

PREVALENCE OF HYDATIDOSIS IN NOMADIC TRIBES OF SOUTHERN IRAN

M. SABERI-FIROUZI, F. KAFFASHIAN,* E. HAYATI,* A.A.
GHADERI,* H. KESHAVARZ,** S. ARSHADI, C. ARSHADI,
M.S.E. SOTUDEHMARAM, M.S. MASSARRAT,*** AND M.A.
GHALAMBOR*

*From the Schools of Medicine,*Paramedical Sciences and **Public Health, Shiraz University of
Medical Sciences, Shiraz, Islamic Republic of Iran, and ***Philips University, Marburg, Germany.*

ABSTRACT

In order to assess the prevalence of *Echinococcus granulosus* (EG) infection (hydatidosis) in nomadic tribes of southern Iran, 1000 individuals from a total population of 112,519 were selected by randomized single blind cluster sampling method and studied from 1994-1995. The study included: (1) a physical examination by a gastroenterologist, (2) abdominal ultrasonography (US), and (3) detection of anti-EG-antibodies (EGA) by an enzyme-linked immunosorbent assay (ELISA) and counterimmunoelectrophoresis (CIE). The statistically significant prevalences were: US: 1.8%, CIE: 6.8%, and ELISA, 13.7%. The rate of infection varied with age, sex, education, occupation, life style, geographical location of tribal subgroups and the frequency of contact with dogs and cattle. The power of agreement between a combination of each two methods were significant as determined by kappa statistics method. The results obtained indicated that a combination of ELISA and CIE was the most reliable method with a high sensitivity and specificity.

MJIRI, Vol. 12, No. 2, 113-118, 1998.

Keywords: *Echinococcus granulosus*, echinococcosis, hydatidosis, nomadic and migrant tribes.

INTRODUCTION

Echinococcosis is a cyclozoonotic parasitic infestation, usually caused by *Echinococcus granulosus* (EG) which is indigenous in developing countries; it may occasionally occur in the western hemisphere.¹⁻⁴

The most important reasons for its high prevalence in the third world countries are: exposure to infected cattle⁵ and dogs, as the main vector, and ingestion of meat, herbs and vegetables infected with EG eggs due to contaminated

fertilizers and, in general, poor sanitary measures. After ingestion of eggs, the larvae are released in the intestinal tract and migrate to the liver, lungs, and various other organs through the portal vein. In the affected organs, protoscoleces develop a protective boundary leading to the formation of a cyst. The cyst harbors numerous protoscoleces that, upon ingestion by dogs, perpetuate the parasitic life cycle. About 80% of the cysts are localized in the abdominal cavity (liver, spleen and kidneys), 8.5% in the lungs and the remaining 11.5% are found in other organs.¹ Unilocular and calcified cysts are easily detected by radiography and ultrasonography.^{6,11,15,17,27,28,31} Infection by the parasite evokes the synthesis of several antibodies, the detection of which is one of the most routinely used methods of diagnosis. Casoni's test^{8,9} with a 92% detection rate is the oldest immunological

Address correspondence to:

Mohammad Ali Ghalambor, Ph.D., Professor of Biochemistry, School of Paramedical Sciences, Shiraz University of Medical Sciences, Shiraz, Islamic Republic of Iran.

Hydatidosis in Southern Iran

procedure which is gradually losing acceptance due to a high rate of false positivity (12%), relative non-specificity, and the danger of causing hypersensitivity and anaphylactic shock.^{9,37} Several immunochemical techniques have been devised including ELISA,^{5,10,21,28,35} CIE,^{7,11,14,16} complement fixation,^{11,40} indirect hemagglutination,^{12,14,16} latex blotting,^{9,25,38} double diffusion,²⁰ a simple and fast dot blotting assay,^{23,24} immunoblotting using an affinity purified heparin-binding lipoprotein fraction (HBLF)²⁵ as the specific antigen and monoclonal antibodies directed against specific antigens 5 and B of EG.³⁴ However, roentgenographic confirmatory test(s) are required.^{4,6,17,27,31} In the most highly specific immunochemical methods, antigen number 5 (arc number 5) in CIE of EG is used as the mono-specific antigen.³⁵ A highly reactive purified antigen preparation for diagnosis of hydatidosis has been reported recently.³⁹

The objective of this study was to determine the prevalence of hydatidosis in nomadic tribes of southern Iran using different methods of detection.

