

Effective Diagnosis of *Schistosomiasis haematobium* by Immunomagnetic Bead ELISA technique Using Super-Paramagnetic Nanoparticles

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ABSTRACT: *Schistosomiasis haematobium* is a serious public health problem in Egypt. Detection of *S. haematobium* antigens is a better immunodiagnostic tool than determination of the antibody level. We developed a novel immunomagnetic bead ELISA based on IgG for detection of E/S antigen in sera of rabbit infected with *S. haematobium*. Detection of E/S in serum gave a sensitivity of 95%, a specificity of 93.7% compared to other parasitic infections group and 100% compared to healthy control group. On the other hand, detection of E/S in urine gave a sensitivity of 91%, a specificity of 93.7% compared to other parasitic infections group and 100% compared to healthy control group. The novel assay appears to be sensitive for detection of schistosomal antigenemia and valuable to judge the efficacy of chemotherapy in murine schistosomiasis.

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1. INTRODUCTION

Human schistosomiasis is a chronic, debilitating parasitic disease and is caused mainly by three species of the genus *Schistosoma*: *S. haematobium*, *S. japonicum* and *S. mansoni* (He *et al.* 2005). More than 600 million people are at risk with about 200 million actually infected in 74 countries mainly in the tropics and subtropics (Ruelas *et al.* 2006). *Schistosomiasis haematobium* is an important public health problem in Africa and the Middle East affecting more than 110 million people in rural, agricultural and peri-urban areas (WHO, 2008). Schistosomiasis is second only to malaria in terms of public health importance (Abdulla *et al.*, 2007). It is associated with a variety of clinical syndromes that may lead to severe morbidity (Bahgat *et al.*, 2010). The ancient Egyptians contracted the disease more than 4000 years ago (El-Zayadi, 2004).

Adult worm pairs of *S. haematobium* are found in copula within venous plexuses surrounding the bladder and ureters. Hundreds of eggs are laid by each female worm per day, and these gradually find their way into the lumen of the bladder (Blanchard, 2004). The disease is characterized by painful micturition, dysuria, hematuria, proteinuria and the presence of schistosome eggs in the urine of infected persons (Bosompema *et al.*, 1996 and Conor *et al.*, 2002). In the later stages immune-mediated granulomatous response to parasite eggs lead to granuloma formation in the lower urinary tract; which is the main cause of the pathology in the bladder (Pearce *et al.*, 2002). Schistosomiasis is

associated with debilitating morbidity manifested by sequelae such as iron deficiency anemia, cognitive impairment, lassitude, growth stunting (Savioli *et al.*, 2004) and predisposition to cancer of the bladder especially in adults (Michaud, 2007).

Routine diagnosis of *Schistosoma haematobium* infections can be done by detection of eggs in urine samples where eggs can be demonstrated because the volume of urine usually screened is relatively large. In addition, urine does not contain fibre-like structures as in stool which may hamper the recognition of eggs (Van Leishout *et al.*, 2000). However, the number of eggs counted in urine is strongly influenced by the protocol of sample collection, as most eggs are found around noon after physical exercise in combination with fluid intake, and in the last drops of micturition (Savioli *et al.*, 2004). Besides, routine microscopic technique is not sensitive enough, as it is difficult to find eggs in the urine of people with a low worm load, those with infections less than one month duration or in patients with chronic infections, where egg production and excretion is low (Lengler *et al.*, 1991).

Schistosoma antibody detection assays, though very sensitive particularly in individuals from endemic areas, do not however differentiate between active and past infection and do not correlate with intensity of infection (Van Leishout *et al.*, 2000). In addition, elevated antibody levels are still detectable many years after treatment (Ross *et al.*, 2001).

