Schistosomiasis is one of the most widespread parasitic infections. The disease is endemic in 75 countries, with some 600 million people at risk of infection. In Egypt, two species, *Schistosoma haematobium* and *S. mansoni* cause urinary and intestinal schistosomiasis, respectively. Despite mass treatment campaigns, schistosomiasis remains endemic and a significant prevalence of the disease still exists. Disease control plans depend mainly on the diagnosis and treatment of infected individuals. Up until now, diagnosis has been dependent on the detection of schistosome eggs in either stool or urine. However, because of low and sporadic egg production, the risk of undiagnosed individuals is tremendous. Undiagnosed individuals remain infected and transmission of the disease continues. In the last few years, many diagnostic techniques have been developed, including ultrasonography, detection of circulating antibodies to semi-purified or fractionated antigens, and detection of parasite circulating antigens in different host body fluids.

Detection of specific antibodies to *S. haematobium* and *S. mansoni* were used to detect antibodies in the Falcon assay screening test (FAST-ELISA) and the enzyme-linked immunoelectrotransfer blot (EITB). Circulating anodic antigen (CAA) and circulating cathodic antigen (CCA) were quantified in serum and urine samples in a sandwich ELISA using monoclonal antibodies. Parasitologically, the prevalence of *S. haematobium* was 7.01% in females and 25.82% in males, giving an overall prevalence of 15.8%. The combination of urine CCA and serum CAA for detecting circulating antigens and the combination of the *S. haematobium* adult worm microsomal antigens (HAMA) FAST-ELISA and the HAMA EITB for detecting antibodies significantly improved the sensitivity of detecting *S. haematobium* circulating antigens and antibodies. Also, including a medical examination as an integral part of field studies and correlating immunodiagnostic results with other clinical and investigational data allowed us to calculate an accurate estimation of *S. haematobium* prevalence in this area of low endemicity.

**Materials and Methods**

**Population.** A 10% systematic sample of houses (every tenth house) in Behbeet village, 50 km south of Cairo, was identified and numbered, and an accurate map was drawn. All occupants more than five years of age were interviewed and invited to participate in the study. Stool, urine, and blood samples were collected from 370 individuals in containers with computer-generated labels. Demographic data from each individual were recorded on data sheets. The investigations were performed in accordance with the Ministry of Health and Human Services guidelines for Clinical Research and Treatment under a protocol approved by the Schistosomiasis Research Project, VACSERA, Egypt, and the Centers for Disease Control and Prevention (CDC-IRB approval # 1381-CDC-IRB).

**Heterologous infection sera.** Positive reference sera and sera from other heterologous diseases were obtained from CDC (Atlanta, GA). Heterologous infection sera were collected from schistosome-free geographic locations. They included the following infection sera: hepatitis from Alaska and Nevada (United States, n = 18), trichinosis from Louisiana (United States, n = 20), cysticercosis from Peru and...
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Mexico (n = 41), filariasis from Haiti (n = 18), amebiasis from Mexico (n = 52), onchocerciasis from Guatemala (n = 14), and echinococcosis from Alaska (United States, n = 19). Schistosoma mansoni infection sera were collected from Brazil and Puerto Rico (n = 23). Both locations are non-endemic for S. haematobium. Schistosoma haematobium infection sera were collected from the Giza and Menia governates of Egypt (n = 18). Both locations are non-endemic for S. mansoni. These sera were collected under the aforementioned study protocol (CDC-IRB approval # 1381-CDC-IRB).

Physical examinations. All participants enrolled in this study underwent a physical examination and submitted two consecutive stool and urine samples for parasitologic examination. Schistosoma mansoni eggs excreted in feces were detected microscopically in four separate slides by the modified Kato slide method. Schistosoma haematobium eggs excreted in urine were detected by filtration with Nucleopore® polycarbonate membranes (Corning Separations, Acton, MA). Egg counts and other data from microscopic observations were recorded on patient data sheets. Patients with active excretion of eggs received a full physical examination for evidence of schistosome-associated disease. Blood samples were drawn, and the patients were immediately treated with 60 mg of praziquantel (Biltricide®; Bayer AG, Leverkusen, Germany) per kg of body weight. Serum samples were frozen at −85°C; urine samples were stored at −35°C with 0.1% sodium azide added as a preservative.

