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Specificity of circulating antigen detection for *schistosomiasis mansoni* in Senegal and Burundi

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Summary The specificity of schistosome circulating antigen detection was determined in negative individuals from two *S. mansoni*- endemic countries, Senegal and Burundi, and compared with results from Dutch control individuals. A nearly absolute specificity was achieved for circulating anodic antigen (CAA) detection in serum, irrespective of the target population or sample pretreatment method. Circulating cathodic antigen (CCA) detection in serum and urine resulted in a lower specificity than serum CAA detection. Apparent large differences in specificity of CCA detection between countries were mainly due to pretreatment methods. Apparently, the alkaline/heating pretreatment method is not as effective as trichloroacetic acid (TCA)-pretreatment in removing (certain) interfering components, which may vary between populations. In view of the development of the urine CCA assay into a noninvasive screening test, a slightly lower specificity may still be acceptable. For precise epidemiological analyses the highly specific serum CAA assay remains the method of choice.

keywords *Schistosoma mansoni*, circulating anodic antigen (CAA), circulating cathodic antigen (CCA), specificity, endemic countries, pretreatment

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Introduction

Assays for the detection of circulating anodic antigen (CAA) and circulating cathodic antigen (CCA) are a valuable alternative to stool examination for the diagnosis of human schistosome infections, and are increasingly used in (sero)epidemiological studies. Both antigens are glycoconjugates associated with the gut of the adult worm and released by the parasite in large amounts into the circulation of the infected host. They can be demonstrated in serum and urine, and their levels are specific and sensitive markers of presence and intensity of infection (Deelder *et al*. 1994; Krijger *et al*. 1994). CCA detection in urine has potential for the development of a noninvasive screening test (Polman *et al*. 1995; Van Etten *et al*. 1997), while serum CAA detection may provide a more direct measure of worm burdens for epidemiological research (Polman *et al*. 1995; Van Lieshout *et al*. 1998).

The cut-off values (concentrations or titres below which the test is considered negative) for these assays have so far mainly been based on results from Dutch individuals who had never been exposed to infection. A widespread standard application of circulating antigen detection assays in epidemiological research and screening programmes critically depends on the validation of these cut-off values in endemic populations. Concomitant infections, and different immunological, nutritional or other parameters may affect the specificity or (after adjusting the cut-off levels) the sensitivity, hence the applicability of the assays. So far, only a limited number of circulating antigen field studies have included negative controls from endemic countries. An important reason for this is the difficulty of defining truly negative controls in endemic areas with the generally available parasitological techniques (De Vlas & Gryseels 1992).

We took advantage of the very focal distribution of *S. mansoni* in Burundi and Senegal to define with reasonable certainty uninfected communities and individuals comparable to nearby endemic communities where circulating antigen studies were performed previously (Polman *et al*. 1995, 1998).

These individuals were used as negative controls to determine the specificity of circulating antigen detection in both *S. mansoni* endemic countries.

Materials and methods

Study populations

The group from Burundi consisted of 108 schoolchildren (6–16 years old; 51 males, 57 females) from Munanira in the highlands, where there is no transmission of *S. mansoni*. In Burundi, *S. mansoni* is confined to the Imbo plain and Lake Cyohoha; *S. haematobium* does not occur at all (Gryseels 1991).

The group from Senegal consisted of 130 individuals (3–75 years old; 62 males, 68 females) from Nguene Sarr in the Louga area. There is no local or nearby transmission source, and cases of schistosomiasis have not been reported so far. *S. mansoni* has only recently been introduced to the northern provinces of Senegal and is still confined to the area of Richard Toll, about 80 km north of Louga (Gryseels *et al*. 1994).

Informed consent was obtained from all participants or their parents. Pregnant women were excluded from the study. Medical care was offered for diagnosed illnesses (e.g. intestinal helminth infections; general complaints).

The group of Dutch controls has been described by Krijger *et al*. (1994) and was used as a reference.

Parasitology

Faecal egg counts were determined for all participants by two stool examinations, each consisting of duplicate 25 mg Kato–Katz slides (Katz *et al*. 1972; Polderman *et al*. 1985). In addition, one stool sample (2–3 g) per individual was examined for *S. mansoni* eggs with highly sensitive qualitative methods, the stools from Burundi according to the Sedimentation-Selective-Filtration (SSF) method (Polderman *et al*. 1994), and those from Senegal with a slightly modified Visser filter method (Visser & Pitchford 1972; Schutte *et al*. 1994). Individuals from Senegal were also checked for *S. haematobium* eggs in urine by the nucleopore filtration technique.