Antigen detection could be used in routine screening for case detection in low transmission

areas or detection of residual infections in very low transmission areas in order to eliminate the parasite reservoir and aid interruption of transmission. In *S. haematobium* infection, antigen levels were found to be significantly correlated with the egg excretion and decreased rapidly following successful treatment (Van Lieshout et al., 1994 and WHO, 2000). Detection of schistosomal antigens in serum and urine is a powerful immunodiagnostic tool and is considered as an alternative to egg counts in faeces (De Jonge et al., 1988; Van Lieshout et al., 1992), and urine (Kremsner et al., 1994). As the sensitivity of both antigen detection and egg counts is limited when the intensity of infection is low (De Jonge et al., 1991), a diagnostic technique with increased sensitivity is desirable. A variety of antigens are secreted and excreted by parasites present in the blood, faeces, urine and other fluids of the infected host. These antigens have potential for use in immunodiagnosis and vaccine development (Abdel-Rahman, 1999).

This study was conducted on 100 *S. haematobium* infected patients from endemic areas in El fayoum Governorate and from out clinic patients and hospital at TBRI and El-Azhar University Hospital. Patients were diagnosed by ERCP or finding characteristic eggs in urine samples collected and 63 patients were infected with other parasites (*Fasciola*, hookworm, hydatid and trichostrongyloides). In addition, 35 individuals of the medical staff at TBRI served as parasite free-healthy negative control.

The magnetic bead immunoassay combines the use of magnetic beads with a high binding capacity as a solid phase and the rapid reaction kinetics of solutions with the simple separation of bound and unbound materials on the solid phase, which provides the chance of enhancing the sensitivity of antigen detection (Gundersen et al., 1992; Ndhlovu et al., 1995).

2. MATERIALS AND METHODS

2.1. Materials:

2.1.1. Animals:

New Zealand white male rabbits, weighing approximately 1.5 kg and about 3 months age, purchased from Rabbit Research Unit (RRU), at Agriculture Faculty, Cairo University. They were housed in the animal house at Theodore Bilharz Research Institute (TBRI), Giza, Egypt, being kept for 4 weeks (wk) (experiment duration) under standard laboratory care at 21°C, 16% moisture, filtered drinking water with additional salts 1cm/ 5 liter and vitamins 1cm/ 10 liter. Diet was 15% protein, 3% fat and 22% fiber purchased from RRU. Animal experiments were carried out according to the

internationally valid guidelines and in an institution responsible for animal ethics.

2.1.2. Parasites:

Adult worms of *S. haematobium* were obtained from the Biological Centre in TBRI for preparation of parasite antigen. This antigen was used to immunize rabbits for the production of polyclonal antibodies. Mature *S. haematobium* worms were recovered by porto-mesenteric perfusion of livers of *S. haematobium* infected mice at 8-12 wk post-infection (PI).

2.2. Methods:

2.2.1. Antigen preparation:

To obtain E/S products, mature worms were washed 6 times in 0.01 M PBS, pH 7.3, at 37°C and maintained for 6 hr (1 mature worm per milliliter) in Roswell Park Memorial Institute (RPMI) 1640 medium, pH 7.3, containing 2% glucose, 30 mM *N*-2-hydroxyethylpiperazine-*N*-2-ethane-sulfonic acid (HEPES), and 25 mg L⁻¹ gentamycin at 37°C (Dalton and Heffernan, 1989). The medium containing the E/S products was then removed and centrifuged at 48,000 g for 30 min at 4°C. The supernatant was filtered, aliquoted, and stored at -20°C until use.

2.2.2. Immunization:

Blood samples were collected from healthy rabbit ears before injection and examined with ELISA for checking for *S. haematobium* antibodies and cross reactivity with other parasites, according to Fagbemi and Guobadia (1995). Each rabbit received an intramuscular injection (i.m) as 1 mg of *S. haematobium* E/S antigens mixed with equal vol. of complete Freund's adjuvant (CFA) (Pierce, Rockford, IL, USA). Booster doses (0.5 mg E/S antigens in equal vol. of incomplete Freund's adjuvant (IFA) (Sigma)) were administered at wk 2, 3 and 4 after the initial dose according to Fagbemi et al. (1995). Test blood samples were withdrawn before the injection of each immunization dose to detect the titer of antibodies produced. When the titer was high, the animal was sacrificed for blood collection, serum preparation and purification.

