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ORIGINAL ARTICLE

The serological study of cystic echinococcosis and assessment of surgical cases during 5 years (2007-2011) in Khorram Abad, Iran

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Abstract

Background: *Echinococcus granulosus* is a cestode whose larval stage causes cystic echinococcosis in wild animals, livestock, and human.

Objective: The aim of this study is to highlight the seroepidemiology of *E. granulosus* infection and surgical cases in the general population of Khorram Abad district, southwest of Iran.

Materials and Methods: Anti-*E. granulosus* antibodies were tested in 617 inhabitants in Khorram Abad by enzyme-linked immunoassay and antigen B. The surgical cases of cystic echinococcosis were investigated in Shohaday-e-Ashayer Hospital of Khorram Abad (as a referral center) from 2007 to 2011.

Results: In total, 95 (15.4%) of the 617 participants (mean age 39.6 ± 17.6 years) had anti-*E. granulosus* antibodies. Prevalence of infection was more in males (60%) than females (40%), and showed statistical significance ($P < 0.001$). High-titer antibodies were most prevalent among the subjects aged 20-29 years. There was significant association between the presence of *Echinococcus* antibodies and the sector of residence, education of volunteers, and occupation ($P < 0.05$). According to hospital records, 58 cystic echinococcosis cases were referred to the hospital during the 5 years. Among the cases, 28 (48.3%) were men and 30 (51.7%) were women. They were between 4 and 74 years of age (36.6 ± 18.9 years). The liver was the organ where most of the hydatid cysts (51.7%) were located, followed by lungs (20.7%).

Conclusion: This is the first report of the seroprevalence and contributing factors for *E. granulosus* infection in the general population in Khorram Abad. The findings confirm the importance of diagnosing human cystic echinococcosis in these regions, given the prevalence rates of surgical cases during the last 5 years, and need further evaluation of the risk factors present.

Key words: Cystic echinococcosis, hospitals records, hydatidosis, seroepidemiology

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Introduction

Echinococcus granulosus is the causative organism of cystic echinococcosis (CE), which is a chronic zoonotic disease of major public health importance. Human CE is known to be endemic in the Mediterranean region, including all countries of the Middle East.[1,2] In Iran, CE is one of the most important parasitic infections of humans and domestic animals.[3,4] Average prevalence rates of 24.41%, 8.51%, 18.89%, and 35.76% have been reported in sheep, goat, cattle, and

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buffalos, respectively.[5] A study showed that the prevalence of CE in camels was 35.21% (233/661).[6] The parasite is transmitted cyclically between livestock as the intermediate hosts and dogs as the definitive host. Stray dogs parasitized with *E. granulosus* have been found in different parts of Iran, with prevalence rates from 5 to 49%.[7] Up to 27.8% of sheepdogs were found to be infected with *E. granulosus* in the rural and urban areas.[8] The most commonly used index of human hydatidosis is annual incidence of hospital surgical cases. Despite the limitation of this method, data on annual rates prove to be of great value for defining public health importance, providing new information about the epidemiology of the disease and documenting its spread in different locations and among various groups at risk.

There have been few seroepidemiology studies reporting the surgical incidence of CE in Iran.[9-12] Due to the importance of this helminth zoonosis and the lack of data about its prevalence in our area, we conducted a seroepidemiological study to assess the prevalence of human CE and the epidemiological factors associated with the spread of the disease. This investigation also reports on the surgical incidence of CE in a general hospital of Khorram Abad district over a period of 5 years (2007-2011).

Materials and Methods

Study area

Khorram Abad, the capital of Lorestan province, is located in the southwest of Iran, bordering with the states of Markazi, Hamedan, Kermanshah, Khuzestan, Ilam, and Isfahan. The estimated population of Khorram Abad is 540,000. The district covers an area of approximately 6233 km^2 [Figure 1]. The study site (48° 21′, 30° 43′) is the largest city in Lorestan province. The area is characterized by a humid equatorial climate and receives most rainfall (annual average: 525 mm) between March and May. The mean annual temperature is 17.2°C.

