Seroepidemiological Survey of Human Hydatidosis in Western Part of Iran

M.R. Zarif-fard*, N. Abshar, M.A. Akhavizadegan and G.R. Motamedi Razi Vaccine & Serum Research Institute, P.O.Box 11365-1558, Tehran, Iran

Summary

A total number of 4138 sera from apparently healthy volunteers living in 8 different provinces of the western part of Iran were collected and tested by a standard ELISA. Of those, 3908 (94.45%) were negative and 230 (5.55%) were positive. The results are monitored according to ethnic groups, sex, age, occupation, training, province and season.

Key words: hydatidosis, human, antigen, ELISA, Echinococcus granulosus

Introduction

Hydatidosis is an important parasitic disease for herbivorous and man caused by the larval stage of *Echinococcus* species. Distribution of the disease is related to its intermediate and definitive hosts (Lukshenko 1971).

E.granulosus lives in the small intestine of carnivores, as infective hosts. Herbivorous and man acquire the larval stage through ingestion of infective eggs shed via the faeces of infected dogs. Human infection may occurs by direct contact with dogs or from contaminated environment. When ruminants are slaughtered their disposed viscera may be eaten by definitive hosts. The adult worm is then developed in their intestines (Lukshenko 1971, Mc connell 1979). Hydatid disease in human is potentially dangerous, organ type and cyst sizes are very important in the final pathogenicity of parasite (Matossian 1977).

Numerous studies based on the detection of humoral response of the host against the parasite have been carried out on development immunodiagnostic test(s) for hydatid disease in man (Laplante 1991, Liu *et al* 1992a, b). Iran is one of the endemic areas in the Middle East. Her provinces, suitable for husbandry, are located around the Zagros mountain including the affected areas (Eslami 1997, Noorja 1988).

In this study the human humoral response against *Echinococcus* antigen, to show a clear feature of the prevalence of the disease in above mentioned areas, by ELISA assay was detected.

Materials and Methods

Samples. 4138 sera from apparently healthy volunteers who living in 8 different provinces in the western part of Iran were randomly collected by cluster population method. The collected sera were frozen at -20C until use.

Solutions and buffers. All the buffers and solutions were prepared in the laboratory according to Deplazes and Felix instruction (1991) and kept in the refrigerator (4C) until use.

Antigen. 96 well microplates (Nunc immunoplates) were coated with hydatid fluid (sheep origin) as antigen. The substrate and conjugate were obtained from Dr.P.Deplazes, University of Zurich, Switzerland.

ELIZA assay. The ELISA assay was carried out according to the method described by Deplazes (1991). Antigen solution was diluted (1:200) in coating buffer. 100ml of diluted antigen was pipetted into each well of 96-well microplate and incubated overnight at 4C. Then the plates were washed with washing buffer. The plates were blocked with second with second buffer. Serum sample dilutions were made 1:200 in blocking buffer was added and incubated for 90 min at 37C.100ml/well detection antibody (conjugate) was added and incubated for 90 min at 37C. The plates were washed in washing buffer, and substrate (100ml/well) was added and incubated for 5-15 min at 37C. Known positive and negative controls were included in all test plates. The optical density (OD) for each test was calculated immediately such as average of negative calibration sera (n1, n2 and n3) multiplied by factor of two.

Statistical Analysis. All data were analyzed statistically by x² test.

Results

The results according to sex, age, ethnic groups and occupation and according to province, locality, season and training are showed in tables 1 and 2, respectively. The analysis of data, showing the significant differences between hydatidosis and sex (P<0.0001), province (P<0.005), locality (P<0.005) and season (P<0.025). The disease was not affected by age, ethnic groups, occupation and training.

Discussion

According to the results of this study (Tables 1,2) the prevalence of hydatid disease in the western part of Iran is 5.6%. Previous studies, which have been done based IFA method, had showed fewer ratios (Arbabi & Masoud 1992). However, specificity and sensitivity of ELISA test could be one of its reasons, as well as high infection rates of carnivores and wild animals (20-45%) in these areas (Eslami 1997, Noorja 1988).