2.2.3. Purification and labeling of IgG polyclonal antibodies (pAb):

Purification of anti-*Schistosoma* IgG PAb sera were performed by ammonium sulphate precipitation according to Nowotny (1979), followed by treatment with caprylic acid treatment (Mckinney and Parkinson, 1987), then by DEAE anion exchange chromatographic method.

Purified anti-E/S *S. haematobium* was mixed with reducing buffer of Bio-Rad high and low molecular weight (M.W) standards then electrophoresed on SDS-PAGE according to method of Laemmli (1970). Testing for reactivity of PAb against different concentrations of *S. haematobium* E/S antigens was

performed by modified indirect ELISA (Engvall and Perlmann, 1971). Standardization of serial dilutions (2.5, 5, 10, 15, 20 and 30 µg/ml) of purified PAb were done by sandwich ELISA. According to Tijssen and Kursta (1984), labeling of PAb was done with horseradish peroxidase (HRP) using periodate method.

2.2.4. Collection of samples from human subjects

This study was conducted on 100 *S. haematobium* infected patients from endemic areas in El fayoum Governorate and from out clinic patients and hospital at TBRI and El-Azhar University Hospital. Patients were diagnosed by ERCP or finding characteristic eggs in urine samples collected and 63 patients were infected with other parasites (*Fasciola*, hookworm, hydatid, *Ascaris* and *Ancylostoma*). In addition, 35 individuals of the medical staff at TBRI served as parasite free-healthy negative control. Urine and blood samples were collected from all cases and sera were separated, aliquoted and kept at (-70°C) until used. They were classified into three groups;

Group A: *Schistosoma* infected group (n=100).

Group B: Other parasites infected groups (n=63). Group B included 10 patients infected with *Hymenolepis nana*, 10 with *Echinococcus granulosus*, 20 with *Fasciola*, 8 with *Ascaris* and 15 with *Ancylostoma*.

Group C: Healthy control group (n=35).

2.2.5. Sandwich enzyme linked immunosorbent assay (Sandwich ELISA).

Sandwich ELISA was based on the original method of Engvall and Perlman (1971) was used with the microplate modification of Venkatesan and Wakelin (1993).

2.2.6. Detection of E/S *Schistosoma* antigens in patient's sera by IMB-ELISA technique.

The microtitration plates were coated with 100 µl/well of anti-*S. haematobium* antibodies coupled with super-paramagnetic nanoparticle. (15 µg/ml for IgG in carbonate buffer 0.06 M, pH 9.6) and incubated overnight at room temperature. Plates were washed 3 times with 0.1 M PBS/T, pH 7.4. The remaining sites in the wells were blocked by 200 µl/well of 2.5% FCS/PBS/T and incubated for 2 hr at 37°C.

The plates were washed 3 times with PBS/T. 100ul of serum samples was pipetted into the wells in duplicate and incubated for 2 hr at 37°C. The wells were then washed 3 times as before. 100µl/well of peroxidase-conjugated polyclonal antibodies of 1/250 for IgG was then added and incubated for 1 hr at room temperature.

2.2.7. Statistical Analysis

The data are presented as mean ± standard deviation of mean ($X \pm SD$). The mean values of each group were calculated from the mean values of individual patients. The mean groups were compared by analysis of variance (Snedecor and Cochran, 1981). The

comparison between various groups was done using either Student's T test or ANOVA. Correlation between the optical density of ELISA technique and the number of ova in urine samples of *Schistosoma* infected human was performed by application of correlation coefficient (r) according to Snedecor and Cochran (1981). The data were considered significant if p values were equal to or less than 0.05. Statistical analysis was performed with the aid of the SPSS computer program (version 6.0 windows).

