, Belo Horizonte (MG), Brazil; phone +55 (0)31 295 3566 (ext. 143), fax +55 (0) 31 295 3115.

plicate slides prepared and examined according to the Kato–Katz method (KATZ *et al.*, 1972).

CCA was assessed by the ELISA described by DE JONGE *et al.* (1990). Before testing, aliquots of urine samples were incubated at 70°C for 30 min in an alkaline buffer (pH 9·6) to prevent non-specific reactions (KRIJGER *et al.*, 1994), resulting in 2-fold dilution of the original samples. The optical densities were measured at 405 nm with a Biorad™ absorbance reader. The lower detection limit of the ELISA in the present study was 2 ng/mL AWA-TCA (the trichloroacetic acid soluble fraction of the adult worm antigen). Initially, concentrations of AWA-TCA were calculated from a standard dilution curve. AWA-TCA contains approximately 3% CCA (VAN DAM *et al.*, 1993, 1994; BERGWERFF *et al.*, 1994). For further analyses, CCA levels were determined.

As both egg output and CCA levels were not distributed normally, non-parametric statistical methods were used to evaluate the results. Both sets of data were characterized by the range and median of the individual means. Kendall's correlation coefficient (W) was used to express the concordance between individual daily egg counts and CCA levels. The correlations between egg counts and CCA levels per collection, and between their individual means, were expressed by Spearman's rank coefficient. The statistical package SPSS/PC+™ version 4·0 for an IBM personal computer was used to perform all calculations.

Table. Results of *Schistosoma mansoni* egg counts and circulating cathodic antigen determinations on seven different occasions in schoolchildren in Brazil

	1	2	3	4	5	6	7
Egg counts[a]							
No. of samples							
Collected	63	65	69	66	67	58	53
Included	63	65	69	64	67	58	53
Positive	89%	89%	94%	88%	91%	95%	96%
Eggs/g[b]	103 (0–3852)	138 (0–7360)	213 (0–10131)	160 (0–6408)	177 (0–7308)	152 (0–7104)	188 (0–4500)
Circulating cathodic antigen							
No. of samples							
Collected	63	65	69	66	65	58	53
Included	55	51	47	59	51	53	45
Positive	95%	100%	96%	95%	98%	96%	93%
Level (ng/mL)[b]	12·1 (0–346)	13·3 (0·8–217)	11·8 (0–275)	14·1 (0–319)	17·1 (0–361)	10·3 (0–316)	6·4 (0–160)
Comparative statistics							
No. of cases							
Included	52	51	47	59	51	47	39
Negative							
By Kato–Katz	6	7	2	8	5	3	2
By CCA-ELISA[c]	3	0	2	3	1	1	2
Spearman[d]	0·75*	0·59**	0·52**	0·64*	0·68**	0·62**	0·44**

[a]Duplicate Kato–Katz smears.
[b]Geometric mean (range in parentheses).
[c]Circulating cathodic antigen enzyme-linked immunosorbent assay.
[d]Spearman's rank correlation coefficient between eggs/g and level of circulating cathodic antigen; *P<0·05, **P<0·01.

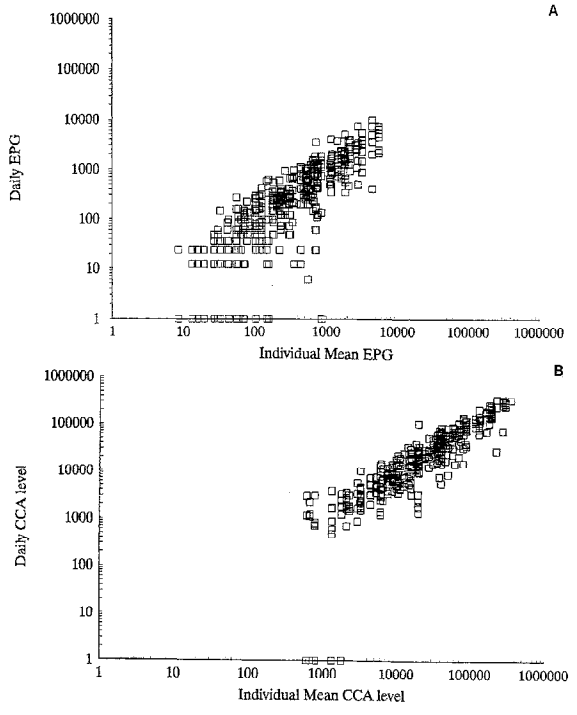

Fig. 1. Schistosomiasis mansoni. Relation between (A) single egg count results (eggs/g, EPG) per collection and (B) single determinations of circulating cathodic antigen (CCA) level (pg/mL) per collection and their respective means. In order to include zero values, data were transformed by adding one (i.e., EPG+1 and CCA+1) and plotted on a logarithmic scale. Due to overlap, the number of symbols does not correspond with the actual number of observations.

