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ORIGINAL ARTICLE

The enzymes and electrolytes profiles in hydatid cyst fluid of naturally infected Iranian domestic ruminants

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Abstract Cystic echinoccoccosis is a zoonotic and prevalent disease which causes health problems and economic looses worldwide. The biochemical components of hydatid cyst fluid (HCF) have important role in metabolism and growth of unilocular hydatid cyst. The aim of the current study was to determine HCF level of enzymes and electrolytes profiles in naturally infected Iranian domestic ruminants. The livers and lungs infected with hydatid cysts were collected and HCF was aspirated from a total of 100 cysts obtained from livers (50 cysts) and lungs (50 cysts) of slaughtered domestic ruminants. Biochemical measurement of HCF was undertaken using colorimetric technique, refractometer, flame photometer, and biuret method. The enzyme levels of HCF were also measured by using appropriate kits. The average HCF level of calcium (Ca) was found to be significant higher in cattle (7.49 ± 1.28 mg/dl) and goats (13.98 ± 5.11 mg/dl) lungs. The average HCF level of phosphorous (P) was significantly higher in cattle livers (0.82 ± 0.02 mg/dl) than other examined animals. Measurement of the average HCF level of magnesium (Mg) was significantly lower in camel lungs (11.8 ± 1.05 mg/dl) than that in cattle livers (10.56 ± 1.73 mg/dl). The highest average HCF levels of Natrium (Na, 122.8 ± 11.91 mEq/l) and Kalium (Ka, 7.18 ± 1.37 mEq/l) were measured in sheep livers. The average HCF level of albumin (Alb) in infected lungs was significantly lower in cattle (0.48 ± 0.01 mg/ml) than camels (0.95 ± 0.05 mg/ml). The average HCF level of total protein (TP) in infected lungs was also lower in sheep (0.51 ± 0.06 mg/dl) than goats (3.21 ± 0.51 mg/dl). The highest average HCF level of creatine phosphokinase (CPK, 1229.25 ± 13.21 U/ml) and lactate dehydrogenase (LDH, 363.62 ± 10.44 U/ml) were measured in infected lungs of camels. It was concluded that HCF level of enzymes and electrolytes had differences in examined Iranian domestic ruminants which may be used to screen and help in characterization and identification of strains of *Echinococcus granulosus*.

Keywords Electrolyte · Enzymes · Hydatid cyst fluid · Ruminant

Introduction

Hydatidosis is the oldest and one of the important helminthic disease in both developing and developed countries caused by metacestode, i.e. hydatid cyst of the dog tapeworm, *Echinococcus granulosus*. The metacestode develops in internal organs of many herbivorous and omnivorous species (Zhang et al. 2003). *Echinococcus granulosus* is the most prevalent specie in several North Africa and Middle East countries, causing significant public health problems (Abdullah et al. 2013). In Iran, the prevalence varies 5–10% for sheep and goats, 20–40% for cattle, and 0.5–7/10^5 for humans in liver (70%), lungs (22%), and other organs (8%) (Yakhchali and Ghargi 2001; Eslami 2006; Yakhchali and Mardani 2011).

The chemical components of hydatid cyst fluid (HCF) play an important role in metabolism and physiology of the metacestode. The various electrolytes were reported in

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HCF (Agosin and Repetto 1965; Kassis and Tanner 1977; Farayha and Smyth 1983; Radfar et al. 2005). The important role of Ca and P are to adjust HCF acidities as calcareous corpuscles (Frayha and Haddad 1980). The HCF level of Sulfur is similar to that of blood in intermediate host (Radfar and Iranyar 2004; Eslami 2006). Organic materials play a critical role in metabolism, defense mechanism and physiology of the metacestode (Yakhchali et al. 2012). Zinc also plays important role in immunity, metabolism and activating the enzymes of the metacestode. Iron and copper influence respiration of the parasite (Ozen et al. 1992; Radfar and Iranyar 2004; Rahdar et al. 2008; Shaafie et al. 1999). There is an inverse relationship between the cholesterol concentration and protoscolices viability (Thompson and Lymbery 1995; Eslami 2006). HCF is germ free; however it is a suitable media for other infective organisms. The HCF level of immunoglobulin and anti-complement factors are similar to the host serum (Frayha et al. 1980; Eslami 2006).

Understanding how parasite grows in the host tissues and what it needs, may be essential to lunch control programs against the parasite (Rahdar et al. 2008). The metabolism and biochemical differences of *E. granulosus* may be the causes of strains formation (Thompson 1991; Shaafie et al. 1999). In the endemic areas, strain identification and discrimination should be carried out on the basis of morphology, biology, biochemistry, immunology and molecular tools where different cycles of transmission exist (Kanwar et al. 1994; Thompson and Lymbery 1995; Kassis and Tanner 1977; Burgu et al. 2000). Thompson and Lymbery (1995), Shaafie et al. (1999) and Macpherson and McManus (1982) clearly indicated biochemical measurements establish a baseline assessment of chemical composition of HCF which applicable to discriminate strain variations of *E. granulosus* in different areas. Thus the present study was aimed to evaluate HCF level of enzymes and electrolyte profiles in naturally infected Iranian domestic ruminants.

