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ELECTROPHORETIC DIFFERENTIATION OF SOLUBLE ANTIGENS FROM *Echinococcus granulosus* ISOLATES USING SDS-PAGE TECHNIQUE

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SUMMARY

A comparison was made by sodium dodecyle suphate polyacrylamide gel electrophoresis SDS-PAGE between somatic hydatid fluid, protoscoleces and *Echinococcus granulous* adults antigens recovered from camel and equine (donkey) origins.

Comparing hydatid fluids indicated that differences were found in the arrangement and molecular weights of developed protein bands. Donkey hydatid fluid showed 3 protein bands having a molecular weight ranging between 44.6-143 K Da. while that of camel origin revealed 7 bands. These bands had variable molecular weights either low (28.8 - 46.7 K Da.) or high (72.4 - 100.0 K Da.). The protein patterns of protoscoleces were most complex in both origins. Protein experession in camel protoscoleces had 12 bands. These bands had variable molecular weights, 8 bands having low molecular weights (19.9 - 64.6 K Da.), the other 4 bands had higher molecular weights (89.1 - 136.8 K Da.). The identified bands among donkey protoscoleces were 8 bands. Only one band had a low molecular weight (53.7 K Da.), while the others were of high molecular weights (67.6 - 157.9 K Da.). Protein profile of *E. granulosus* somatic antigens revealed that both isolates had equal number of protein bands. All the stained protein bands were relatively similar except two bands having low molecular weight 10.5 & 26.3 K Da. However, one band characterized camel sample had a high molecular weight (105.3 K Da.).

INTRODUCTION

Echinococcus granulosus, the causative agent of unilocular hydatidosis, whose larval stages (hydatid) in some animals and man has a cosmopolitan distribution, with low to high degree of endemicity. In several regions of the world, Echinococcosis is a serious public health,

zoonotic and economic problems (Abd El-Sadek, 1992; El-Bassiouny et al., 1995, Himonas et al., 1994, Watson-Jones et al., 1997 and Derbala and Zayed, 1997 b).

The success of hydatidosis control has not been wholly achieved. This partial failure could be due to the existence of different isolates (strains or substrains) as well as presence of uncommon intermediate hosts such as horses, donkeys and pigs (Hassan, 1991; Derbala and Zayed, 1997 a and Derbala and Zayed, 1997 b).

The occurrence of these criteria has been discussed in Egypt (Derbala and Moustafa, 1998), where DNA restriction endonuclease analysis and isoenzymatic variant patterns have been described.

Morphological similarity of hydatid fluid, protoscoleces and adult stages of *E. granulosus* subspecies, as well as the risk resulting from dog infection during the period of experimental infection stimulated the worker to investigate protein analysis using SDS-PAGE electrophoresis of hydatid fluid, protoscoleces and adult worm of *E. granulosus* of both equine (donkey) and camel origins and to identify the specific protein constituents and the phenotypic variability of these different populations.

MATERIAL AND METHODS

- Hydaid fluids (HF) were separately obtained from individual hydatid cysts of both infected camel and donkey. HF were concentrated in a dialysis bag against polyethelene glykol, M.W., 8000, (Sigma Co., USA) plus protein inhibitors, protease inhibitors, 2 mM (10mg/100 PBS-pH, 7.2) for overnight at 4°C. Prote concentrations were measured in the obtain HF as described by Bradford (1976).

- For the liberation of protoscoleces, hyda sands were digested by incubation at 37°C, w gentle shaking in the digestion solution (0.2 fresh pepsin 1:10.000 Sigma. Co., USA) Hank's saline solution pH 7.3 for 15-45 m After sedimentation, protoscoleces were wash three times in PBS pH 7.2. Protease inhibi was added as mentioned above. The suspens was subjected to sonication for 5 min. interv ten times with cooling in ice bath. sonication was carried out using Gellenka ultrasonic instrument at 24 Amplitude. homogenates were cooled centrifuged 10.000g for 30 min. Protein concentration measured according to Bradford (1976).

