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BIOCHEMICAL AND PARASITOLOGICAL STUDIES ON HYDATID CYSTS IN FARM ANIMALS IN BENI-SUEF GOVERNORATE

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SUMMARY

This study was performed in order to investigate the prevalence and the variations of some biochemical parameters in the serum and hydatid cyst fluids of camel, sheep and cattle infected with cystic forms of Echinococcus granulosus. The blood samples and hydatid cysts were collected from the animals slaughtered in Beni-Suef abattoir. The hydatid fluid and cystic germinal layer extracts were compared in these animals using Slap gel electrophoresis. The results indicated that the prevalence of hydatidosis was highest in camel (22%) followed by sheep (7.14%), and cattle (2.4%). The most affected organ was the lung in both camel and cattle where liver was most affected in sheep. The biochemical results indicated quantitative variations in the levels of total protein, AST, ALP, CPK enzymes, direct bilirubin, and magnesium in hydatid fluids. In addition to these parameters, there were also, quantitative variations in the levels of serum globulin, phosphorus and ALT. Sheep and cattle isolates showed a similarity in the biochemical composition of hydatid cyst fluids and identical electrophoretic patterns in cystic protein of germinal layer which were differed markedly from those of camel. These results suggest the existence of sheep and camel strains in Egypt.

INTRODUCTION

Hydatidosis is a common zoonotic parasitic disease caused by the dog tape worm, Echinococcus granulosus, and its larval stage, the hydatid cyst. The disease is worldwide, causing serious public health problems (Schantz, 1990).

It affects sheep and cattle leading to considerable economic losses due to condemnation of infected organs during the meat inspection (Kimberling, 1988). Camel also, has attracted much interest as an intermediate host of E. granulosus, it has an important role as a reservoir of infection in man (Eckert, et al., 1989). In human beings, the disease cause hepatic and pulmonary lesions, as well as allergic manifestation due to rupture of the cyst (Ichhpujani and Bhatia, 2002).

Diagnosis of hydatidosis is still an unresolved problem, serological tests using crude antigens are sensitive, and however, their specificity is not satisfactory (Sadjjadi, et al., 2007). Numerous studies have provided evidence that E. granulosus is characterized by extensive genetic variation comprising a number of strains that differ in biological features, such as intermediate host specificity, developmental rate, and infectivity to humans (Lavikainen, et al., 2003).

The biochemical characters of hydatid cyst were studied by several authors. Khorsandi and Tabibi (1978), analysed hydatid cyst fluid by using electrophoresis, immunoelectrophoresis, and biochemical tests. El-Zayyat, et al. (1999), has evaluated immunoblot analysis of hydatid cyst fluid of camel origin for diagnosis of human cystic echinococcosis.

Extracts of laminated layer taken from sheep cysts were found to be more important marker of the disease status (Taherkhani, et al., 2007). Alkaline phosphatase (AP), extract from sheep hydatid cyst was examined as an antigen for immune-diagnosis of human cystic echinococcosis (Mahmoud and abo-Gamra, 2004). They concluded that extract from cyst membrane is more specific than that of cyst fluid by using immunoblot analysis.

However, biochemical studies are useful in differentiating strain variations of E. granulosus in different countries (Shaafie, et al., 1999; Kumaratilake, et al., 1979; McManus and Macpherson, 1984). The strains of E. granulosus were studied by Harieche, et al. (1998) using electrophoretic techniques for iso enzyme analysis of protoscoleces. The differences in biochemical composition and metabolism

between different strains of hydatid cyst were also, studied by McManus (1981).

Human cases in Egypt are of the camel/dog strain, and camels are important hosts for the transmission of human hydatidosis (Azab, et al., 2004). However, sheep play the important role in dissemination of the disease. This is due to the fact that their cysts are the highly fertile ones as compared to other animal intermediate hosts. So, the risk cycle in hydatidosis is being sheep-dog-man (Haridy, et al., 2000).

The strain characterization is particularly important in regions where more than one species of livestock intermediate host exists and where there is the possibility of different cycles of transmission and sources of infection for humans (Thompson and Lymbery, 1995).

So, the aim of the present study was to investigate the prevalence of hydatidosis among cattle, sheep, and camels in Beni-Suef governorate. Also, to evaluate the biochemical profiles of hydatid cyst fluids from different hosts for identification of strain variations of E. granulosus.

