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CHARACTERIZATION OF HYDATID CYST FLUID FROM RUMINANTS AND HUMANS BY SDS-PAGE IN PUNJAB, PAKISTAN

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ABSTARCT

Polypeptide analysis of purified hydatid cyst fluid (HCF) from ruminants (sheep, goat, cattle, buffalo and camel) and human beings was performed by sodium dodecyl sulphate polyacrylamide gel (12%) electrophoresis (SDS-PAGE) under reduced conditions. HCFs from different species revealed variable segregation pattern of proteins not only within the different hosts of the parasites but also in different types of HCF. In fertile HCF, five to three protein bands were observed in different intermediate hosts of the parasite viz., buffalo (269, 166, 89, 59 and 25kDa), goat (46, 29, 22 and 18kDa), sheep (209, 138 and 63kDa), cattle (269, 89 and 59kDa), camel (195, 166 and 123kDa) and human beings (195, 138, 21 and 6kDa). In sterile HCF of different species, less number of protein bands was found as compared to fertile cysts. The two protein bands were observed in sterile HCF collected from camel (195 and 141kDa), cattle (27 and 18 kDa), buffalo (43 and 27kDa) and human being (30 and 21kDa), whereas only one polypeptide band found in sheep (123kDa) and goat (78kDa). SDS-PAGE analysis of hydatid cyst fluid collected from different intermediate hosts of *Echinococcus granulosus* exhibited variable profiles of proteins. The present study suggests that SDS-PAGE alone is not a reliable diagnostic tool both in ruminants and human, it should be coupled with recent molecular techniques.

Key words: *Echinococcus granulosus*, polypeptides, SDS-PAGE, human beings, ruminants, fertile and sterile cysts.

INTRODUCTION

Hydatidosis caused by metacestode (larvae) of *Echinococcus granulosus* in livestock and human beings is an important global public health problem (Sadjjadi, 2006). Significant economic losses to meat industry are due to formation of hydatid cysts in internal organs (Lightowlers *et al*., 1984). Protoscoleces (infective form) of parasite are present only in fertile cysts and not in infertile (Kamenetzky *et al.,* 2000). In many areas the disease is being diagnosed in increasing number, whilst in other areas it is re-emerging due to collapse of public health programmes associated with socio-economic changes (Shafiq *et al*., 2005).

Prevalence of *E. granulosus* can be made by proper sampling from abattoir but difficult in areas where home slaughtering is practised or not under veterinary supervision. The development of proper diagnostic serological techniques will help in diagnosis and control of hydatid disease (Moro *et al*., 1997). In routine, sero-diagnostic tests are used but have limitations of cross reactivity. These limitations are eliminated by SDS-PAGE and Western blotting (Doiz *et al*., 2001).

In view of situation, present plan was to have preliminary information on protein profiling of both fertile and sterile hydatid cyst fluids from different species of animals and human beings by SDS-PAGE. Data obtained will be useful to find out some common proteins to be used in western blotting for accurate diagnosis of such drastic disease.

MATERIALS AND METHODS

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) technique of protein profiling was used under denaturing conditions as described by Laemmeli (1970).

Parasitic Samples: Both fertile and sterile hydatid cyst fluids were collected aseptically from intermediate hosts of the parasite viz., buffalo, cattle, goat, sheep, camel and human beings in Punjab, Pakistan.

Antigen Preparation: Hydatid cyst fluid (HCF) samples were prepared according to the method provided by Rogan et al. (1991) with little modifications. The HCFs (fertile and sterile) from various intermediate hosts were centrifuged at 10,000 rpm for 30 minutes and supernatants were separated for analysis. Samples were mixed with loading dye (Bromophenol Blue 1% (2 Drops), Glycerol 2 ml, β-Mercaptoethanol 1ml) to prepare the working dilutions (10ml) for gel electrophoresis. For this purpose, 100µl of each of fluid

samples, 50 µl of loading dye was added in new eppendorf tubes. The samples were gently but thoroughly mixed using vortex and heated in a boiling water bath for 5 minutes to denature the proteins. A 40µl of each of the sample was loaded in to the well of the gel.

Gel preparation: Polyacrylamide gel was prepared by following Laemmli (1970). Proteins were resolved on 12% gel. Perfect protein marker™ 10-225 KDa, catalog number 69079-3 manufactured by Novagen was used.