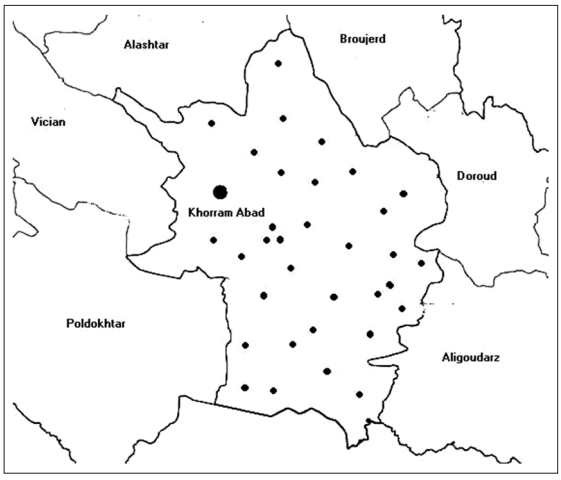

Figure 1: Regions in Khorram Abad district from where the sera were collected for testing for human cystic echinococcosis

Serum samples

A total of 617 sera samples were collected from randomly examined individuals (234 males and 383 females) attending Khorram Abad district health center from July to November 2011. Of the 617 people who gave blood samples, 432 lived in urban and 185 were in rural areas. The demographic details of the respondents and data relating to their demographic characteristics including age, gender, place of residence, occupation, history of disease, and level of education were recorded.

Preparation of antigen

Antigen B was prepared from the hydatid cyst fluid of *E. granulosus*, collected from cysts developed in the liver of sheep as previously described.[13] In brief, 100 ml of hydatid cyst fluid was dialyzed overnight against 5 mM acetate buffer (pH 5) at 4°C. The samples were centrifuged at 50,000 ×*g* for 30 min to remove the albumin. The supernatant was removed and the pellet was dissolved in 0.2 M phosphate buffer (pH 8). Saturated ammonium sulfate was used to remove the globulin from the sample. The sample was boiled in a water bath for 15 min and centrifuged at 50,000 ×*g* for 1 h to isolate antigen B.

Serological testing

Serological evaluation of samples was done by enzyme-linked immunosorbent assay (ELISA) using antigen B. One hundred microliters of hydatid cyst fluid (5 μg/ml antigen B) was dispensed into the wells of microtiter plates that were then incubated overnight at 37°C. Excess protein binding sites were blocked at 37°C for 120 min by 3% skimmed milk diluted in phosphate-buffered saline (PBS) with 0.1% Tween 20. After the wells were washed three times with PBS/0.1% Tween 20, serum samples (diluted 1:50) were added and plates were incubated for 90 min at 37°C. Following another wash, 100 μl of horseradish peroxidase-conjugated antihuman immunoglobulin (diluted 1:1000) was added to each well and the plates were incubated for an additional 60 min at 37°C. After another washing step, the bound antibodies were detected by the addition of chromogen/sustrate (100 μl/well of OPD, 0.025% H_2O_2 in 0.1 M acetate buffer, pH 5). After the color had developed for 30 min, the reaction was stopped with 1 mM sulfuric acid. The plates were read on ELISA reader (STAT-FAX-2100, OHAIO, USA) at 495 nm. The cut-off point was set as 2 SD above the mean of controls (healthy individuals from non-endemic area).

Collection of records of hydatid cyst patients

All the cases that were referred to Shohaday-e-Ashayer Hospital of Khorram Abad (as a referral center) with diagnosis of echinococcosis and underwent surgery for hydatid cyst between October 2007 and November 2011 were included. Information regarding patients' demographic characteristics, cyst location, and history of disease was collected.

Statistical analysis

All statistical analyses were carried out using SPSS for windows version 16.0. Statistical evaluation was performed by Chi square test and $P < 0.05$ was considered significant.

Ethical consideration

Approval of the study protocol was obtained from the Ethical Review Board of Lorestan University of Medical Sciences. Written informed consent was obtained from all the study participants or their parents/guardians.

Results

Prevalence of antibodies and associated risk factors

Antibodies to *E. granulosus* were detected in 617 subjects aged 3-97 years (mean 39.6 ± 17.6 years), with an overall seroprevalence rate of 15.4%. The seroprevalence rate was substantially higher among the people aged 20-29 years (n = 31, 21.8%) than in older subjects (50-59 years, n = 17, 17.3%; and ≥60 years, n = 13, 16.9%) (P = 0.017). High-titer antibodies were most prevalent among those in the age group 2-29 years, suggesting that recent or current exposure to *Echinococcus* is most common in this age group. Seropositivity was more prevalent in males (60.0%) than in females (40.0%), and showed statistical significance ($P < 0.001$).

