Original Research Article

SDS-PAGE Protein Pattern and Antigenicity Cross Reaction of Human Schistosomes

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Abstract: Schistosomiasis is one of the most important neglected tropical diseases. Its control depends on treatment with the available drug praziquantel. No vaccine exists despite the intense search for molecular candidates and adjuvant formulations over the last three decades. The present study aimed to compare the antigenic protein structures of *Schistosoma mansoni, S. haematobium*, *Fasciola. hepatica* and *Echinococus granulosus* hydatid cyst and to find out shared antigens among these species which could be recognized by *S. mansoni* antibodies. Antigenic protein structures were recognized through the use of Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) after Coomassie blue staining. Antigenic differences between the detected polypeptides and positive *S. mansoni* serum were performed using Western blotting. The SDS-PAGE profiles of the tested parasites revealed many polypeptides ranging from 15-206 kDa. Some of these proteins were shared between all the examined parasites e.g.: 52-47 and 15-25 kDa. Some ranged from 42-38 kDa were shared with both examined schistosomes and hydatid cyst fluid. Bands ranged from 58-55 kDa were common in *S. mansoni, S. haematobium* and *F. hepatica.* The protein bands of about 60 kDa crossly reacted with *S. mansoni* serum and detected in all used antigens. The detected immunoreactive proteins from other helminthes could be used to develop potential vaccine against schistosomiasis.

Keywords: Platyhelminthes, SDS-PAGE, Western Blotting, *Schistosoma* Vaccine

Introduction

Schistosomiasis remains one of the most prevalent diseases in the world especially in the developing countries (King, 2009). It infects between 391 and 600 million people in 74 developing countries in the tropics and sub-tropics and close to 800 million, mostly children, are at risk (King, 2010). The causative agents are several dioecious trematodes, of the family Schistosomatidae, most notably *Schistosoma mansoni*, *S. haematobium* and *S. japonicum* (King, 2009; 2010). *S. mansoni* is the most prevalent species being endemic in 55 countries e.g., Arab peninsula, Egypt, Libya, Sudan, Sub-saharan Africa, Brazil, some Caribbean islands, Suriname and Venzuela (Chitsulo *et al*., 2000). *S. haematobium*, which is the causative agent of urogenital schistosomiasis, is endemic in 53 countries in Africa and the Middle East; while *S. japonicum* is endemic in China, Indonesia and the Philippines (WHO, 2012). Schistosomiasis control strategies are mainly based on chemotherapy. A single anti-schistosome drug, Praziquantel (PZQ), is readily available. Despite its low cost and self-limiting side-reactions, the drug has only been offered to less than 13% of the target population (WHO, 2012). Praziquantel is highly effective in treatment of light and moderate infections. However, in areas of high endemicity and transmission and/or intensive PZQ mass administration, PZQ cure rates are almost insignificant (Colley *et al*., 2014). Many consider that the best long-term strategy to control schistosomiasis is through immunization combined with drug treatment (Bergquist, 2002). An anti-

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schistosomiasis vaccine that induces even a partial reduction in worm burdens could considerably reduce the parasite pathology and limit its transmission (Chitsulo *et al*., 2004).

An effective vaccine against schistosomiasis is an alternative and desirable tool to help in the disease control; however, the complex schistosome life cycle and the complexity of its interaction with the host immune system turn vaccine development into a difficult task. Neither drug development nor vaccine development is sufficient to ensure the success of schistosomiasis control programs. As a matter of fact, the combination of an effective vaccine together with chemotherapy is the strategy of choice. Many studies demonstrated that without a more sensitive diagnosis test, able to detect individuals with low parasite burden, no control strategy will achieve the desirable result which nowadays is diseases elimination (Fonseca *et al*., 2013). One of the main reasons hindering the development of a vaccine against schistosomiasis is the entrenched dogma stating protection is dependent on the generation of type 1-immune responses. This belief was based on preponderance of Interferon-gamma (IFN-γ) released by bronchoalveolar leukocytes, total lung tissue and lung-draining lymph nodes in Radiation-Attenuated (RA) cercariae-vaccinated mice (Wynn *et al*., 1994; Wilson *et al*., 1996). More importantly, several studies using knockout mice conclusively demonstrated that the optimal protection in the RA vaccine model is dependent on the induction of both type-1 and type-2-associated immune responses (Wynn and Hoffmann, 2000; Tallima *et al*., 2015). It is imperative to use type 2-, not type 1-inducing cytokines or molecules as adjuvants to the schistosome-derived antigens used for vaccination (El Ridi and Tallima, 2015).

