

DAY-TO-DAY FLUCTUATION OF SCHISTOSOME CIRCULATING ANTIGEN LEVELS IN SERUM AND URINE OF HUMANS INFECTED WITH *SCHISTOSOMA MANSONI* IN BURUNDI

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Abstract. Day-to-day fluctuations of both circulating anodic antigen (CAA) and circulating cathodic antigen (CCA) in serum and urine were examined simultaneously in a group of *Schistosoma mansoni*-infected individuals from Burundi and compared with each other and with fecal egg count fluctuations. Significant correlations were found between fecal egg counts and circulating antigens (CAA and CCA) and between circulating antigen levels in serum and urine samples. The cumulative percentage of positive results after three samplings was highest for urine CCA detection, followed by fecal egg counts, serum CCA, serum CAA, and urine CAA detection, respectively. It was demonstrated that circulating antigen levels in both serum and urine showed less fluctuation than fecal egg counts, except for urine CAA levels. The serum CAA detection assay in particular, although less sensitive in this low endemic area in Burundi, gave very constant measurements over a period of one week. Our results indicate that detection of circulating antigens in a single serum or urine sample provides a quantitatively more stable diagnosis of *S. mansoni* infection than fecal egg counts based on a single stool examination.

Most control programs and epidemiologic studies on *Schistosoma mansoni* infection are based on the microscopic detection and quantification of parasite eggs in feces with the Kato-Katz technique.¹ Its relatively low sensitivity for light infections combined with high fluctuations in egg excretion makes this method less appropriate for use, especially in low-to-moderate endemic areas.^{2–4} Generally, fecal egg counts do not provide the direct measure of worm burden needed to answer important standing questions on the dynamics of schistosome infections, immunity, morbidity, and control.^{4–6}

The detection of circulating anodic antigen (CAA) and circulating cathodic antigen (CCA) in serum and urine may be a valuable alternative to parasitologic examination because circulating antigen levels are specific and sensitive markers of presence and intensity of schistosome infection.^{7–13} Antigen detection in urine is promising for the development of a simple, noninvasive, field-applicable screening test. (e.g., dipstick).¹⁴ For epidemiologic studies, antigen detection in serum may provide the much needed direct measure of worm burden.¹⁵

Both for operational screening and epidemiologic research, it is desirable to rely on a single one-sample test. The high variability in egg excretion requires repeated sampling and multiple counts to obtain reliable results.^{16–18} To further evaluate the potential of circulating antigen detection, it is thus important to study in how far antigen levels in serum and urine fluctuate, as compared with fecal egg counts. A few studies on fluctuation of antigen levels have been conducted.^{11, 12, 19–21} However, this is the first study in which day-to-day fluctuations of both CAA and CCA in serum as well as urine are examined simultaneously, in one group of *S. mansoni*-infected individuals from Burundi, and compared with each other and with fecal egg count fluctuations.

MATERIALS AND METHODS

Study population. Fifty individuals from the Rusizi Plain, an *S. mansoni*-endemic region in Burundi, were included in

this study. The area and epidemiologic details have been described by Gryseels and Nkulikyinka.²² The study group consisted of 25 females and 25 males, who had been diagnosed as positive in a preliminary population survey based on single 25-mg Kato-Katz slides. Their ages ranged from seven to 70 years (median = 18.5 years); 26 persons were less than 20 years old. All selected individuals volunteered to participate in the study after having been duly informed. The study was approved by the Ethical Commission within the Burundi Ministry of Health.

Parasitology. Stool samples were collected three times (days 1, 4, and 7) within one week. Fecal egg excretion was determined by duplicate 25-mg Kato-Katz examinations on each stool sample, on the day of collection.²³

Collection and treatment of serum and urine samples. Blood and urine samples were collected three times, simultaneously with stool samples. Blood samples were centrifuged and the serum was carefully removed. Sodium azide (0.4% [w/v]) was added to the urine samples as a preservative. Both serum and urine samples were stored frozen at -20°C , transported on dry ice to the Netherlands, and stored at -20°C until use.

