

Comparative Concentrations of Some Chemical Elements in the Hydatid Sand of *Echinococcus granulosus* Cysts from Various Animals in Libya

L.O. Elmajdoub, W.A. Rahman, W. Mustafa Fadzil and S.A. Mohd Nor

School of Biological Sciences, Universiti Sains Malaysia,
11800 Minden, Penang, Malaysia

Abstract: A study was conducted to compare the concentrations of some chemical elements in the hydatid sand of *Echinococcus granulosus* cysts. The cysts were recovered from sheep, camels and cattle from Libya. The TM3000 Microscope was used to estimate the concentrations of these elements. The highest rate was recorded for carbon in lung camel (59.5%) followed by oxygen in sheep liver (43.5%). Phosphorus and sodium were present in small amounts. However, calcium was reasonably high in the liver and lung of cattle (11.2%, 10.9% respectively). There are high statistical significances ($p < 0.01$) between sheep and cattle, as well as camels and cattle for calcium concentration. However, for carbon, phosphorus, and sodium, the difference between the three animals is of low significance ($p < 0.05$). Differences in oxygen for all three animals were not significant.

Key words: Chemical Elements • Hydatid Sand • *Echinococcus Granulosus* Cysts • Animals • Libya

INTRODUCTION

Cystic echinococcosis is amongst the most important parasitic zoonoses and remains a public health and economic problem of global concern [1]. Hydatid disease is endemic in Libya, where many domestic animals act as intermediate hosts of *Echinococcus granulosus* [2, 3]. In Libya, the zoonotic importance of the parasite was proved [4-6]. The larva of *E. granulosus* develops as a fluid bladder by a cyst wall. Protoscoleces have derived from germinal layer, which grew toward the cysts cavity, through a mechanism which is not fully understood [7, 8]. Normally the protoscolex is invaginated into the posterior region until external factors stimulate evagination [9]. Usually the larval hooklet characteristics remain unchanged by passage through the final host [10].

Criteria used in the recognition of specific strains have all emphasized the use of morphological details, particularly of the protoscoleces and hooklets [11]. However, previous studies had shown that the concentrations of some chemical elements in cysts are useful in strain differentiation of *E. granulosus* [10].

The aim of the present work is to estimate the concentrations of carbon, oxygen, calcium, sodium and phosphorus in the hydatid sand of *E. granulosus* cysts

recovered from camel, sheep and cattle in Libya, using the TM3000 Microscope. Differences in the concentrations of these elements were then compared in the respective animals.

MATERIALS AND METHODS

Collection of Hydatid Cysts: Hydatid cysts of *E. granulosus* were obtained from animals slaughtered in the government abattoirs from Yefern (south-western Libya), Tripoli (north-western Libya), Misurata (northern Libya) and Elbedia (north-eastern Libya). Hydatid fluid was aspirated from 8 cysts from sheep (4 from liver and lung), 9 from camel (4 from lung and 5 from liver) and 8 from cattle (4 from liver and 4 from lung).

Preparation of Hydatid Sand: Hydatid sand samples from different cysts were selected. Hydatid sand samples were subjected to repeated centrifugation and the resultant pellet was washed using phosphate buffer. They were then fixed in 3% (v/v) glutaraldehyde in 0.1M of the phosphate buffer. The resuspended samples were centrifuged two times for 5 min at 1000 rpm. The supernatant was discarded and the pellet was resuspended in 1% osmium tetroxide prepared in the

Table 1: Percentages of chemical elements from liver and lung from infected hosts.

Chemical Element	Liver			Lung		
	Sheep	Camel	Cattle	Sheep	Camel	Cattle
Carbon	50.76%	53.13%	51.2%	52.24%	59.49%	42.09%
Oxygen	43.51%	41.45%	32.98%	39.59%	33.75%	40.4%
Calcium	4.13%	3.8%	11.16%	5.77%	4.71%	10.9%
Phosphorus	0%	0%	4.65%	1.74%	2.61%	5.66%
Sodium	0.38%	0.73%	0%	0%	0%	0%

Table 2" Mean and std. error of concentration of chemical elements in the respective animals.

Mean± S.D	Carbon			Oxygen		
	Sheep	Camel	Cattle	Sheep	Camel	Cattle
	51.5±1.47	55.86±1.62	46.6±3.86	41.5±1.06	38.1±1.94	36.7±1.88
	↔ NS			↔ NS		
		↔ *			↔ NS	
	↔ NS			↔ NS		

NS= Non-significant, *= $P \leq 0.05$

	Calcium			Phosphor			Sodium		
	Sheep	Camel	Cattle	Sheep	Camel	Cattle	Sheep	Camel	Cattle
Mean± S.D	4.95±1.4	4.2±0.5	11.02±1.8	.8±.75	1.1±.5	5.2±1.8	.2±.11	.4±.2	0
	↔ NS			↔ NS			↔ NS		
		↔ **			↔ *			↔ *	
	↔ **			↔ *			↔ *		

NS= Non-significant, *= $P = 0.05$, $P = 0.01$

phosphate buffer for 1 hour. Samples were allowed to air-dry at room temperature on the stubs with a double-sided sticky tape and viewed by Hitachi Analytical Table Top Microscope TM3000. This microscope offers the possibility to integrate an EDX system for full-scale elemental analysis of crystal substances from the samples. Data was analyzed using the software SPSS v. 19 for windows.