3. RESULTS

3.1. Assessment of purified rabbit anti-*Schistosoma* E/S products PAb:

By using 12.5% SDS-PAGE technique under reducing condition, 50% ammonium sulphate-precipitated protein showed that, most of the albumin was removed from rabbit anti-*Schistosoma* E/S products PAb. Precipitated proteins appeared as several bands. Further purification of whole immunoglobulins (Igs) by 7% caprylic acid precipitation and DEAE anion exchange chromatographic method removed the remaining non-IgG proteins. The purified IgG was represented by H- and L- chain bands at 53 and 31 KDa respectively (Fig. 1).

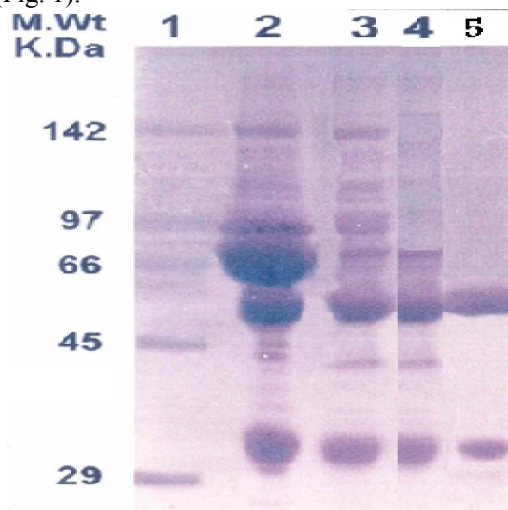


Figure 1: 12.5% SDS-PAGE of E/S of anti-*S. haematobium* IgG antibodies before and after purification (stained with Coomassie blue).

Lane 1: Mw of standard protein.

Lane 2: Anti-*S. haematobium* IgG antibodies before purification.

Lane 3: Precipitated proteins after 50% ammonium sulfate treatment.

Lane 4: Purified IgG antibodies after 7% caprylic acid treatment.

Lane 5: Purified IgG antibodies after ion exchange chromatography.

3.2. Reactivity of pure PAb to *Schistosoma* E/S products:

The reactivity of PAb was determined by indirect ELISA, gave a strong reactivity to *Schistosoma* E/S till dilution 1:250 (Fig. 2).

3.3. Standardization of anti- *Schistosoma* E/S PAb. Determination of the optimum concentration of coating with anti- *S. haematobium* antibody.

The optimization of various reagents were assayed by sandwich ELISA. The optimum concentration of purified IgG PAb was 15 µg/ml, whereas conjugated IgG PAb was 1:250 dilutions (Fig. 3).

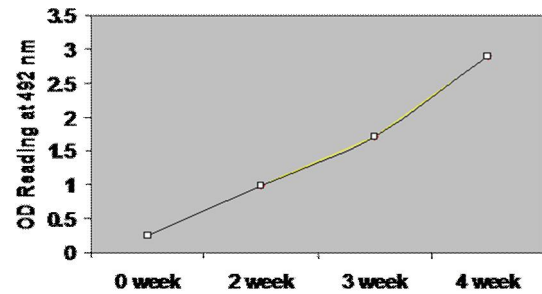


Figure 2: Reactivity of rabbit anti-*S. haematobium* antibodies (diluted 1/250) against *S. haematobium* E/S antigens by indirect ELISA.