Before testing in an ELISA, samples were diluted (1:4 for serum, and 1:2 for urine) in alkaline buffer and heated to 70°C to remove interfering components.²⁴

Determination of antigen levels. Circulating anodic antigen levels in serum and urine were determined by ELISA as described by Deelder and others,⁷ using anti-CAA monoclonal antibody 120-1B10-A both as capture antibody and as alkaline phosphatase-conjugated second antibody. The lower detection limit of this ELISA was approximately 1 ng/ml of the trichloroacetic acid (TCA)-soluble fraction of schistosome adult worm antigen (AWA-TCA). Samples were tested in two-fold dilution series at an initial dilution of 1:4 for sera and 1:2 for urines. Samples with a CAA concentration greater than the lower detection limit were considered positive. These cut-off values were determined by Krijger and others²⁴ using Dutch control individuals.

Circulating cathodic antigen levels in serum and urine were determined according to the method of De Jonge and others²⁵

TABLE 1

Percentages of positive results, ranges, and geometric means per assay determined at three different timepoints per assay*

	1	2	3
Fecal egg counts			
% positive	85.4	79.2	77.1
Geometric mean	99.6	92.3	99.6
Range	0–13,720	0–10,720	0–6,520
n	48	48	48
Serum CAA			
% positive	69.8	65.1	65.1
Geometric mean	3.3	3.3	3.3
Range	0–196.7	0–207.8	0–218.2
n	43	43	43
Serum CCA			
% positive	78.4	78.4	70.3
Geometric mean	5.0	4.5	3.3
Range	0–78.2	0–87.4	0–72.6
n	37	37	37
Urine CAA			
% positive	38.1	16.7	14.3
Geometric mean	0.06	0.02	0.02
Range	0–3.7	0–4.4	0–1.3
n	42	42	42
Urine CCA			
% positive	89.5	94.7	86.8
Geometric mean	13.1	14.3	10.8
Range	0–298.6	0–226.1	0–259.9
n	38	38	38

* CAA = circulating anodic antigen; CCA = circulating cathodic antigen; n = number of tested cases. Geometric means and ranges are expressed as eggs per gram of feces (fecal egg counts) or as nanograms per milliliter (CAA and CCA in serum and urine).

using anti-CCA IgG3 monoclonal antibody 54-5C10-A as capture antibody and IgM monoclonal antibody 8-3C10 as the biotinylated second antibody. Streptavidin–alkaline phosphatase was used as the enzyme label. Samples were tested in two-fold dilution series at an initial dilution of 1:4. The lower detection limit of this ELISA was approximately 2 ng/ml of AWA-TCA. Cut-off values based on Dutch controls were applied according to Krijger and others:²⁴ serum samples with CCA concentrations > 1.52 ng/ml, and urine samples with a CCA concentration greater than the lower detection limit were considered positive.

Antigen levels in both serum and urine were expressed as concentrations of pure CAA or CCA (ng/ml). They were calculated with a four-parametric curve fitting method, using a standard dilution curve of AWA-TCA, which contains approximately 3% (w/v) CAA and 3% (w/v) CCA.²⁶

Statistical methods. Since egg output and circulating antigen concentrations were not normally distributed, data were log-transformed and parametric statistical methods were used to describe and evaluate the results. To allow for zeros in the analyses, log-transformation was applied after adding a value equal to half the detection limit of the particular assay to the data. Data were characterized by geometric means and ranges. To calculate the association between egg output and antigen levels of the different assays, the sum of three measurements was calculated per assay and the Pearson's correlation test was applied. Cumulative frequencies of positive findings were calculated by taking the mean of the percentages of positive results after one, two,

TABLE 2

Coefficients of correlation (Pearson correlation; $P < 0.001$ for all coefficients) between fecal egg counts and circulating antigens (CAA and CCA) and between circulating antigen levels in serum and urine samples*

	Serum CAA	Serum CCA	Urine CAA	Urine CCA
Fecal eggs	0.85 (43)	0.76 (37)	0.67 (42)	0.77 (37)
Serum CAA		0.86 (33)	0.72 (37)	0.83 (33)
Serum CCA			0.68 (31)	0.79 (30)
Urine CAA				0.60 (31)

* CAA = circulating anodic antigen; CCA = circulating cathodic antigen. Values in parentheses are the number of cases.

and three samplings, respectively. Thus, the cumulative percentage of positive results after one sampling was calculated by taking the average of the percentages of positives at timepoints 1, 2, and 3; the cumulative percentage of positive results after two samplings by taking the average of the percentages of positive results at timepoints 1 + 2, 1 + 3, and 2 + 3. For the cumulative frequency after three samplings, the total percentage of positives of timepoints 1 + 2 + 3 was taken.