RESULTS

Percentages of the various chemical elements found in the hydatid sand of cysts from different animals are shown in Table 1.

The highest rate was recorded for carbon in camel lung (59.5%) followed by oxygen in sheep liver (43.5%). The elements phosphorus and sodium were present in small amounts. However, calcium was reasonably high in the liver and lung of cattle (11.2%, 10.9% respectively).

There are high statistical significances ($p < 0.01$) between sheep and cattle, as well as camel and cattle for calcium concentration (Table 2). However, for carbon, phosphorus, and sodium, the difference between the three animals is of low significance ($p < 0.05$). Differences in oxygen for all three animals were not significant.

There was a high significant difference ($P \leq 0.01$) between organs (liver and lung) for sodium concentration of all three animals, but none between organs for the other elements of all three animals.

DISCUSSION

Most of previous taxonomy descriptions of *E. granulosus* cysts had been based on morphological characteristics using scanning electron microscopy [11, 12, 5, 13]. However, a new approach to determine the concentration of chemical elements from crystallic and other substances found in hydatid sand could be a useful tool in separating different strains of the parasite [10].

Antoniou and Tselentis (1993) showed the presence of crystalloid structures on the surface of protoscoleces of hydatid cysts. However, [12] did not report on the estimations of chemical elements concentration present in the hydatid cyst. In the present study, concentrations of carbon, oxygen, calcium, sodium and phosphorus seemed to vary according to host species, although how this information could be useful in future taxonomical procedures needs to be investigated further. However, calcium concentration in cattle seemed to be higher than in sheep and camel, and thus explained the sterility of the examined hydatid cysts in cattle. However, concentrations of sodium and phosphorus were generally low in all animals, which is suggestive of crystalline substances belonging to calcareous substances, which consisted mainly of calcium and carbon.

In conclusion, differences in concentrations of chemical elements in the hydatid sand of *E. granulosus* seemed apparent. Further investigations need to be carried out to assess how these differences in chemical element concentrations could be useful in future taxonomic studies.

REFERENCES

1. Thompson, R.C.A., A.J. Lymbery and C.C. Constantine, 1995. Variation in *Echinococcus* towards a taxonomic revision of the genus. *Advanced Parasitol.*, 35: 145-176.
2. Dar, F.K. and S.C. Taguri, 1979. Epidemiology and epizootiology of hydatidosis in the Libyan Jamahiriya and recommendations for a programmer of surveillance and control of the disease. *Garyonis Medical J.*, 2: 11-15.
3. Shaafie, L.A., A.H. Khan and K. Rambabu, 1999. Biochemical profiles of hydatid cyst fluids of *Echinococcus granulosus* of human and animal origin in Libya. *J. Helminthol.*, 73: 255-258.
4. Tashani, O.A., L.H. Zhang, A. Jegi and D.P. McManus, 2002. Epidemiology and strain characteristics of *Echinococcus granulosus* in the Benghazi area of eastern Libya. *Annals of Tropical Medicine and Parasitol.*, 96: 369-381.
5. Khalifa, M.R., N.A.M. Mazen, A. Marwan and L.O. Elmajdoub, 2004. Light and scanning electron microscopy of the hooklets of protoscoleces of hydatid cysts infecting sheep and camel from Misurata, Libya and their possible role in parasite strain recognition. *J. Egyptian German Society for Zool.*, 44: 11-19.
6. Elmajdoub, L.O., K.M. Elhoti, and N.M. Hadid, 2007. Prevalence of hydatid disease in slaughtered livestock animals from Misurata abattoirs, Libya. *J. Union. Arab of Biol.*, 28: 163-174.
7. Galindo, M., M.J. Gonzalez, and N. Galanti, 2002. *Echinococcus granulosus* protoscoleces formation in natural infections. *Biological Res.*, 35: 365-371.
8. Martinez, C., R. Paredes, R.P. Stock, A. Saralegui, M. Andreu, C. Cabezon, R. Ehrlich and N. Galanti, 2005. Cellular organization and appearance of differentiated structures in developing stages of the parasitic platyhelminth *Echinococcus granulosus*. *J. Cellular Biochemistry*, 94: 327-335.
9. Rogan, M.T. and K.S. Richards, 1987. *Echinococcus granulosus*: changes in the surface ultrastructure during protoscoleces formation. *Parasitol.*, 94: 359-367.
10. Yildiz, K. and S. Gurcan, 2009. The detection of *Echinococcus granulosus* strains using larval rostellar hook morphology. *Turkiye Parazitoloji Dergisi*, 33: 199-202.
11. Smith, S.A. and K.S. Richards, 1991. Ultrastructure and microanalyses of the protoscoleces hooks of *Echinococcus granulosus*. *Parasitol.*, 103: 267-274.
12. Antoniou, M. and Y. Tselentis, 1993. Studies on *Echinococcus granulosus* using the scanning electron microscope. I. Preparations of the parasite for infection of the final host. *Parasitology Res.*, 79: 537-542.
13. Almeida, F.B., R. Siva, R.H. Neves, M.M. Goncalves, E.L. Romani and J. Silva, 2009. Morphological and morphometric studies on protoscoleces rostellar hooks of *Echinococcus granulosus* from Peru visualized by several microscopic techniques. *Neotrop. Helminthol.*, 3: 65-72.