It is not unusual to find common molecules between species of various parasite genera, families, or phyla. The sharing of molecules able to elicit immune responses between different species of various genera is known as antigenic community and it is responsible for antigenic cross-reactivity. The sharing of molecules among organisms is an expected finding because there are many molecules, such as enzymes, hormones, receptors, etc., that have been conserved during evolution. This has special relevance for the identification of molecules with potential for drug or vaccine development effective against different species or genera of organisms (Losada *et al*., 2005).

The identification of proteins common to *F. hepatica* and *S. mansoni* could provide targets for developing drugs or vaccines that can be simultaneously effective against both organisms. The fatty acid-binding proteins (FABP-Fh15 and Sm14) have been the only *F. hepatica/S. mansoni*-common proteins exploited as a potential dual-vaccine. However, because *F. hepatica* and *S. mansoni* are parasites with great antigenic complexity, it is expected that they possess common proteins other than FABPs that might contribute to serological cross-reactivity (Vilar *et al*., 2003).

Experimental co-infection with *E. granulosus* and *S. mansoni* showed marked reduction in hepatic granuloma size with absence of concentric fibrosis. Thus the effects of helminthes on infections with other pathogens are complex and dependent on many factors such as the helminthes species, co-infecting pathogen, protective and pathological immune mechanisms (Elwakil *et al*., 2007).

The present study aimed to compare the antigenic protein structures of *S. mansoni*, *S. haematobium*, *F. hepatica* and *E. granulosus* hydatid cyst and to find out shared antigens among these species which could be recognized by *S. mansoni* antibodies through the use of western blot technique and which could be considered as a base for a vaccine for protection against these parasites.

Materials and Methods

Antigens Preparation

S. mansoni and S. haematobium Antigens

Soluble Adult Worm Antigens (AWA)

Adult worms of *S. mansoni* and *S. haematobium* (Egyptian strain) were provided by *Schistosoma* Biological Supply Program Unit (SBSP), Theodor Bilharz Research Institute (TBRI), Giza, Egypt. They were obtained from experimentally infected mice and golden hamester. They were homogenized, sonicated then centrifuged at 20000 rpm for one hour at 4°C. Its protein contents were measured and stored at -20°C until used (Deelder *et al*., 1976).

Soluble Egg Antigen (SEA)

The eggs of *S. mansoni* and *S. haematobium* were provided by TBRI. They were suspended in 4°C Phosphate Buffer Saline (PBS) at a concentration of 100,000 eggs mL^{-1}. The eggs were homogenized on ice, centrifuged at 200 rpm for 20 min. SEA was ultracentrifuged for 90 min at 100,000 rpm at 4°C. Its protein contents were measured and stored at -20°C until used (Lewis, 1998).

F. hepatica Antigens

Total Soluble Extract of Fasciola Adult Worm (TSE)

Adult *Fasciola* worms were recovered from the liver of freshly slaughtered sheep at local abattoirs. Worms were washed in 0.01 mM cold PBS, homogenized and

complemented with Phenylmethylsulfonyl Fluoride (PMSF) one mM and leupeptine at 0.5 µg mL^{-1} (as protease inhibitors). TSE was centrifuged at 12.000 rpm for 30 min and stored at -20°C after its protein contents estimation, until used. TSE of *Fasciola* was obtained according to Strauss *et al.* (1997).

Fasciola Adult Worm Vomit (AWV)

Fasciola worms were washed in 0.9M NaCl at room temperature. They were incubated with fresh cold 0.9M NaCl at 4°C for 45 min. The worms were again incubated at 37°C for 30 min and the supernatant with evident black intestinal content was collected, centrifuged at 12,000 g for 30 min and stored at -20°C until used, after measuring its protein contents (Planchart *et al*., 2003).

