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# Circulating anodic and cathodic antigen in serum and urine of mixed *Schistosoma haematobium* and *S. mansoni* infections in Office du Niger, Mali

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**Summary** In Office du Niger, an area endemic for both *Schistosoma haematobium* and *S. mansoni* in Mali, circulating anodic (CAA) and cathodic (CCA) antigen detection assays were performed on pre-treatment serum and urine samples from two villages, Rigandé and Siguivoucé, and compared with egg counting methods. The highest prevalence was obtained with the urine-CCA assay which also had the highest sensitivity to *S. haematobium, S. mansoni* or mixed infection. A single urine-CCA assay was as sensitive as repeated egg counts (one stool + two urine examinations per individual). When the different assays were tested in parallel, several combinations including assays on serum were found to be highly sensitive. As urine sampling is widely accepted, urine assays will be used for further monitoring these villages one and two years after chemotherapy.

**keywords** Schistosomiasis, *Schistosoma haematobium*, *Schistosoma mansoni*, circulating anodic antigen, circulating cathodic antigen, Mali.

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## Introduction

Assays for the detection of circulating schistosome adult worm antigens (circulating anodic and cathodic antigen – CAA & CCA) provide a powerful alternative method for the diagnosis of schistosomiasis (Deelder *et al.* 1994). The serum-CAA assay can be adequately performed in a normally equipped public health laboratory in Mali (De Clercq *et al.* 1995). Studies by Van Lieshout *et al.* (1992) indicate that the diagnostic performance could be improved by parallel testing for CAA and CCA in serum and urine. We compared CAA and CCA assays on pre-treatment serum and urine samples in Office du Niger, a vast perennial irrigation area (960,000 hectares) in Mali (West Africa), where rice culture is predominant and which is endemic for both *Schistosoma haematobium* and *S. mansoni*.

Since 1982, Office du Niger has been one of the main intervention areas of the National Schistosomiasis Control Programme (NSCP), where a control strategy based mainly on mass chemotherapy with single 40 mg/kg doses of praziquantel has been used. In mixed infection areas, the parasitological determination of *S. haematobium* and *S. mansoni* infections requires both urine and stool examination. Although very specific, the reliability of these methods is limited because of day to day fluctuation of egg excretion, low sensitivity in low endemic areas, overestimation of cure rates etc. Stool sample collection may be difficult for cultural reasons. The objective of the study was to assess if antigen

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detection could provide a useful alternative to urine and stool egg counts.

## Material and methods

### Study population

In September 1993, in 4 villages of the Office du Niger not yet covered by the NSCP, a preliminary survey of 726 individuals of all ages living in the sub-districts of Niono (Rigandé and Siguivoucé) and Kolongo (Bougounam, Siguinogué), indicated higher prevalences of urinary and intestinal schistosomiasis. Two months later a survey was initiated in the neighbouring villages of Rigandé and Siguivoucé.

Most inhabitants are from the same ethnic group (Mossi) and share the same principal canal. Transmission is permanent and water contact very intense, particularly among children and women, due to the recreational and domestic activities. *Bulinus truncatus* and *Biomphalaria pfeifferi* are the local intermediate hosts. In each large village (> 400 people) about 240 individuals were registered. The heads of households were asked to bring family cards for the selection of the study population. Cards were collected and drawn randomly. When a household was drawn, all its members were recorded for the study. The draw was discontinued when a total of 240 individuals had been reached.

### Experimental design

*Parasitological techniques*

431 urine (205 in Rigandé and 226 in Siguivoucé), 324 stool (149 in Rigandé and 175 in Siguivoucé) and 348 blood samples (156 in Rigandé and 192 in Siguivoucé) were collected. For each individual, 10 ml urine was filtered (Nuclepore) and examined microscopically. Individuals with a negative urine examination were re-examined the following day; one Kato slide (41.6 mg) per person was prepared and examined microscopically to count the eggs. Only one stool could be examined as people are very reluctant to handle stools or to be seen handing in specimens and frequently suffer from constipation due to their diet. After parasitological examination, mass treatment with a single 40 mg/kg dose of praziquantel was offered to the population.

*Serological techniques*

One ml of urine and 5 ml of blood (serum separated) were collected and stored at −20°C until use. All circulating antigen detection assays were performed in the laboratory in Bamako (Mali). Circulating anodic antigen (CAA) was determined by ELISA according to Deelder *et al.* (1989), except that a biotinylated conjugate (120-1B10-A/biotin) was used on the samples from Rigandé. Circulating cathodic antigen (CCA) was determined by ELISA according to de Jonge *et al.* (1990). The assays were performed on 348 individuals from both villages. With both methods (egg counting and ELISA) prevalence and intensity were determined. Sensitivity of the antigen detection assays was calculated with reference to the parasitological egg counts and since the serum-CAA assay is considered 100% specific (Van Lieshout *et al.* 1992), the sensitivity of each diagnostic test was also assessed with reference to the combined results of repeated egg counts and serum-CAA.