Circulating antigen detection

Ninety-eight serum and 108 urine samples were collected in Burundi, 130 serum and urine samples in Senegal. Before testing in ELISA, serum and urine samples were pretreated to remove interfering components. Samples from Burundi were diluted in alkaline buffer and heated to 70 °C (alkaline/heating), and samples from Senegal were treated with 4% trichloroacetic acid (TCA). Although both pretreatment methods are reported to work equally well (Krijger *et al*. 1994), we chose to use the same pretreatment procedures as used in the studies in nearby endemic areas (Polman *et al*. 1995, 1998). However, for urine CCA detection, we cross-checked by submitting each series of samples also to the alternative pretreatment method.

Circulating anodic antigen (CAA) in serum and circulating cathodic antigen (CCA) levels in serum and urine were determined by ELISA (Deelder *et al*. 1989; De Jonge *et al*. 1990). Antigen concentrations were calculated with a four parametric curve fitting method, using a standard dilution curve of the TCA-soluble fraction of schistosome adult worm antigen (AWA-TCA), which contains approximately 3% (w/w) CAA and 3% (w/w) CCA.

The lower detection limit of the serum CAA ELISA was approximately 1 ng/ml of AWA-TCA (= 0.03 ng CAA/ml). The cut-off value for this ELISA is equal to the lower detection limit of the assay both after pretreatment with TCA and after alkaline/heating (Krijger *et al*. 1994). Serum samples were tested in twofold dilution series at an initial dilution of 1 : 4. Urine CAA levels were not determined in this study, as results with CAA detection in urine have so far been relatively poor (Polman *et al*. 1995, 1998).

The lower detection limit of the CCA ELISA was approximately 2 ng/ml of AWA-TCA (= 0.06 ng CCA/ml). The cut-off value for the urine CCA ELISA (based on 102 Dutch control urine samples) is equal to 2.46 ng CCA/ml after pretreatment with TCA, and equal to the lower detection limit after pretreatment with alkaline/heating. For the serum CCA ELISA, the cut-off values (based on 106 Dutch control sera) are 1.52 ng CCA/ml after pretreatment with alkaline/heating and 1.14 ng CCA/ml after TCA pretreatment, respectively (Krijger *et al*. 1994). Samples were tested in twofold dilution series, at an initial dilution of 1 : 4 (urines pretreated with TCA and sera) or 1 : 2 (urines pretreated with alkaline/heating).

Results

None of the individuals from Burundi or Senegal had *S. mansoni* eggs in their stools, neither with the Kato-Katz nor with the SSF or Visser filter method. No *S. haematobium* eggs were found in the urine samples from Senegal.

The circulating antigen detection results are presented in Table 1. All individuals were negative for serum CAA, except one in Burundi. This positive serum was from a 15-year-old boy with no specific characteristics; the level was 0.16 ng/ml CAA, just above the cut-off level. For serum CCA, 30 false positives were found in Burundi and 10 in Senegal; for urine CCA, 91 false positives were found in Burundi and two in Senegal. The specificity of the serum CAA ELISA was thus 99% in Burundi and 100% in Senegal. For the serum CCA ELISA the specificity was 92.3% in Senegal (with TCA pretreatment) and 69.4% in Burundi (with alkaline/heating). For the urine CCA ELISA, the specificity was 98.5% in Senegal (TCA) and only 15.7% in Burundi (alkaline/heating). After switching the pretreatment

Table 1 Specificity of CAA and CCA detection in serum and CCA detection in urine samples pretreated with alkaline/heating or TCA in *S. mansoni*-negative individuals from Burundi and Senegal. Cut-off levels are based on Dutch control individuals, and chosen such that a specificity of at least 98% was achieved (Krijger *et al*. 1994)

		Specificity[%]		
Assay	Cut-off [ng antigen/ml]	Dutch Controls	Burundese Controls	Senegalese Controls
Serum CAA				
ALK	*	98.0	99	–
TCA	*	100	–	100
Serum CCA				
ALK	1.52	98.0	69.4	–
TCA	1.14	98.0	–	92.3
Urine CCA				
ALK	*	98.0	15.7	23.8
TCA	2.46	98.8	95.4	98.5

ALK, alkaline/heating pretreatment; TCA, TCA pretreatment; *****, lower detection limit

procedures, the specificity for the urine CCA ELISA with TCA pretreatment was 95.4% in Burundi, and only 23.8% with alkaline/heating in Senegal. We could not examine this effect for the serum CCA ELISA due to insufficient amounts of serum.