Hydatid Cyst Antigen

Preparation of Crude Hydatid Fluid (CHF) Antigen

Hydatid cysts were removed from lungs of freshly slaughtered camels. Its fluid was aspirated and centrifuged at 2000 rpm for 20 min. The supernatant was dialyzed overnight against 0.1 mM PBS at 4°C, transferred to a sterile tube and stored at -20°C until use, after measuring its protein contents (Nasrieh and Abdel-Hafez, 2004).

The Serum Samples

Sera were obtained from *S. mansoni* experimentally infected mice provided by TBRI to be used in western blot against the previously prepared antigens. Negative or normal control sera were obtained from non *Schistosoma* infected mice. All mice were free for other parasites.

Sodium Dodecyl Sulphate Poly-Acrylamide Gel Electrophoresis (SDS-PAGE)

For separation of proteins, discontinuous SDS-PAGE with 12.5% concentration under denaturated conditions was carried out as described by Laemmli (1970) using a mini gel. One part of antigens were diluted with two parts (v/v) of sample buffer [2% SDS, 5% (v/v) b-mercaptoethanol, 0.1% bromophenol blue and 0.25% (v/v) glycerol in 62.5 mM Tris-HCl, pH 6.8] and then boiled for three minutes in a water-bath. The solution was mixed and then degassed by attaching the flask to a vacuum for 15 min and finally poured into the mould as a separating gel (Biogene). The gel was allowed to set and then the stacking gel was poured on top of the running gel with the comb in place. The wells were filled with 30 µL antigen extracts. Non-prestained Molecular Weight (MW) standards (wide range, Bio Basic Inc Canada) were incorporated into the control gel lanes to determine the relative molecular weights of the resolved proteins. Electrophoresis was carried out on a vertical gel slab (Bio-Rad mini gel unit®, Bio-Rad, USA) in 12% polyacrylamide gel and 5% stacking gel, under reducing condition at 150 V for approximately 1.5 h. The recovered gel was either stained using Coomassie® blue R-250 to visualize protein bands or transferred to Immobilon-p transfer membrane to perform immunobloting.

Western Blotting or Immunoelectroblotting

Following SDS-PAGE, the antigens were electrophoretically transferred to Immobilon-p transfer membrane (Millipore Corporation, Bedford, USA) for 1.5 h at 100 V in a transfer buffer (0.025 M Tris-glycine; pH 8.3, 20% v/v methanol) using a Mini Trans-Blot® Transfer Cell (Bio-Rad) to identify antigenic subunits within parasite's antigens by immunoblotting as described by Towbin *et al.* (1979). The membrane was blocked with 5% Bovine Serum Albumin (BSA) powder in 0.1 M PBS. The membranes were separately incubated overnight at 4°C with serum of S*chistosoma* infected and non-infected mice, diluted 1:100 and 1:200 in 1% (BSA) in PBS, 1% Tween 20. The reactions were developed using alkaline phosphatase-conjugated goat anti-human IgG (H&L chain) (Sigma), diluted 1:1,000 in 1% BSA, PBS, 0.005 M Tween 20 and incubated for four hours at room temperature. The transferred antigens were visualised by staining with 3.3', 5, 5' Tetra Methylebenzidine (TMB; Kirkegard and Perry Laboratories, USA). Molecular Weight (MW) estimates were made by comparing the motility of the tested samples with that of the standard protein mixture the standard protein mixture (Amersham pharmacia biotech. USA). Molecular mass of both the SDS-PAGE and western-blot was determined using Gel-ProAnalyzer package (Media Cybernetica; 1993). Both expirements were repeated three times.

Results

Using SDS-PAGE for protein analysis of the tested antigens, *S. mansoni* Adult Worm Antigen (AWA), revealed the presence of nine polypeptides. Their MW ranged from 206 to 15 kDa. The major protein bands were at MW of 206.4, 103.2, 68.2, 56.6, 48.5, 39.2, 31.8, 25.4 and 15.9 kDa (Fig. 1: Lane 1). While *S. mansoni* SEA revealed the presence of seven visible protein bands. Their MW ranged from 74 to 15 kDa. The major bands were at MW of 73.9, 47.8, 42.0, 33.6, 33.4, 26.4 and 15.7 kDa (Fig. 1: Lane 2).

