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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.

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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 cattle[5] 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.

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

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	$x^2 = 1.3$ df = 1.0 P-value = 0.24		$x^2=2.20$ df = 1.00 P-value = 0.11	

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		$x^2 = 4.40$ df = 6.00 P-value = 0.61		$x^2 = 7.36$ df = 6.00 P-value = 0.28		NS NS NS		$x^2 = 2.89$ 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] Kenya[31] and Turkana,[28,35] Africa.

A study conducted by Nasseh and Khadivi[40] 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 disconcordance 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,

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		$x^2 = 1.48$ df = 1.0 P-value = 0.22		$x^2 = 1.0$ df = 1.0 P-value = 0.28		NS NS NS		$x^2 = 1.15$ 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 Sumalia, Algeria, Moracco, Tunisia and Libya.

Nasseh and Khadivi[40] 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 Dehdashti[21] 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*.

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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.

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