MATERIALS AND METHODS

One thousand individuals (442 males and 558 females) with an age range between 1-80 years (mean = 36±15.9) were selected by a randomized, cross-sectional, and clustering sample collection method. Each subject was interviewed by a trained epidemiologist and completed a questionnaire, followed by a physical examination (PE) by a gastroenterologist. Then abdominal ultrasonography (US) was performed by a portable Model Kertz Technick sonograph. A blood sample (10 mL) was drawn by a venojector syringe, placed in a heparinized test tube, mixed and stored at 4°C. Sera were prepared, kept at -20°C and used for ELISA and CIE procedures.

The results of PE and US were compared and evaluated by clinicians and the immunochemical data were interpreted by immunologists, followed by statistical evaluation using the statistical package for social sciences (SPSS) computer software program. The power of agreement between results of a combination of each two methods, i.e., ELISA vs. CIE, ELISA vs. US and CIE vs. US was determined by kappa statistical method.³⁷

RESULTS

The prevalence of echinococcal infection was assessed by immunochemical and clinical procedures in 1000 individuals and the prevalences obtained by various methods were: US, 1.8%; CIE, 6.8% and ELISA, 13.7% (Table I). As indicated, a combination of ELISA-CIE, US-CIE and US-ELISA gave rates of 5.0%, 1.8% and 2.4%, respectively. Hydatidosis was more prevalent in females than males with

Table I. Prevalence of hydatidosis using CIE, ELISA and ultrasonography.

Method	Positive cases	%
US	18	1.8
CIE	68	6.8
ELISA	127	13.7
ELISA + CIE	50	5.0
US + CIE	12	1.8
US + ELISA	24	2.4
US + ELISA + CIE	6	0.6

* Total number of cases tested = 1000

US = Ultrasonography

CIE = Counter-current immunoelectrophoresis

ELISA = Enzyme - linked immunosorbent assay.

Table II. Prevalence of hydatidosis according to sex.

Method	Males (n = 442)		Females (n=558)	
	No. of positives	%	No. of positives	%
CIE	25	5.7	43	7.7
ELISA	52	11.8	58	15.2
US	4	0.9	8	1.4
ELISA + CIE	20	4.5	30	5.4
Statistical indices	$\chi^2 = 1.3$ df = 1.0 P-value = 0.24		$\chi^2 = 2.20$ df = 1.00 P-value = 0.11	

a ratio of about 1.3 in the order mentioned (Table II).

In addition, there was a positive correlation in terms of the prevalence of echinococcosis between sexes (Table II) as well as age (Table IV). The relationship between education and prevalence of the disease is shown in Table IV. The prevalence was much higher in illiterates as compared with educated people. As cattle and dogs play an important role in transmission of hydatidosis, individuals with different occupations were subjected to all methods of diagnosis and the data summarized in Table V demonstrates that the prevalence was the highest in shepherds and carpet weavers, respectively. The effect of dog contact clearly showed the role of this animal in the transmission of hydatidosis (Table VI). In order to confirm these data, the prevalences obtained by different methods of diagnosis were compared two-by-two using kappa statistical method. The power of agreement between the results of each two methods are listed in Table VII. As indicated in Table VII-A, ELISA-US combination had 100%, CIE-US (Table VII-B) 47%, and CIE-ELISA 27% agreement, respectively (Table VII-C). Statistical powers of agreement between the results of each combination of two methods were: 5% for ELISA-CIE, 2.4% for US-ELISA, 1.8% for US-CIE, and 0.6% for US-ELISA-CIE, respectively.

Table III. Prevalence of hydatidosis according to age.

Age	No. tested	CIE		ELISA		Ultrasonography		CIE + ELISA	
		No. of positives	%	No. of positives	%	No. of positives	%	No. of positives	%
<15	77	7	9.1	7	9.1	1	1.2	5	6.5
15 - 29	274	20	7.3	37	13.5	2	0.7	15	5.5
30 - 44	339	22	6.5	48	14.2	1	0.2	18	5.3
>45	306	19	6.2	45	14.7	8	2.6	12	3.9
Statistical indices		t = 0.17 df = 996 P-value = 0.83		t = 1.08 df = 996 P-value = 0.28		t = 2.95 df = 996 P-value = 0.009		t = 0.96 df = 996 P-value = 0.34	

Table IV. Relationship between the prevalence of hydatidosis and education.