MATERIALS AND METHODS

Collection of samples:-

A total of 406 carcasses (100 camels, 124 cattle, and 182 sheep) were examined for hydatidosis in Beni-Suef abattoirs during the period from March, 2010 to March 2011. The detected cysts were isolated, and transferred in plastic bags for laboratory investigations.

2-Blood samples:

Blood samples were collected from each slaughtered animal having hydatid cysts. Sera were prepared and preserved at -20°C.

Laboratory investigations:-

1- Hydatid cyst fluid:

The fluids of fresh cysts were withdrawn using sterile plastic syringes, then centrifuged at 1500 rpm for 30 minutes. The supernatant fluid was collected and stored at -20°C for biochemical examinations.

2-Germinal layer:

The inner layer of each fertile cyst was removed and suspended in PBS, sonicated and stored at -20°C for biochemical examinations.

Biochemical examinations:

Supernatant hydatid cyst fluid (HCF), the germinal layer (GL), and serum samples from infected ten animals (camels, cattle, and sheep), were subjected to biochemical investigations. HCF and GL were used for slap gel electrophoresis according to **Davis (1964)**.Serum samples and HCF were used for biochemical tests according to table, 1.

Table 1: Procedures adopted for serum biochemical analysis.

Parameter	Reference
Total protein	Sonnenwirth and Jarett (1980)
Albumin	Drupt (1974)
Total bilirubin	Jendrassiki(1938)
Direct bilirubin	Jendrassiki(1938)
Glucose	Werner, et al. (1970)
Aspartate aminotransferase (AST)	Reitman and Frankel (1957)
Alanine aminotransferase (ALT)	Reitman and Frankel (1957)
Alkaline phosphatase (ALP)	Kilichling and Freiburg (1951)
Creatinine Phospho Kinase(C.P.K.)	Kilichling and Freiburg (1951)
Calcium (Ca)	Glinder and King (1972)
Phosphorus (P)	Kilichling and Freiburg (1951)
Magnesium(Mg)	Neilly and Nelly (1956)

Statistical data were analyzed using T-student test according to **SPSS 14(2006).**

RESULTS

The prevalence of hydatidosis in camels, sheep and cattle, and the distribution of hydatid cysts in body organs were shown in tables 2 and 3 respectively. The level of serum biochemical parameters of camel, cattle and sheep infected with cystic echinococcosis were shown in table, 4. The mean levels of serum total protein, globulin, AST, ALT, ALP and CPK of infected animal serum were significantly higher than control group, whereas direct bilirubin, P, Mg and Ca were significantly lower than control. The other parameters analyzed were not significantly different between the groups.

Table 5 showed the level of hydatid fluids biochemical parameters of camel, cattle and sheep infected with cystic echinococcosis. The level of total protein, AST, ALP, and CPK were found to be significantly lower whereas direct bilirubin and magnesium were found to be significantly higher in the cyst fluids of sheep and cattle compared with camels.

Slap gel electrophoresis of hydatid fluids and germinal layer collected from camel, cattle and sheep infected with cystic echinococcosis was shown in table 6. There was a significant variation between mean RV% in HCF of sheep, cattle and camel

isolates except in band-3 which showed a similarity in the three species of animals.

Table 2: The prevalence of hydatidosis in camels, sheep and cattle in Beni-Suef Governorate

Animal species	Examined	Positive	Percentage
Camels	100	22	22%
Sheep	182	13	7.14%
Cattle	124	3	2.4%
Total	406	38	9.36%

Table 3: The distribution of hydatid cysts in body organs.

Animal species	Total positive	Lung		Liver		Mixed	
		No.	%	No.	%	No.	%
Camels	22	12	54.54%	6	27.27%	4	18.18%
Sheep	13	4	30.77%	6	46.15%	3	23.07%
Cattle	3	3	100 %	0	0 %	0	0 %

Table 4: Biochemical parameters of serum collected from camel, cattle and sheep infected with cystic echinococcosis.