Several demographic characteristics (sector of residence, education of volunteers, and occupation) were significantly associated with the presence of *Echinococcus* antibodies in unadjusted analysis [Table 1].

Interestingly, living in rural regions was a putative risk factor. The highest seropositive individuals inhabited the rural area (38.9%), while the lowest incidence was in the urban region (5.3%).

Annual hospital records of CE cases

The records of surgical Shohaday-e-Ashayer Hospital, as the only referral center for surgery of hydatid cysts, were collected for a 5-year period of 2006-2010. Their ages ranged from 4 to 74 years (mean age 36 years and 6 months). Of these, 28 (48.3%) were men and 30 (51.7%) were women. Regarding the patients' age, 26 percent of cases were recorded in 20-29 years old [Table 2]. The liver was the most common organ of CE, accounting for 51.7% of the cases. Liver cyst involvement was seen in males (60.7%) more than in females (43.3%). The lungs were the second most frequently involved organ infected (20.7%) [Figure 2].

In a remarkable number of patients (98.3%), hydatid cysts were located only in a single organ (liver, lung, kidney, femur, pelvis, and peritoneum), and in 1.7% of the patients, they were located in other organs also (liver and spleen). In patients receiving surgical treatment, the mean hospital stay was 9.6 days (1-33 days).

Discussion

CE is one of the most important zoonotic diseases caused by a parasite of the class Cestoda. Man, sheep, and cattle, serving as intermediate hosts, contract the infection by ingesting the eggs. The released embryo crosses the intestinal wall to get deposited in the liver, lungs, and other organs where the larval form of the parasite develops.[2] Surgery is considered to be the best treatment option, although it increases the risk of intraoperative spillage of protoscoleces.[14] The disease has a worldwide distribution and is endemic in several countries including Kenya, Turkey, and China.[15] In different parts of Iran, hydatidosis is a common disease, and the prevalence rates of 2-63% in definitive host dog and 1.5-70% in the intermediate hosts including goat, cattle, sheep, and camels have been reported.[6,10,16-19]

This study identifies the prevalence of CE in Khorram Abad during July-November 2011. The prevalence of human *Echinococcus* infection in this survey was 15.4%, and it confirms that Khorram Abad has a comparable infection rate with other areas in which echinococcosis is considered to be highly endemic. A study conducted by Rafiei *et al.*[19] reported 13.8% seropositivity among the nomadic people in southwest of Iran. Saberi-Firoozi *et al.*[20] studied the prevalence of hydatid cyst and reported *Echinococcus* antibodies (13.7%) among the people in southern Iran. The prevalence of CE in the present study is similar to the rates reported in the previous studies. The present study shows that the seropositivity rate was significantly higher in males (60%) than females (40%). The reasons for such a high prevalence could be attributed to different behavioral attitudes and the close contact of males with their dogs.

The results of this investigation provide an indication of the prevalence of hydatid cyst among the subjects aged 20-29 years (21.8%). According to Sarkari *et al.*,[11] the prevalence of hydatidosis is in the 30-39 years age group.

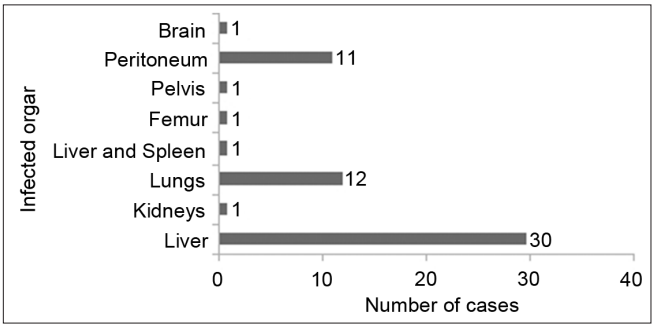

Figure 2: Infected organs by hydatid cysts as reported in Shohaday-e-Ashayer Hospital in Khorram Abad from 2007 to 2011

Table 1: Characteristics of people investigated by enzyme-linked immunosorbent assay for human cystic echinococcosis