Concerning adult *S. haematobium* Soluble Worm Antigen (AWA) revealed eight polypeptides; which

polypeptide MW ranged from 190 to 16 kDa. These bands were corresponding to Molecular Weights (MW) of 186.1, 73.1, 57.4, 50.2, 45.0, 40.0, 25.2 and 16.9 kDa (Fig. 1: Lane 3). At the same time nine polypeptide bands were observed in fractionation of *S. haematobium* SEA. Their MW was ranged from 196 to 15 kDa. These bands were corresponding to MW of 195.8, 71.9, 56.4, 50.0, 42.0, 31.7, 31.3, 25.2 and 15.3 kDa (Fig. 1: Lane 4). *F. hepatica* Total Soluble Extract (TSE) and Adult Worm Vomit (AWV) revealed multiple components via SDS-PAGE. The TSE revealed four major protein fractions with MW of 63.0, 51.7, 25.0 and 14.9 kDa while that of *Fasciola* AWV antigen revealed six major protein fractions at MW of 90.0, 70.5, 61.5, 55.0, 47.5 and 25.0 kDa (Fig. 1: Lane 5 and Lane 6 respectively).

Fractionation of CHF antigen using SDS-PAGE revealed eleven bands at MW of 80.5, 70.0, 64.8, 59.4, 47.5, 42.0, 38.0, 34.0, 32.8, 24.2 and 17.0 kDa (Fig. 1: Lane 7).

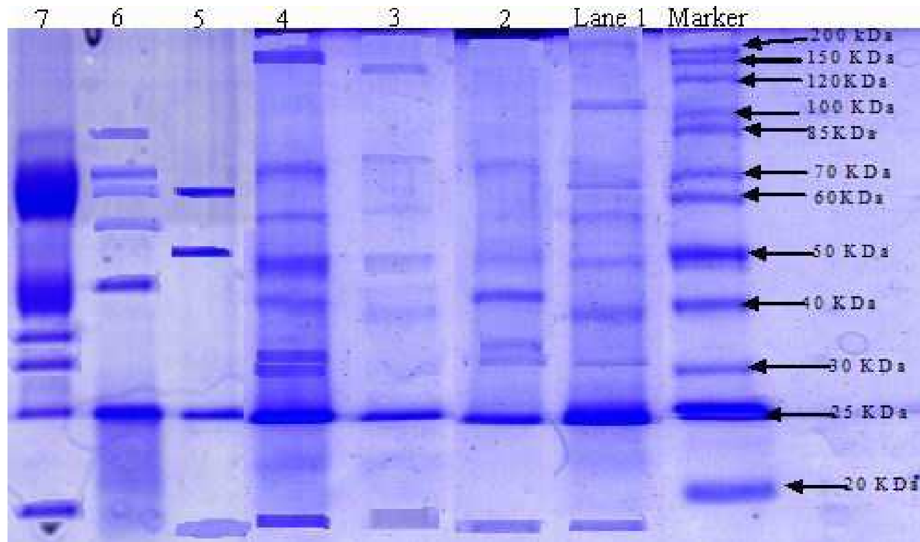

Fig. 1. Polypeptide composition of the tested antigens by SDS-PAGE stained with Commassie brilliant blue, M: Molecular weight marker (M); Lane 1: soluble AWA of *S. mansoni*; Lane 2: SEA of *S. mansoni*; Lane 3: Soluble AWA of *S. haematobium*; Lane 4: SEA of *S. heamatobium*; Lane 5: *F. hepatica* TSE; Lane 6: *F. hepatica* AWV; Lane 7: Hydatid Cyst Fluid (CHF)