Preparation of resolving gel: Resolving gel used for segregation of proteins was 12.5%. Before casting the gel for polymerization the glass plates of gel assembly were placed together using 1mm thick spacers. Resolving gel (Acrylamide-Bisacrylamide(30%) 8.0ml, Tris-HCl (pH8.8) 3.0M 3.4ml, SDS(10%) 0.2ml, Distilled water 8.4ml, TEMED 14µl and Ammonium per sulphate 250µl) solution was poured in between the glass plates assembled in the gel assembly leaving 0.5 inch vacant at the top for stacking gel. Distilled water (100µl) was layered at the top of the gel to avoid dryness, give a flat surface and remove the oxygen from the surface of the gel as it inhibits polymerization. Gel was then left undisturbed at room temperature for 20minutes for polymerization.

Stacking gel preparation: Stacking gel used was 4.5%. Once the resolving gel was polymerized the overlying water was removed by tilting the gel assembly upside down. Then the stacking gel (Acrylamide-Bisacrylamide (30%) 0.94ml, Tris-HCl (pH 6.8) 1.0M 0.35ml, SDS (10%) 55µl, Distilled water 4.9ml, TEMED 5µl and Ammonium per sulphate 230µl) was poured at the top of resolving gel. A comb was inserted in the stacking gel to obtain required number of wells for sample loading. The gel was kept at room temperature for 15 minutes for polymerization.

Gel electrophoresis: The gel plates were fixed in the electrophoresis chamber and were placed in the electrophoresis tank filled with electrophoresis buffer (3g of Tris, 14.4g of glycine and 1g SDS per liter distilled water, pH 8.4) to a volume so as the bottom of the gel was dipped in it. The central area of the electrophoresis chamber was filled completely with the same buffer. 10µl protein marker was loaded in the first well and 40µl of each of the samples was loaded in consecutive wells with the help of micropipette. The electrophoresis was performed for 6 hours and stopped one centimeter above the bottom of gel length at a current supply of 170 V in a cooling chamber maintained at 4^{o}C.

Staining: After the electrophoresis, gel was removed from the plates carefully. Two glass plates were separated from each other using a spatula and gels were transferred to staining solution (125mg Coomassie Brilliant Blue G-250, 112.5ml methanol, 22.5ml glacial acetic acid and 112.5ml distilled water). The box containing gel and staining solution was placed on shaker with constant agitation for four hours.

De-staining: Stained gel was placed in de-staining solution (112.5ml methanol, 22.5ml glacial acetic acid and 112.5ml distilled water) with constant agitation until background became transparent and protein bands visualized in the form of blue colored light and dark bands. After de-staining, the image was captured by Gel Documentation System.

RESULTS

Protein profiles of hydatid cyst fluids along with known protein markers segregated for polypeptides using sodium dodecyl sulphate polyacrylamide gel (SDS-PAGE) electrophoresis on 12% resolving gel are presented at plates 01 and 02. Protein markers of catalog number 69079-3 manufactured by Novagen depicted nine polypeptide bands having molecular weights 225, 150, 100, 75, 50, 35, 25, 15 and 10kDa, respectively. The log molecular weights (log MW) and relative flow (Rf) values of above mentioned protein markers are shown in Table 01. Standard curve plotted between log MWs of protein markers and their Rf values is displayed in Fig. 01. The MWs of unknown polypeptides of hydatid cyst fluids (HCF) from different species calculated using standard curve of known protein markers are shown in Table 02.

Sterile HCF of sheep revealed only one polypeptide band of 123kDa (log MW 2.09, Rf 0.1927). Fertile HCF from sheep exhibited different polypeptide patterns. In one case, there was only one peptide band of 63kDa (log MW 1.8, Rf 0.289) where as in other cases two bands were observed having MW 209kDa (log MW 2.32, Rf 0.1084) and 138kDa (log MW 2.14, Rf 0.1686). Sterile HCF of goat revealed only one polypeptide band of 78kDa (log MW 1.89 and Rf 0.265). Fertile HCF from goat exhibited four polypeptide bands, molecular weights of polypeptides calculated were 46kDa (log MW 1.66, Rf 0.3373), 29kDa (log MW 1.46, Rf 0.4096), 22kDa (log MW 1.34, Rf 0.4457) and 18kDa (log MW 1.25, Rf 0.4819).