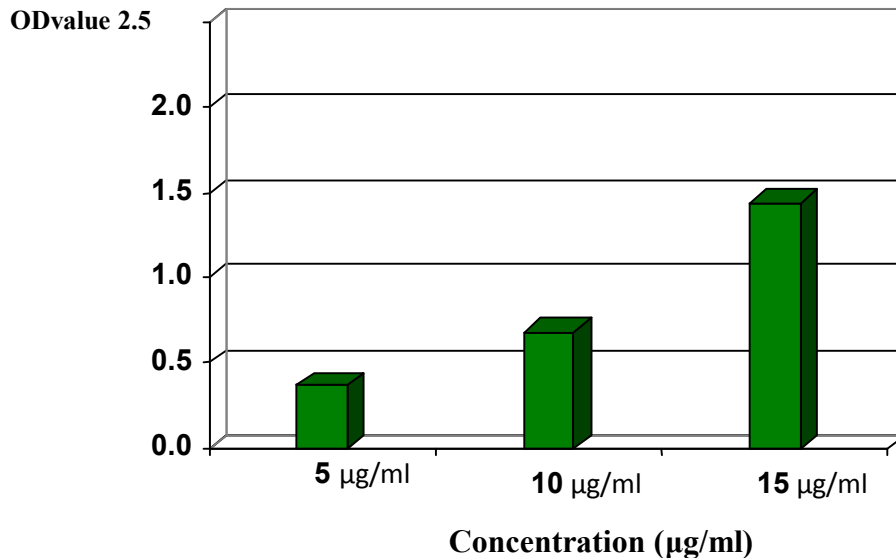


Figure 3: Determination of the optimum concentration of purified anti- *Schistosoma* IgG antibody used as capture antibody in sandwich ELISA..

3.4 Detection of *S. haematobium* antigen in human samples using sandwich ELISA

The *S. haematobium* antigen level was measured as optical density (OD) reading at 492 nm. As mean (X) OD of each group \pm standard deviation (SD).

In order to determine the cut off point for positivity or the line of demarcation between positive and negative results, the mean OD reading of negative controls + SD of the mean were estimated. Tested samples showing OD values > cut off value were considered positive for *S. haematobium*.

3.4.1. Detection of E/S *S. haematobium* antigen in serum

The cut off value equals was 0.428. when detecting E/S antigen in serum, the results were

positive in 87 cases (87%) of group A, while 13 cases were negative (13%). In group B (patients with other parasitic infections) 8 cases were detected as positive (3 with fascioliasis, 2 with hydatidosis, 1 with ancylostoma infection, 1 with ascariasis and 1 with *H. nana* infection), while the other 55 cases were negative. All healthy control patients were negative. The sensitivity of *S. haematobium* E/S antigen detection in serum was found to be 87%. However, the specificity was found to be 100% compared to the healthy control and 87.3% compared to the patients with other parasitic infections. There is a highly statistical significant difference between the positivity in schistosomiasis infected group and the other two tested groups ($P < 0.001$) (Table 1).

Table 1: *S. haematobium* E/S antigen level in serum in different groups

Infected Groups	Positive cases				Negative cases			
	Males(No.)	X ± SD	Females (No.)	X ± SD	Males (No.)	X± SD	Females (No.)	X± SD
Healthy control (n= 35)	-	-	-		22	0.373±0.12	13	0.331±0.28
Schistosoma (n= 100)	54	1.44±0.34	33	0.99±0.22	10	0.283±0.22	3	0.297±0.17
Fasciola (n= 20)	1	0.799±0.13	2	0.532±0.13	14	0.321±0.16	3	0.303±0.19
Hydatid (n= 10)	1	0.882±0.30	1	0.562±0.29	5	0.329±0.27	3	0.301±0.24
H.nana (n=10)	1	0.903±0.21	-	0.689±0.22	7	0.298±0.11	2	0.297±0.12
Ascaris(n=8)	1	0.978±0.19	-	0.732±0.37	4	0.305±0.19	3	0.280±0.15
Ankcylostoma (n=15)	1	0.793±0.16	-	0.803±0.14	7	0.277±0.18	7	0.266±0.30

3.4.2. Detection of E/S *Schistosoma* antigen in urine samples of the study groups

The calculated cut off OD value is 0.392. When detecting E/S antigen in urine, the results were positive in 84 cases (84%) of group A, while 16 cases were negative (16%). In group B (patients with other parasitic infections) 10 cases were detected as positive, 3 with fascioliasis, 2 with hydatidosis, 2

case with ancylostoma infection, 2 with ascariasis and 1 with *H. nana* infection, while the other 53 (84.13%) cases were negative. All healthy control patients were negative. The sensitivity of *S. haematobium* E/S antigen detection in urine was found to be 84%. However, the specificity was found to be 100% compared to the healthy control and 84.1% compared to the patients with other parasitic infections (Table 2).