Biochemical profiles	Camel		Cattle		Sheep	
	Control	Infected	Control	Infected	Control	Infected
Total protein (g/dl)	4.8 ± 0.45	6.2 ±0.92**	4.6 ±0.13	6.8 ±0.95**	5.1 ±0.94	6.9 ±1.2*
Albumin (g/dl)	2.3 ±0.12	2.1 ±0.15	2.4 ±0.11	1.8 ±0.09*	2.1 ±0.13	2.3 ±0.011
Globulin (g/dl)	2.5 ±0.19	4.1 ±0.13**	2.2 ±0.08	5.0 ±0.35**	3.0 ±0.25	4.6 ±0.73*
T. bilirubin (mg%)	1.5 ±0.09	0.87 ±0.09*	1.2 ±0.07	1.11 ±0.07	1.0 ±0.08	1.4 ±0.06
D. bilirubin (mg%)	0.35 ±0.02	0.06 ±0.01**	0.25 ±0.09	0.23 ±0.016	0.3 ±0.05	0.18 ±0.01
Glucose(mg%)	68 ±0.95	64 ±0.95	74 ±1.33	77 ±2.33	68 ±3.4	79 ±2.9
AST(Iu/ml.)	20.3 ±0.13	54.15 ±0.79**	21.5 ±1.15	84.3 ±3.2**	25.4 ±1.15	76.4 ±2.9**
ALT(Iu/ml.)	10.3 ±0.14	10.50 ±0.65	10.5 ±0.19	14.22 ±0.39*	10.5 ±0.85	17.21 ±0.39*
(Iu/ml.) ALP	85 ±0.98	147 ±0.34**	92 ±2.3	144 ±8.1**	82 ±2.1	140 ±2.33**
C.P.K.(Iu/ml.)	25 ±0.85	55 ±0.99**	18 ±0.91	44 ±0.95**	14 ±0.75	55 ±1.2**
Ca (mg/dl.)	10.5 ±0.87	9.9 ±0.79	10.5 ±0.75	9.4 ±0.84	10.5 ±0.94	10.4 ±0.39
P (mg/dl.)	5.5 ±0.35	3.9 ±0.17*	6.3 ±0.92	3.9 ±0.13**	5.5 ±0.19	5.1 ±0.18
Mg (mg/dl.)	3.9 ±0.16	1.8 ±0.09*	3.6 ±0.32	1.3 ±0.085*	4.0 ±0.23	2.1 ±0.36*

Data expressed as mean ±SE. *significantly different from control at (P<0.05).
**Significantly different from control at (P<0.01).

Table 5: Biochemical parameters of hydatid fluids collected from camel, cattle and sheep infected with cystic echinococcosis.

Biochemical profiles	Host origin		
	Camel	Cattle	Sheep
(g/dl) Total protein	9.2±0.19 *	8.4±0.18	7.8±0.33
Total bilirubin (mg%)	1.35±0.11	1.40±0.19	1.37±0.18
Direct bilirubin (mg%)	0.18±0.05 *	0.31±0.02	0.33±0.05
Glucose(mg%)	54±3.1	55±2.1	56±1.2
(Iu/ml.)AST	80.4±1.9 *	68.6±2.0	72.2±1.8
ALT(Iu/ml.)	11.6±1.2	10.33±0.8	11.3±0.9
(Iu/ml.) ALP	158±2.4 *	139±1.9	141±2.03
C.P.K. (Iu/ml.)	77±2.2*	62±2.1	60±1.33
Ca (mg/dl.)	9.6±0.76	9.0±0.72	9.4±0.65
P (mg/dl.)	3.2±0.31	3.1±0.17	4.3±0.14
Mg (mg/dl.)	0.9±0.17*	1.4±0.12	1.6±0.1

Data expressed as mean ±SE. *significantly different from each other at (P<0.05).

Table 6: Percent Raw volumes slap gel electrophoresis of hydatid fluids and germinal layer collected from camel, cattle and sheep infected with cystic echinococcosis.

Bands	Hydatid cyst fluid			Germinal layer		
	Camel	Cattle	Sheep	Camel	Cattle	Sheep
1	23±0.59	25±1.20	26±0.50	21±0.85	22±0.94	28±0.92
2	30±1.20	17±0.92*	20±1.15	15±0.21	15±0.40	12±0.34
3	24±0.95	25±0.39	24±0.17	32±0.79*	26±0.55	26±0.26
4	14±0.85*	22±0.75	24±0.65	25±0.87	23±0.46	14±0.46
5	9±0.33	11±0.19	6±0.15*	5±0.24*	9±0.65	15±0.92*
6				2±0.15*	5±0.30	5±0.33

Data expressed as mean ±SE. *significantly different from each other at (P<0.05).