Antigen preparation. The preparation of MAMA and HAMA was performed according to the method of Tsang and others. Briefly, microsomal antigens from S. mansoni or S. haematobium adult worms were purified by differential centrifugation, urea extraction, molecular sieving, and ionic-exchange chromatography.

FAST-ELISA. The FAST-ELISA was performed to detect antibodies to MAMA antigens. The Falcon Assay Screen-centrifugation, urea extraction, molecular sieving, and ionic-pore excreted in urine were detected by filtration with Nucleopore® polycarbonate membranes (Corning Separations, Acton, MA). Egg counts and other data from microscopic observations were recorded on patient data sheets. Patients with active excretion of eggs received a full physical examination for evidence of schistosome-associated disease. Blood samples were drawn, and the patients were immediately treated with 60 mg of praziquantel (Biltricide®; Bayer AG, Leverkusen, Germany) per kg of body weight. Serum samples were frozen at −85°C; urine samples were stored at −35°C with 0.1% sodium azide added as a preservative.

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with the homologous antigen (HAMA) showed an enhanced MAMA in the FAST-ELISA. The FAST-ELISA screening for the CCA ELISA. Samples with concentrations above the lower detection limit were considered positive.

The intensity of infection, as measured by egg output, decreased in group ≥ 20 years of age. In the 10–14-year-old age group, there was a statistically significant difference (P = 0.05) in the intensity of infection between males and females. The geometric mean egg output in this age group was 17.9 epml for males and 7.1 epml for females. A similar trend was seen in the 5–9-year-old age group. Other helminths identified included Hymenolepis nana, 6; Enterobius, 4; Ancylostoma duodenale, 2; Ascaris, 1; Taenia saginata, 1; and Strongyloides, 1.

**Parasitologic data.** Four hundred thirty-four residents of Behbeet village (n = 4,438) were invited to enroll in this 10% survey study. Of these participants, 370 donated blood, urine, and stool, giving a total compliance of 85.2%. One hundred seventy-two were male and 198 were females, with a median age of 23.6 years (range = 5–75). Parasitologic examination demonstrated the presence of live S. haematobium eggs in the urine of 67 individuals (18.1%) and S. mansoni eggs in stool of two individuals (0.54%). Most of the S. haematobium-infected participants (59) showed an egg output of less than 50 eggs/ml (epml). Egg output ranged from 1 to 153 epml; 71.6% of those excreting eggs were males and 28.4% were females. Figure 1 depicts the parasitologic data in relation to the age of the infected villagers.

**Results**

**Immunodiagnostic data: assay specificity.** Heterologous parasitic infection sera (n = 240) from persons residing in schistosome-free geographic locations were tested for antibody cross-reactivity with HAMA and for antigens recognized in the CAA and CCA assays. The heterologous sera had a reactivity less than 1.2 U/μl in the HAMA FAST-ELISA and failed to react with GP23 in the CAA assay (Figure 2). The ELISA showed that serum CAA and urine CCA were not detected in the tested sera except in 20% (1) of Onchocerca volvulus–infected individuals and 20% (3 of 14) of Wuchereria bancrofti–infected individuals and 20% (3 of 14) of Onchocerca volvulus–infected individuals in the CAA and CCA assays, respectively. Thus, at the cutoff levels used in the present study, the specificities of the serum CAA and urine CCA assays were 97.8% and 95.5%, respectively. In a population infected with W. bancrofti or O. volvulus, the specificities of the assays would decrease to about 80%. For the antibody assays, the HAMA FAST-ELISA, and the HAMA EITB, the specificities were 100%.

**Clinical data.** Analysis of the clinical data in relation to TCA), which contains approximately 3% (w/w) CAA and CCA. The lower detection limits for circulating antigen were 0.3 ng/ml of AWA-TCA for the CAA ELISA and 0.7 ng/ml CCA. The lower detection limits for circulating antigen were therefore 94.0%. By screening with the HAMA FAST-ELISA and the EITB, all egg-passing individuals were detected and the sensitivity of antibody detection reached 100% (Table 1). Interestingly, the MAMA EITB detected a substantial percentage (19.6%) of the participants possessing antibodies to the species-specific band GP30, thus indicating a current or previous infection with S. mansoni.

**Table 1.** Detection of antibodies in Schistosoma haematobium egg-positive patients (n = 67) by FAST-ELISA with MAMA and HAMA and by EITB with HAMA.

