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SODIUM DODECYL SULPHATE POLYACRILAMIDE GEL ELECTROPHORESIS (SDS-PAGE) FOR DIFFERENTIATION OF DIFFERENT SCHISTOSOME AND FASCIOLID SPECIES

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Abstract

Fasciola and *Schistosoma* spp. are digenetic trematodes that have a major detrimental impact on human health worldwide. It is not unusual to find common molecules among parasites of different species, genera, or phyla. In this study Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was used to identify the common proteins of adult *Fasciola* and *Schistosoma* spp. Adult *Fasciola* spp, were collected from the bile ducts of naturally infected cattle and sheep. *Schistosoma mansoni* and *S. haematobium* adults surface antigens were prepared in Theodor Bilharz Research Institute were used. Results showed sharing bands between all worms, which have the same molecular weight of 36 kDa. On the other hand, band with molecular weight 41 kDa was sharing between them except *F. hepatica*. Bands at 48 and 170 kDa were sharing between all species of *Fasciola* spp and *Schistosoma mansoni*.

Key words: *F.* gigantica, *F. hepatica, S. mansoni, S. haematobium*, electrophoresis, Bands.

Introduction

It is not unusual to find common molecules between species of various helminth genera, families, or phyla. The sharing of molecules is able to elicit immune responses between different species of various genera known as antigenic community; is responsible for antigenic cross-reactivity (Losada *et al,* 2005).

Fasciola and *Schistosoma* spp. are digenetic trematodes that have a major detrimental impact on animal and human health worldwide (Chen and Mott, 1990; Savioli *et al,* 2002; Mas-Coma, 2005). Identification of proteins common to *Fasciola* and *Schistosoma* spp could provide targets for developing drugs or vaccines that can be simultaneously effective against both organisms (Ramajo *et al,* 2001; Vilar *et al,* 2003; Ramos *et al,* 2009). *Fasciola hepatica* and *S. mansoni* have evolved in similar ways to avoid the immune responses of their hosts (Mc Manus and Dalton, 2006).

The aim of the present study was to identify the common proteins of *F. hepatica*, *F. gigantca*, Intermediate form and *S. mansoni* and *S. hmatobium* adult worms through the analysis of adult worm crude extracts by Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

Material and Methods

Preparation of *F. hepatica, F. gigantic*a and intermediate form adult worms: Adult *Fasciola hepatica*, *F. gigantic* and intermediate form flukes were collected from the bile ducts of the naturally infected cattle and sheep slaughtered at slaughter-houses. The flukes were washed repeatedly with 0.1 M phosphate-buffered saline (PBS), to eliminate all traces of blood and bile. One worm of each type was used to obtain the total soluble extract. *F. hepatica*, *F. gigantica* and Intermediate form somatic antigens were prepared using the methods described by Hillyer and De Weil (1977) and Mansour *et al.* (1983). Antigens were stored at -20°C until used.

Preparation of *S. mansoni* and *S. haematobium* adult worm surface antigen (AWSA): Adult worm surface antigens were prepared

in Theodor Bilharz Research Institute, Giza, from necropsied mice 56 days after infection to recover adult worms by perfusion from the portal system. Worms were washed repeatedly with PBS and then stored at -20^{o}C until used (McLaren *et al*, 1978).

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE): The protein patterns were analyzed using SDS-PAGE in the first dimension. Somatic antigens were separated by SDS-PAGE (Laemmli, 1970) using a miniprotein II cell (Bio-Rad). The antigens were heated in a water bath at 100°C for 10 min., and then added to each well of a 10% stacking gel and 12% separating gel. SDS-PAGE was carried out at 60, and 120 V, for 20, and 90 min, respectively. Gels were stained with 0.05% Coomassie brilliant blue and silver staining (Sigma Chemistry).

Molecular weights of proteins were determined by comparing their migration distance against that of a known molecular marker. For molecular weight determination of proteins LabImage (2006) program was used.