## Results

The parasitological and serological prevalences and intensities of infection are shown in Table 1. The 2 villages have the same epidemiological profile: high prevalences of infections but low overall egg load. *S. haematobium* infections over 50 ep 10 ml were found in 25% and 33% of the cases, and *S. mansoni* infections over 100 epg in 27% and 22% of the cases in Rigandé and Siguivoucé, respectively. Highest prevalence was obtained with the urine-CCA assay in both villages (> 90%). Intensities, measured as the geometric mean titre of the positive samples, were comparable in the 2 villages for all the serological tests except the serum-CAA titre which was two times higher in Siguivoucé than in Rigandé.

The sensitivity of the different tests is shown in Table 2. In both villages, the urine-CCA assay was found to be the most sensitive test to diagnose an infection due to *S. mansoni, S. haematobium* and also a mixed infection. The evaluation of the sensitivity of different assay combinations is shown in Table 3. CCA urine & CAA urine, CCA urine & CCA serum, and CCA urine & CAA serum were found to be the most sensitive assay combinations (sensitivity > 90%).

Taking the combined results of egg counts and serum-CAA, both considered 100% specific, as a reference, a

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**Table 1** Prevalences and intensities of *Schistosomia* infection in two villages in Office du Niger, Mali

| | Rigandé | | Siguivoucé | |
|---|---|---|---|---|
| | Prevalence % | Intensity GM | Prevalence % | Intensity GM |
| *S. haemat.*† | 72* | 17 | 83** | 25 |
| *S. mansoni*†† | 69# | 72 | 64## | 96 |
| Mixed inf. | 54 | | 55 | |
| CAA-serum§ | 62 | 121 | 87 | 271 |
| CCA-serum§ | 67 | 18 | 63 | 19 |
| CAA-urine§ | 70 | 10 | 81 | 15 |
| CCA-urine§ | 95 | 253 | 96 | 215 |

GM, geometric mean of positive samples or titres. †*Schistosoma haematobium*: based on 2 urine samples (10 ml). Number of urine samples: 205 in Rigandé & 226 in Siguivocé. *of which 54% and **51% were mixed infections. Number of stool samples: 149 in Rigandé & 175 in Siguivoucé. ††Schistosoma mansoni: based on a single stool examination (41.6 mg) # of which 78% and ## 86% were mixed infections. §Serological tests performed in 156 individuals from Rigandé and 192 from Siguivoucé.

single urine-CCA assay was found to be as sensitive as repeated egg counts (one stool and two urine examinations). The increase in sensitivity obtained when combining CCA urine & CAA urine was only marginal (Table 4).

## Discussion

In mixed infection areas, the parasitological determination of *S. haematobium* and *S. mansoni* infections requires examination of both urine and stool samples. Antigen detection could thus provide a useful alternative, as only a single urine (or serum) sample is needed. Detection of CAA and CCA in urine of intestinal and urinary schistosomiasis patients in previous studies (de Jonge *et al.* 1989) suggested the usefulness of urine samples for non-invasive immunodiagnosis of the disease. Studies by Van Lieshout *et al.* (1991, 1993 & 1994) confirmed that there was little circadian variability in urine CAA or CCA levels and that the test could be used to monitor the efficacy of schistosomiasis chemotherapy, though levels of CAA in urine decreased more slowly after successful therapy. However, in general the concentration of CAA

**Table 2** Sensitivity of the different circulating antigen assays in two villages from the Office du Niger

| | | Sensitivity of the tests (%)* | | | |
|---|---|---|---|---|---|
| Villages | Type of infection | CAA serum % (**) | CAA urine | CCA serum | CCA urine |
| Rigande | *S. haematobium* | 31 (26) | 54 (28) | 42 (26) | 96 (27) |
| | *S. mansoni* | 40 (15) | 53 (28) | 60 (15) | 94 (16) |
| | Mixed inf. | 76 (75) | 77 (74) | 81 (75) | 93 (77) |
| Siguivouce | *S. haematobium* | 78 (46) | 72 (46) | 41 (46) | 94 (47) |
| | *S. mansoni* | 93 (15) | 93 (15) | 53 (15) | 100 (15) |
| | Mixed inf. | 96 (88) | 87 (95) | 77 (87) | 97 (95) |

