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Full Length Research

The prevalence and biochemical characters of hydatid cyst in sheep and goats slaughtered at El-Karhga, New-Valley Governorate, Egypt

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A total of 987 carcasses of sheep (459) and goats (528) of different ages, in the period of June, 2012 – June, 2013, reared in different localities at El-Kharga city (in and out the abattoir), where 37 (8.06%) of sheep and 29 (5.49%) of goats were found harbored hydatid cyst liver, and lung were the most infected organ in sheep at rate of 4.57% and 2.61%, respectively, but in goats the lung was the most predilection site than liver in rate of 3.03% and 2.46% respectively, both liver and lung were cystic, in sheep only in rate of 0.65%, and the under develop cyst recorded in kidney of sheep 0.28%. Sheep fell in age category of 3 year and above were more infected (15.29%) compared to 1 to 3 year (4.23%) and below 1 year (0%) old. Likewise, the prevalence in goats was 7.89%, 3.79% and 0% respectively in the same age groups. Fertility of cyst was highest in sheep than goats in rate of 62.16% and 58.62%, respectively. The biochemical analysis revealed significant increase in protein level of lung cysts extract ($P < 0.01$) than liver cyst extract in sheep and goats. While liver cyst extract revealed significant increase in both of, creatinine ($P < 0.01$), calcium ions ($P < 0.01$) and magnesium ions ($P < 0.05$) than lung cyst extract in both sheep and goats, while cholesterol and triglyceride revealed significant increase in liver cyst extract of sheep and no change in liver cyst extract of goats. The differences in chemical component values of cyst extract in both sheep and goat and cystic organ in the same animal may pointed some strain variability in parasite metabolism, growth rate or even strain variation.

Key words: *Echinococcus granulosus,* hydatidosis, hydatid cyst, sheep, goats.

INTRODUCTION

Hydatidosis is a major parasitic disease of veterinary and public health importance throughout the world where the disease has great economic and zoonotic importance because it affects almost all the domestic animals (sheep, goats, cattle, Buffaloes, horses, pigs) and human as intermediate hosts. Food animals are obligatory hosts in the life cycle of the parasite where the dog-sheep cycle has been reported to be predominant for *E. granuloses*, (Altintas et al., 1999), where the degree of hydatid infection in food animals helps to indicate the risk to man with *E. Granuloses* via dogs, sheep and goats play a very important role in the epidemiology of hydatidosis in Egypt where sheep and goats are still slaughtered traditionally and carcass wastes are easily accessible to stray dogs and other wild carnivores (Haridy et al., 2000). Hydatidosis caused by metacestode of adult worms of echinococcus where the fertility of the cysts is very necessary to be determined, because it gives an idea about the diversity and level of threat in a particular area from different species of *Echinococcus*, especially the species that are dangerous for the general public. *E. granulose* and its metacestode (hydatid cyst) in

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herbivores and humans have been recognized as the most important helminthes zoonoses with great economic and public health significances in developing countries (Schantz, 1990). Sheep and goats is the most important intermediate host, where scolices from these animals are most highly infective for dogs (Jobre et al., 1996). Infection with the adult stage of *E. granulosus* is generally asymptomatic and non-pathogenic to the canid host but the infection with the larval stage of *E. granuloses* can be pathogenic depending on the localization, size of cyst, and intensity of infection in the animals or human intermediate host (Kaufamann, 1996). Biochemical studies are useful in differentiating strain variations of *E. granulosus* in different countries, biochemical studies on hydatid cyst from different host origins (sheep & goat) can provide valuable information for characterizing and determining of strains of *E. granulossus* in this study area (Leder and Waller, 2008). Therefore the objective of the present paper is to determine of the current prevalence of hydatidosis, evaluate the biochemical profiles of hydatid cyst fluids and protoscolices from different hosts (sheep, goat) and estimation of the financial annual losses in small ruminants slaughtered in the study area.