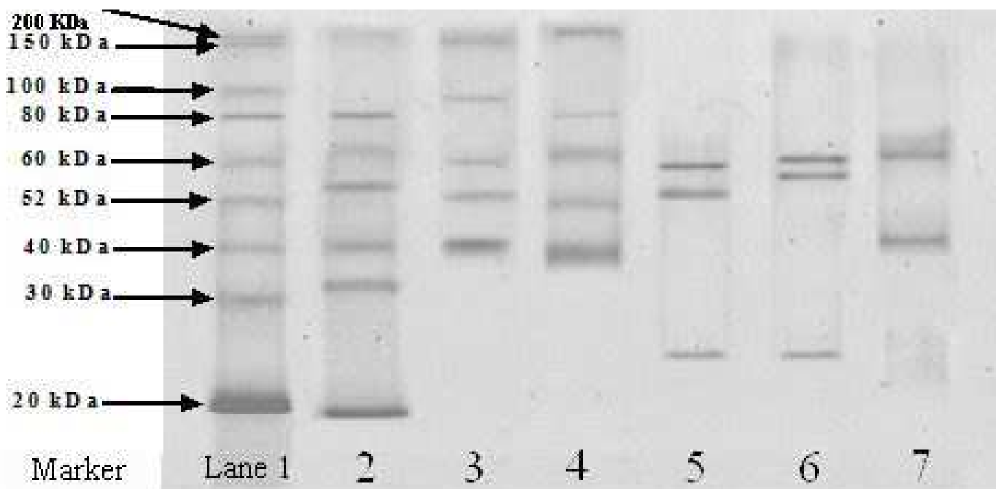

Fig. 2. Immunoblot reaction of the crossly reacted tested antigens using *S. masoni* serum; Lane 1: Protein bands of *S. mansoni* AWA; Lane 2: protein bands of *S. mansoni* SEA; Lane 3: Protein bands of *S. haematobium* AWA; Lane 4: Protein bands of *S. haematobium* SEA; Lane 5: Protein bands of *F. hepatica* TSE; Lane 6: protein bands of *F. hepatica* AWV; Lane 7: Protein bands of CHF

Western Blotting (WB) of *S. mansoni* AWA and SEA showed that, *S. mansoni* positive serum recognized the bands with MW of approximately 200, 150, 100, 80, 60, 52, 40, 31 and 20 kDa in AWA (Fig. 2: Lane 1) and 200, 80, 63, 54, 40, 33 and 19 kDa in SEA (Fig. 2: Lane 2). Immunoblot of *S. haematobium* AWA and SEA against *S. mansoni* positive serum showed cross reaction with the bands of approximately 184, 95, 59, 52 and 40 kDa in AWA (Fig. 2: Lane 3) and 195, 79, 63, 50 and 38 kDa in SEA (Fig. 2: Lane 4).

Electrophoretically separated polypeptides from *F. hepatica* TSE and AWV antigens were transferred to nitrocellulose membrane and immunoblotted against *S. mansoni* positive serum. From these polypeptides, three polypeptides at MW of 59, 52 and 23 reacted crossly with *S. mansoni* positive serum in case of TSE (Fig. 2: Lane 5). While in AWV, polypeptides at MW of 60, 55 and 23 kDa crossly reacted with the serum (Fig. 2: Lane 6). Western blotting of CHF against *S. mansoni* positive serum showed cross reactions at only two polypeptides at MW of 61 and 40 kDa for CHF (Fig. 2: Lane 7). Negative or normal control sera did not show any reaction with the antigens or extracts used in WB.

Discussion

The current study clarified the antigenic similarity between *S. haematobium*, *S. mansoni*, *F. hepatica* and *E. granulosus* hydatid cyst from the aspect of molecular weight using SDS-PAGE and from the aspect of immune reaction using WB technique against *S. mansoni* positive serum. The examination of these crude extracts, instead of more simplified material, had the advantage of increasing the chance of detecting the most abundant proteins expressed by these parasites (Hamilton *et al*., 1998) and that could be relevant with respect to the aim of the current study.