Cumulative frequency distributions of the coefficient of variation (CV) values (standard deviation of the three measurements divided by the mean of these three measurements) were plotted as a way to express fluctuation independent of the survey method. To analyze the day-to-day fluctuations of egg counts and circulating antigen levels in more detail, one-way analysis of variance was performed, and the intraclass correlation coefficient was calculated according to Fleiss.²⁷

RESULTS

To allow strict comparison between different days, individuals with missing values were excluded from the analysis, for each assay separately. Table 1 summarizes the day-to-day results of fecal egg counts and serum and urine antigen levels for the three different samplings. As shown in Table 2, circulating antigen levels in serum and urine were significantly correlated ($P < 0.001$), both with egg counts and with each other.

The cumulative percentages of positives with increasing number of examinations by stool examination as well as by circulating antigen detection, are shown in Table 3. After three samplings, the highest percentage of positive results was found by urine CCA detection, followed by fecal egg counts, serum CCA, serum CAA, and urine CAA detection, respectively.

The cumulative frequency distributions of CV-values were calculated per test and plotted in one graph to get a comparative picture of day-to-day fluctuations of fecal egg counts and serum and urine antigen levels (Figure 1). This graph indicates that CAA and CCA levels in serum and CCA levels in urine showed less fluctuation than egg counts (higher percentage of cases with low CV values). Levels of CAA in serum were more constant than all the others.

TABLE 3

Cumulative frequencies of positive findings in each assay, after respectively one, two, and three measurements*

	% positive		
	1	2	3
Fecal egg counts	80.6	89.6	93.8
Serum CAA	66.7	70.6	74.4
Serum CCA	75.7	83.8	89.2
Urine CAA	23.0	34.9	42.8
Urine CCA	90.3	94.8	97.4

* CAA = circulating anodic antigen; CCA = circulating cathodic antigen; % positive = mean of the percentages of positive results after one, two and three samplings, respectively (see Materials and Methods).

To assess and compare the daily variations of egg output and circulating antigen levels more precisely, intraclass correlation coefficients (according to Fleiss²⁷) were computed for each test, as indicators of day-to-day fluctuation (Table 4). It is again shown that serum CAA levels remained very stable over a period of one week, followed by urine CCA, serum CCA, egg count, and urine CAA levels, respectively.

DISCUSSION

For a reliable diagnosis and appropriate interpretation of quantitative data, it is important to know whether antigen levels are stable during an active infection. In an animal study on *S. japonicum*-infected rabbits, day-to-day variation of antigen levels was shown to be minimal.¹⁹ In 15 Egyptian schistosomiasis patients, no significant circadian variability in urine CAA levels was observed.¹² In 20 patients from Zaire with *S. mansoni* infections, serum antigen levels showed less day-to-day fluctuation than egg counts.¹¹ Recently, Van Etten and others studied the day-to-day fluctuation of urine antigen levels in *S. mansoni*-infected individuals from Burundi, and found a higher variation in egg counts than in CCA levels.²⁰ In urines of 31 *S. haematobium*-infected school children from Gabon, egg output showed less day-to-day variation than circulating antigen levels.²¹

In the present study, day-to-day fluctuations of both CAA and CCA in serum as well as in urine and fecal egg count fluctuations were examined simultaneously in one group of *S. mansoni*-infected individuals from Burundi. In this way, daily variations of circulating antigen levels and egg output could be analyzed and compared in a comprehensive and precise way.

As indicators of day-to-day variation, the cumulative CV values as well as the intraclass correlation coefficients were determined for each test. With both methods it was shown that antigen levels in serum and urine showed less fluctuation than fecal egg counts, except for urine CAA levels. A relatively high fluctuation in urine CAA levels of Burundese individuals was also found by Van Etten and others.²⁰ In addition, the observed urine CAA levels were low, and only a small percentage of positives was found by urine CAA detection in comparison with the other assays. In agreement with previous observations,^{10, 15, 20, 24} these findings do not support the use of the current urine CAA detection assay for a reliable diagnosis of *S. mansoni*.

