URINE CIRCULATING SOLUBLE EGG ANTIGEN IN RELATION TO EGG COUNTS, HEMATURIA, AND URINARY TRACT PATHOLOGY BEFORE AND AFTER TREATMENT IN CHILDREN INFECTED WITH SCHISTOSOMA HAEMATOBIUM IN KENYA

ANTHONY I. KAHAMA, ADEL E. ODEK, RUTH W. KIHARA, BIRGITTE J. VENNERVALD, YERI KOMBE, TITUS NKULILA, CHRISTOPH F. HATZ, JOHN H. OUMA, AND ANDRE M. DEELDER

Department of Parasitology, Leiden University Medical Centre, Leiden, The Netherlands; Division of Vector Borne Diseases, Ministry of Health, Nairobi, Kenya; Danish Bilharziasis Laboratory, Copenhagen, Denmark; Kenya Medical Research Institute, Nairobi, Kenya; Regional Hospital, Morogoro, Tanzania; Swiss Tropical Institute, Basel, Switzerland

Abstract. A cohort of 117 school children infected with Schistosoma haematobium was followed-up after therapy with praziquantel (0, 2, 4, 6, 12, and 18 months) and various infection and morbidity parameters (egg counts, hematuria, soluble egg antigen [SEA] in urine, and ultrasonography-detectable pathology) were quantified. At the onset of the study, 97% of the children were positive for S. haematobium with a geometric mean egg count of 45.7 eggs/10 ml of urine. Eighty-one percent of the children were positive for SEA in urine with a geometric mean SEA concentration of 218.8 ng/ml of urine. Ninety-two percent and 56% of the children were microhematuria positive and macrohematuria positive, respectively. Two months after treatment, all infection and morbidity indicators had significantly decreased. Reinfection after treatment as determined by detection of eggs in urine was observed by four months post-treatment while the other parameters remained low. The clearance of SEA was slower than that of egg counts while pathology resolved at an even slower pace. Levels of SEA and egg output showed similar correlations with ultrasound detectable pathology; these correlations were better than the correlation between hematuria and pathology.

In Kenya, schistosomiasis haematobia is endemic mainly along the coastal plain with a few scattered foci in the Eastern, Central, Western, and Nyanza provinces. So far, there have been no reported cases of other human schistosome species in this region. In areas where the disease is endemic, there are risks of urinary tract pathology that manifests clinically as hematuria, proteinuria, and pathology of the urinary tract. Long-term infection is associated with iron deficiency and anemia and with a predisposition to cancer of the bladder. Recent studies have found a significant negative association between S. haematobium infection and mental capabilities in school children.

The global objective in schistosomiasis control is the reduction of morbidity rather than the total eradication of the disease. Though the intensity of infection is thought to reflect the severity of the disease, this relationship is still elusive and differs within an infected community and between different geographic areas. In addition, there are currently no direct or indirect measures that can predict disease development. Such tools, if developed, could have a direct application in morbidity control within a primary health care setting. Measurement of genus-specific antigens is increasingly being used to try to quantify both infection and morbidity. Measurement of circulating anodic antigen (CAA) and circulating cathodic antigen (CCA) has been applied with significant associations being found between levels of these antigens and pathology as measured by the reagent strip index (RSI). However, the dynamics of these antigens in S. haematobium infections (especially serum CCA) are complex since some studies have been unable to detect this antigen. This antigen was reported for the first time by Kremser and others in 1994 in the urine of S. haematobium-infected individuals. Additionally, levels of CAA in urine are low and no correlations between CAA/CCA levels and actual pathologic lesions as determined by ultrasound have been recorded.

We have recently described a specific and sensitive assay that detects soluble egg antigen (SEA) in the urine of S. haematobium-infected children and observed that the levels of this antigen were significantly correlated with intensity of infection as measured by egg counts and with pathology as determined by ultrasonography. In this study, we examine the dynamics of SEA in urine in a group of school children before and after treatment and compare these with intensity of infection, hematuria, and ultrasound-detectable pathology.