Table 2: Results of E/S *Schistosoma* antigen detection by sandwich ELISA in urine

Infected Groups	Positive cases				Negative cases			
	Males (No.)	X± SD	Females (No.)	X± SD	Males (No.)	X± SD	Females (No.)	X± SD
Healthy control (n= 35)	-	-	-		22	0.344±0.13	13	0.322±0.21
Schistosoma (n= 100)	74	1.33±0.26	10	1.07±0.18	12	0.307±0.17	4	0.299±0.14
Fasciola (n= 20)	2	0.500±0.19	1	0.737±0.15	14	0.259±0.27	3	0.305±0.17
Hydatid (n= 10)	1	0.658±0.22	1	0.594±0.22	5	0.289±0.21	3	0.209±0.22
H.nana (n=10)	1	0.543±0.20	-	0.682±0.11	8	0.268±0.15	1	0.301±0.12
Ascaris (n=8)	1	0.669±0.11	1	0.622±0.39	3	0.311±0.13	3	0.288±0.14
Ankcylostoma (n=15)	1	0.513±0.22	1	0.609±0.15	6	0.306±0.21	7	0.239±0.30

3.5-Detection of E/S *S. haematobium* antigen in serum and urine using IMB- ELISA technique.

3.5.1. Detection of E/S *S. haematobium* antigen in serum samples

The presence of *S. haematobium* E/S antigen in serum samples of the different studied groups was evaluated by nanomagnetic beads method. The mean OD value of *S. haematobium* infected group (2.01±0.32) was significantly higher than that of other parasites group (group B).

Out of 100 schistosomiasis cases, 95 cases gave positive results, while 5 cases gave negative results, giving a sensitivity of 95%. All the 35 healthy

controls (group C) were negative being below the cut off value for E/S positivity giving a 100% specificity of the procedure, comparing to infected group. In group B (patients with other parasitic infections) 4 cases were detected as positive (2 with fascioliasis, 1 with hydatidosis and 1 case with Ankylostoma infection), while the other 59 (93.7%) cases were negative giving specificity of the procedure of 93.7% to group B. The P-value is < 0.001 which means that there is a statistical significance in positivity between schistosomiasis group and other infection groups (Table 3).

3.5.2. Detection of *S. haematobium* E/S antigen in urine samples

Detection of *S. haematobium* E/S antigen in urine by sandwich ELISA using immunomagnetic bead technique. The OD cut off value is 0.402. The result shows that out of 100 schistosomiasis infected cases, 91 cases (91%) were positive giving a sensitivity of 91%. On the other hand, only 4 cases (6.3%) of group B (2 with fascioliasis, 1 with hydatidosis and 1 case with ancylostoma infection) were positive giving a specificity of 93.7%, with measurement of agreement of 0.834 compared to this group. While none of the healthy controls gave positive results giving a specificity of 100% with kappa agreement 0.839 within this group. There is a statistically significant ($P < 0.001$) difference in the positivity between group A and the other two groups (B & C) (Table 4)

3.6. Correlation between number of egg in urine and E/S antigen in serum of *S. haematobium* infected group A.

There was a strong positive correlation between number of egg/10ml urine and E/S antigen in serum samples ($r = 0.687$; $P < 0.001$) of *S. haematobium* infected group. Figure (4) shows that there is a direct relation between the infection intensity and OD value for *S. haematobium* E/S detection by sandwich ELISA.

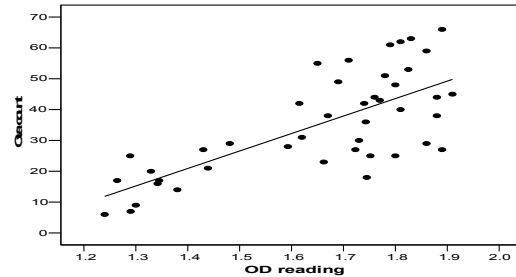


Figure 4. Correlation between number of egg/10ml urine and *S. haematobium* E/S antigen in serum of *S. haematobium* infected group A ($r = 0.687$; $P < 0.001$).