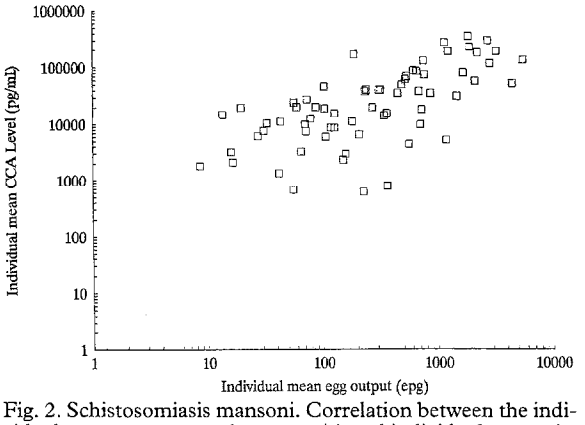

Fig. 2. Schistosomiasis mansoni. Correlation between the individual mean egg counts (epg=eggs/g) and individual mean circulating cathodic antigen (CCA) level determinations plotted on a logarithmic scale. Due to overlap, the number of symbols does not correspond with the actual number of observations.

Results

The results are summarized in the Table. The apparently lower number of CCA determinations compared to stool examinations was due mainly to the exclusion of

ELISA results which did not comply with the criteria of reliability of the assay. However, for 73% of all children included, at least 5 samples of both urine and faeces could be used for further analysis. Individual means of the 7 egg counts ranged from 9 to 5510 eggs/g (median 309). Individual means of the CCA levels ranged from 609 to 350700 pg/mL (median 1979).

The relationship between the single test results and individual means for both egg counts and CCA levels is shown in Fig. 1. In order to include zero values on a log scale, the values were transformed by adding one unit. There was a strong association between individual measurements and mean values in both sets of data. This association appeared to be stronger with the CCA, as there was less variation in these measurements than in the single egg counts.

There were more negative results with the Kato–Katz method than the ELISA. The total number of false negative results in all surveys and cases was 33/346 (9·5%) for the Kato–Katz method and 12/346 (3·5%) for the ELISA. The CCA ELISA therefore appeared to be significantly more sensitive than the Kato–Katz technique (χ^2=9·39, P<0·01).

Kendall's W was 0·8 for the single egg count (n=20) and 0·88 for CCA levels (n=13). This test included only those subjects without any missing result for both the ELISA and egg count on all collection days. As mentioned before, due to non-compliancy or, to some extent, exclusion of technically unsatisfactorily CCA determinations, with more than 80% of the subjects some results were missing.

Spearman's rank correlation between individual mean egg counts and individual mean CCA levels was 0·69 (P<0·001) (Fig. 2).

Discussion

Previous studies have shown that ELISAs for the detection of CAA and CCA are powerful tools for the qualitative and quantitative diagnosis of human schistosomiasis, and for the follow-up of chemotherapy. Urine-based assays are particularly promising for the development of population-based, non-invasive screening methods. For that purpose, a dipstick format has recently been developed (VAN ETTEN *et al.*, 1994). The reliability of an assessment of the day-to-day fluctuation of antigen urine levels is essential to determine whether a single specimen test would be adequate for screening and diagnosis. This study is one of the first to assess the fluctuation of CCA in urine of individuals infected with *S. mansoni*, and also the first such study in Brazil.