Materials and methods

Animals sampling

The livers and lungs infected with hydatid cysts of slaughtered domestic ruminants, i.e. cattle (*Bos taurus*), sheep (*Ovis aries*), goats (*Capra hircus*) and camels (*Camelus dromedarius*) were collected. The animals were also examined according to ethical considerations in animal studies consistent with recommendations of the American Veterinary Medical Association and Model Code of Practice for the Welfare of Animals. The HCF was aspirated using sterile disposable syringes from a total of 100 cysts (50 cysts of lungs and 50 cysts of per each animal) (Thompson and Lymbery 1995; Yakhchali et al. 2012). The HCF was subjected for centrifugation to remove the protoscolices (1500 rpm for 30 min, +4 °C). The inhibitor of enzyme denaturation, i.e. Phenylmethylsulfonyl fluoride (PMSF, 5.0 mmol) was also added to HCF and stored at −20 °C until laboratory analysis (Yakhchali and Mardani 2013).

Measurement of HCF level of electrolytes

Analyses of calcium (Ca), phosphorus (P) and magnesium (Mg) HCF levels were performed using colorimetric methods (Randox, Antrim, UK; Stanbio, Boerne, USA) (15). HCF levels of Na and K were evaluated using Flame photometer and Na–K standards (ZistChimi, Iran).

Measurement of HCF level of enzymes

HCF specific gravity (Sg), total amount of protein (TP) and albumin (Alb) were evaluated using densitometer, refractometer and biuret method, respectively. The activity of alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine phosphokinase (CPK) (Roche Diagnostics, Mannheim, Germany), and aspartate aminotransferase (AST) were measured (Enzymatic Assay XpressBio, USA).

Statistical analysis

Statistical evaluation of HCF level of enzymes and electrolytes were performed using One-Way ANOVA with confidence interval of 95% (SPSS 11.5 software). A probability score of ≤ 0.05 was regarded as significant.

Results

The average HCF level of the enzymes and electrolytes from naturally infected Iranian domestic ruminants are tabulated in Tables 1 and 2.

The average HCF level of Ca had significant difference in lungs of cattle (7.49 ± 1.28 mg/dl) and goats (13.98 ± 5.11 mg/dl) ($P < 0.05$). No significant difference was found with regard to the average HCF level of Ca in lungs of sheep (11.56 ± 1.71 mg/dl) and cattle (7.49 ± 1.28 mg/dl) ($P > 0.05$). The average HCF level of P was higher in liver of cattle (0.82 ± 0.02 mg/dl) than other ones; however, in contrast, the average HCF level of Mg was lower in lungs of camels (11.8 ± 1.05 mg/dl) than the liver of cattle (10.56 ± 1.73 mg/dl) ($P < 0.05$). The highest average HCF level of Na (122.8 ± 11.91 mEq/L) and K (7.18 ± 1.37 mEq/L) were found in infected livers

Table 1 The average serum level of electrolytes profiles, i.e. Ca, K, Mg, Na, P in hydatid cyst fluids of Iranian domestic ruminants (n = 48, mean ± SD)

Animals	Organs	Sg (pH 7)	Ca (mg/dl)	K (mEq/l)	Na (mEq/l)	P (mg/dl)	Mg (mg/dl)
Cattle	Liver	1.34 ± 0.33	12.62 ± 2.58	1.66 ± 0.21	110.3 ± 11.25	0.82 ± 0.02^{Sig}	10.56 ± 1.73
	Lung	1.34 ± 0.41	7.49 ± 1.28^{Sig}	2.65 ± 0.43	107.15 ± 10.11	0.62 ± 0.02	11.61 ± 1.01
Sheep	Liver	1.33 ± 0.19	11.56 ± 1.71	7.18 ± 1.37	122.8 ± 11.91	0.74 ± 0.03	8.96 ± 0.96
	Lung	1.33 ± 0.12	4.13 ± 0.703	6.47 ± 1.49	108 ± 10.36	0.51 ± 0.06	10.78 ± 1.48
Goats	Lung	1.38 ± 0.14	13.98 ± 5.11^{Sig}	5.48 ± 0.92	112.27 ± 9.32	0.74 ± 0.01	9.43 ± 0.94
Camels	Lung	1.34 ± 0.31	12.34 ± 5.44	6.39 ± 1.12	108.82 ± 9.71	0.46 ± 0.04	11.8 ± 1.05^{Sig}

Sg specific gravity, *Sig* significant ($P < 0.05$)

Table 2 The average serum levels of total protein (TP), albomine (Alb), and enzymes of HCFs of domestic ruminants (n = 140, mean ± SD)

