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Isoenzymatic pattern of glucose-6-phosphate dehydrogenase and isocitrate dehydrogenase in Iranian *Echinococcus granulosus*

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ABSTRACT

Morphology and genetic studies of *Echinococcus granulosus* have indicated that there are 2 different strains of this parasite in Iran, ovine and camel. However, no study has been carried out to date on the phenotypic characterization of this parasite. In the present study the electrophoretic pattern of glucose-6-phosphate dehydrogenase (G6PD) and isocitrate dehydrogenase (ICD) was demonstrated. In this study isolates of ovine and camel hydatid cysts were collected from slaughterhouses across Iran. Hydatid fluid and hydatid sand were separated and collected. The fluid was concentrated and the protoscoleces from the sand were extracted. The amount of total protein in protoscoleces and concentrated hydatid fluid was determined. Electrophoretic pattern of extract was indicated by SDS-PAGE. Non-denaturating electrophoresis was also used for study of electrophoretic pattern of G6PD and ICD; so that major and minor enzyme activities were indicated. Densitometry of electrophoretic pattern indicated 2 major bands for each of these enzymes in camel and sheep with the same pattern in the extract of protoscoleces and hydatid fluid. Based on the fact that the band of enzymes in each of 2 isolates has different molecular patterns; we propose that these represent the 2 different strains (sheep-dog and camel-dog) of this parasite in Iran.

Key words: *Echinococcus granulosus*, enzyme, phenotype, camel, sheep

Introduction

Echinococcus granulosus is the causative agent of unilocular hydatidosis, a disease that can result in serious consequences and which is an economical and hygienic problem to animals and to humans (HOSSEINI and ESLAMI, 1998).

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Because of the variations in infection potency for humans and animals, length of period (FRENCH et al., 1982), severity of disease (THOMPSON and LYMBERY, 1995), response to treatment (McMANUS and BRYANT, 1982), identification of the strains of *E. granulosus* was considered as an important element perquisite in control programs of the World Health Organization and Food and Agriculture Organization of the United Nations (HOSSEINI and ESLAMI, 1998; SILES-LUCAS and CUESTA-BANDERA, 1996).

Genetic and morphological characterizations of Iranian *E. granulosus* by ZHANG et al. (1998), revealed 2 different strains (sheep-dog and camel-dog). On the basis of studies on the cytochrome complex 1 and NADH dehydrogenase it was revealed that there are 2 strains for *E. granulosus* in Iran (ZHANG et al., 1998). Until recently, few phenotypic studies have been done in Iran. SILES-LUCAS et al. (1996) have studied the phenotype of Iranian *E. granulosus* by SDS-PAGE. In the present study we investigate the extract of *E. granulosus* and hydatid fluid for the electrophoretic mobility pattern of 2 enzymes, glucose-6-phosphate dehydrogenase (G6PD) and isocitrate dehydrogenase (ICD), by non-denaturating PAGE and with larger sample sizes.

Despite its importance very few countries have performed detailed investigations on this parasite. Since the epidemy of this parasite is alarming in Iran (HOSSEINI and ESLAMI, 1998) the need for an elaborate study of *E. granulosus* is immediate and crucial.

Materials and methods

Liver and lung from 19 infected sheep and 15 infected camels were collected from different slaughterhouses across Iran and transferred to the parasitology laboratory of the School of Veterinary Medicine, University of Tehran. Hydatid fluid was collected by sterilized needle and the hydatid sand was washed three times by phosphate buffer solution (PBS). Samples of hydatid fluid and sand were refrigerated at -70 ^{o}C. Hydatid fluid was concentrated approximately to 0.1 level of its initial volume by dialysis tube and polyethylene glycol (PEG) powder. Hydatid sands were lyzed by multiple freeze-thaw in 10 mL Hank's saline solution with glass beads so that the lysis was facilitated by the release of the protoscoleces (SILES-LUCAS et al., 1996). Before dialysis 0.1 M and 6.8 mM TLCK were added to both of them.

These procedures were followed by sonication of the protoscoleces for protein extraction. Eight drops of Tris-HCL buffer, pH 7 were added to 1 mL of this extract before a second sonication. Supernatant fluid was harvested by centrifugation for subsequent analysis.

A) Crude protein concentration measurement: Bradford assay was done according to Bradford protein assay (BRADFORD, 1976).

B) SDS-PAGE of the protein: 3% stacking gel (Tris-HCL buffer 0.5 M, pH 6.8) and 7% resolving gel (Tris-HCL buffer 1.5 M, pH 8.8) were prepared. The running was achieved over a period of 5 h by 70 Volts.