PATIENTS AND METHODS

Study population. School children from two schools in the Kaloleni division of the Coast province of Kenya were selected for this study. This area is endemic for S. haematobium with no reported case of infection with S. mansoni. A more detailed description of the study area and population has described elsewhere. Two schools were selected for the study, Tsunguni with an initial prevalence of 86% and Kibaokiche with a prevalence of 77%. From the group previously described, 117 children participated in all follow-up examinations: 52 from Tsunguni and 65 from Kibaokiche of whom 59 were boys and 58 were girls (age range = 6–15 years, median = 9 years).

Informed consent was provided by the pupils’ parents, the education office, and the local administration. All the children found positive at the start and at the end of the study were treated with praziquantel at a dose of 40 mg/kg of body weight. The study protocol was approved by the Ministry of Health’s Ethical Review Committee of the Ministry of Health of Kenya and the Danish Central Medical Ethics Committee.

Collection and parasitologic examination of urine. For the determination of prevalence and intensity of infection, S. haematobium eggs were quantified on a minimum of three consecutive days before therapy and at months 2, 4, 6, 12,
and 18 after treatment. Urine was collected in each of the surveys between 10:00 AM and 2:00 PM. A duplicate 10-ml aliquot was filtered from each urine sample using 15-mm polycarbonate filters (Nuclepore®; Costar Europe Ltd., Badhoevedorp, The Netherlands), which were then placed on a labeled slide and examined under a microscope within 6 hr. The mean egg counts of the two filters were recorded. The individual infection status was determined by taking the mean egg count for a minimum of three days.

Microhematuria was evaluated semi-quantitatively using reagent strips (Hemastix®; Ames, Bie and Bernsten, Copenhagen, Denmark) and the results were ranked as negative, trace, +, ++, or +++ according to the manufacturer’s instructions. Macrohematuria was scored as positive on urine samples with a reddish-brown color or altogether bloody. A 10-ml urine sample for the SEA assay was collected in a Polysorb tube (Nunc, Roskilde, Denmark) once within the three-day period and placed in a cool box with ice. The samples were frozen within 6 hr and stored at −20°C until use.

Clinical examination. Ultrasound investigations were performed on all children using an ultrasound machine (SSD-500, 3.5 MHz; Aloka Co., Ltd., Tokyo, Japan) by two physicians who had been trained for this purpose. The assessment of bladder pathology followed the procedure described previously. Briefly, bladder wall thickening, mass or polyp formation and dilatation of the ureter were considered as pathology. The results were recorded according to the Cairo classification on a standard form.

Sandwich ELISA. A monoclonal antibody-based sandwich ELISA was used for the quantification of SEA in urine of the infected children as described previously. The lower detection limit for this ELISA was 16–31 ng/ml and samples with absorbency values above the mean of the buffer rows plus two standard deviations were considered positive for SEA. The assay has a specificity of 93% and a sensitivity of 90% and shows little reactivity with S. mansoni-infected and negative control urine samples. For urinary egg counts > 49 eggs per 10 ml of urine, the assay showed a sensitivity of 100%, while for a lower positive egg count the sensitivity was > 70%.

Statistical analysis. Egg counts and SEA concentrations were log-normally distributed (this was confirmed by logarithmic transformation log(x + 1) of the egg counts and SEA values); thus, parametric methods were used to evaluate results. Students’ t-test was applied accordingly to test for differences and the Pearson’s and Spearman’s correlation coefficients (as applicable) were computed to examine the associations between the SEA concentrations and other parameters.

RESULTS

For standardized comparison of results, only those children with complete data sets on egg counts, hematuria, pathology, and SEA were selected. The infection rates as determined by various parameters in this group are summarized in Table 1. Before treatment, 97% of the children were excreting S. haematobium eggs in their urine, with a geometric mean egg output of 45.7 eggs/10 ml of urine (range = 0–1,000 eggs/10 ml of urine); 16 (13.8%) children had egg counts > 1,000. Ninety-two percent of the children had dipstick-detectable hematuria and 56% had heavy hematuria. Eighty-one percent of the children were positive by the SEA-ELISA with a geometric mean SEA concentration of 218.8 ng/ml (range = 0–37,349 ng/ml). Fifty-eight percent of the children had overall ultrasonography-detectable pathology before treatment.