MATERIALS AND METHODS

Study area

The study was conducted in El-Kharga city (In and out the abattoir) at New-Valley Governorate (in the western Egyptian desert). In the period from June 2012 – June 2013. This area is a depression that lies between the Nile, Sudan and Libya with its capital at the Kharga Oasis where the rainfall is almost absence throughout the year and the ground water is the main source of water.

Animals

This study was carried out prospectively on sheep and goats slaughtered in and out of the abattoir in El-Kharga city where all organs of the slaughtered animals was carefully inspected for hydatid cyst either by visual or palpation and when necessary one or more incision was carried out in order to detect small hydatid cyst (under develop), where the numbers, ages, sex and all observed data of the slaughtered animals and the organ infected with hydatid cyst were recorded and the animals under the study were classified into three groups according to the age, below 1 year, 1 - 3 year and above 3 years.

Sampling

In the abattoir, meat inspection was carried out on different organs of each slaughtered animals particularly in those organs in which the cyst is more likely to be appeared. Each organ was assessed macroscopically either by visual inspection or palpation and when necessary one or more incision was carried out in order to detect small hydatid cysts (under develop). The infected organs from each positive animal were collected and recorded the data about them and the hydatid cyst were carefully cut with knife and collected in plastic packages containing 10% formalin as preservative (forming very less or no residue in cysts, thus facilities staining and mounting of protoscolices) and send for parasitological laboratory.

Parasitological examination

The cysts were carefully incised with knife for confirmation the diagnoses and examined for protoscolices, to assess their fertility by viability test and the cysts were discriminated into three categories (Fertile, viable and non viable), according to Larrieu et al. (2001), where they recorded that fertile cyst containing protoscolices and germinal membrane while viable cyst containing germinal membrane only and non viable don't containing any of them.

Viability test procedure

From the total positive animals each cyst was carefully incised and examined for protoscolices, to assess their fertility. Fertile cysts were subjected to viability test. A drop of the sediment containing the protoscolices was examined microscopically on glass slide covered with cover slip to observe amoeboid like peristaltic movements with 40x objective according to Soulsby (1982). For clear visualization a drop of 0.1% eosin solution was added to equal volume of protoscolices in hydatid fluid on microscope slide the principle viable protoscolices should completely or partially exclude the dye (No color) while the dead ones take it up (bright yellow) (Macpherson, 1985).

Histological examination

This test for differentiation between *echinococcosis* cyst and *Taenia hydatigenia* and indicated the morphological characters' of echinococus cyst by formalin- fixed materials of cyst and processed by staining method, the acid-Schiff positive, cellular laminated layer with or without nuclear materials and nucleated germinal membrane characteristic for *echinococcosis.*

Biochemical examination

The cysts were transferred to the parasitological laboratory and their fluids were aspirated aseptically, centrifuged at 1500 rpm at 4°C for 30 min and the supernatants analyzed for various biochemical parameters including glucose, total protein, urea nitrogen.