Statistical analysis: Data were tabulated and analyzed through computer using the statistical package for social science (SPSS) version 20.

Table 1: Preparation of 12% and 5% SDS-PAGE

5% separating gel	12% separating gel	Reagents
11	9.9	H2O
2.6	12.0	Stock (1)
….	7.5	Stock (2)
2.0	…..	Stock (3)
0.1	0.3	10% SDS
0.1	0.3	10% ammonium per-sulfate
0.006	0.012	TEMED

Results

In the present study, with SDS-PAGE electrophoretic analysis of somatic and excretory antigens of adults *S. haematobium, S. mansoni, F. hepatica, F. gigantica* and Intermediate form had several bands were used. (Tab. 2, Fig.1):

The antigenic components of somatic extract of *S. haematobium, S. mansoni, F. hepatica, F. gigantica and Fasciola* intermediate form adult worms. The extracts of all these worms had some sharing bands between each other and each one had specific bands.

The sharing bands between all of them were one band which has the same molecular weight in five worms which was 36 kDa. The band with molecular weight 41 kDa was sharing between them except *F. hepatica*. Bands at 48 & 170 kDa were sharing between all species of *Fasciola* and *S. mansoni*.

There were specific bands to each genus and even to each species as for *Schistosoma haematobium* and *S. mansoni* bands at 5, 26, 100, and 150 kDa. The sharing bands between *S. haematobium* and *F. hepatica* were at 29 kDa. In *S. mansoni* bands at 32, 56, 65 and 71 kDa were specific.

The band at 14 kDa was specific for the *Fasciola* species while bands at 115 and 75 kDa were specific for both the *F. hepatica* and *Fasciola* intermediate form species. In *F. hepatica* and *F. gigantic* species band at the 30 kDa was specific.

Table 2: SDS-PAGE analysis of somatic antigens of adults of *Schistosoma* and *Fasciola* species

F. gigantica Mol. W	*F.* intermediate form Mol. W	*F. hepatica* Mol. W	*S. haematobium* Mol. W	*S. mansoni* Mol. W
225	225	225	225	225
170	170	170		170
			150	150
170	115	115		
			100	100
	75	75		
				71
				65
	48	48		48
	41		41	41
48	36	36	36	36
41				32
36	30	29	29	
30			26	26
	14	14		
			5	5
14				