Cure rate in this study was defined as the percentage of the children excreting eggs before treatment and having zero egg counts eight weeks after treatment; in both schools the cure rate was 64.6%.

The prevalence and intensity of infection as determined by egg counts significantly decreased (P < 0.0005) two months after treatment (Table 1). Four months post-treatment, the prevalence of infection began to increase, with the upward trend continuing with ultimately 68% of the children being reinfected at the end of the study. This change was highly significant (P < 0.0005). The prevalence of heavy infections, i.e., ≥ 50 per 10 ml of urine (Figure 1) decreased significantly two months after treatment and remained low up to the 18 month timepoint. Although no difference was noted in the prevalence and intensity of infection between

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>12</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean egg output (no./10 ml of urine)</td>
<td>45.7</td>
<td>1.4</td>
<td>1.6</td>
<td>2.5</td>
<td>5.6</td>
<td>14</td>
</tr>
<tr>
<td>(41–91)</td>
<td>(1.3–1.6)</td>
<td>(1.4–2.0)</td>
<td>(1.8–3.4)</td>
<td>(3.8–8.3)</td>
<td>(8.8–22.2)</td>
<td></td>
</tr>
<tr>
<td>Mean SEA (ng/ml)</td>
<td>97 (13.8)†</td>
<td>34</td>
<td>40</td>
<td>40</td>
<td>57</td>
<td>68 (8.5)†</td>
</tr>
<tr>
<td>(127.7–380.7)</td>
<td>(4.1–10.4)</td>
<td>(2.3–5.3)</td>
<td>(5.0–11.6)</td>
<td>(6.3–18.6)</td>
<td>(9.0–23.8)</td>
<td></td>
</tr>
<tr>
<td>Microhematuria (% positive)</td>
<td>92</td>
<td>44</td>
<td>23</td>
<td>25</td>
<td>53</td>
<td>54</td>
</tr>
<tr>
<td>Heavy hematuria (% positive)</td>
<td>56</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>15</td>
<td>27</td>
</tr>
<tr>
<td>Pathology (% positive)</td>
<td>58</td>
<td>21</td>
<td>18</td>
<td>4</td>
<td>29</td>
<td>51</td>
</tr>
<tr>
<td>* Values for egg counts and SEA are geometric means, confidence intervals, and percentage positive; values for hematuria and pathology are percent positive.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>† Percentage of children with ≥1,000 eggs per 10 ml of urine.</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
the two schools before treatment, the post-treatment pattern was strikingly different as prevalence and intensity of infection remained low in Tsunguni while reinfection set in almost immediately in Kibaokiche. Before treatment, females were more heavily infected than males although the difference was not statistically significant; however, the situation was reversed 18 months later with more males being reinfected than females.

The number of children positive for SEA in urine significantly decreased two months after treatment \( (P = 0.02) \) and decreased further at four months post-treatment \( (P = 0.001) \) (Table 1). There was a sharp decrease in the geometric mean SEA concentration two months after treatment but with minimal change four months after treatment. However, the prevalence and levels of urine SEA increased significantly \( (P = 0.007) \) six months after treatment. For a better understanding of the kinetics of SEA concentrations, the levels were categorized arbitrarily into different classes as follows: light (16–99 ng/ml), moderate (100–999 ng/ml), and heavy \( (\geq 1,000 \text{ ng/ml}) \). The prevalences of moderate and heavy infections were 38% and 35%, respectively, but after treatment the levels decreased and remained low, particularly for the heavy SEA levels. It was interesting to note that we continued to detect SEA two and four months after treatment even as the levels of egg output were extremely low. The overall and geometric mean concentration of SEA were higher in older children \( (\geq 10 \text{ years old}) \) before treatment but 18 months after treatment the situation was reversed. The SEA-ELISA showed that more children in Tsunguni were positive than in Kibaokiche.