This study was not designed to investigate the sensitivity or specificity of the methods used. However, some conclusions can be reached relating to these characteristics. CCA was detected by ELISA in 96% of the urine samples from proven infected cases, while *S. mansoni* eggs were detected in 90% of the (duplicate) Kato–Katz slides. These results show that the CCA assay is at least as sensitive as stool examination and confirm the diagnostic capacity of the urine-based CCA ELISA. The concordance between the single assays, as expressed by Kendall's W, shows a stronger association between repeated CCA measurements than between repeated egg counts. This suggests that CCA levels in urine in this study showed less day-to-day variation than egg counts. These findings agree with the results reported from a study of individuals from Burundi infected with *S. mansoni* (see VAN ETTEN *et al.*, 1996). Thus, for epidemiological studies, the urine CCA ELISA appears to be a valuable tool and an attractive alternative to serum-based assays. This is encouraging for the further development and use of a simple format, such as reagent strips, with a single urine specimen for population screening. However, it should be borne in mind that the ELISA is a more expensive and more elaborate assay, so that for the time being methods such as the Kato–Katz technique remain the methods of choice.

References

Barreto, M. L., Silva, J. T. F., Mott, K. S. & Lehman, J. S., jr (1978). Stability of faecal egg excretion in *Schistosoma mansoni* infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **72**, 181–187.

Bergwerff, A. A., Van Dam, G. J., Rotmans, J. P., Deelder, A. M., Kamerling, J. P. & Vliegenthart, J. F. G. (19940. The immunologically reactive part of immunopurified circulating anodic antigen from *Schistosoma mansoni* is a threonine-linked polysaccharide consisting of 6)-[β-D-GlcpA-(113)]-β-D-GalpNAc-(11 repeating units. *Journal of Biological Chemistry*, **269**, 31510–31517.

Deelder, A. M., De Jonge, N., Boerman, O. C., Fillié, Y. E., Hilberath, G. W., Rotmans, J. P., Gerritse, M. J. & Schut, D. W. O. A. (1989). Sensitive determination of circulating anodic antigen in *Schistosoma mansoni* infected individuals by an enzyme-linked immunosorbent assay using monoclonal antibodies. *American Journal of Tropical Medicine and Hygiene*, **40**, 268–272.

Deelder, A. M., Qian, Z. L., Kremsner, P. G., Acosta, L., Rabello, A. L. T., Enyong, P., Simarro, P. P., Van Etten, E. C. M., Krijger, F. W., Rotmans, J. P., Fillié, Y. E., De Jonge, N., Agnew, A. M. & Van Lieshout, L. (1994). Quantitative diagnosis of *Schistosoma* infections by measurement of circulating antigens in serum and urine. *Tropical and Geographical Medicine*, **46**, 233–238.

De Jonge, N., Fillié, Y. E., Hilberath, G. W., Krijger, F. W., Lengeler, D. H., de Savigny, D. H., Van Vliet, N. G. & Deelder, A. M. (1989). Presence of the schistosome circulating anodic antigen (CAA) in urine of patients with *Schistosoma mansoni* or *Schistosoma haematobium* infections. *American Journal of Tropical Medicine and Hygiene*, **41**, 563–569.

De Jonge, N., Kremsner, P. G., Krijger, F. W., Schommer, G., Fillié, Y. E., Kornhelis, D., Van Zeyl, R. J. M., Van Dam, G. J., Feldmeier, H. & Deelder, A. M. (1990). Detection of the schistosome circulating cathodic antigen by enzyme immunoassay using biotinylated monoclonal antibodies. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **84**, 815–818.

De Vlas, S. J. & Gryseels, B. (1992). Underestimation of *Schistosoma mansoni* prevalences. *Parasitology Today*, **8**, 274–277.

Feldmeier, H., Nogueira-Queiroz, J. A., Peixoto-Queiroz, M. A., Doehring, E., Dessaint, J. P., De Alencar, J. E., Dafalla, A. A. & Capron, A. (1986). Detection and quantification of circulating antigen in schistosomiasis by monoclonal antibody. II. The quantification of circulating antigens in human schistosomiasis mansoni and haematobium: relationship to intensity of infection and disease status. *Clinical and Experimental Immunology*, **65**, 232–243.

Katz, N., Chaves, A. & Pellegrino, J. (1972). A simple device for quantitative stool thick-smear technique in schistosomiasis mansoni. *Revista do Instituto de Medicina Troipical de São Paulo*, **14**, 397–400.

Kestens, L., Mangelshots, K., Van Marck, E. A. E., Gigase, P. L. J. & Deelder, A. M. (1988a). Clearance of artificial immune complexes consisting of circulating cathodic and monoclonal antibodies in *Schistosoma mansoni* infected mice. *Annales de la Société Belge de Médecine Tropicale*, **68**, 241–254.