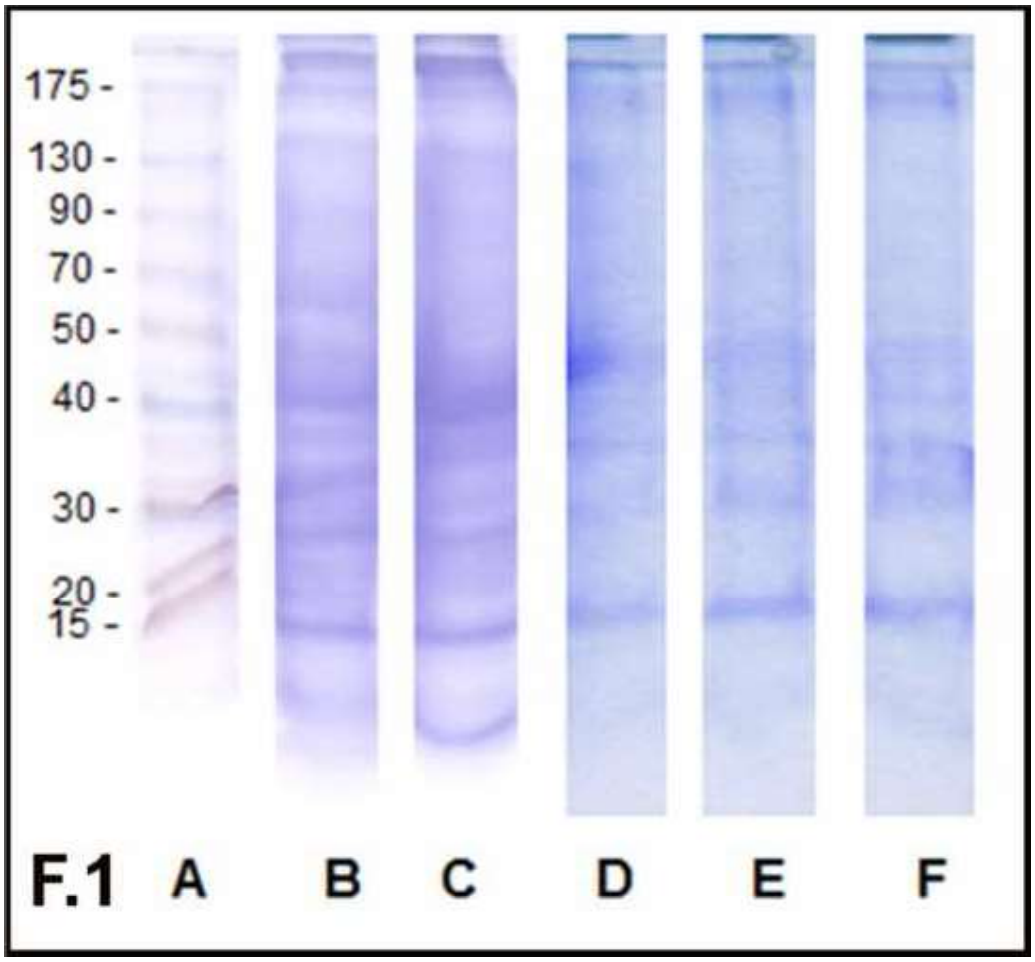

Fig. 1: SDS-PAGE analysis of somatic antigens of adult worms of *Schistosoma* species and *Fasciola* species. A: molecular weight marker, B: *Schistosoma mansoni*, C: *Schistosoma haematobium*, D: *Fasciola hepatica*, E: *Fasciola* intermediate form and F: *Fasciola gigantica*

Discussion

In view of our aim to identify proteins that are common in *Schistosoma haematobium, Schistosoma mansoni, Fasciola hepatica, Fasciola gigantica and Fasciola* intermediate form adult worms in the present work obtained soluble extracts from whole adult worms. Although complex mixes of proteins make for difficult electrophoresis, 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, and that could be relevant with respect to the aim of our study. As is well known, a simpler composition like, for instance, the excretory/ secretory products result in much lower protein yields, which are highly specific and useful for immunodiagnosis (Espino *et al,* 1987; Carnevale *et al,* 2001; Salimi-Bejestani *et al,* 2005; Arias *et al,* 2007).

Studies have shown that cross-resistance, between *Fasciola* and *Schistosoma* spp. in several hosts. Hillyer and De Weil (1977) demonstrated that adult *Fasciola hepatica* infection induce significant resistance to subsequent challenges with *S. mansoni* in mice. Similarly mice with mature primary infection with *S. mansoni* were found to show significant resistance to challenge infection with *F. hepatica* (Hillyer, 2005). Moreover, Christensen *et al*. (1987) showed that mice infected with immature stages of *Fasciola hepatica* showed resistance to infection of *S. mansoni*. Cross resistance had also been shown between schistosome species and liver flukes in farm animals.

Also, the electrophoretic analysis of schistosomes and *Fasciola* species to detect similar bands responsible for cross reactivity between them showed false positive diagnosis of patients. In the present study, the SDS-PAGE analysis of *Schistosoma* and *Fasciola* species showed that each of them had several bands, some of these bands are sharing and others are specific to each parasite.

As regards *Fasciola gigantica,* the somatic proteins had 7 bands with molecular weights of 170, 48, 41, 36, 30 and 14 kDa, and this is more or less similar to the results obtained by Meshgi *et al*. (2008) who stated in his study the difference between *Fasciola* species somatic and excretory -secretory antigens, they found that *F. gigantica* somatic proteins had 11 major protein bands with molecular weights of 18, 22, 24, 33, 36, 42, 46, 57, 60, 62 and 68 kDa, which is similar to the present results in band at 36 kDa.