Discussion

Hydatidosis is a zoonotic parasite of worldwide in distribution; it is hyperendemic in most North African countries and in several areas of East Africa. The disease is a major public health problem that affects also the human welfare and economy in these areas. Camels appear to act as the intermediate in some parts of North and East Africa (Develoux, 1996). In our study, the

prevalence of hydatid cyst was highest in camels (22%), followed by sheep (7.14%), and cattle (2.4%). Nearly similar results were obtained by Rahman, et al. (1992) in Egypt, Elmahdi, et al. (2004) in Sudan, Dada and Belino (1978) in Nigeria and Latif, et al. (2010) in Pakistan.

Camels like sheep graze close to the ground over a very wide areas where the abundance of stray dogs and a chance of ingesting contaminated herbages are high. In addition, the lack of hygienic measures in camel management and improper hygienic disposal of infected offal's, which serve as a focus for further spread of hydatidosis.

Concerning the location of hydatid cysts, the lung and liver were the most predilection sites than other organs. This might be attributed to the narrow size of lung capillaries and spongy texture of lung tissues, where the oncosphere retained and developed to mature cyst (Al-Rashed, et al., 1994), whereas Aba-yazed (1982) concluded that liver serves as a primary barrier in the body after penetration of intestinal wall by oncosphere of Echinococcus granulosus. Indeed, it has been proven by several studies that each strain is characterized by sites of predilection. The preferential localization of hydatid cysts in the lung of camels in the present study was in accordance with observations made by many authors, Saad, et al. (1983) in Sudan, Wachira, et al. (1993) in Kenya, Develoux, et al. (1985) in Niger, and Yena, et al. (2002) in Mali, but not with those of EL Mogdad (1984) in Mauritania, who reported similar infection rates in liver and lungs of camel. On the other hand, sheep strain is characterized by the liver locations as observed by Oudna, et al. (2006) in Tunisia and Salem, et al. (2011) in Mauritania.

Biochemical substances within hydatid cysts play a definitive role in the metabolism, physiology and immunology of cystic echinococcosis (Thompson and Lymbery, 1995; Shaafie, et al., 1999). The quantitative variation in the metabolism and the biochemical composition of hydatid fluids reflect strain variation in different intermediate hosts which may relate to their possible infectivity to man (McManus, 1981, Shaafie. et al., 1999; and McManus and Macpherson, 1984). Moreover, the development of the same strain or species of Echinococcus in different species of intermediate hosts may also cause shifts in the metabolism essential for parasite survival in different environments (Thompson, 1991; Thompson and Lymbery, 1995).

This study showed that the mean levels of serum total protein, globulin, AST, ALT,

ALP, and CPK of infected animal serum were significantly higher than the normal range, whereas direct bilirubin, P, and Mg were significantly lower than the normal range. The other parameters analyzed were not significantly different between the groups. The increased level of total protein and globulin may be due to production of antibodies (Ayaz, et al., 2007). High level of AST, ALT, ALP and CPK may be due to liver disfunction as a result of hydatidosis (Radfar, et al., 2008).

The mean levels of electrolytes (Ca, P, and Mg) in serum were higher than hydatid cyst fluid. The low level of Ca, P, and Mg was also observed by (Ayaz, et al., 2007 and Radfar, et al., 2008). The important role of Ca and P are for preventing the acidity of hydatid cyst fluid and they are found as calcareous body inside the hydatid cyst (Frayha and Haddad, 1980).

These results indicated that the entrance of electrolytes to cyst is based on parasite requirement. The complex layer of cyst has an important role in the transformation of nutritional material from serum to cyst. Knowledge of parasite nutrition behavior can help us to drug treatment in in operative cyst via selection of effective drug and adherence of them to biological material that promote distribution of drug to the cyst. Beside, identification of cyst fluid composition helps us to recognize of the hydatid cyst from non-parasitic cyst and explanation of different strain of parasite in one endemic area.