Discussion

Nearly absolute specificity was found for the serum CAA ELISA in both study groups, confirming the results from Dutch negative controls and previous field studies on serum CAA (De Jonge *et al*. 1988; Krijger *et al*. 1994; El-Morshedy *et al*. 1996; Håkangård *et al*. 1996). The unique polysaccharide structure of CAA (Bergwerff *et al*. 1994) appears to guarantee an invariably high specificity for the serum CAA assay, and the cut-off level based on Dutch controls can thus be safely used as a standard for widespread epidemiological use. This cut-off level is equal to the lower detection limit of the assay, leaving the possibility of increasing the sensitivity of the serum CAA test without affecting specificity. Preliminary results indeed indicate that further improvement of the serum CAA test performance resulted in a decrease of the lower detection limit to 0.3 ng/ml of AWA-TCA (= 9 pg CAA/ml), while maintaining a specificity of at least 98% in Dutch controls as well as Senegalese and Burundese negatives (GJ Van Dam, unpublished results).

The results for the CCA assays were not as clear-cut as for CAA. It is known that CCA detection may give false positive results, probably due to the fact that the polysaccharide structure of CCA contains repeating units of Lewis-X trisaccharide. This molecule is a common epitope on human anti-inflammatory cells (e.g. granulocytes) and a possible cause of cross-reactions with other infections (Van Dam *et al*. 1994). Therefore, cut-off levels for the CCA ELISA are set higher than the actual lower detection limit of the assay (Krijger *et al*. 1994). According to our results however, the cut-off levels in use with the alkaline/heating pretreatment would still be far too low. To achieve a specificity of at least 98% with this method, cut-off levels would have to be set at such a high level that sensitivity would drop considerably. Applying the adjusted cut-off levels to the endemic Burundi data reported by Polman *et al*. (1998) would lead to a decrease in the percentage of positives from 97.4% to 73.3% for urine CCA, and from 89.2% to 64.8% for serum CCA. The cut-off levels in use with TCA pretreatment on the other hand, would hardly, if at all, have to be adjusted. Although cross-checking was not possible for serum CCA detection, the results suggest that this assay was subjected to the same problem with alkaline/heating pretreatment. In the Dutch negative controls, there was no difference in specificity between both pretreatment techniques (Krijger *et al*. 1994). Our present results in negatives from Burundi and Senegal indicate that this conclusion is not generally valid. Apparently, the alkaline/heating pretreatment method is not as effective as TCA-pretreatment at removing certain interfering components, the composition and levels of which may vary between populations. A likely explanation is the higher frequency of various infectious diseases, and the consequent presence of more anti-inflammatory cells sharing the Lewis-X epitope with CCA, in urine and blood of *S. mansoni* negative individuals in Africa.

In conclusion, our results further corroborate that the serum CAA assay is highly specific, irrespective of the target population or the pretreatment method used. Moreover, a further increase of the sensitivity of the assay by decreasing the lower detection limit may be possible without affecting specificity. The assay is thus confirmed as a highly valuable alternative to egg counts, with potential for even further improvement.

CCA detection in Burundi and Senegal resulted in more false positive reactions. However, for a noninvasive screening test such lower specificity may be acceptable. Important differences were observed between Dutch and African negative controls with the alkaline/heating pretreatment, but not with TCA pretreatment. For CCA detection, the sample pretreatment method of choice would thus be TCA pretreatment, as this method appears to be more efficient and consistent in removing interfering substances.

More generally, this observation clearly shows that there may be considerable differences in test performance when using Dutch controls or local negatives. It is thus important to include – if possible – sufficiently large panels of negative samples from (various) endemic areas in method development. As negative controls within an endemic community are diffi-

cult to define, the approach we used, taking advantage of the focal distribution of *S. mansoni*, may be applied to obtain negative controls from nearby uninfected but otherwise comparable communities.

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