Macrohematuria and microhematuria decreased significantly eight weeks after treatment and few children showed any heavy hematuria until one year after treatment (Table 1). The clearance of microhematuria continued with significant reductions at two and four months post-treatment \( (P < 0.0005 \text{ and } P = 0.001) \). A sharp increase in the number of children with microhematuria was observed 12 months after treatment but the increase at 18 months post-chemotherapy was not significant. More males than females had heavy hematuria both before and after treatment, but this difference was not statistically significant. An equal proportion of the males and females were positive for microhematuria before treatment, while eight weeks after treatment a significantly higher number of females had hematuria compared with males.

Lower urinary tract lesions before treatment were observed in 58% of the children. At this time, more children in Tsunguni than in Kibaokiche had overall pathology, but no difference was observed between boys and girls. However, more of the older children showed pathology than the younger ones. Urinary tract lesions after treatment seemed to clear slowly compared with egg counts (Figure 2) two months post-treatment with the downward trend continuing up to the six months timepoint. However, a sharp increase was observed at 12 months and at 18 months post-treatment 51% of the children had developed pathologic lesions in the lower urinary tract. Resolution and reappearance of pathology paralleled the clearance and reappearance of SEA and that of egg counts (Figure 2).

Significant correlations were observed between SEA and egg counts (Table 2) before and after treatment \( (r = 0.7, P < 0.0005 \text{ and } r = 0.6, P < 0.0005) \), respectively. Significant correlations were also found between SEA with hematuria \( (r = 0.3, P < 0.003 \text{ and } r = 0.5, P < 0.0005) \) and ultrasound detectable pathology \( (r = 0.3, P < 0.003 \text{ and } r = 0.4, P < 0.0005) \).

**DISCUSSION**

This study has examined the dynamics of (circulating) SEA levels in urine before treatment and its clearance and reappearance after treatment in comparison to egg counts,
pathology as determined by ultrasonography, and hematuria. In this study group, SEA levels in urine cleared slower than egg counts, but after reinfection, egg output increased much faster. Consistent with SEA, urinary tract lesions also cleared at a much slower rate. Cure rates two months after treatment determined either by egg counts or SEA were similar.

We have previously described an assay detecting SEA in the urine of *S. haematobium*-infected individuals and have found significant correlations with egg counts, hematuria, and ultrasound-detectable pathology. 9,13 In the current study, similar correlations were also observed both before therapy with praziquantel and 18 months after therapy. Clearance of SEA from the urine was slower compared with egg counts and levels increased significantly six months after treatment, about two months later than egg counts; SEA levels continued to increase in parallel with egg counts and overall pathology. It was observed that SEA could be demonstrated in significant amounts even when egg count levels were very low. This could be interpreted as the activity of live eggs in the bladder wall. In addition, in other studies it has been suggested that praziquantel kills only the mature eggs while immature eggs remain unaffected and develop to maturity. 14-16 Such eggs may either be excreted to the outside or remain trapped in the tissues and this could account for the continued detection of SEA after treatment, even when the egg counts are very low. However, further studies are needed to evaluate the importance of these findings in relation to pathology.

It is striking that six months after treatment levels of SEA increased significantly while egg counts were still low and pathologic lesions continued to be cleared even further. This could indicate the secretion of SEA by live eggs trapped in the bladder wall; eggs are not yet excreted through the bladder wall, which could explain the low egg counts in urine. At this time, the pathologic lesions on the bladder wall are still being cleared while new ones, undetectable by ultrasound, are forming. This could explain the discrepancy between the high levels of SEA compared with egg output and pathology.

Resolution of lower urinary tract lesions after treatment was slow and paralleled the clearance of SEA compared with egg counts. This also corresponds to the clearance in those with heavy egg output (≥ 50 eggs/10 ml of urine) and that of high SEA levels (≥ 1,000 ng/ml of urine). Similar correlations were found between egg counts and SEA levels with pathology; these correlations were stronger than the correlation between hematuria and pathology. This is a positive indication that the SEA detection may be used both as an infection and morbidity indicator. However, more studies should be carried out to evaluate the application of SEA levels in urine as a possible predictor for the predisposition to severe urinary tract pathology.