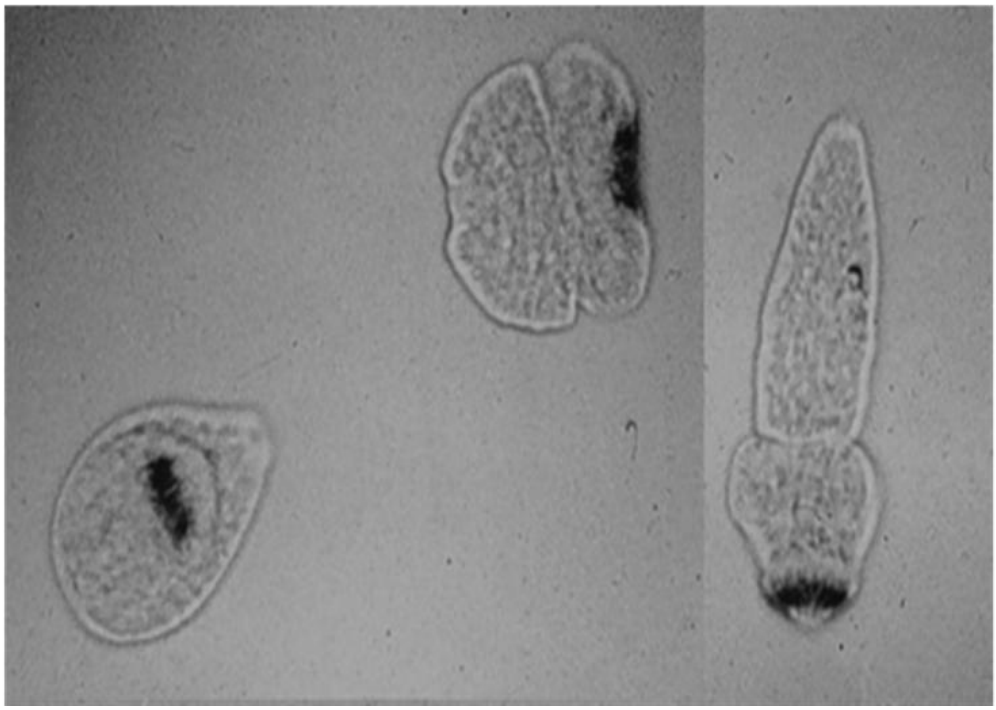

Figure 1. Hydatid sand fluid, show multiple protoscolices invigilated at left and evaginated (middle and right) in saline.

Uric acid, triglycerides, cholesterol, creatinine, sodium, potassium, calcium and phosphorus, as follows: glucose by the glucose oxides method; total protein by the Biuret method; urea nitrogen (UN) by diacetyl monoxim method, uric acid by the phosphotungstic acid (PTA) method; triglycerides by the enzymatic procedure of (McGowan et al., 1993), cholesterol by a modified Abdel-Kendall/Levey-Brodie (A-K) method, creatinine by Jaffe method, phosphorus by ammonium molybdate method. The concentration of sodium and potassium were measured using flame photometry (FLM2, Bach-Simpson Ltd, Ontario, Canada). Samples were analyzed for calcium by atomic absorption. (Shimadzo AA-670, Shimadzu Corporation,Kyoto, Japan).

RESULTS

Fertile cysts were subjected to viability test where a drop of the sediment containing the protoscolices was examined microscopically on glass slide covered with cover slip to observe amoeboid like peristaltic movements, and with used eosin stain, the viable cyst exclude the stain as shown in Figure 1, (showing multiple protoscolices with evaginated in right and left of picture), where incidence of hydatid cyst in slaughtered sheep and goats illustrated in Tables 1a, 1b, 2 and Figure 2, this resulted revealed that 37 out of 459 of sheep (8.06%), harboring hydatid cyst and 29 out of 528 of goats (5.49%), were infected with hydatid cyst. In relation to aged animal as in Table 3 and Figure 3, revealed high incidence of infection in age of 3 year to up and 1 - 3 year in sheep (15.29% ,4.23%, respectively) and goats (7.89%, 3.79% respectively), while the study revealed negativity of all slaughtered animals below one year age in both sheep and goats. In relation to fertility of hydatid cyst, the result indicated that slightly higher in sheep (62.16%) compared to goats (58.62%), and no difference in the viable cyst in sheep (24.32%) and goats (24.13%), but the non viable cyst were predominant in goats (17.24%) than in sheep (10.8%). While the underdeveloped cyst were found in sheep only (2.70%), as in Table 4. In relation to location of cyst in the organ illustrated in Table 5, indicated that the liver and lung were the more predilection sites for hydatid cyst in both sheep and goats where in sheep the highest incidence were noticed in liver followed by the lung (4.57%, 2.61% respectively), but in goats the highest incidence of hydatid cyst were observed in the lung followed by the liver (3.03%, 2.46%), respectively. Biochemical analysis of different hydatid cysts are illustrated in Tables 6 and 7, that indicated the total protein of lung cysts in sheep and goats higher value significantly ($P < 0.01$) than liver. While in the liver cyst were found creatinine ($P < 0.01$), calcium ions ($P < 0.01$) and magnesium ions ($P < 0.05$) are statistically increase

Table 1a. Month-wise and location of slaughter incidence of hydatidiosis in sheep.