Education	No. tested	CIE		ELISA		Ultrasonography		CIE + ELISA	
		No. of positives	%	No. of positives	%	No. of positives	%	No. of positives	%
None	664	46	6.9	100	15.1	9	1.3	23	5.0
Elementary	203	13	4.6	26	12.8	2	1	10	4.9
Intermediate	58	4	6.9	4	6.9	0	0.0	3	5.2
High school	46	2	4.3	4	8.6	0	0.0	2	4.3
Associate degree	12	1	8.3	1	8.3	0	0.0	12	8.3
Statistical indices Total = 938		$\chi^2 = 4.40$ df = 6.00 P-value = 0.61		$\chi^2 = 7.36$ df = 6.00 P-value = 0.28		NS NS NS		$\chi^2 = 2.89$ df = 6.00 P-value = 0.82	

DISCUSSION

The prevalence of echinococcosis was studied in 1000 subjects selected from a total population of 112,519 by a randomized cluster sampling method and surveyed during the periods when the nomadic tribes were settled in Fars-or neighboring provinces-in southern Iran. These nomadic tribes represent 9.8% of the entire Iranian nomadic population who are at a high risk due to their specific culture as well as occupation (mostly shepherds and farmers) and life style.

Analysis of the results revealed a prevalence of 1.8% with US which was completely in agreement with those of ELISA as confirmed by other workers from field trials conducted in Kuwait,³⁰ Tunisia,^{27,37} Libya,^{6,32} Kenya³¹ and Turkana,^{28,35} Africa.

A study conducted by Nasseh and Khadivi⁴⁰ on 17,600 randomly selected patients showed a prevalence of 0.02% (352 positive cases from all patients tested). The prevalence reported in their study belongs to a region of Iran where 75% of the population live in rural areas (who are mostly shepherds, farmers and carpet weavers), but seems to be very low, most probably due to the use of Casoni and complement fixation tests and routine X-rays which are

much less sensitive than the procedures used in the present study.

Immunochemical diagnostic tests yielded higher prevalences, 6.8% for CIE and 13.7% for ELISA. Similar studies carried out in other parts of the world have also confirmed that ELISA is the most sensitive method of assay giving the highest prevalence. The areas tested were Turkana,³⁵ 16.4%; Jordan,³³ 5.7%; and Tunisia,^{27, 37} 7.7%. The reasons for the observed discordance of the prevalence by different methods are: (1) the difference in sensitivity, ELISA being the most sensitive and US the least; (2) small cysts with less than 2.0 mm diameter are not easily detectable by portable sonography, and (3) some cysts which are present in extra-abdominal organs may be completely undetectable by US. However, regardless of size, these cysts are mostly capable of evoking antibody responses resulting in seropositivity by immunochemical techniques, such as ELISA and CIE. These results are in agreement with those reported by other investigators in Tunisia,^{27,37} Libya^{6,32} and Kenya.³¹ Furthermore, our data as well as those of others have established that shepherd dogs play a major role as vectors in the transmission of hydatidosis. Similar studies performed in Sumalian nomadic muslims,

Hydatidosis in Southern Iran

Table V. Relationship between hydatidosis and occupation.

Occupation	No. tested	CIE		ELISA		Ultrasonography		CIE + ELISA	
		No. of positives	%	No. of positives	%	No. of positives	%	No. of positives	%
Shepherd	373	23	6.1	51	13.6	5	1.35	16	4.2
Farmer	3	0	0.0	1	33.2	0	0.0	0	0
Carpet weaver	394	29	7.4	60	10.2	4	1.0	21	5.3
Civil service	65	0	0.0	0	0.0	0	0.0	0	0
Student	59	5	8.5	6	10.2	0	0.0	4	6.7
Jobless	22	2	9.1	1	4.5	0	0.0	1	4.5
Housewife	84	9	10.7	18	21.4	2	2.3	7	8.3

Table VI. Relationship between dog contact and the prevalence of hydatidosis.