<table>
<thead>
<tr>
<th>No. antibody positive/</th>
<th>No. egg positive</th>
<th>56/67</th>
<th>66/67</th>
<th>63/67</th>
<th>67/67</th>
</tr>
</thead>
<tbody>
<tr>
<td>% positive</td>
<td></td>
<td>83.6</td>
<td>98.5</td>
<td>94.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

* MAMA = S mansoni adult worm microsomal antigen; HAMA = S haematobium adult worm microsomal antigen; EITB = enzyme-linked immunoelectrotransfer blot. 
† A reaction greater than 10 U/μl is considered positive. 
‡ A reaction greater than 1.2 U/μl is considered positive. 
§ Antibody recognition of GP23 is considered positive. 
¶ Positive in the FAST-ELISA and/or EITB assay with HAMA as antigen.
Table 2

<table>
<thead>
<tr>
<th>No. of individuals</th>
<th>epml = 0</th>
<th>epml &lt; 20</th>
<th>epml = 20–50</th>
<th>epml &gt; 50</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean epml</td>
<td>0</td>
<td>7.0</td>
<td>26.9</td>
<td>91.4</td>
<td>21.3</td>
</tr>
<tr>
<td>Range epml</td>
<td>0</td>
<td>1–18</td>
<td>20–38</td>
<td>55–153</td>
<td>1–153</td>
</tr>
<tr>
<td>Serum CAA</td>
<td>131 (43%)</td>
<td>23 (51%)</td>
<td>8 (57%)</td>
<td>6 (75%)</td>
<td>37 (55%)</td>
</tr>
<tr>
<td>Serum CCA</td>
<td>18 (6%)</td>
<td>7 (16%)</td>
<td>4 (29%)</td>
<td>4 (50%)</td>
<td>11 (16%)</td>
</tr>
<tr>
<td>Urine CAA</td>
<td>29 (10%)</td>
<td>3 (7%)</td>
<td>2 (14%)</td>
<td>2 (25%)</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Urine CCA</td>
<td>90 (29%)</td>
<td>32 (71%)</td>
<td>12 (86%)</td>
<td>8 (100%)</td>
<td>52 (78%)</td>
</tr>
</tbody>
</table>

* epml = eggs/ml of urine; CAA = circulating anodic antigen; CCA = circulating cathodic antigen. For definitions of other abbreviations, see Table 1.

parasitologic results revealed that the presence of the disease is significantly correlated with some clinical manifestations and symptoms such as hematuria (26.9%), leukocyturia (60.0%) (as indicated by the urine strips), and urinary tract complaints currently or during the last six months before physical examination (35.8%) (Table 3). These parameters and others listed in Table 4 were also significantly correlated with the EITB-HAMA results. Meanwhile, urine analysis of 68% of the persons excreting eggs showed significant leukocyturia (n = 46; P < 0.001); 86% (n = 48) of the leukocyturia-positive donors tested positive for circulating antigen (urine CCA) (P < 0.01).

Estimation of prevalence. In parasitologically negative subjects (n = 303), the percentage of seropositive individuals with reactivity in the FAST-HAMA more than 1.2 U/ml and recognizing the *S. haematobium*-species specific GP23 band in the EITB assay was 40.5% (n = 123). Meanwhile, 57% (n = 172) of the parasitologically negative villagers tested positive for serum CAA or urine CCA. Clinically, 59 of the 303 parasitologically negative persons were recorded as having almost the same schistosomiasis-indicative symptoms observed in the infected participants group, that is, hematuria, leukocytosis, and current urinary tract complaints. The percentage of persons who excreted no eggs, but had circulating antigens and antibodies and reported clinical findings, was 19.5%. Therefore, integrating the clinical and immunodiagnostic data with the conventional parasitologic data gave an estimated overall prevalence in this area of 37.6% instead of 18.1%.

**DISCUSSION**

Diagnosis of schistosomiasis has mainly depended upon finding ova in patients’ excreta. Serologic testing has been used to enhance our ability to detect the disease. However, none of the studies reported in the literature applied a multi-diagnostic approach combining antibody and circulating an-
tigen detection with clinical data to accurately estimate the prevalence of the disease.