Two polypeptide bands were observed on electrophoresis of sterile HCF of cattle with molecular weights of 27kDa (log MW 1.43, Rf 0.4216) and 18kDa (log MW 1.25, Rf 0.4819). In fertile HCF of cattle three polypeptide bands were found having MW of 269kDa (log MW 2.43, Rf 0.0722), 89kDa (log MW 1.95, Rf 0.2409) and 59kDa (log MW 1.77, Rf 0.3012). Sterile HCF of buffalo revealed two polypeptide bands of 43kDa (log MW 1.63 and Rf 0.3493) and 27kDa (log MW 1.43 and Rf 0.4216). Fertile HCF from buffalo showed five protein bands with MW of 269kDa (log MW 2.43, Rf 0.0722), 166kDa (log MW 2.22, Rf 0.1445), 89kDa (log

MW 1.95 and Rf 0.2409), 59kDa (log MW 1.77 and Rf 0.3012) and 25kDa (log MW 1.4, Rf 0.4337). Two polypeptides of 195kDa (log MW 2.29, Rf 0.1204) and 141kDa (log MW 2.15 and Rf 0.1686) were segregated in resolving gel on electrophoresis of sterile HCF collected from camel. Only two polypeptides were observed in fertile HCF of camel however, there was difference in molecular weights of polypeptides. In one case MW was 195kDa (log MW 2.29, Rf 0.1204) and 166kDa (log MW 2.22, Rf 0.1445), where as in other case MW of 195kDa (log MW 2.29, Rf 0.204) and 123kDa (log MW 2.09 and Rf 0.1927) were recorded.

Electrophoretic pattern of proteins of sterile as well as fertile HCF collected from human is presented at plate 01 (3,lane A,B) and plate 02 (7, lane A,B) respectively. Sterile HCF of human revealed two polypeptide bands of 30kDa (log MW 1.47 and Rf 0.0.4096) and 1kDa (log MW 1.32, Rf 0.4578). Fertile HCF from human exhibited four polypeptides with MW of 195kDa (log MW 2.29, Rf 0.1204), 138kDa (log MW 2.14 and Rf 0.1686), 21kDa (log MW 1.32, Rf 0.4578) and 6kDa (log MW 0.76, Rf 0.6506).

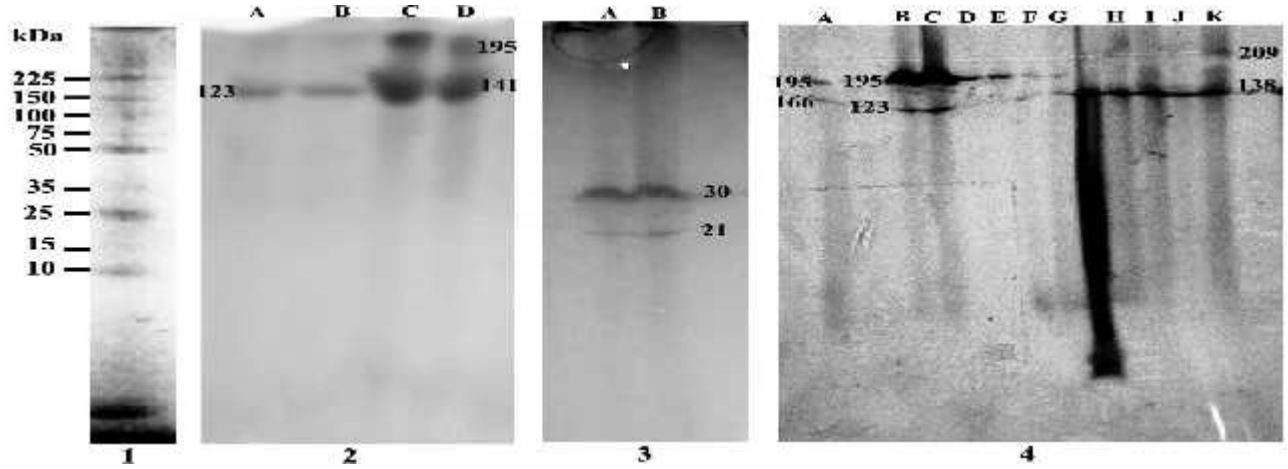

Plate 1. Polypeptide analysis of hydatid cyst fluid by sodium dodecyl sulphate polyacrylamide gel electrophoresis using 12% resolving gel. 1: Protein markers of known molecular weights used as standard 2: Lanes A,B Hydatid cyst fluid (fertile) of sheep Lanes C,D Hydatid cyst fluid (fertile) of camel 3: Lanes A,B Hydatid cyst fluid (sterile) of human isolate 4: Lanes A,B,C,D,E,F and G Hydatid cyst fluid (fertile) of camel 4: Lanes H,I,J,K Hydatid cyst fluid (fertile) of sheep isolate.