Table 3: Detection of E/S *S. haematobium* antigen in serum using IMB- ELISA technique

Infected Groups	Positive cases				Negative cases			
	Males (No.)	X± SD	Females (No.)	X± SD	Males (No.)	X± SD	Females (No.)	X± SD
Healthy control (n= 35)	-	-	-		22	0.301±0.12	13	0.333±0.16
Schistosoma (n= 100)	74	2.01±0.32	21	1.22±0.27	2	0.281±0.10	3	0.261±0.17
Fasciola (n= 20)	1	0.621±0.14	1	0.518±0.19	14	0.211±0.22	4	0.267±0.13
Hydatid (n= 10)	-	0.819±0.23	1	0.771±0.22	6	0.245±0.22	3	0.288±0.25
H.nana (n=10)	-	0.442±0.21	-	0.506±0.18	8	0.196±0.21	2	0.215±0.15
Ascaris (n=8)	-	0.671±0.14	-	0.707±0.23	5	0.222±0.16	3	0.191±0.13
Ankylostoma(n=15)	1	0.512±0.16	-	0.416±0.15	7	0.188±0.14	7	0.230±0.22

Table 4: Detection of *S. haematobium* E/S antigen in urine with IMB-ELISA technique

Infected Groups	Positive cases				Negative cases			
	Males (No.)	X± SD	Females (No.)	X± SD	Males (No.)	X± SD	Females (No.)	X± SD
Healthy control (n= 35)	-	-	-		22	0.339±0.19	13	0.345±0.21
Schistosoma (n= 100)	74	1.88±0.31	17	1.12±0.22	5	0.298±0.11	4	0.261±0.17
Fasciola (n=20)	1	0.555±0.19	1	0.483±0.13	14	0.199±0.20	4	0.255±0.11
Hydatid (n= 10)	-	0.798±0.30	1	0.666±0.25	6	0.301±0.26	3	0.288±0.25
H.nana (n=10)	-	0.623±0.29	-	0.608±0.16	8	0.246±0.19	2	0.215±0.15
Ascaris (n=8)	-	0.701±0.16	-	0.672±0.32	5	0.299±0.19	3	0.278±0.18
Anklostoma(n=15)	1	0.653±0.19	-	0.569±0.13	7	0.292±0.11	7	0.299±0.32

There was also a strong positive correlation between number of egg/10ml urine and *S.*

haematobium E/S antigen in urine samples of *S. haematobium* infected group ($r = 0.417$; $P < 0.001$)

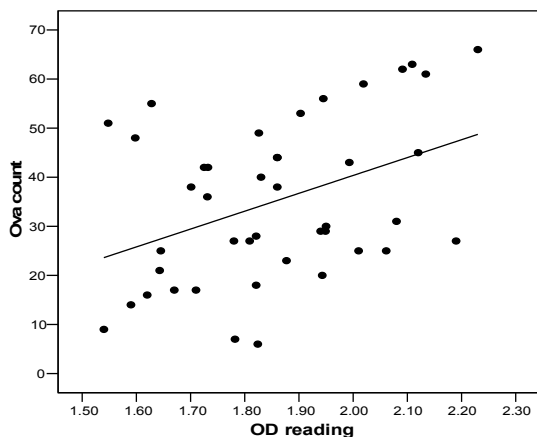


Figure 5: Correlation between number of egg/10ml urine and *S. haematobium* E/S antigen in urine of *S. haematobium* infected group A ($r=0.417$; $P < 0.001$).

4. DISCUSSION

Schistosomiasis is one of the most important parasitic diseases in tropical areas. Approximately 200 million people worldwide currently suffer from the infection, which causes more than 500,000 deaths each year (Zhou et al., 2005) with 20 million exhibiting severe symptoms (Zhang et al., 2007).

Definitive diagnosis of urinary schistosomiasis is carried out by the detection of eggs in urine.

Nevertheless, the parasitological methods of diagnosis have low sensitivity in patients with the acute phase of the illness or with low-intensity infection (Corachan, 2002). In addition, day-to-day and circadian variation in egg excretion may lead to incorrect estimates in prevalence and intensity of infection (Salah et al., 2006).