Animal	Sheep				
Date	No. of examined	No. of infected	Site of slaughter.		%
			In abattoir	Out abattoir	
June	9	--	--	9	0%
July	12	1	1	11	8.33%
August	8	-	---	8	0%
September	15	----	2	13	0%
October	370	32	10	360	8.6%
November	2	--	---	2	0%
December	10	2	-----	10	20%
January	7	--	----	7	0%
February	9	2	-----	9	22.2%
March	3	--	----	3	0%
April	6	-	1	5	0%
May	8	--	----	8	0%
Total	459	37	14	445	8.06%

Table 1b. Month-wise and location of slaughter incidence of hydatidiosis in Goats.

Animal.	Goat				
Date.	No of examined	No of infected	Site of slaughter		%
			In abattoir	Out abattoir	
June	7	1	----	7	14.28%
July	5	--	-----	5	0%
August	15	2	----	15	13.33%
September	20	1	-----	20	5%
October	410	22	5	405	5.36%
November	5	---	----	5	0%
December	9	1	---	9	11.11%
January	12	1	----	12	8.33%
February	6	--	----	6	0%
March	20	--	2	18	0%
April	12	1	---	12	8.33%
May	7	--	---	7	0%
Total	528	29	7	521	5.49%

Table 2. Prevalence of hydatid cyst in sheep and goat slaughtered in El-Kharga, New-Valley Governorate.

Species	No of examined	No of positive	Percent %
Sheep	459	37	8.06%
Goat	528	29	5.49%

than of lung, while cholesterol, ($P < 0.01$), triglyceride ($P < 0.05$) were significant increase in liver cyst of sheep only and no significant difference in liver cyst of goats.

Discussion

The presence of the adult worms in dogs and the larval stages in sheep and goats lead to the widespread geographical distribution of this disease which may attributed to the worldwide availability of susceptible hosts, the increasing of environmental pollution and the variability of origin of animals, mode of grazing and other environmental factors. (Saif, 2001; Ali et al., 2003). In our survey, the majority of slaughtered animals were bread with practical relationship between animal and dogs, different hydatid cysts were investigated also under developed cysts were recorded in sheep which might be

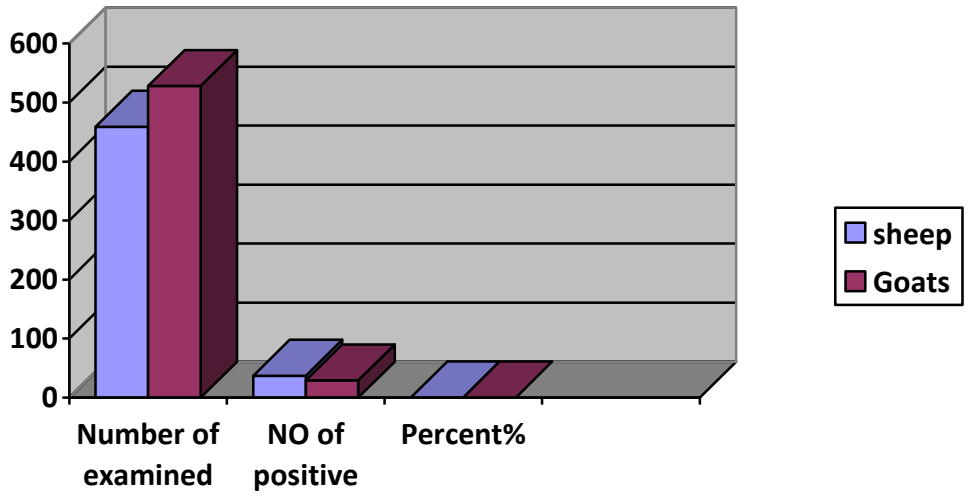

Figure 2. Indicated the number of animal, positive and percent of infection.