In the present study, fractionation of *S. mansoni* AWA revealed nine polypeptide bands ranged from 206.4 to 15.9 KDa. While seven visible protein bands were detected on SDS-PAGE analysis of *S. mansoni* SEA with MW from 73.9 to 15.7 kDa. These results showed some similarity to that done by Chacon *et al.* (2002) who revealed nine specific molecules of *S. mansoni* AWA: 155, 122, 97, 90, 50, 36, 31 and 25 kDa. The finding of the current study also coincided with Basyoni and Abd El-Wahab (2013) study who reported seven major protein bands of the fractioned *S. mansoni* AWA with MW of 92, 70, 67, 54, 44, 30 and 20 kDa. The findings concerning SEA fractionation were to some extent related to the findings of Lukacs and Boros (1991) who showed that *S. mansoni* SEA was separated into 15 to 22 different protein bands. The protein bands were divided into nine different fractions (<21 and >200 kDa). Stadecker *et al.* (2001) stated that, the best studied and most abundant egg component of *S. mansoni* is the Sm-p40 antigen representing approximately 10% of SEA. In addition, Soliman *et al.* (2003) used SDS-PAGE to analyze soluble worm antigens, cercarial antigen preparations and soluble egg antigens of *S. mansoni* and authors reported that 32 kDa was a chief band of these three fractionated antigens.

In the present study; immunoblot of *S. mansoni* AWA and SEA with *S. mansoni* serum revealed intense reaction recognizing most polypeptides bands obtained in SDS-PAGE. Antigenically active components in *S. mansoni* AWA antigen on reaction with homologous serum were nine polypeptides at MW of 200, 150, 100, 80, 60, 52, 40, 31 and 20 kDa. While those of *S. mansoni* SEA were at MW of 200, 80, 63, 54, 40, 33 and 19 kDa. Coinciding with these results, when immunobloting tests employed to detect *S. mansoni* AWA antibodies, an immunogenic fraction with a MW of 31-32 kDa (Sm 31/32) was considered to be the most frequently documented fraction. Thus it is considered of high diagnostic importance and could therefore be used as a serologic marker (Valli *et al*., 1999; Basyoni and Abd El-Wahab, 2013). At the same time, Vendrame *et al.* (2001) reported that when sera of *S. mansoni* infected patients were analyzed by immunobloting against both *S. mansoni* AWA and SEA elucidated an intense reaction identifying the bands of 204, 194, 164, 152, 130, 115, 92, 67, 55, 50, 40, 36, 31, 25 and 22 kDa.

Ludolf *et al.* (2014) by using two dimensional electrophoresis and WB techniques were able to identify 47 different antigenic proteins in *S. mansoni* AWA and SEA, a slightly larger number when compared to previous *S. mansoni* serological-proteomic studies.

In present study, SDS-PAGE electrophoresis of *S. haematobium* AWA revealed polypeptides of 186.1, 73.1, 57.4, 50, 45.0, 40.0, 25.2 and 16.9 kDa. While in *S. haematobium* SEA fractionation nine bands were observed with MW of 195.8, 71.9, 56.4, 50.0, 42.0, 31.7, 31.3, 25.2 and 15.3kDa. These findings were closely similar to the findings of Hayunga *et al.* (1981) who observed the bands of MW at 85, 60, 43 and 30 kDa on fractionation of *S. haematobium* AWA. Also these results somehow came in line with the results obtained by Hillyer and Pacheco (1986) who demonstrated six distinct bands with MW of 131, 76, 74, 55, 40 and 25 kDa on analyzing the protein components of *S. haematobium* adult worm antigen preparation. Concerning the bands obtained for *S. haematobium* SEA were came in line with Gaafar *et al.* (1993) who revealed seven bands ranged from 84 to 28 kDa namely, 84, 63, 57, 55, 40, 30 and 28 kDa. While Mahfouz *et al.* (2011) detected bands ranged from 106-18.5 kDa.

The findings of current study revealed that many polypepetides of the soluble *S. haematobium* adult

worm antigen namely (184, 95, 59, 52 and 40 kDa) and *S. haematobium* SEA (195, 79, 63, 50 and 38 kDa) were recognized by *S. mansoni* serum, reflecting that, the two *Schistosoma* species share common antigens, confirming numerous previous studies. Hayunga *et al.* (1981) showed that the majority of *S. haematobium* AWA proteins were recognized by *S. mansoni* sera and that, the only 25 kDa was *S. haematobium* specific. Aronstein and Strand (1983) reported that the majority of soluble AWA recognized by sera from *S. haematobium* infected patients were also recognized by sera from *S. mansoni* infected patients.