C) Staining and destaining of SDS-PAGE gel: Gels were stained with 25% commassie blue R250 in 95% ethanol. After staining gels were destained by alcohol and acetic acid (30/70, V/V) (SILES-LUCAS et al., 1996).

D) Analysis of G6PD and ICD by non-denaturating PAGE: In order to present the activity of the 2 enzyme proteins, separation of the crude lysate was performed on 10% non-denaturating gel (LAEMMLI, 1970). At the end of running gels were sliced. A slice from each sample was placed in G6PD staining solution (30 mg NADP disodium salt, 20 mg Nitro Blue Tetrazolium, 2 mg Phenazin Meta Sulfate, 200 mg G6P disodium salt, 10 mg $MgCl_2$. $4H_2O$, 25 mL Tris-HCl buffer, and 90 mL distilled water) (CUTILLAS et al., 1993) and the other was placed in ICD staining solution (20 mg NADP disodium salt, 20 mg Nitro Blue Tetrazolium, 3 mg Phenazin Meta Sulfate, 5 mg Naisocitrate. H_2O, 20 mg $MgCl_2$. $4H_2O$ and 85 mL distilled water) (HIKIMAC, 1964). The slices were then incubated at 37 °C for 1 h. After washing with distilled water the bands were scanned by densitometer LRE pheroterons model at 595 nm. The bands corresponding to G6PD and ICD appeared as major and minor peaks, indicating the major and minor enzymatic activity, respectively.

Results

BRADFORD assay of protein in the concentrated hydatid fluid and extracted protoscoleces indicated greater than 100 µg/mL of protein in samples. The SDS-PAGE analysis of these samples revealed several bands (Fig. 1), with different molecular weights.

Non-denaturating PAGE analysis of concentrated hydatid fluid and extract of protoscoleces, followed by G6PD and ICD specific staining, indicated these bands as major and minor peaks for sheep (Figs 2. and 3.) and camel (Figs 4. and 5.) as measured by densitometry. Average of G6PD major bands number in the sheep and camel isolates were 1.89 ± 0.32 and 1.86 ± 0.36, respectively. Furthermore, the values for minor bands number were 2.84 ± 0.5 for sheep and 1.88 ± 0.34 for camel isolates. On the other hand, while there is no minor band in the camel isolates, mean ICD major bands number for sheep and camel isolates were 2.78 ± 0.55 and 1.81 ± 0.4, respectively. Average of ICD minor bands number for the sheep isolates was 3.79 ± 0.71. All values are shown as Mean ± SD.

Discussion

Electrophoretic mobility of major G6PD and ICD bands were different in the 2 strains (camel and sheep). Although several minor G6PD bands in these 2 strains had similar

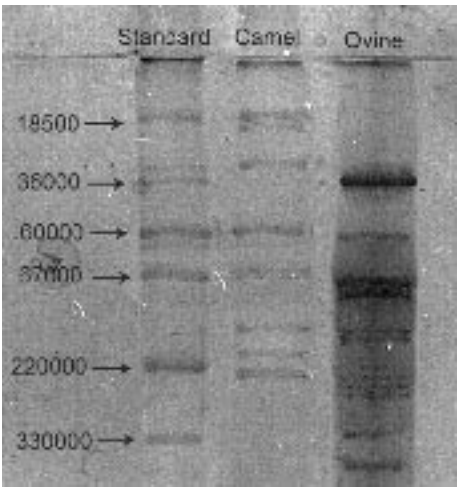

Fig. 1. Electrophoretic pattern of protoscoleces extract for camel and ovine as compared to standard by SDS-PAGE

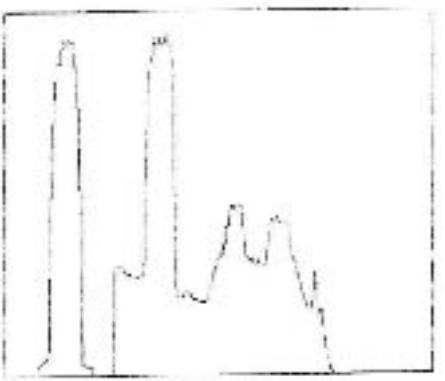

Fig. 2. Densitometric curve of glucose 6-phosphate dehydrogenase (G6PD) in extract of camel protoscoleces in 595 nm. There are 2 major and 2 minor bands.

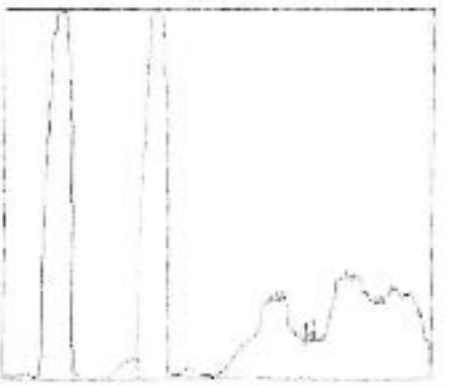

Fig. 3. Densitometric curve of glucose 6-phosphate dehydrogenase (G6PD) in extract of ovine protoscoleces in 595 nm. There are 2 major and 3 minor bands.