Animals	Organs	TP (mg/ml)	Alb (mg/ml)	ALT (U/L)	AST (U/L)	CPK (U/L)	LDH (IU/ml)
Cattle	Liver	0.15 ± 0.07	0.53 ± 0.02	6098.56 ± 15.63	5710/95 ± 15.63	615.9 ± 8.21	205.35 ± 5.32
	Lung	2.61 ± 0.75	0.48 ± 0.01^{Sig}	5242.31 ± 9.17	4288.57 ± 13.49	760.68 ± 9.15	233.25 ± 6.23
Sheep	Liver	0.82 ± 0.09	0.64 ± 0.01	5117.63 ± 8.47	5250.26 ± 11.17	770.89 ± 9.19	249.14 ± 6.48
	Lung	0.51 ± 0.06	0.51 ± 0.02	5190.66 ± 8.68	3475.95 ± 17.86	731.76 ± 9.12	212.51 ± 6.11
Goats	Lung	3.21 ± 0.51	0.68 ± 0.01^{Sig}	7512.06 ± 18.76	6091.74 ± 11.12	624.82 ± 8.99	142.48 ± 3.28
Camels	Lung	1.39 ± 0.06	0.95 ± 0.05	5189.22 ± 13.71	4995.74 ± 9.08	1229.25 ± 13.21	363.62 ± 10.44

ALT alanine aminotransferase, *AST* aspartate aminotransferase, *CPK* creatine-phosphokinase, *Sig* significant ($P < 0.05$)

of sheep ($P < 0.05$). In addition, the average Sg of HCF was approximately similar in all examined animals ($P > 0.05$).

The lowest average HCF level of Alb was significantly measured in lungs of cattle (0.48 ± 0.01 mg/dl) ($P < 0.05$). The average HCF level of TP was also significantly lower in lungs of sheep (0.51 ± 0.06 mg/dl) than goats (3.21 ± 0.51 mg/dl) ($P < 0.05$). The highest average HCF levels of CPK (1229.25 ± 13.21 U/ml) and LDH (363.62 ± 10.44 U/ml) were evaluated in infected lungs of camels ($P < 0.05$).

Discussion

In different intermediate hosts, metabolism of hydatid cyst and biochemical differences may cause a range of changes in the parasite species and/or sub-species (Kassis and Tanner 1977; King 1995). This fact play important role in metabolism, immunity and physiology of the metacestode which explain the existence of more than one strain in a region (Araxie et al. 1962; Thompson 1991; Thompson and Lymbery 1995; Sharbatkhori et al. 2011; Shaafie et al. 1999).

The average Sg of HCF was approximately within the same range in all examined animal. This finding was not in agreement with Eslami (2006) who reported it was in the range of 1.007–1.015 (pH 7.2–7.4). This may be due to the role of pH, HCF constituents, metabolic status and the age of each hydatid cyst (King 1995). The average HCF level of electrolytes was not within the same levels in infected lungs and livers of examined animals. According to Radfar et al. (2012), biochemical profiles of HCF did not relate to cyst location. In addition, Farayha and Smyth (1983) noted the concentration of ions and other chemical components in HCF of sheep had a wide variation in range. Radfar and Iranyar (2004) reported the highest average HCF level of Ca (4.47 ± .9 mmol/L), Na (148.6 ± 10.03 mmol/L), K (6.6 ± 1.6 mmol/L) and Mg (1.36 ± 0.4 mmol/L) in lungs of camels. Shaafie et al. (1999) and Radfar et al. (2012) indicated, in contrast, biochemical profiles of HCF had no significant differences in camels, cattle, goats and sheep origins. Frayha et al. (1980) and Eslami (2006) reported the average HCF levels of Ca, Mg, Na, and Chlorine (Cl) was higher in hydatid cyst than those in protoscolices. While Radfar et al. (2005) and Izadi and Ajami (2006) reported the average HCF levels of Ca, P, Mg, Na and K were approximately higher in sheep than other examined animals and humans. This was also in line with Farayha and Smyth (1983) who noted ions and other chemical components had a wide variation in concentration of HCF and protoscolices contents.

In this study, average HCF levels of TP and Alb had the highest amounts in lungs of goats and camels. Izadi and Ajami (2006), in contrast, revealed Alb had no significant difference in different intermediate hosts. While HCF level

of Alb had lower amount in sheep origin. Radfar et al. (2005) reported the main HCF levels of proteins were belonged to Alb and Globulin. Ozen et al. (1992) also reported Alb and Toxalbumin from HCF.

In the present study, the average HCF levels of CPK and LDH were significantly highest in infected lungs of camels. While the highest average HCF levels of AST and ALT were measured in infected lungs of goats. Izadi and Ajami (2006) reported the existence amounts of ALT in animals and humans. In another research, the highest activities were recorded for alkaline phosphatase (AP), LDH, AST, and ALT (Rahdar et al. 2008). According to Izadi and Ajami (2006), gamma-glutamyl transferase (GGT) had lower activity in sheep due to low level of Alb.

According to Frayha and Haddad (1980), there are quantitative and qualitative differences in metabolism of hydatid cysts of various animals throughout the world. This is probably due to complex geographic strain differences besides of biochemical and physiological features. Thus it was confirmed that more than one strain exist in examined animals and may be informative way to discriminate strains for effective prescribing anthelminthic drugs. Moreover, it clearly uncovered that biochemical, physiological, metabolic differences, protoscolices contents, and geographic strain distribution may help to distinguish the source of human infection.

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