In the present work, fractionation of *F. hepatica* TSE revealed the presence of four major protein bands at MW of 63.0, 51.7, 25.0 and 14.9 kDa while that of *Fasciola* AWV antigen revealed the presence of six major protein bands at MW of 90.0, 70.5, 61.5, 55.0, 47.5 and 25.0 kDa. These findings somehow agreed to some previous studies that recorded the presence of eight protein bands in *F. hepatica* TSE with molecular weights ranging from 25.5-48 kDa (Allam *et al*., 2002) and from 62 to 18 kDa (Meshgi *et al*., 2008). Meanwhile De Almeida *et al.* (2007) detected seven protein bands for *F. hepatica* TSE with MW of 57, 44-46, 38, 10, 9 and 8 kDa. For the *F. hepatica* AWV, they observed four polypeptide bands which were at MW of 8, 12, 15 and 24 kDa. On the other hand, Sabry *et al.* (2010) reported that resolving of TSE antigen showed MW bands at 62, 60, 58, 37, 12.5 and 6.5 kDa, while resolving of the AWV antigen showed bands with MW 56, 37, 29 and 12.5 kDa.

The current study clarified the cross reactivity of *F. hepatica* TSE and AWV antigen with *S. mansoni* serum by WB technique; the bands recognized by the serum were 59, 52 and 23 kDa for TSE and 60, 55 and 23 kDa for AWV. The results of the present study were in agreement with those of Hayunga *et al.* (1981) who revealed degree of immunologic identity between *S. mansoni* and *F. hepatica*. They observed that a protein from *F. hepatica*, with an approximate MW of 60.0 kDa, reacted with *S. mansoni* infection serum and this antigen might be biochemically similar to the prominent 60.0 kDa MW peak found in both *S. mansoni* and *S. haematobium*. Cross reaction between *F. hepatica* TSE antigen and patients with schistosomiasis *mansoni* was recorded at MW of 45 KDa and 57 KDa by Farghaly *et al.* (2009) and at MW of 37, 32, 29 and 21 kDa by Sabry *et al.* (2010). This cross reaction might be due to the fact that, *F. hepatica* and *S. mansoni* have evolved in similar ways to avoid the immune responses of their hosts (McManus and Dalton, 2006).

Maghraby *et al.* (2009) observed heterologous resistance between *S. mansoni* and *F. hepatica*. A low molecular weight common component of 14 kDa isolated from *S. mansoni* stimulates a protective response against both *S. mansoni* and *F. hepatica* infections. Such a dual-purpose vaccine, aimed primarily for veterinary use against an economically important disease such as fasciolosis, might represent an attractive route for the development of a vaccine against schisotsomiasis (Hillyer, 2005). In this respect Boukli *et al.* (2011) used two dimentional-Polyacrylamide Gel Electrophoresis (PAGE) technique and revealed considerable similarity in the proteomic maps of *F. hepatica* and *S. mansoni* with respect to the number of spots detected and their MW. They identified 28 immunoreactive proteins that were common to both adult *F. hepatica* and *S. mansoni* and they suggested that some of the identified proteins could be used to develop vaccines against both fascioliasis and schistosomiasis.

Resolving of CHF antigen by SDS-PAGE revealed bands at 80.5, 70.0, 64.8, 59.4, 47.5, 42.0, 38.0, 34.0, 32.8, 24.2 and 17.0 kDa MW. The data found in this study were somehow in agreement with those obtained in a previous study conducted by Khalifa *et al.* (2005), they reported that SDS-PAGE of CHF revealed polypeptides of 140, 130, 116, 106, 97, 86, 56, 48, 38, 29, 24, 20, 16,12, 8 kDa. In the same time Saha *et al*. (2011), detected polypeptides of 72.8, 66.5, 60.2, 41.0, 25.1, 19.0 and 8 kDa. On the other hand, Tabatabaie *et al.* (2013), revealed bands with MW ranged from 8 to 150 in which the sharpest bands with MW of 67 kDa on fractionation of CHF and a fewer number of bands with MW of 12, 16 and 24 on fractionation of Ag-B. The two major antigens in hydatid fluid of camel (the lipoprotein antigen-5 and antigen-B) that was detected by Tabatabaie *et al.* (2013), also were detected in the present work. Antigen-5 consisted of two major subunits of molecular mass 38 and 20 KD and antigen-B involved three subunits; 24, 16 and 8 kDa.