- Adult worms of *E. granulous* camel and equ (donkey) origins were obtained experimental infection of puppies. Two gro of puppies were infected separately with vi protoscoleces of camel and donkey hyd cysts (Himonas et al., 1994). Sixty days infection, *Echinococcus granulosus* worms of camel origin were obtained those of donkey origin, were obtained 70 post infection. The worms of each animal washed several times in 0.01M phosphate b saline PBS pH 7.4 and homogenized with pH 7.4 at 4°C until a uniform suspension obtained. The homogenates were centrifug 10.000g for 30 minutes. The supernatants

collected and protein concentrations were measured in both *E. granulosus* of equine and camel origins as described by Bradford (1976).

Fractionation of somatic proteins, antigens and molecular weight determination by Sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis according to (Laemmli, 1970):

The molecular weights for homogenous preparation of hydatid fluids, protoscoleces and adult *E. granulosus* of both camel and equine (donkey)origins were determined using sodium dodecyl sulphate polyacrylamide gel electrophoresis using BIO-RAD MINE PROTEIN apparatus. Gels containing 3% stacking gel, 10% acrylamide were prepared from a stock solution of 30% by weight of acrylamide and 0.8 by weight N, N' - bis - methylene acrylamide.

The final concentration in the separating gel was as follows: 0.375 M Tris-HCL, pH 8.8 and 0.1% SDS. The gels were polymerized chemically by the addition of 0.0125% by volume of tetramethyl ethylene diamine (TEMED) and ammonium persulphate. The stacking gels of 3% acrylamide and a length of 1 cm containined 0.125 M Tris-HCL, pH 6.8 and 0.1% SDS. They were polymerized chemically as mentioned for the separating gel. The electrode buffer, pH 8.3 contained 0.025 M Tris, 0.192 M glycine and 0.1% SDS. The samples (0.02 - 0.03 ml) contained the final concentrations (final sample buffer): 0.0625 M Tris-HC1 pH 6.8, 2% SDS, 10% glycerol, 5% B-mercaptoethanol and 0.001% bromophenol blue as the dye. The protein concentration of each sample was 70µg. The protein samples were completely dissociated by boiling for 3 min. in a water bath. Protein molecular weight standards (Sigma Co. USA) included α-Lactalbumin (14.4 K. Da.): Soybean trypsin inhibitor (20.0 K Da.), Carbonic anhydrase (30.0 K Da.): Ovalbumin (43.0 K Da.); Bovine serum albumin (67.0 K Da.) and Phosphorylase b (94.0K Da.) were used as reference standards in parrallel with the used samples. Electrophoresis was carried out with a current of 40 mA per gel until the bromophenol blue marker reached the bottom of the gel (2 hours). The proteins were fixed in the gel overnight with 5% methyl alcohol and 7.5% acetic acid V/V (50 ml methyl alcohol, 75 ml acetic acid and completed to one liter with distilled water. The slab gels were stained for 2 hrs at room temperature with Comassie Brilliant Blue (R 250) (0.25% W/V Comassie blue powder dissolved in destained solution). Gels were then destained with destaining solution (45% methanol, 5% glacial acetic acid and 50% distilled water) with several changes, till the bands became clear and dried in a slab gel drier (Model 443, BIO-RAD).

A calibration curve was constructed by plotting the electrophoretic mobilities of the calibrated proteins against the logarithms of their corresponding molecular weight (Fig. 1). For molecular weight determination, the electrophoretic mobility of the unknown was measured and its corresponding molecular weight was interpreted from the calibration curve.

$$\text{Electrophoretic mobility} = \frac{\text{distance of protein migration}}{\text{gel length after destaining}} \times \frac{\text{gel length before staining}}{\text{distance of dye migration}}$$

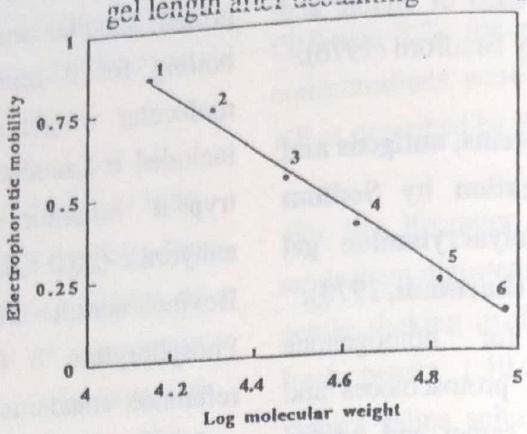


Fig. (1): Calibration curve for molecular weight determination by SDS polyacrylamide gel electrophoresis.