Wanchai *et al*. (1997) found that *F. gigantica* somatic proteins showed 14 bands, their molecular weights are 94,70, 86, 60, 54, 46, 38, 36, 30, 27, 24, 17, 14.4 kDa which is similar to the present results in bands 14, 30, and36 kDa.

In contrast, *F. gigantica* shows only five major protein bands of 57.6, 54, 48, 29 and 27 kDa according to Allam *et al*. (2002) with one band sharing with the present study at 48 kDa and similar band at 29 kDa.

On the other hand, Maghraby *et al.* (2007) found that *F. gigantica* worms homogenate, gave 8 bands which were 177.300 - 156.810 - 110.330 - 105.950 - 102- 64.084 - 56.403 - 45.893, with no similar bands with our present study.

As regards *F. hepatica* the somatic proteins had 8 major peptide bands with molecular weights of 170, 115, 75, 48, 36, 29 & 14 kDa. Allam *et al*. (2002) demonstrated the presence of 8 bands with smaller molecular weights (48, 45, 43.5, 37, 33, 29, 27 & 25.5 kDa.), with two bands similar to the present study at 48 and 29 kDa. However, Mesghi *et al*. (2008) found that the major *F. hepatica* somatic antigens or proteins detected by SDS PAGE were 8 with molecular weight of 18, 22, 24, 33, 36, 42, 46 & 62 kDa with one band similar to the present results at 36 kDa.

De Almeida *et al*. (2007) demonstrated that *F. hepatica* somatic antigens had the following bands (2, 8, 9, 10, 31-33, 36, 38, 44-46, 49, 53, 57, 61, 65, 75-81, 87-93 and

107 kDa with two sharing bands with the present results at 36 and 75 kDa.

As regards *S. haematobium* somatic proteins had 8 bands with molecular weights of 150, 100, 41, 36, 29, 26 and 5 kDa. Sharaf (2015) recognized several bands with molecular weights of *: 170 , 150 , 133 , 123, 95, 84, 78, 74, 65, 52-54, 45-47, 42, 37, 34 ,28-30, 26 ,23 , 20, l3 ,11 and 5* kDa with two bands similar to our results at 26 and 150 kDa., while Bahia *et al*. (2010) identified several bands with molecular weights of 255, 230, 210, 150kDa with one band similar to the present results at 150 kDa.

As regards *S. mansoni* the somatic proteins had 13 bands with molecular weights of 170, 150, 100, 70, 65, 56, 48, 41, 36, 32, 26 and 5 kDa. Hirsch *et al*. (1997) found that the major bands in *S. haematobium* somatic antigens were 170, 115, 76, 65, 64-59, 54-56,47-51.5, 46, 40 - 32, 29, 10kDa, with several sharing bands with the present results at 32, 48 , 56, 65, and170kDa. Sulahian *et al.* (2005) reported that the western blot profiles obtained with sera from patients with confirmed schistosomiasis yielded numerous bands between 40 and 200 kDa. Therefore, bands of 65, 70, 80, 95, 110, and 120 kDa were considered diagnostic with one sharing band with the present results at65 kDa. Carvalho *et al*. (2011) found that bands were at 200, 100, 43 and 18 kDa with one sharing band with the present results at 100 kDa, while Italo *et al*. (2005) found that bands molecular weights in kilo-Daltons were as follows *8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 20, 24, 28, 31, 34, 39, 52, 63, 82* kDa but, without similar bands to the present findings.

Conclusion

The outcome data showed that the detection of the somatic antigens of the adults *Fasciola* spp. and *Schistosoma* spp. had protein bands were more or less similar to the results obtained in other studies.

The difference in the results might be due to the use of different techniques of SDS-PAGE electrophoresis.

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