The systematic purification of microsomal antigens from adult worms has allowed the development of rapid, highly specific, and sensitive assays such as the FAST-ELISA and EITB for detecting antibodies in schistosomiasis. When MAMA was used in the FAST-ELISA, the sensitivity and specificity were 98% and 99%, respectively, in detecting sera infected with *S. mansoni*. The EITB is capable of differentiating infections caused by the three species of the genus *Schistosoma* of major importance in humans. Antibody reactivity with GP30 of MAMA identifies a *S. mansoni* infection, GP18, GP23, or GP29 of *S. japonicum* adult worm microsomal antigens (JAMA) identifies *S. japonicum*, and GP23 of HAMA identifies *S. haematobium*. Thus, the FAST-ELISA and EITB were used as screening and confirmatory assays, respectively, for prior or present schistosomiasis.

In this study, detection of schistosome-specific antibodies directed to microsomal antigens in an *S. haematobium*-endemic area was evaluated using both MAMA and HAMA. The sensitivity of the MAMA FAST-ELISA was 83.6%, whereas the HAMA FAST-ELISA screening was able to detect a significantly higher percentage (*P* = 0.001) of the infected persons (98.5%). When screening was done with the HAMA FAST-ELISA and EITB, all egg-passing individuals were detected and the sensitivity of antibody detection reached 100%. Therefore, in *S. haematobium*-endemic areas, screening with HAMA, rather than MAMA, in the FAST-ELISA would increase the sensitivity of detecting egg-excreting persons. The EITB confirmatory test is not only capable of determining the infecting species, but also complements the FAST-ELISA in detecting all infected persons. Only *S. haematobium* is known to be endemic in Behbeet; consequently, stool samples were usually overlooked and only urine examination was performed. The detection of a substantial percentage (19.64%) of the participants possessing antibodies to *S. mansoni* GP30 certainly emphasizes the importance of immunodiagnosis for proper patient management. Therefore, treatment based solely on the presence of ova is inadequate and allows patients with low egg outputs to remain undiagnosed and continue to transmit the disease. A new assay format that could combine the simplicity of the FAST-ELISA and the capability of the EITB to determine the species would be very useful in field studies.

The different sensitivities achieved with different circulating antigen assays were recorded in other studies with *S. mansoni* infections. The urine CCA assay was capable of identifying 52 of the 67 active cases (78%), followed by the serum CAA assay with 37 cases (55%), while 87% of the active cases were positive for at least one of these two assays. The improvement of sensitivity by parallel testing on more than one antigen has been reported previously in a study of Egyptian schistosomiasis patients. The sensitivity of the four individual circulating antigen assays reported by van Lieshout and others in 1992 was much higher than the data reported in the present study and matched those of Van Lieshout and others in 1994. This is consistent with the previous observation that the sensitivity of the assays is not only dependent on the intensity of infection, but also on the prevalence in the area where the patients come from. However, it is possible that the apparent association of assay sensitivity with prevalence of infection is actually due to parasite strain differences in the production of CAA and CCA.

Recently, circulating antigen detection was also applied in a newly established, heavily *S. mansoni*-infected community in the northern part of Senegal, where fecal egg excretion was found in 91% of the population. Similar to our study, the urine CCA assay was found to have the highest sensitivity, immediately followed by the serum CAA assay, while CAA could only be demonstrated in 55% of the urine samples and in remarkably low concentrations. Results were explained by a possible difference in clearance dynamics of this antigen due to the very recent nature of the infection in the study population. The fact that in our study, with longstanding infections and low egg excretion, levels of CAA in urine were also found to be very low indicates that other mechanisms may play a role in this phenomenon.

Based on the results of this study, we conclude that the combination of urine CCA and serum CAA for detecting circulating antigens and the combination of the HAMA FAST-ELISA and the HAMA EITB for detecting circulating antibodies significantly improves our ability to detect *S. haematobium* circulating antigens and antibodies. With the increased sensitivity of the assays, one can determine the prevalence of infection with higher accuracy. In addition, this study highlights the value of integrating immunodiagnostic tools and clinical findings. The clinical findings of hematuria, leukocyturia, and urinary tract complaints were significantly associated with egg-positive patients. The clinical findings of hematuria, leukocyturia, and urinary tract complaints, tender abdomen, and supra-pubic tenderness were significantly associated with antibody-positive patients. Therefore, including these clinical findings as a key to look closely for evidence of infection is important in patient management. Correlating immunodiagnostic results with other clinical and investigational data should allow us to provide recommen-
dations to physicians practicing in rural endemic areas, which in turn would significantly enhance the efficiency of the national anti-schistosomiasis control program in Egypt.

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