1) α-Lactalbumin (14.400);
2) Soybean trypsin inhibitor (20,000);
3) Carbonic anhydrase (30,000);
4) Ovalbumin (43,000);
5) Bovine serum albumin (67,000);
6) Phosphorylase b (94,000).

RESULTS

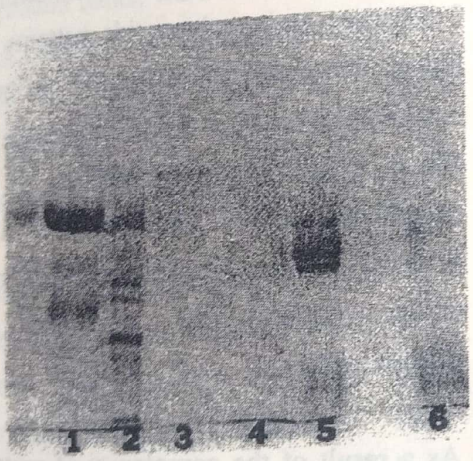
Fractionation of HF, protoscoleces and *E. granulosus* whole worm antigens obtained from camel and equine (donkey) origins by using SDS-PAGE technique was carried out.

Seven clear protein bands of camel hydatid fluid were observed at the level of 28.8, 34.7, 38.0, 46.8, 72.4, 61.3 & 100.0 K Da. However, the distribution of these bands was 4 bands having low molecular weights ranged between (28.8 - 46.8 K Da.), while the other bands had hi molcular weights (72.4, 81.3 & 100.0 K D respectively). HF of the other sub-species h only 3 bands consisting of one having a l molecular weight (44.6 K Da.) compared with other two bands which had higher molecu weights, Table 1.

Results revealed variations between protein ba of hydatid fluids of both origins. These variati represented by the number, distribution and molecular weights, table 1. The electrophor patterns of the camel samples ranged between & 28.8 K Da. with 7 bands, while those of

Table 1: Molecular masses (K Da.) of total protein from HF, protoscoleces and adult *E. granulosus* antigens resolved by SDS-PAGE.

HF		Protoscoleces		Adult E. granulosus	
camel	donkey	camel	donkey	camel	donkey
100.0	143.2	136.8	157.9	105.3	97.4
81.3	128,3	125.9	150.8	94.7	81.6
72.4	44.6	102.3	131.2	78.9	68.4
46.8		89.1	107.1	73.7	26.3
38.0		64.6	89.1	15.8	10.5
34.7		54.9	79.4		
28.8		38.0	67.6		
		34.7	53.7		
		29.5			
		26.3			
		22.9			
		19.9			



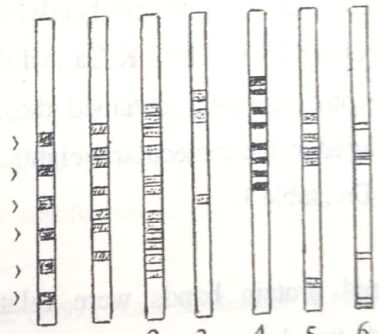


Fig. (2): Comparative study of the total proteins of HF, protoscoleces and *E. granulosus* worms by SDS-PAGE and Comassie stain. (a) standard

1) Camel HF
2) Camel protoscoleces
3) Donkey HF
4) Donkey protoscleces
5) *E. granulosus* of camel origin
6) *E. granulosus* of equine (donkey origin).

donkey were 143.2 - 44.6 K Da. where only three bands were noticed.