Table 3. Age-wise incidence of hydatid cyst in sheep and goats.

Animal	Sheep			Goat		
Age	No. of examined	No. of positive	Percent %	No of examined	No of positive	Percent %
Below year	29	---	0%	10	---	0%
1-3 year	260	11	4.23%	290	11	3.79%
3 year to up	170	26	15.29%	228	18	7.89%
Total	459	37	8.06%	528	29	5.49%

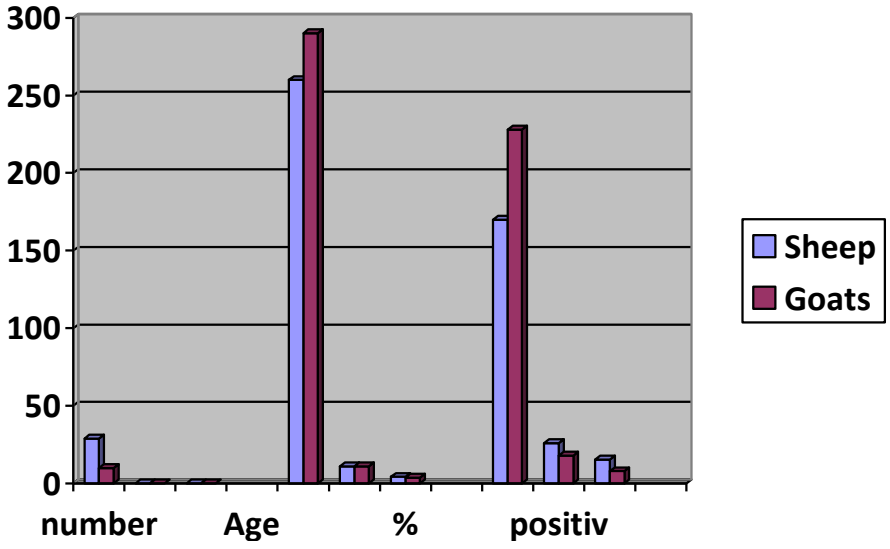

Figure 3. Indicated the relationship of infection with age.

due to immunological response of the host which might prevent extension of cyst (Larrieu et al., 2001; Torgerson, 2002; Torgerson and Budk, 2003, Lahmar et al., 2004). The data of the present study indicated that the rate of

Table 4. Fertility-wise distribution of hydatid cyst in sheep and Goats.

	No of positive	Fertile		Viable		Non viable		Under develop	
Sheep	37	23	62.16%	9	24.32%	4	10.8%	1	2.70%
Goats	29	17	58.62%	7	24.13%	5	17.24%	--	0%

Table 5. Location and type of hydatid cyst in slaughtered sheep and goats.

Animal	Organ	No of animal examined	Hydatid cyst.		Fertile		Viable (Sterile)		Non viable		Under develop	
			No	%	No	%	No	%	No	%	No	%
Sheep	Liver	459	21	4.57%	14	66.7	6	28.6	1	4.76	-	0
	Lung	459	12	2.61%	9	75	3	25	----	0	-	0
	Liver and lung	459	3	0.65%	----	0	----	0	3	100	-	0
	Kidney	459	1	0.28%	-	0	--	40	----	0	1	100
Goats	Liver	528	13	2.46%	7	53.8	4	30.8	2	15.4	--	0
	Lung	528	16	3.03%	10	62.5	3	18.8	3	18.8	-	---
	Liver and lung	528	---	----	----	---	----	----	----	----	-	---
	Kidney	528	---	0%	----	---	----	-----	----	-----	---	0

Table 6 . Biochemical parameters of hydatide cyst in sheep.