Kestens, L., Mangelschots, K., Van Marck, E. A. E., Gigase, P. L. J. & Deelder, A. M. (1988b). *Schistosoma mansoni*: impaired clearance of model immune complexes consisting of circulating anodic antigen and monoclonal IgG1 in infected mice. *Parasitology Research*, **74**, 356–362.

Kremsner, P. G., Enyong, P., Krijger, F. W., De Jonge, N., Zotter, G. M., Thalhammer, F., Muhlschlegel, F., Bienzle, U., Feldmeier, H. & Deelder, A. M. (1994). Circulating anodic and cathodic antigen in serum and urine from *Schistosoma haematobium*-infected Cameroonian children receiving praziquantel: a longitudinal study. *Clinical Infectious Diseases*, **18**, 408–413.

Krijger, F. W., Van Lieshout, L. & Deelder, A. M. (1994). A simple technique to pretreat urine and serum samples for quantitation of schistosome circulating anodic and cathodic antigen. *Acta Tropica*, **56**, 55–63.

Lambertucci, J. R., Dias, E. P., Rocha, R. S., Pedrosa, E. R. P. & Katz, N. (1983). O valor dos ovos mortos como criterio de avaliação de cura parasitologica na esquistossomose mansoni. *Revista da Sociedade Brasileira de Medicina Tropical*, **16**, 158–161.

Lima, E., Costa, M. F. F., Rocha, R. S. & Katz, N. (1984). Avaliacao da estabilidade na contagem de ovos de *Schistosoma mansoni* pelo metodo de Kato–Katz em uma zona endemica da esquistossomose. *Revista da Sociedade Brasileira de Medicina Tropical*, **17**, 7–12.

Polderman, A. M., Mpamila, K., Manshande, J. P. & Bouwhuis Hoogerwerf, M. L. (1985). Methodology and interpretation of parasitological surveillance of intestinal schistosomiasis in Maniema, Kivu Province, Zaire. *Annales de la Société Belge de Médecine Tropicale*, **65**, 243–249.

Teesdale, C. H., Fahringer, K. & Chitsulo, L. (1985). Egg count variability and sensitivity of a thin smear technique for the diagnosis of *Schistosoma mansoni*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **79**, 369–373.

Van Dam, G. J., Seind, J., Rotmans, P., Daha, M. R. & Deelder, A. M. (1993). *Schistosoma mansoni* circulating anodic antigen but not circulating cathodic antigen interacts with complement component. *European Journal of Immunology*, **23**, 2807–2812.

Van Dam, G. J., Bergwerff, A. A., Thomas-Oates, J. E., Rotmans, J. P., Kamerling, J. P., Vliegenthart, J. F. G. & Deelder, A. M. (1994). The immunologically reactive O-linked polysaccharide chains derived from circulating cathodic antigen isolated from the human blood fluke *Schistosoma mansoni* have Lewis X as repeating unit. *European Journal of Biochemistry*, **225**, 467–482.

Van Etten, L., Folman, C. C., Eggelte, T. A., Kremsner, P. G. & Deelder, A. M. (1994). Rapid diagnosis of schistosomiasis by antigen detection in urine with a reagent strip. *Journal of Clinical Microbiology*, **32**, 2404–2406.

Van Etten, L., Engels, D., Drijger, F. W., Nkulikyinka, L., Gryseels, B. & Deelder, A. M. (1996). Fluctuation of schistosome circulating antigen levels in urine of individuals with *Schistosoma mansoni* infection in Burundi. *American Journal of Tropical Medicine and Hygiene*, **54**, 348–351.

Van Lieshout, L., De Jonge, N., El Masry, N. A., Mansour, M. M., Krijger, F. W. & Deelder, A. M. (1992). Improved diagnostic performance of the circulating antigen assay in human schistosomiasis by parallel testing for circulating anodic and cathodic antigens in serum and urine. *American Journal of Tropical Medicine and Hygiene*, **47**, 463–469.

WHO (1993). *The Control of Schistosomiasis; Second Report of the WHO Expert Committee*. Geneva: World Health Organization, Technical Report Series, no. 830.

Received 27 June 1996; revised 7 October 1996; accepted for publication 8 October 1996