*= the sensitivity of the tests was determined on the basis of 2 urine and 1 stool examination
(**) = No of samples

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**Table 3** Sensitivity of the different combinations of two circulating antigen detection assays (%)

| | CAA serum & CCA serum | CAA urine & CCA serum | CAA serum & CAA urine | CCA serum & CCA urine | CAA serum & CCA urine | CCA urine & CAA urine |
|---|---|---|---|---|---|---|
| Rigandé | | | | | | |
| *S. haematobium* | 54 | 69 | 96 | 100 | 96 | 100 |
| *S. mansoni* | 60 | 71 | 57 | 93 | 93 | 93 |
| Mixed inf. | 87 | 90 | 87 | 94 | 94 | 95 |
| Siguivoucé | | | | | | |
| *S. haematobium* | 80 | 76 | 89 | 96 | 96 | 96 |
| *S. mansoni* | 93 | 100 | 100 | 100 | 100 | 100 |
| Mixed inf. | 95 | 89 | 97 | 96 | 99 | 99 |

in urine is relatively low in comparison with the concentration of CCA in urine (Krijger *et al.* 1994).

Although good results were obtained with the CCA-ELISA assay in urine in *S. mansoni* (Van Lieshout et al. 1992) and *S. intercalatum* infections (Kremsner *et al.* 1993), the application of this assay in *S. haematobium* infections is still controversial (de Jonge *et al.* 1989; Kremsner *et al.* 1994). Our data in a mixed *S. haematobium*/*S. mansoni* infection area indicate a very satisfactory sensitivity of the urine-CCA assay. A number of combinations of urine and serum assays were also found to be very sensitive. By referring to the combined results of egg counts and serum-CAA ('gold standard'), the increase in sensitivity obtained by combining the two urine assays was however not as great as the improvement observed by Van Lieshout *et al.* (1992). The differences in sensitivity and intensity observed in the CAA assays between the two villages were probably due to the use of a different conjugate as detecting antibody on the samples from Rigandé. In our study, CCA detection in urine either alone or in combination with other antigen detection tests (such as the urine CAA assay) was the most appropriate diagnostic method.

In Senegal, in a recent *S. mansoni* epidemic focus (Polman *et al.* 195), the highest prevalence and sensitivity were also obtained with the urine-CCA assay. In Egypt, where several combinations of assays were shown to improve diagnosis, the combination of the two assays on urine yielded a sensitivity of 94% for mixed infections and 93% for *S. mansoni* infections (Van Lieshout *et al.* 1992).

In conclusion, several assay combinations, including assays on serum samples, were found to be very sensitive. As urine sampling is highly acceptable in endemic areas, the combination of the two assays on urine was used for further monitoring the situation in these villages at two months, one year and two years after mass treatment with 40 mg/kg praziquantel. Unpublished results indicate that the highest prevalences were obtained by the urine-CCA assay, that at one year after treatment highest sensitivity was obtained by the

**Table 4** Sensitivity of the different diagnostic tests with reference to the combined results of urine-stool egg counts and serum-CAA

| | Sensitivity | | | | | |
|---|---|---|---|---|---|---|
| | CAA serum %[1] | CAA urine | CCA serum | CCA urine | CCA urine & CAA urine | Repeated egg counts[2] |
| Rigande | 67 (99) | 74 (110) | 70 (104) | 95 (141) | 97 (143) | 96 (142) |
| Sisguivouce | 89.5 (171) | 82 (156) | 63 (121) | 96 (184) | 98 (187) | 94 (180) |
| Both villages | 80 (270) | 78 (266) | 66 (225) | 96 (325) | 97 (330) | 95 (322) |

[1]No of positive samples. [2]one stool and two urine examinations.

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urine-CAA test (or the combination of CCA & CAA in urine) and at two years after treatment by the urine-CCA test (or the combination of serum-CAA and urine-CCA). These preliminary results are still being analysed.

Further studies also need to be done on the specificity of the urine-CCA, particularly in endemic areas, as this may be lower than for the serum-CAA. However, field approaches to this question are quite demanding as identification of false-positive antigen detection tests requires a very thorough parasitological examination to distinguish these from parasitological false-negatives.

The urine-CCA assay is considered a suitable candidate for a non-invasive diagnostic test. Recently, a reagent strip assay (dipstick) for detection of CCA in urine of *S. mansoni*-infected individuals has been developed with encouraging results (van Etten *et al*. 1994).

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