No. examined	Total	Statistical analysis		*P* value†
		Seropositive (%)	Seronegative (%)	
Age group (years)				
0-9	2 (2.9)	14 (87.5)	16	0.017
10-19	0 (0.0)	52 (100.0)	52	
20-29	31 (21.8)	111 (78.2)	142	
30-39	15 (12.3)	107 (87.7)	122	
40-49	17 (15.5)	93 (84.5)	110	
50-59	17 (17.3)	81 (82.7)	98	
≥60	13 (16.9)	64 (83.1)	77	
Education				
Illiterate	15 (18.3)	67 (81.7)	82	0.026
Primary	22 (22.7)	75 (77.3)	97	
Secondary	47 (16.5)	238 (83.5)	285	
More than high school	11 (7.2)	142 (92.8)	153	
Occupation				
Housewife	18 (18.3)	251 (94.3)	269	<0.001
Student	11 (5.7)	181 (94.3)	192	
Farmer	20 (59.3)	23 (83.5)	43	
Other	27 (23.9)	86 (76.1)	113	
Residency				
Rural	72 (38.9)	113 (61.1)	185	<0.001
Urban	23 (5.3)	409 (94.3)	432	

†Significance by the χ^2 test

Table 2: Distribution of human cystic echinococcosis cases by age and sex reported in Khorram Abad hospital during 5 years (2007-2011)

	No. infected	Total		*P* value†
		Male	Female	
Age group (years)				
0-9	1	0	1	0.017
10-19	7	2	9	
20-29	6	9	15	
30-39	4	6	10	
40-49	3	3	6	
50-59	4	2	6	
≥60	3	8	11	
Total	28 (48.3%)	30 (51.7%)	58 (100%)	

†Significance by the χ^2 test

Several risk factors for hydatidosis have been identified in human population, but inconsistent results are abundant. Sex, for example, was suggested to be associated with both increased and decreased risk of infection.[21] Young age and poor sanitation are the additional factors contributing to *Echinococcus* exposure in some communities.

As regards residency, a significant difference was found between rural life and urban life (38.9% vs. 5.3%, respectively). Some investigations have found that the risk for developing *Echinococcus* infection is higher in rural areas than in urban areas.[19] In earlier studies, some provinces in Iran such as West Azerbaijan, Kermanshah, and Mazandaran demonstrated a significant difference with the place of living. It was observed that most of the affected people were farmers or housewives living in rural areas.[5] This study confirms that inhabitants of rural communities may be heavily exposed to *Echinococcus*. The presence of an unrestrained dog population and adequate climate and environmental conditions for egg survival contribute to the high seroprevalence rate observed.

We found, however, a positive association between seropositivity of *Echinococcus* and education level ($P = 0.026$). The high prevalence of antibody titer in people with primary education may be associated with socioeconomic status.

An important result of this study is the high seropositivity rate (59.3%) of hydatid cysts in the farmers. Possible reasons that could be demonstrated are the presence of dogs in the household of most farmers and their close contact with *Echinococcus* eggs. Women as housewives showed high prevalence of echinococcosis. This might be due to that they works along with their husbands in the farmland and also help with sheep and cattle raising.

The present study is the first comprehensive investigation of surgical cases of hydatid cyst and seroepidemiology of human echinococcosis in Khorram Abad. In Iran, the surgical CE rate is reported to be 2/100,000 and human infection rate is more than 1% of the total population.[22] The annual surgical cases of human hydatidosis admitted

with diagnosis of CE were investigated during the 5-years (2007-2011). According to on the hospital records, females (51.7%) were found to be more infected with *Echinococcus* compared to males (48.3%). This sex difference is similar to that reported in previous studies for the CE cases.[15] The highest rate of surgical case of hydatidosis has been reported in the age group of 20-29 years. In Palestine, the surgical incidence of CE increased with age reduction in the 11-20 year age group.[23] The differences in the incidence rates of surgical cases could be attributed to the close contact with dogs and different occupations. On the other hand, given that hydatid cysts grow very slowly, this leads to a relatively slow course and delayed presentation, making it a disease of middle-aged people.

Our finding shows that different organs were affected, but the most common sites of location of hydatid cysts are liver and lung. Majority of the cysts detected were located in the liver.[5]

Conclusion

In spite of numerous studies conducted on this, evidence-based and standardized approaches are still needed to define appropriate strategies for the epidemiological evaluation, immunodiagnosis, and clinical management of CE, among other aspects. Therefore, we conducted to investigate the seroprevalence of human echinococcosis and the prevalence rate of CE based on the hospital records during a 5-year period in Khorram Abad district.

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