In current study, Immunoblotting of CHF against *S. mansoni* serum identified two major discrete antigenic fractions: 61 and 40 kDa in HCF antigen and no polypeptides were detected in MW bands corresponding to Ag-B. Coinciding with these findings, many studies reported that, the cluster of bands (35-38 kDa) of CHF crossly reacted with sera from patients with other parasitic infections including schistosomiasis and fascioliasis (Rott *et al*., 2000; Al-Olayan and Helmy, 2012). The cross reactivity of these polypeptides might be attributed to its phosphorycholine epitope, first described by Shepherd and McManus (1987). Another explanation for the detected cross reactivity was that the chronic parasite diseases produce a discharge of highly immunogenic substances into the host organism, which elicit cross-reactive or non-specific

immune response, as in case of the schistosomiasis and hydatidosis (Ishida *et al*., 2003). The finding concerning immunoblotting of Ag-B was fairly similar to previous study conducted by Al-Olayan and Helmy (2012), who observed that, the polypeptides at 24-22, 16 and 8 kDa which corresponding to Ag-B showed 100% specificity as they did not react with sera from patients with other parasitic diseases. The explanation of the detected cross reactivity in the examined parasites antigens when immunoblotted against *S. mansoni* serum might be due to the fact that, *F. hepatica* and *S. mansoni* had evolved in similar ways to avoid the immune responses of their hosts. Therefore, it was not surprising that several of the common proteins identified between *F. hepatica* and *S. mansoni* antigens as they had biological functions related to immune evasion mechanisms. Another explanation for the cross reactivity among these parasites was that, the chronic parasite diseases produced a discharge of highly immunogenic substances into the host. These substances provoked cross reactive or nonspecific immune response such as in case of the schistosomiasis, fascioliasis and hydatidosis. On the other hand, it was significant to point out that an immunologic fraction with MW of 31-32 kDa was considered to be the most frequently documented fraction in *S. mansoni* AWA and it was specifically reacted with the *S. mansoni* serum.

Conclusion

Using SDS-PAGE analysis of antigens of *S. mansoni* (AWA, SEA), *S. haematobium* (AWA, SEA), *F. hepatica* (TSE, AWV) and Hydatid cyst (CHF) revealed many shared proteins between the examined parasites extract. It was noticed that, the polypeptides ranged from 73-68 kDa and that ranged from 42-38 kDa were shared with the AWA, SEA of both examined schistosomes and CHF. Bands ranged from 58-55 kDa were common in *S. mansoni* AWA, *S. haematobium* (AWA, SEA) and *F. hepatica* AWV. Bands within the range of 52- 47 and 26-22 kDa were detected in all tested antigens. Bands with MW of 34-31 kDa were observed in *S. mansoni* (AWA, SEA), *S. haematobium* SEA and CHF. Bands ranged between 15- 17 kDa were shared with all tested antigens except for *F. hepatica* AWV. Using WB; the protein bands with MW of about 60 kDa crossly reacted with *S. mansoni* serum and detected in antigens of *S. mansoni* (AWA, SEA), *S. haematobium* (AWA, SEA), F. *hepatica* (TSE, AWV) and CHF. These immunoreactive proteins could be used to develop dual vaccine against human schistosomes. Using a more sensitive staining method, e.g., silver staining was recommended to check whether their main bands still can match the bands in western blot. This could help to compare the 60 kDa band with different method and should highlight this protein in different gels. Also it could show whether the band was only one protein or several proteins with similar molecular weight.

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Author's Contributions

Nahed A.A. EL-Ossily: Carried out the experiments and data-analysis.

Doaa A. Yones: Data-analysis, manuscript preparation and publication.

Mohamed El-Salahy M.M. Monib and Ahmed S.A. Hassanin and Khalifa M.A. Refaat: Manuscript revision.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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