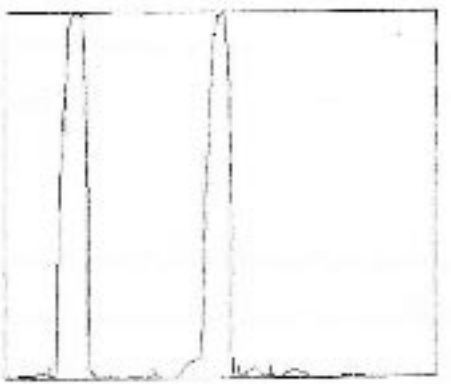

Fig. 4. Densitometric curve of glucose Isocitrate Dehydrogenase (ICD) in extract of camel protoscoleces in 595 nm. There are 2 major bands.

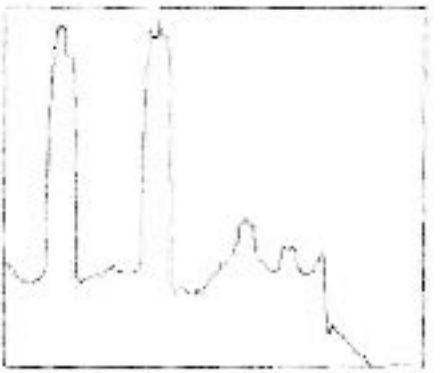

Fig. 5. Densitometric curve of Isocitrate Dehydrogenase (ICD) in extract of ovine protoscoleces in 595 nm. There are 2 major and 2 minor bands.

electrophoretic mobility, these minor bands were not categorized in the enzyme group. In this respect, it has been documented that only the major bands represent the enzyme group. On the other hand, it was hypothesized that the main difference between camel and sheep strains of *E. granulosus* lies in the gene cluster of enzyme, as reflected in this study as major band with different electrophoretic mobility (SILES-LUCAS and CUESTA-BANDERA, 1996).

ZHANG et al. (1998) have characterized sheep-dog (genotype 1 or JI) and camel-dog (genotype 6 or J6) in Iran. Our phenotypic study complemented this previous genotypic study. Also, genotypic studies could determine the genetic differences by means of restriction digestion and hybridization with specific gene probes, but they could not differentiate various strains of *E. granulosus*.

The study by SILES-LUCAS et al. (1996) on secretory and somatic proteins of *E. granulosus* indicated that SDS-PAGE of *E. granulosus* extracts and hydatid fluid was not adequate to distinguish between the different *E. granulosus* strains. In fact, as the present study indicated (Fig.1) high concentration of proteins in *E. granulosus* extracts and hydatid fluid was revealed in the SDS-PAGE. In addition, as SILES-LUCAS et al. (1996), mentioned, due to the complexity of the protein profiles obtained from the protoscoleces even electrophoretic separation by SDS-PAGE could not establish clear differences between different isolates. However, these researchers indicated that isoenzymatic pattern study of *E. granulosus* extracts and hydatid fluid was suitable to identify the different strains.

Hence, at the present study ICD and G6PD were studied; the former is a regulatory enzyme in the Krebs's cycle while the latter is a key enzyme in the pentose-phosphate pathway. As SILES-LUCAS et al. (1996) used isoenzymatic pattern for the study of *E. granulosus* strains, the present study included 2 isoenzymes of Iranian *E. granulosus*. Moreover, we used a large sample size that enabled us to distinguish the difference with a high level of confidence. Our present study used hydatid cyst of natural parasite. However, SCHANTZ et al. (1976) indicated that although electrophoretic pattern of *E. granulosus* and hydatid fluid of murine model infected with sheep and horse *E. granulosus* did not show any difference with the natural hosts. In spite of this fact, study on the effects of non-specific host and various passages on these hosts by electrophoresis are necessary.

This assertion is in agreement with observations made by HOSSEINI and ESLAMI (1998), the isoenzymatic study of MACPHERSON and McMANUS (1984), as well as the genetic studies of McMANUS and SMYTH (1982) and McMANUS and BRYANT (1986), in that all of them showed 2 different strains for Iranian *E. granulosus*.

A significant number of human isolates belong to the ovine genotype. On the other hand, sheep-dog genotype is more frequent than the other genotypes including sheep-dog, goat-dog and camel-dog (ZHANG et al., 1998). To date, no report of infection in humans by camel strain has been reported. However, one should not exclude the possibility of infection in humans by this strain. In conclusion, our findings provided important insights to the control of hydatidosis and protection of public health.