Contact status	No. tested	CIE		ELISA		Ultrasonography		CIE + ELISA	
		No. of positives	%	No. of positives	%	No. of positives	%	No. of positives	%
Yes	812	59	7.3	116	14.3	10	1.2	44	5.4
No	182	9	4.8	21	11.2	2	1	6	3.3
Statistical indices Total tested = 1000		$\chi^2 = 1.48$ df = 1.0 P-value = 0.22		$\chi^2 = 1.0$ df = 1.0 P-value = 0.28		NS NS NS		$\chi^2 = 1.15$ df = 1.0 P-value = 0.28	

who do not keep dogs due to religious faith, have shown a prevalence of 0.0%, while non-muslim endemic patterns prevail in residents of Somalia, Algeria, Morocco, Tunisia and Libya.

Nasseh and Khadivi⁴⁰ have reported a sex preference of EG infection for females with a ratio of 1.20 in northeastern Iran, in agreement with a ratio of 1.30 obtained in the present study. The results of a similar study conducted in Jordan showed a ratio of girls to boys (age range of 5-17 years) of approximately 4:1. In addition, the prevalence of hydatidosis in 1,085 university students (age range 18-24) tested gave a female to male ratio of 3:1.^{33,34} The relationship between hydatidosis and age reported herein shows a maximum prevalence in those with an age of 45 years or older (15%) and a minimum in children below 5 years (9%) which are concordant with the results reported by Macpherson and Romig.³¹ Moreover, the results of a field study carried out in Tunisia also confirms our data showing that the prevalence of hydatidosis in children of about five years of age was 3.5% and the age of 39 years.³⁷

Bastani and Dehdashti²¹ in an accurate retrospective roentgenographic study of 120 cases mostly from nomadic tribes settled in southern Iran demonstrated that the prevalence of hydatidosis correlated with education, i.e., 15.2% in illiterates compared with 10.2% in educated individuals. Occupation seems to be the most determining factor in the prevalence of echinococcosis. Housewives

who deal more frequently with contaminated meat, vegetables and herbs (21.4%), shepherds (33.6%) and farmers (13.6%) had the highest prevalence rates. The results obtained in the present study are in complete agreement with those of Biffin et al.^{5,41} in an evaluation of ELISA for the diagnosis of hydatid disease. The prevalence in a selected study population was 4% which was much lower than that of veterinary factory workers in Poways (8%).⁴ Individuals engaged in occupations in which contact with animals and contaminated foods is minimal such as teachers and civil service workers, had almost no apparent infection—a prevalence of 0.00%. However, a similar study in university students in Jordan gave a prevalence of 5.16%, but no data were reported for people with other occupations.³³

According to the data presented herein, the most sensitive method is ELISA and the most specific is CIE. To compensate for technical errors affecting accuracy and reliability, a combination of ELISA and CIE is recommended for the diagnosis of hydatidosis. When such a combination is used concomitant with a reliable clinical method, e.g., ultrasonography, highly reliable results will be obtained.¹⁴ All patients with positive sonographic results are seropositive by ELISA (100% of cases) due to the presence of antibodies against specific antigens of *Echinococcus granulosus*.

ACKNOWLEDGEMENTS

This research was supported by a grant from Shiraz

Table VII. The power of agreement between each two methods used.

A. Ultrasonography and ELISA.

		ELISA		Total
		Positive	Negative	
US	Positive	12	0.0	12
	Negative	125	813	988
	Total	137	813	100

B. Ultrasonography and CIE.

		CIE		Total
		Positive	Negative	
US	Positive	5	7	12
	Negative	63	925	988
	Total	68	932	1000

C. CIE and ELISA.

		ELISA		Total
		Positive	Negative	
CIE	Positive	50	18	68
	Negative	87	845	932
	Total	137	863	1000

University of Medical Sciences (SUMS). Shiraz, Iran.

The authors wish to express their appreciation to Dr. M. Amiri, Deputy Chancellor for Health Services, SUMS for supplying first aid and primary care medications.

The authors would also like to express their gratitude to the Department of Rural Services of southern Iran for providing transportation and coordinating all of the field trips to a large number of tribal communities.

Special thanks are due to Mr. M. Shahbazi, a predoctoral student of Anthropology at Washington University, St. Louis, Missouri, USA for his assistance in communication with subjects at the sites of the portable medical set-up and his zealous efforts for supervising the audiovisual programs during this study.