The direct evaluation of pathology currently relies on ultrasonography and various studies have used this approach in an epidemiologic setting. 17 However, this requires trained physicians and thus may be difficult to use routinely. To achieve better morbidity control, a simple tool that can be used in the field is needed. Kremsner and others 7 have found significant correlations between adult worm antigen detection (CAA and CCA) and RSI (a combined reagent strip measurement of hematuria, proteinuria, and leukocyturia) as an indicator of pathology. Our study appears to be the first that has examined the dynamics of SEA detection and pathology and the results seem promising.

In conclusion, the quantification of SEA in urine, although

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**Figure 2.** Relationship between pathology as determined by ultrasonography and soluble egg antigen (SEA). Open bars = mean SEA levels in ng/ml of urine; hatched bars = mean egg counts/10 ml of urine. The line indicates pathology prevalence. Error bars show 95% confidence intervals.

**Table 2**

<table>
<thead>
<tr>
<th>Soluble egg antigen</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>12</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine eggs</td>
<td>0.7+</td>
<td>0.2</td>
<td>-0.03</td>
<td>0.2</td>
<td>0.3</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td><em>P</em> &lt; 0.0005</td>
<td><em>P</em> = 0.03</td>
<td>NS</td>
<td><em>P</em> = 0.007</td>
<td><em>P</em> = 0.002</td>
<td><em>P</em> &lt; 0.0005</td>
</tr>
<tr>
<td>Hematuria</td>
<td>0.3‡</td>
<td>0.07</td>
<td>-0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td><em>P</em> &lt; 0.0005</td>
<td>NS</td>
<td>NS</td>
<td><em>P</em> = 0.034</td>
<td><em>P</em> = 0.003</td>
<td><em>P</em> &lt; 0.0005</td>
</tr>
<tr>
<td>Pathology</td>
<td>0.3‡</td>
<td>0.1</td>
<td>-0.04</td>
<td>-0.02</td>
<td>0.16</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td><em>P</em> = 0.003</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td><em>P</em> &lt; 0.0005</td>
</tr>
</tbody>
</table>

* NS = not significant.

† By Pearson’s correlation coefficient.

‡ By Spearman’s correlation coefficient.
relatively new, has the potential of being developed as a simple tool for the assessment of morbidity in *S. haematobium* infections. However, this must await the simplification of the assay into a dipstick-like technique that would be easier to apply in the field. A dipstick-like assay has already been developed in our laboratory for the detection of CCA and field studies have shown promising results. However, the present study is part of a project aimed at developing assays that could be used for the diagnosis and morbidity assessment in *S. haematobium* infections; in this project, an ELISA that sensitively detects eosinophil cationic protein (ECP) in urine of *S. haematobium*-infected individuals has been developed and urine ECP was found to correlate well with other parameters of infection. Thus, we need to validate our results against the other tools for assessment of infection and morbidity, among which are the assays for the circulating adult worm antigens and the ECP.

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Authors’ addresses: Anthony I. Kahama and Andre M. Deelder, Department of Parasitology, Leiden University Medical Centre, PO Box 9605, 2300 RC Leiden, The Netherlands. Adel E. Odek, Ruth W. Kihara, and John H. Ouma, Division of Vector Borne Diseases, Ministry of Health, PO Box 20750, Nairobi, Kenya. Birgitte J. Ven-
nervald, Danish Bilharziasis Laboratory, Jaegersborg Allé ID, DK-2920 Charlottenlund, Denmark. Yeri Kombe, Kenya Medical Research Institute, PO Box 20977, Nairobi, Kenya. Titus Nkulila, Mbeya Consultant Hospital, PO Box 419, Mbeya, Tanzania. Christoph F. Hatz, Swiss Tropical Institute, PO Box, CH-4002 Basel, Switzerland.

Reprint requests: Andre M. Deelder, Department of Parasitology, Leiden University Medical Centre, PO Box 9605, 2300 RC Leiden, The Netherlands.

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