To overcome this problem, several immunological tests have been developed for diagnosis of schistosomiasis (Rabello et al., 2002).

Moustafa et al. (1998) reported that antigen detection assays may facilitate earlier diagnosis than antibody tests, as production of detectable levels of specific immunoglobulin needs time.

In this study we have demonstrated that by using a magnetic beads assay, which can utilize larger sample volumes, a higher sensitivity can be achieved for detection of *Schistosoma* infections in serum and urine samples as compared with ELISA.

We obtained E/S products from mature worms. E/S was used with complete and incomplete Freund's adjuvants for immunization of rabbits for preparation of anti-*Schistosoma* polyclonal antibodies. Adjuvants are usually used in immunization protocols in animals for many reasons. They can provide a depot for the immunogens at the site of injection allowing for slow, prolonged release of the immunogen in the

animal and more important, they provide a mean of enhancing the immune response to the antigen.

By using 12.5% SDS-PAGE technique under reducing condition, 50% ammonium sulphate-precipitated protein showed that, most of albumin was removed from rabbit anti-*Schistosoma* E/S products PAb. Precipitated proteins appeared as several bands. Further purification of whole Igs by 7% caprylic acid precipitation and DEAE anion exchange chromatographic method removed the remaining non-IgG proteins. The purified IgG was represented by H- and L- chain bands at 53 and 31 kDa respectively. The reactivity of PAb was determined by indirect ELISA, gave a strong reactivity to *Schistosoma* E/S till dilution 1:250. The optimization of various reagents were assayed by sandwich ELISA. The optimum concentration of purified IgG PAb was 20 mg/ml, whereas conjugated IgG PAb was 1:250 dilutions.

This yield of PAb was reasonable in comparison with the yield of purified immunoglobulin from any biological fluid following similar purification procedures (Bride et al., 1995; Yang and Harrison, 1996). The reactivity of the purified PAb was tested by indirect ELISA. It is of note that ELISA has been described as a valid test for detection of rabbit antibodies to fluke antigens, and has been the technique receiving most attention as an immunodiagnostic method for various parasitic infections (Espino and Finlay, 1994).

Antigen detection assay in serum is generally performed by sandwich ELISA (Wescott et al., 1983; Chen and Mott, 1990; Hillyer et al., 1992; Fagbemi and Guobadia, 1995; Sampaio-Silva et al., 1996).

The ES-IMB-ELISA was comparable to that obtained with the ES-ELISA when the same samples were tested. Use of IMB offers the potential advantage of improving the sensitivity of the assay. The magnetic bead immunoassay combines the use of magnetic beads with a high binding capacity as a solid phase and the rapid reaction kinetics of solutions with the simple separation of bound and unbound materials on the solid phase, which provides the chance of enhancing the sensitivity of antigen detection (Gundersen et al., 1992; Ndhlovu et al., 1995). E/S detection in the serum samples of the tested groups by sandwich ELISA using nanomagnetic, out of 100 Schistosomiasis cases 95 gave positive results, while 5 gave negative results, giving a sensitivity of 95%. On the other hand, E/S detection in the serum samples of the tested groups using sandwich ELISA only revealed a sensitivity of 87%. E/S detection in the urine samples of the tested groups by sandwich ELISA using nanomagnetic. Out of 100 schistosomiasis infected cases 91 (91%) were positive giving a sensitivity of 91%. On the other

hand, E/S in urine samples using sandwich ELISA only revealed a sensitivity of the procedure was 84%. These results suggest that in the groups studied, The IMB-ELISA is found to provide higher specificity and sensitivity compared to a microplate-based ELISA technique (Gessler *et al.*, 2006; Conlan *et al.*, 2008).

In conclusion, the IMB-ELISA appears to be a sufficiently sensitive and feasible assay for detection of schistosomal antigenemia and valuable to judge the efficacy of chemotherapy in murine schistosomiasis. And the evaluation of its potential use in human schistosomiasis is in progress.

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