We would also like to acknowledge Drs. D. Mehrabani and M. Ghaneyan for their participation in the field trips.

The authors are indebted to Mrs. Shirazi, Mr. M.A.

Shahbazi, Mr. Gholipour and Mrs. Doroodchi for their assistance in this research. The assistance of Miss F. Faramarzi in typing of the manuscript is highly appreciated.

REFERENCES

1. Saidi F: Surgery of hydatid disease. New York: W.B. Saunders Co., pp. 2-4, 1976.
2. Donovan SM, Mickiewicz N, et al: Imported echinococcosis in southern California. *Am J Trop Med Hyg* 53 (6): 668-671, 1995.
3. Jenkins DJ, Power K: Human hydatid cyst in south Wales and the Australian Capital Territory. *Med J Aust* 164 (1): 18-21, 1996.
4. Palmer SR, Biffin AH, et al: Control of hydatid disease. *Wales Br Med J* 16 (312): 674-675, 1996.
5. Biffin AH, Jones AM, et al: Human hydatid disease: evaluation of an ELISA for diagnosis and population screening of a control programme. *J Med Microbiol* 39 (1): 48-52, 1993.
6. Shambes MK, Macpherson CN, et al: Prevalence of human hydatid disease in northern Libya. *Ann Trop Med Parasitol* 86 (1): 381-386, 1992.
7. Mansicefo S, et al: Simplified CCIEP with commercially produced antigen on cellulose acetate membrane for the diagnosis of hydatidosis. *Trans Roy Soc Trop Med Hyg* 47: 260-261, 1980.
8. Ardehali S, et al: Evaluation of counter immunoelectrophoresis, cross immunoelectrodifusion and agar gel diffusion for human hydatid cyst disease. *Tran Roy Soc Trop Med Hyg* 71: 481-485, 1977.
9. Lass N, et al: The immunodiagnosis of hydatid disease. Postoperative evaluation of the skin test and four serological tests. *Annal Allergy* 31: 430-436, 1973.
10. Sloan L, Schneider S, Rosenblatt J: Evaluation of enzyme-linked immunoassay for diagnosis of cysticercosis. *J Clin Microbiol* 33 (12): 3124-3128, 1995.
11. Babba H, Messedi A, et al: Diagnosis of human hydatidosis: comparison between imagery and six serologic techniques. *Am J Trop Med Hyg* 50 (1): 64-68, 1994.
12. Nejruh FM: Usefulness of indirect haemagglutination (IHA) and enzyme-linked immunosorbent assay (ELISA) in the diagnosis of human hydatidosis. *East Afr Med J* 66 (5): 310-314, 1989.
13. Ito A, Schantz PM, Wilson F, et al: A new serodiagnostic marker for differentiation of active and inactive cases of alveolar hydatid disease. *Am J Trop Med Hyg* 52 (1): 41-44, 1995.
14. Parija SE: Recent trends in serodiagnosis of hvdatid disease. *Southeast Asian J Trop Med Public Health Suppl* 22: 371-376, 1991.
15. Behir AA, Chour H, et al: Echotomographic and serological population based study of hydatidosis in central Tunisia.