Table 1. Frequency and relative frequency of ELISA according to variables

Results of ELISA		Negative		Positive		Total	
Variables		No.	%	No.	%	No.	%
	Male	1723	95.3	85	4.7	1808	100
Sex	Female	2185	93.8	145	6.2	2330	100
	Total	3908	94.4	230	5.6	4138	100
	>20	785	94.4	47	5.6	832	100
	21-40	1840	94.3	111	5.7	1951	100
Age	41-60	901	94.9	84	5.1	949	100
	>60	382	95	24	5	402	100
	Total	3908	94.4	230	5.6	4138	100
	Turk	1990	94.8	110	5.2	2100	100
	Kord	1301	93.9	85	6.1	1386	100
Ethnical	Lour	501	94.2	31	5.8	232	100
Groups	Other	116	96.7	4	3.3	120	100
_	Total	3908	94.4	230	5.6	4138	100
	Staff	496	95	26	5	522	100
į	Worker	377	94	24	6	401	100
	Farmer	323	94.7	18	5.3	341	100
Occupation	Housekeeper	1778	93.6	121	6.4	1899	100
	Hunter	25	92.6	2	7.4	27	100
	Carpet-weaver	33	94.3	2	5.7	35	100
	Other	876	95.9	37	4.1	913	100
	Total	3908	94.4	230	5.6	4138	100

It is clear from the results reported that prevalence of hydatid disease was affected by sex, in female this was significantly higher (P<0.0001) than male. Because of, it is likely, women work in the farm and are more exposed to the animals than men. It is confirmed by previous study (Zarif-fard & Masoud 1998). Similar results were obtained in rural population of Ardabi, Eastern Azarbijan and kermanshah specially in summer and autumn. Direct contact with dogs, handling farm animals and face

less public health could be important reasons for high prevalence of hydatidosis in these areas.

This study confirms and extends previous report showing that prevalence of hydatid disease is influenced by sex and locality (Zarif-fard & Masoud 1998). According to our observations, prevalence of the disease is not affected by age, ethnic groups, occupation and training. Differences between our results and the other reports could be to follow on more populations and areas.

Table 2. Frequency and relative frequency of ELISA according to variables

_	Negative		Positive		Total	
Variables		%	No.	%	No.	%
Ardebil	720	91.7	38	8.3	785	100
E.Azabaijan	648	91.4	64	8.6	748	100
W.Azarbaijan	360	97	11	3	371	100
Eilam	775	94.7	43	5.3	818	100
Kordestan	399	95.4	19	4.6	418	100
Hamedan	352	94.9	19	5.1	371	100
Lourestan	495	95	26	5	521	100
Total	3908	94.4	230	5.6	4138	100
Town	2383	95.4	114	4.6	2497	100
Village	1499	93	112	7	1611	100
Total	3882	94.5	226	5.5	4108	100
Spring	853	94.5	50	5.5	903	100
Summer	837	92.4	69	7.6	906	100
Automn	1573	95.3	78	4.7	1651	100
Winter	645	95.1	33	4.9	678	100
Total	3908	94.4	230	5.6	1508	100
Illiterate	1410	93.5	98	6.5	1508	100
Primary.S	1276	94.4	76	5.6	1352	100
Middle.S	999	95.4	48	4.6	1047	100
Collage	223	96.5	8	3.5	231	100
Total	3908	94.4	230	5.6	4138	100
	Ardebil E.Azabaijan W.Azarbaijan Eilam Kordestan Hamedan Lourestan Total Town Village Total Spring Summer Automn Winter Total Illiterate Primary.S Middle.S Collage	Ardebil 720 E.Azabaijan 648 W.Azarbaijan 360 Eilam 775 Kordestan 399 Hamedan 352 Lourestan 495 Total 3908 Town 2383 Village 1499 Total 3882 Spring 853 Summer 837 Automn 1573 Winter 645 Total 3908 Illiterate 1410 Primary.S 1276 Middle.S 999 Collage 223	Ardebil 720 91.7 E.Azabaijan 648 91.4 W.Azarbaijan 360 97 Eilam 775 94.7 Kordestan 399 95.4 Hamedan 352 94.9 Lourestan 495 95 Total 3908 94.4 Town 2383 95.4 Village 1499 93 Total 3882 94.5 Spring 853 94.5 Summer 837 92.4 Automn 1573 95.3 Winter 645 95.1 Total 3908 94.4 Illiterate 1410 93.5 Primary.S 1276 94.4 Middle.S 999 95.4 Collage 223 96.5	Ardebil 720 91.7 38 E.Azabaijan 648 91.4 64 W.Azarbaijan 360 97 11 Eilam 775 94.7 43 Kordestan 399 95.4 19 Hamedan 352 94.9 19 Lourestan 495 95 26 Total 3908 94.4 230 Town 2383 95.4 114 Village 1499 93 112 Total 3882 94.5 226 Spring 853 94.5 50 Summer 837 92.4 69 Automn 1573 95.3 78 Winter 645 95.1 33 Total 3908 94.4 230 Illiterate 1410 93.5 98 Primary.S 1276 94.4 76 Middle.S 999 95.4 48 Collage 223 96.5 8	Ardebil 720 91.7 38 8.3 E.Azabaijan 648 91.4 64 8.6 W.Azarbaijan 360 97 11 3 Eilam 775 94.7 43 5.3 Kordestan 399 95.4 19 4.6 Hamedan 352 94.9 19 5.1 Lourestan 495 95 26 5 Total 3908 94.4 230 5.6 Town 2383 95.4 114 4.6 Village 1499 93 112 7 Total 3882 94.5 226 5.5 Spring 853 94.5 226 5.5 Spring 853 94.5 50 5.5 Spring 853 94.5 50 5.5 Summer 837 92.4 69 7.6 Automn 1573 95.3 78 4.7 Winter 645 95.1 33 4.9 Total 3908 94.4 230 5.6 Illiterate 1410 93.5 98 Primary.S 1276 94.4 76 5.6 Middle.S 999 95.4 48 4.6 Collage 223 96.5 8 3.5	Ardebil 720 91.7 38 8.3 785 E.Azabaijan 648 91.4 64 8.6 748 W.Azarbaijan 360 97 11 3 371 Eilam 775 94.7 43 5.3 818 Kordestan 399 95.4 19 4.6 418 Hamedan 352 94.9 19 5.1 371 Lourestan 495 95 26 5 521 Total 3908 94.4 230 5.6 4138 Town 2383 95.4 114 4.6 2497 Village 1499 93 112 7 1611 Total 3882 94.5 226 5.5 4108 Spring 853 94.5 50 5.5 903 Summer 837 92.4 69 7.6 906 Automn 1573 95.3 78 4.7 1651 Winter 645 95.1 33 4.9 678 Total 3908 94.4 230 5.6 1508 Illiterate 1410 93.5 98 6.5 1508 Primary.S 1276 94.4 76 5.6 1352 Middle.S 999 95.4 48 4.6 1047 Collage 223 96.5 8 3.5 231