For the three other assays, antigen levels showed less fluctuation than fecal egg counts. Serum CAA levels in partic-

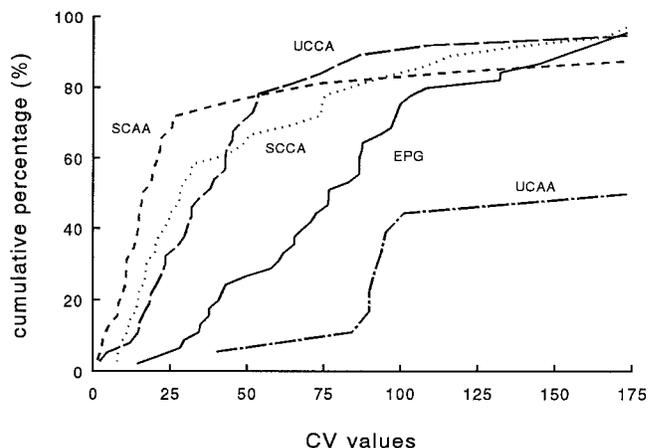


FIGURE 1. Cumulative frequency distribution of individual coefficient of variation (CV) values per assay. — = EPG; - - - = serum (S)CAA; ... = serum (S)CCA; - - - = urine (U)CCA; - · - · = urine (U)CAA. EPG = eggs per gram of feces; CAA = circulating anodic antigen; CCA = circulating cathodic antigen.

ular were very stable over a period of one week. This indicates that in this respect, it is better to rely on a single one-sample antigen detection test, especially CAA detection in serum, than on fecal egg counts based on a single stool examination (i.e., duplicate 25-mg Kato smears of one stool sample).

A low day-to-day fluctuation in serum CAA levels does not automatically exclude fluctuations over longer periods, but this applies to all analytes. Up to now, only one (animal) study has concerned with long-term fluctuations in antigen levels,²⁸ and further research on this topic is needed.

With urine CCA detection, more individuals were found positive than with egg counts and the other antigen detection assays, in single samples as well as in the cumulative results of three samplings. Urine CCA detection was found to be the most sensitive circulating antigen detection assay in previous studies as well.^{15, 20} However, in a preliminary evaluation of endemic controls in Burundi, doubts have arisen on the presumed high sensitivity of the urine CCA detection assay in this population; these findings, which still have to be confirmed, suggest a much lower specificity of urine CCA detection than for serum CAA detection (Polman K and others, unpublished data). Therefore, the observed high positivity rates of urine CCA detection should still be interpreted with caution.

Lower percentages of positive results by serum CAA detection compared with stool examination have been previ-

TABLE 4

Intraclass correlation coefficients and 95% confidence intervals (according to Fleiss²⁷) for each assay*

	R	95% CI
Fecal egg counts	0.77	0.66–0.85
Serum CAA	0.97	0.94–0.98
Serum CCA	0.88	0.81–0.93
Urine CAA	0.61	0.46–0.74
Urine CCA	0.91	0.86–0.94

* R = intraclass correlation coefficient (according to Fleiss); 95% CI = 95% confidence interval; CAA = circulating anodic antigen; CCA = circulating cathodic antigen.

ously observed, particularly in low-intensity infections.^{9,29} In our study, geometric mean egg counts were 90–100 eggs per gram of feces, and the percentage of positive results as detected by serum CAA ELISA was 70–75%, which is consistent with observations by De Jonge and others.⁹ In this particular study, cases were included on the basis of a positive, single, 25-mg Kato-Katz examination result, which may have negatively biased the relative CAA sensitivity (e.g., exclusion of monosexual infections). In addition, this lower sensitivity as compared with egg counts may be due to an efficient antibody response and rapid clearing of immune complexes.⁹ It is therefore not certain whether further improvement of the test performance will overcome this problem.

Our results indicate that in this low-endemic area in Burundi, detection of CAA in a single serum sample is somewhat less sensitive, but provides a quantitatively more stable diagnosis of *S. mansoni* infection than fecal egg counts based on a single stool examination. For urine CCA detection, sensitivity seems higher and day-to-day fluctuation is relatively low. However, the potentially lower specificity might partially invalidate these findings.

Acknowledgments: We thank Dr. S. le Cessie and Dr. S. de Vlas for statistical advice.

Financial support: This study was supported by the Netherlands Organization for Scientific Research in the Tropics (NWO/WOTRO) and the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases (TDR).

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