Hydatidosis in Southern Iran

- Acta Trop 49 (2): 149-153, 1991.
16. Hira PR, Sheueiki HM, et al: Counterimmunoelectrophoresis using arc 5 antigen for the rapid diagnosis of hydatidosis and comparison with the indirect hemagglutination test. *Am J Trop Med Hyg* 36 (3): 592-597, 1987.
 17. Milka N, Larouze B: Echocardiographic and serological screening for hydatidosis in a Tunisian village. *Am J Trop Med Hyg* 35(4): 815-817, 1986.
 18. Bonifaoino R, Mlagor R, et al: Seroprevalence of *Echinococcus granulosus* infection in a Uruguayan rural human population. *Trans Roy Soc Trop Med Hyg* 85 (6): 769-772, 1991.
 19. Hira PR, Sweiki HM, et al: Cystic hydatid disease: pitfalls in diagnosis in the Middle East endemic area. *J Trop Med Hyg* 96 (6): 363-369, 1993.
 20. Planchart S, Botto C, et al: Evaluation of double diffusion, enzyme immunoassay and immunoblotting techniques for the diagnosis of human hydatid disease in tropical areas. *Rev Inst Med Trop Sao Paulo* 36 (3): 205-210, 1994.
 21. Moro PI, Guevara A, et al: Distribution of hydatidosis and cysticercosis in different Peruvian populations as demonstrated by an enzyme-linked immunoelectrotransfer blot (EITB) assay. *Am J Trop Med Hyg* 51 (6): 851-855, 1994.
 22. Wen H, Craig PS: Immunoglobulin G subclass responses in human cystic and alveolar echinococcosis. *Am J Trop Med Hyg* 5 (16): 741-748, 1994.
 23. Ayadi A, Dutoti E, et al: Specific diagnostic antigens of *Echinococcus granulosus* detected by western blotting. *Parasite* 2(2): 119-123, 1995.
 24. Mistrello G, Gentili M, et al: Dot immunobinding assay as a new diagnostic test for human hydatid disease. *Immunol Lett* 47 (1-2): 79-85, 1995.
 25. Barbieri M, Steria S, et al: High performance latex reagent for hydatid serology using *Echinococcus granulosus* lipoprotein antigen fraction purified from cyst fluid in one step. *Int J Parasitol* 23 (5): 565-572, 1993.
 26. Leggatt GR, McManus DR: Identification and diagnostic value of a major antibody epitope on the 12 kDa antigen from *Echinococcus granulosus* (hydatid disease) cyst fluid. *Parasite Immunol* 16 (2): 87-96, 1994.
 27. Bchir A, Larouze B, et al: Echotomographic and serological population based study of hydatidosis in central Tunisia. *Acta Trop* 49 (2): 149-153, 1991.
 28. Craig PS, Zeyhle E, et al: Hydatid disease: research and control in Turkana. II. The role of immunological techniques for the diagnosis of hydatid disease. *Trans Roy Soc Trop Med Hyg* 80 (2): 182-192, 1986.
 29. Barbieri M, Severi MA, et al: Use of specific antibody and circulating serum levels in the hydatid immunodiagnosis of asymptomatic population. *Int J Parasitol* 24 (7): 937-942, 1994.
 30. Hira PR, Behbahani K, et al: Hydatid liver disease: problems in diagnosis in the Middle East endemic area. *Ann Trop Med Parasitol* 82 (4): 357-361, 1988.
 31. Macpherson CNL, Romig T: Portable ultrasound scanner versus serology in screening for hydatid cyst in a nomadic population. *Lancet* 1: 259-261, 1987.
 32. Khan ALL, Elasageyer MM: Seroepidemiological study of human hydatid disease in Libya using finger prick blood samples. *Ann Trop Med Parasitol* 84 (5): 537-539, 1990.
 33. Abo-Shehada MM: Some observations on hydatidosis in Jordan. *J Helminthol* 67 (3): 248-252, 1993.
 34. Liu D, Rickard MD, et al: Assessment of monoclonal antibodies to *Echinococcus granulosus* antigen 5 and antigen B for detection of human hydatid circulating antigens. *Parasitol* 106 (1): 75-81, 1993.
 35. Kenny GV, MacCabe RJ: Seroepidemiology of hydatid disease in the nonintervention area of northeast Turkana. *Ann Trop Med Parasitol* 87 (5): 451-457, 1993.
 36. Holman CDJ: Epidemiological program for computer and calculations. *Am J Epidemiol* 129: 154-158, 1984.
 37. Milka N, Larouze B: Serologic survey of human hydatid disease in high risk population from Central Tunisia. *Am J Trop Med Hyg* 33 (6): 1184-1189, 1984.
 38. Mistrello G, Gentili H, et al: Dot blot immunoassay as a new diagnostic test for human hydatid disease. *Immunol Lett* 47 (1-2): 79-85, 1995.
 39. Abdel-Aal TM, et-Hadiv HM, et al: Studies on the most reactive purified antigen for immuno-diagnosis of hydatid disease. *J Egypt Soc Parasitol* 26 (2): 279-303, 1996.
 40. Nasseh GA, Khadivi B: Epidemiological and clinical aspects of echinococcosis in East Iran. *J Trop Med Hyg* 78 (6): 120-122, 1975.
 41. Biffin AH, Craig PS, Walters TM: Control of hydatid disease in Wales. *Brit Med J* 312 (7032): 674-675, 1996.