References

Arbabi, A., Masoud, J.(1992). Seroepidemiological study in Hamadan prevince by IFA. M.S. thesis, School of Public Health, Medical Sience, Tehran University (In persian).

Deplazes, P., Gottstein, B.(1991). A monoclonal antibody against *Echinococcus* antigen. *Journal of Parasitology* 103: 41-49.

Eslami, A.(1997). Cestoda. In: Veterinary Helmintology, Vol 2. (In persian).

Laplante, J.(1991). Serological mass screening of alveolar Echinococcosis in france-comte (Abs.english). Archive de la hydatidosis 30:825-829.

LIU, D., Liyhtowlers, M.W. and Rickard, M.D.(1992a). Evaluation of a monoclonal antibody-based competition ELISA for the diagnosis of human hydatidosis. *Journal of Parasitoligy* 104:357-361.

LIU, D., Rickard, M.D. and Lightowlers, M.W.(1992b). Further characterization of monoclonal anthibody to *Echinococcus granulosus* antigen 5 and Antigen B. *International Journal of Parasitology* 22(3):391-394.

Lukshenko, N.P.(1971). Problems of epidemiology and prophylax of alveococcosis (multilocular. Echinococcosis) A general review-with particular reference to the U.S.S.R. *International Journal of Parasitology* 1:125-134.

McConnell, J.D., Green, R.(1979). The control of hydatid disease in Tasmania. *Australian Veterinary Journal* 55:140-145.

Matossian, R.M., Rickard, M.D. and Smyth, J.D.(1977). Hydatidosis: a global problem of increasing importance. *Bulletin of the world Healt Organization* 155(4):507.

Noorja, N.(1988). Hydatidosis-Echinococcosis. Ph.D. Thesis. School of Public Health. Medical Science. Tehran University. (In persian).

Zarif-fard, M.R., Massoud, J.(1998). Study of *Echinococcus granulosus* and *Echinococcus multilocularis* infections in conidiae in Ardabile province of Iran. *Archives of Razi Institute* 48-49:47-52.