In the present study, we compared biochemical parameters of hydatid cyst fluids in the natural intermediate hosts (sheep, cattle, and camel) which may assist in the identification and characterization of the strain of E. granulosus prevailing in Egypt. The level of total protein, AST, ALP, and CPK were found to be significantly lower whereas direct bilirubin and magnesium were found to be significantly higher in the cyst fluids of sheep and cattle compared with camels. On the other hand, there was no significant difference between biochemical parameters of hydatid fluids from sheep and cattle.

Shaafie, et al. (1999) found that the concentration of triglycerides and proteins were significantly elevated in the cyst fluids of sheep compared with the other studied. Radfar and Iranyar (2004) found that the level of glucose, creatinine and calcium were significantly lower in the cyst fluids of sheep, goats, cattle and humans compared with camels. Whereas, Izadi and Ajami (2006) found quantitative variations in the

levels of Calcium, Triglycerides, Cholesterol, Creatinin, Albumin, AST and Creatinine Phosphokinase (CPK), in different hydatid cyst fluids of human and animal origin. They suggested existence of more than one strain of Echinococcus granulosus in human and other domestic animal intermediate hosts.

In the present study, quantitative similarities in the biochemical profiles of hydatid fluids in cystic echinococcosis from sheep, and cattle, and quantitative variation from that of camels, suggest the existence of two different strains of E. granulosus in Egypt.

The electrophoretic pattern of hydatid cyst fluids (HCF) and germinal layer (GL) was recognized by 5 and 6 protein bands respectively, expressed as raw volume percent (RV%) using Slap gel electrophoresis. There was a significant variation between mean RV% in HCF of sheep, cattle and camel isolates except in band-3 which showed a similarity in the three species of animals. This result suggested presence of a common antigenic band in the different HCF. Concerning the germinal layer, a significant variation was found between the mean RV% of camel isolates and that of sheep and cattle isolates in band-3 and band-6.

The results of biochemical profiles of HCF and the electrophoretic pattern of GL, suggest the existence of sheep and camel strains of E. granulosus in Egypt. Similar results were obtained by Le Riche and, Sewell (1978), Shaafie, et al. (1999) in Libya, and Radfar and Iranyar (2004) in Iran.

We can conclude that a combination of livestock vaccination with an effective vaccine against the sheep strain of E. granulosus, and dog anthelmintic treatment, could achieve the goal of hydatid control in the long term.

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دراسات بيوكيميائية وطفيلية على الحويصلات المائية في حيوانات المزرعة بمحافظة بني سويف

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أجريت هذه الدراسة من أجل معرفة نسبة الاصابه وكذلك التغيرات في بعض القياسات البيوكيميائية للسيرم وسوائل الحويصلات المائية في كل من الإبل والأغنام والماشية المصابة بحويصلات ديدان المشوكات الحبيبيه. لذلك تم تجميع عينات الدم والحويصلات المائية من الحيوانات المذبوحه في مجزر محافظة بني سويف. وتمت مقارنة سوائل ومستخلص الطبقه الداخليه للحويصلات المائية باستخدام التحليل الكهربي.

وقد دلت النتائج الى أن معدل الاصابه بالحويصلات المائية كانت أعلى في الجمال (٢٢%)، تليها الأغنام(٧,١٤%)، ثم الماشيه (٢,٤%). وكان أكثر أعضاء الجسم اصابه هو الرئه في كل من الإبل والماشية، بينما كان الكبد هو أكثر الأعضاء اصابه في الأغنام.

وأشارت النتائج الى وجود تغيرات بيوكيميائية كميه في مستويات البروتين الكلي وانزيمات الأسبارتيت والألكلاين فوسفاتيز والكرياتينين فوسفوكينيز وكذلك البيليروبين المباشر

والماغنسيوم في سوائل الحويصلات المائية، وبالاضافه الى هذه القياسات كانت هناك أيضا تغيرات كميه في مستويات الجلوبيولين والفوسفور وانزيم الألكلاين ترانسفيريز في السيرم.

وقد أظهرت عينات الأغنام والماشيه وجود تشابه في التركيب الكيميائي لسوائل الحويصلات وكذلك في أنماط التحليل الكهربي للبروتين في مستخلص الطبقه الداخليه للحويصلات المائية والتي تختلف بشكل ملحوظ عنها في الجمال. وقد أوضحت هذه النتائج وجود سلالات للطفيل خاصه بالأغنام والإبل في مصر.