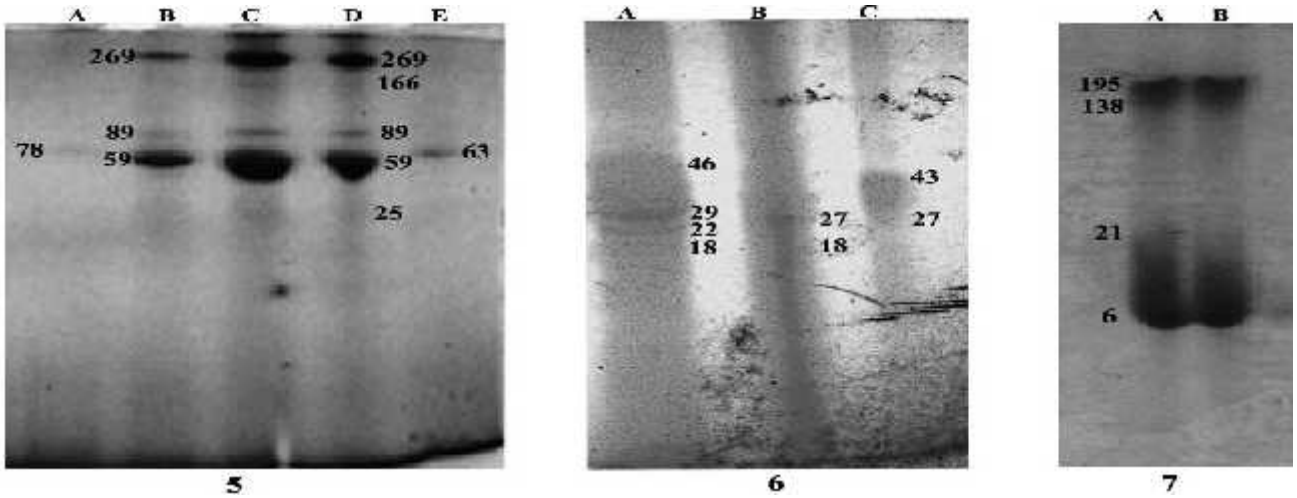

Plate 2. Polypeptide analysis of hydatid cyst fluid on 12% resolving gel by sodium dodecyl sulphate polyacrylamide gel electrophoresis 5: Electorphoretogram of proteins of hydatid cyst fluid from various intermediate hosts Lane A: Sterile hydatid cyst fluid of goat Lane B: Hydatid cyst fluid (fertile) of cattle Lanes C,D: Hydatid cyst fluid (sterile) of buffalo Lane E: Hydatid cyst fluid (fertile) of sheep 6: Lane A: Hydatid cyst fluid (fertile) of goat B: Hydatid cyst fluid (sterile) of cattle C: Hydatid cyst fluid (sterile) of buffalo 7: Lanes A,B Hydatid cyst fluid (fertile) of human.

Table 1. Log molecular weights and relative flow values of known protein markers resolved on 12% gel.

Band No.	Molecular weight (kDa)	Rf value	Log molecular weight
1	225	0.095	2.352
2	150	0.164	2.176
3	100	0.22	2
4	75	0.267	1.875
5	50	0.325	1.698
6	35	0.39	1.544
7	25	0.435	1.397
8	15	0.512	1.176
9	10	0.58	1

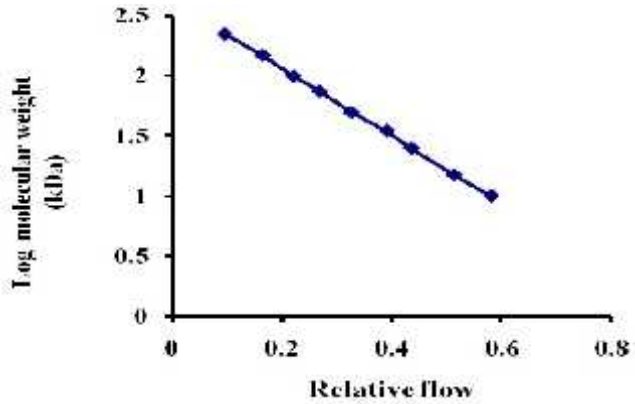

Figure 1. Standard curve based on log mol. wt. and relative flow (Rf) values of known protein markers resolved on 12% gel by SDS-PAGE

Table 2. Different polypeptide profiles obtained from hydatid cyst fluid of various isolates of different intermediate hosts on 12% resolving gel by SDS-PAGE.