Acknowledgments

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References

BRADFORD, M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein - dye binding. Anal. Biochem. 72, 248-254.

CUTILLAS, C., B. RODRIGUEZ, P. GERMAN, D. GUEVARA (1993): Isoenzymatic pattern of glucose 6-phosphate dehydrogenase from Ascaris suum. J. Helminthol. 67, 226-232.

FRENCH, C. M., G. S. NELSON, M. WOOD (1982): Hydatid disease in the Turkana district of Kenya. I. The background to the problem with hypotheses to account for the remarkably high prevalence of the disease in man. Ann. Trop. Med. Parasitol. 76, 425-437.

HIKIMAK, K. (1964): Histochemical studies on dehydrogenase and diaphorases with special preference to their specificity. II. Improvement in the method from the histochemical demonstration of specific oxidative enzymes. Acta. Jpn. 13, 67-84.

HOSSEINI, S. H., A. ESLAMI (1998): Morphological and developmental characteristics of *Echinococcu granulosus* derived from sheep, cattle and camel in Iran. J. Helminthol. 72, 333-341.

LAEMMLI, U. K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680-685.

McMANUS, D. P., C. BRYANT (1986): Biochemistry and Physiology of Echinococcus. In: The Biology of Echinococcus and Hydatid disease (Thompson, R. C. A., Ed.) Allen and Unwin press. London. pp.114-142.

McMANUS, D. P., J. D. SMYTH (1982): Intermediary carbohydrate metabolism in protoscoleces of *Echinococcus granulosus* (horse & sheep strains) and *Echinococcus multilocularis*. J. Parasitol. 84, 351-366.

MACPHERSON, C. N. L., D. P. McMANUS (1984): A comparative study of *Echinococcus granulosus* from human and animal hosts in Kenya using isoelectric focusing and isoenzyme analysis. Int. J. Parasitol. 12, 515-521.

SCHANTZ P. M., C. COLLI, A. CRUZ-REYES, U. PREZIOSO (1976): Sylvatic Echinococcus in Argentina. II. Susceptibility of wild carnivorous to *Echinococcus granulosus* and host-induced morphological variation. Tropenmed. Parasitol. 127, 70-78.

SILES-LUCAS, M., M. C. BENITO, C. CUESTA-BANDERA (1996): *Echinococcus granulosus* genomic and isoenzymatic study of Spanish strains isolated from different intermediate hosts. Vet. Parasitol. 63, 273-282.

SILES-LUCAS, M., C. CUESTA-BANDERA (1996): *Echinococcus granulosus* in Spain: strain differentiation by SDS-PAGE of somatic and excretory, secretory proteins. J. Helminthol. 70, 253-257.

THOMPSON, R. C. A., A. J. LYMBERY (1995): *Echinococcus* and Hydatid disease. Allen and Unwin press. London. pp. 141-151.

ZHANG, L., A. ESLAMI, S. H. HOSSEINI, D. P. McMANUS (1998): Indication of the presence of two distinct strains of *Echinococcus granulosus* in Iran by mitochondrial DNA markers. Am. J. Trop. Hyg. 59, 171-174.

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SAŽETAK

Morfološka i genetska istraživanja trakavice *Echinococcus granulosus* pokazala su da postoje 2 različita soja ovog parazita u Iranu, ovčji i devin. Međutim, do danas nije istraživana fenotipska karakterizacija ovog parazita. U ovom radu je pokazan elektoforetski uzorak glukoza-6- fosfat dehidrogenaze (G6PD) i izocitrat dehidrogenaze (ICD). Sakupljane su hidatidne ciste iz ovaca i deva s klaonica diljem Irana. Hidatidna tekućina i hidatidni pijesak odvajani su i sakupljeni. Hidatidna tekućina bila je koncentrirana, a iz hidatidnog pijeska izdvojeni su protoskoleksi. Određivana je ukupna količina proteina iz protoskoleksa i koncentrirane hidatidne tekućine.

Elektroforetski uzorak ekstrakta izdvojen je pomoću SDS-PAGE metode. Ne-denaturirajuća elektroforeza je također primijenjena u istraživanju elektroforetskog uzorka G6PD i ICD, tako da su zabilježene najveće i najmanje enzimne aktivnosti. Densitometrijom elektroforetskog uzorka dobivene su po dvije velike trake u oba enzima i u ovčjem i u devinom izolatu, identične u ekstraktu protoskoleksa i hidatidne tekućine. Na temelju nalaza različite molekulske mase enzima u svakom izolatu smatra se da je riječ o dvama različitim sojevima.

Ključne riječi: *Echinococcus granulosus*, enzim, fenotip, deva, ovca