Concerning the protein pattern recovered from protoscoleces, the data presented in table 1 revealed a complexity in their protein expression. Camel protoscoleces were represented with 12 stained protein bands while those of the other species consisted of 8 bands (Fig. 2). Camel samples showed 8 protein bands of low molecular weights while one band (53.7 K Da.) was found in case of equine sample. It was noticed that only two bands were relatively similar in the electrophoretic patterns of protoscoleces (54.9 & 53.7 K Da.) and (64.6 & 67.6 K Da.) obtained from camel and donkey samples, respectively. One band was exactly similar in both protoscoleces profile (89.1 K Da.).

Regarding *E. granulosus* somatic antigens, the distribution of the electrophoretic patterns of camel islates consisted of 5 protein bands. These bands varied betwen 15.8 - 105.3 K Da., while the profile of the other species contained the same number but varied in the molecular weights to be 10.5 - 97.4 K Da., table 1.

All the stained protein bands were relatively similar except two low molecular weight bands (10.5 & 26.3 K Da.) among the equine samples. However, one band could characterize the camel isolate of *E. granulosus* having the highest molecular weight (105.3 K Da.).

DISCUSSION

Fractionation of different tested proteins, antigens through SDS-PAGE gel-electrophoresis show relatively large numbers of different prote bands. These bands varied or of closely relat molecular weights. These variations may refle the antigenicity of these proteins and could considered a suitable guide for comparison differentiation (Aly, 1993) of the different strain

In the present study, the stained protein patte of camel and equine (donkey) isolates varied their distribution, number of protein bands a their molecular weights. Such results agreed wi those obtained by Siles-Lucas and Cuesy Bandera (1996) when they studied t electrophoretic variations between ovine a swine populations.

HF of camel samples contained 7 varied prote bands, however equine sample recovered 3 ban only. Therefore, it is possible to establish at lea 3 different basic polypeptide patterns among th HF of camel sample. Moreover, one protein ban was found relatively similar among the patterns HF in both species. In addition HF of equi sample did not show bands between 28.8 and 44 K Da. comparing to camel sample. Such findi could increase the possiblity of stra differentiation.

As a result of the complexity of protein profil obtained from protoscoleces, 12 & 8 prote bands were obtained from camel and donk sampels respectively. The results revealed th only 2 bands had similar molecular weights (89 K Da.) within their band profiles. Equine samp did not show bands between 19.9 and 53.7 K D So, it is possible to distinguish the variou

extracts of protoscoleces. In this respect, the electrophoretic analysis of protoscoleces protein has been used previously as a criterion to evaluate intraspecific variation in *E. granulosus* for ovine and equine protoscoleces (McManus & Barret; 1985). Moreover, McManus & Parkhouse (Cited by McManus & Bryant, 1986) found that electrophoretic analysis of protein from ovine and equine protoscoleces showed many differences between these two isolates. It is apparent to be more satisfactory to use partial protein extracts from protoscoleces, as demonstrated by McManus & Barret (1985).

Dealing with SDS-PAGE electrophoretic analysis of adult *E. granulosus* whole worm antigens, the results showed that the two subspecies had equal number of protein bands. Inspite of the similarity of the protein bands, their molecular weights had varied. Two protein bands were of low molecular weights which characterized the equine (donkey) samples while one protein band has a high molecular weight that may be of value in discriminating the other subspecies from camels.

The results may allow us to suggest the existence of two different protein patterns for *E. granulosus* isolates. These patterns were found in HF, protoscoleces and adult *E. granulosus*. The subgrouping agreed with the genetic studies undertaken by Derbala & Moustafa, (1998). Similar results were obtained by Siles-Lucas et al. (1988) who mentioned that three Spanish substrains of *E. granulosus* had been exsisted which had biochemical, electrophoretic and genetic variations. Moreover, Hassan, (1991) noticed marked differences in the biochemical analysis between larval stages, hydatid fluids of camel, pig and sheep as well as their adult stages. Thererfore, the existence of different substrains were suggested.

Electrophoresis in polyacrylamide gels is a sensitive analytical tool for characterization of antigenic particles of proteins and molecular weights determination. It could be concluded that SDS-PAGE electrophoretic analysis could be used as a criterion to evaluate intraspecific variation in *E. granulosus* subsepecies or substrains.

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