Parameters	Hydatid cyst in lung	Hydatid cyst in liver
Glucose mmol/l	1.67±0.614	1.032±0.502
Cholesterol mmol/l	0.107±0.044	*8.331±6.028
Urea nitrogen mmol/l	0.473±0.79	0.627±3.56
Uric acid mmol/l	0.099±0.15	0.099±0.06
Total protein g/l	**31.336±6.099	15.218±14.067
Triglycerides mm/l	0.128±0.054	*0.321±0.13
Creatinine mmol/l	17.956±15.53	**48.267±22.872
Na mmol/l	4.52±0.30	4.35±0.92
Ca mmol/l	0.173±0.077	**0.524±6.496
Mg mmol/l	0.091±0.31	*1.077±0.35
K mmol/l	9.85±0.49	5.17±0.36

infection by hydatid cysts in goats was lower than in sheep (5.49%, 8.06%, respectively). This can explained by the fact that goats usually feed on the upper parts of plants and shrubs, while sheep hosts feed on the ground grass that increase the chance of swallowing the echinococcus eggs and may related to host immunity (Pandey et al., 1988).

The liver and lung were the predilection site of infection in both sheep and goats. These findings were similar to the observations reported by Farah et al. (1984), Al-Yaman et al. (1985) and coincide with those of Pandey et al. (1988), where lungs are the most predominant organs. In addition, the two organs were infected either alone or together. Al-Abbassy et al. (1980), Ali et al. (2003). In our survey sheep and goats less than one year of age were negative. These findings substantiate what have been reported by Al- Abbassy et al. (1980), and may attributed to a cestode egg in general requires at least one year's time before the hydatid cyst stage grows sufficiently to produce protoscolices capable of infecting the carnivore host, Smyth and McManus (1989). This age variation can be translated into differential exposure to infection because older livestock may have been exposed to more infective stages (Ibrahim et al., 2008). The older animals were highly infected while the younger ones had low rate of infection, that agree with Pandey et al. (1988), and may attributed to two factors. Firstly, higher age reflects a much longer period of risk of infection. Secondly, the chances of detecting cysts at meat inspection are higher in aged animals due to the bigger size of the cyst and higher infection rate where Tashani et al. (2002) reported that young camel, sheep

Table 7. Biochemical parameters of hydatid cyst in Goats.

Parameters	Hydatid cyst in lung	Hydatid cyst in liver
Glucose mmol/l	0.77±5.52	0.85±1.82
Cholesterol mmol/l	1.635±14.86	1.922±0.46
Urea nitrogen mmol/l	0.675±0.327	0.61±0.58
Uric acid mmol/l	0.015±0.012	0.023±0.017
Total protein g/l	*96.27±1.95	92.02±0.63
Triglycerides mmol/l	1.691±11.61	1.753±3.86
Creatinine mmol/l	16.001±0.02	*20.001±0.01
Na mmol/l	4.42±0.30	4.35±0.92
Ca mmol/l	0.173±0.077	**0.524±0.496
Mg mmol/l	0.345±0.214	*1.019±0.816
K mmol/l	7.09±0.85	5.82±0.15