Gel No.	Isolates	Lanes	Band No.	Rf value	Log mol. weight	Mol. wt. (kDa)
2	Sheep(sterile)	A,B	1	0.1927	2.09	123
	Camel(sterile)	C,D	1	0.1204	2.29	195
			2	0.1686	2.15	141
3	Human(sterile)	A,B	1	0.4096	1.47	30
			2	0.4578	1.32	21
4	Camel (fertile)	A	1	0.1204	2.29	195
			2	0.1445	2.22	166
	Camel (fertile)	B-G	1	0.1204	2.29	195
			2	0.1927	2.09	123
	Sheep (fertile)	H-K	1	0.1084	2.32	209
			2	0.1686	2.14	138
	Goat (sterile)	A	1	0.265	1.89	78
	Cattle (fertile)	B	1	0.0722	2.43	269
			2	0.2409	1.95	89
			3	0.3012	1.77	59
5			1	0.0722	2.43	269
			2	0.1445	2.22	166
	Buffalo (fertile)	C,D	3	0.2409	1.95	89
			4	0.3012	1.77	59
			5	0.4337	1.4	25
	Sheep (fertile)	E	1	0.289	1.8	63
	Goat (fertile)	A	1	0.3373	1.66	46
			2	0.4096	1.46	29
			3	0.4457	1.34	22
6			4	0.4819	1.25	18
	Cattle (sterile)	B	1	0.4216	1.43	27
			2	0.4819	1.25	18
	Buffalo (sterile)	C	1	0.3493	1.63	43
			2	0.4216	1.43	27
7	Human (fertile)	A,B	1	0.1204	2.29	195
			2	0.1686	2.14	138
			3	0.4578	1.32	21
			4	0.6506	0.76	6

DISCUSSION

Polypeptide analysis of purified hydatid cyst fluids of different species (Sheep, goat, cattle, buffalo, camel and human) was performed under reduced conditions to evaluate number of polypeptides and their molecular weights in each species. Both number of polypeptides and molecular weights observed in our study were not similar. Variable segregation pattern of proteins was observed. Fractionation of fertile purified fluids of sheep (209, 138 and 63kDa), cattle (269, 89 and 59kDa) and camel (195, 166 and 123kDa) revealed three proteins in each case but their molecular weights varied. According to present results proteins of HCF are variable as have been reported by Sabry (2007) in research on diagnosis of hydatidosis in human and animals. Similar findings on variation of total protein patterns of protoscoleces of *E. granulosus* were reported by Jiang *et al.* (1998). In addition difference among crude and purified hydatid cyst fluid of bovine and swine isolates were most complex in the findings of Siles-Lucas and Cuesta-Bandera (1996). Derbala (1998) performed polypeptide analysis of camel HCF and observed seven polypeptide bands with variable molecular weights. The findings of Derbala, (1998) vary with reference to molecular weights of polypeptides, as only three proteins were present in electrophoretograms of camel HCF in present study.

Polypeptides separated by SDS-PAGE differed in cyst fluid samples of cattle and sheep in the findings of Itagaki *et al.* (1994) which support present results. Segregation patterns of proteins were also variable in goat, human and buffalo isolates. Number of polypeptide bands ranged from three to five. However, a common protein having molecular weight of 166kDa was also found in isolates of buffalo and camel. Similarly a protein with molecular weight of 138kDa was common in sheep and human isolates. Common proteins have been reported by Burgu *et al.* (2000), while working on immunodiagnosis of hydatidosis in sheep and humans by western blotting. The molecular weight of this protein was 116 kDa, a little lower than the proteins observed in preset findings.

The protein of *E. granulosus* from sheep liver as investigated by Doganay *et al.* (2004) ranged between 68.24 and 8kDa, quite different from our results of SDS-PAGE conducted on HCF of sheep. Simsik and Koroglou (2004) analysed HCF and detected 6 bands from sheep. Concluding on the basis of present experiments profile of HCF proteins collected from different species differs from one geographical region to another. Similar comments have been documented by Shambesh *et al.* (1995); Siles-Lucas and Cuesta-Bandera (1996), in their work on partial characterization of cyst fluid of buffaloes.

The protein patterns of protoscoleces (PSC) by SDS-PAGE performed on HCF exhibited variable results in number and molecular weight of polypeptides, both in protoscoleces and hosts. Therefore it was concluded that diagnosis of hydatidosis in human and animals can not be relied only on detection of polypeptides. For exact diagnosis serological as well as molecular techniques like western blotting may be used for assistance.

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