and cattle less likely to be infected than their older ones and that agreement with Ibrahim and Craig (1998), who said that the prevalence increase with age in sheep and cattle. This can be explained by the fact that cysts in younger animals are still in infancy and take several months to develop. Haridy et al. (2006). Studied prevalence between camels, goats, sheep, pigs, cows and buffaloes, reported significant differences between animals regarding liver and lungs infection. The fertility rate is substantially higher in the two animals; it was higher in sheep (62.16%), if compared to that observed by Al-Yaman (1985), (8.0%), and at limit with the results of Al-Abbassy et al. (1980), (39.4%), while it was low if compared to 88.2% obtained by Hassonah and Behbehani (1976). The fertility in goats (58.62%) was higher compared to that reported by Al-Abbassy et al. (1980) (2.0%), and Al-Yaman et al. (1985), (28.6%). *E. granulossus* exists as a complex of intraspecific variants or strains which differ from one to another in a variety of characteristics (different infectivity towards various host species as well as in their transmission patterns, Mac et al. (1986). Different strains of *E. granulose* might cause the variation in fertility rate in various environmental regions, McManus and Smyth (2006). Hydatid cyst fluid is not a simple substance; it is composed of many inorganic and organic materials also biochemical parameters reflect the quantitative differences in the metabolism of hydatid parasite in respected to the sites of parasitism, Radfar and Iranyar (2004). The decreasing or increasing of biochemical values provide the preventive role of the parasite cyst capsule to exchange these substances which may explain in somehow the parasite overcome the immunity of the intermediated hosts and that in contrast to result of previous studies carried by, Nash and Al- Janabi (1980), Bowles and McManus (1993), where they recorded that substances exchange takes place through this capsule may reflect or suggested a various degree of parasitic growth rate or activity. So biochemical analysis of fertile and sterile hydatid cyst fluid (HCF) was conducted to detect various components where biochemical substances of hydatid cysts play a definite role in the metabolism, physiology and immunology of echinococosis cysts also variation in these parameters reflect strains variation in different hosts (Bowles and McManus, 1993; Thompson and Lymbery, 1995). In addition to that Radfar and Iranyar (2004), said that variation in these parameters reflected the relation between intermediate host and parasite. The current study revealed increase in total protein level of lung cysts extract fluid and may be essential to overcome the action of lung and heart pumping rate but not for liver also the increasing reflect the importance of protein in the catabolism and anabolism activities (Gutteridge and Coombs, 1982), but Dow et al. (1996), attributed that for the mixed production of it by host and parasite. Glucose parameters indicated no change in cyst fluid extract in both sheep and goat and various organs (liver, lung) but its occurrence indicated the presence of glycolysis and glycologenesis cycles related to energy production with parasite cyst (Radfar and Iranyar, 2004). Increased level of cholesterol in liver cooperation with lung may be related with its function as a structural component, and also related to the colonized site of the parasite as it increased in liver more than of lung and that agree with many previous study (Dow et al., 1996; Radfar and Iranyar, 2004).

-Triglyceride appearance is expected as it the most abundant sterol molecule within parasite, and as an energy source with long term metabolite fuel storage with physical protection (Gutteridge and Coombs, 1982; Dow et al., 1996).

-Creatinine level reflects the Ammonia metabolism and energy production according to Dow et al. (1996). Urea and uric acid refer to the presence of urea cycle which is essential to eliminate the toxic level of ammonia through amino acid and nucleotide metabolism of cystic parasite (Dow et al. 1996).

-Calcium ions is an important in ATP production and ATPase activities and lately Mg ions act as a cofactor for many enzymes-catalyzed reaction (Dow et al., 1996).

-The present study revealed significant variation of biochemical parameters in different hydatid cyst fluids extract of both intermediate hosts (sheep and goats) and may be attributed to many factors of intermediate host

like age, environmental diversity in different regions and different strains of *E. granulose*, Arbabi and Hooshyar, 2006),

Conclusion

The detection of *E. granulose* hydatid cysts in animals paved the way for known more about human infections. One must keep in mind that the infected human hydatidosis is typically asymptomatic except a few cases of long standing and heavy infections that may be fatal. No doubt that this zoonotic disease needs local and national collaboration, at least in the Arab Countries to minimize its incidence and prevalence. The effective approach to control this disease would be done by eliminates the source of infection i.e. the larval stages in sheep and goats, the disease controlled programmed is limited due to lack of sensitive and specific methods of diagnosing hydatidosis in livestock prior to slaughter, Therefore, the present study was carried out to record the prevalence of hydatidosis in sheep and goats and biochemical characters of hydatide cyst. So may be said this analysis will be further useful for immunological studies, diagnostic tests and may be helpful to find out various strains of *E. granulose* in Egypt.

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