

Original paper

Seroprevalence of human hydatid cyst: a cross sectional study in a rural areas of Zahedan, southeastern Iran

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ABSTRACT. Hydatidosis or cystic echinococcosis (CE) is one of the most common zoonosis diseases. Iran is one of the endemic regions in terms of this disease. For the first time, the present study was conducted to determine the seroprevalence of hydatid cyst in Zahedan rural areas due to the importance of human CE and lack of information in this region. The present study was performed on 551 people referred to seven rural health centers in Zahedan during 2019–2020. Serum samples were collected and analyzed by indirect ELISA method using recombinant antigen B subunit B8/1. Results were analyzed by SPSS (version 22) software and Chi-square test. The CE seroprevalence was 4%. The most positive cases were in the age group of 10–30 years. The highest infection was reported in homemakers. A significant relationship (P -value<0.05) was only reported between the seropositivity to hydatid cyst and the presence of dogs in the environment. The present study's findings indicated human hydatid cyst in rural areas of Zahedan is a health problem; moreover, the control and prevention principles and analysis of various epidemiological aspects of this disease should be considered.

Keywords: seroprevalence, hydatid cyst, rural, Zahedan, Iran

Introduction

Human hydatidosis is a parasitic disease caused by the larval stage of *Echinococcus granulosus*. It is also a common disease between humans and animals [1]. The excreted egg from the definitive host spreads to the metacestode larval stage or hydatid cyst in the visceral organs of the intermediate hosts. Hydatid cysts can form in any part of the body, including the liver, lungs, bones, spleen, kidneys, but they are more common in the liver than in other organs. The time interval between infection and the onset of symptoms can vary from a few months to several years, depending on the cyst's number, location, and stage. The disease is widespread in the world, and if left untreated, it can

sometimes be fatal [2]. Significant outbreaks of disease have been reported in parts of the world, including South of Australia, New Zealand, Africa, Greece, Spain, and the Middle East [3]. Echinococcosis is endemic in all countries of the Iranian plateau, such as Iran, Pakistan and Turkey [4]. The seroprevalence of cystic echinococcosis (CE) in different provinces of Iran has been reported at 1.6% to 20% [5,6].

Zahedan is the capital of Sistan and Baluchestan province, which is located in southeastern Iran. This city is one of the deprived areas of Iran. There is a possibility of the hydatid cyst infection among the residents of urban and rural areas of Zahedan due to risk factors including keeping livestock, presence of stray dogs, illegal slaughter, and livestock supply.

Also, sufficient information on the epidemiology of hydatidosis and the prevalence of the disease in southeastern parts of Iran is not available. Due to the mentioned reasons, conducting this study for the first time on the rural population of this area seems necessary.

Materials and Methods

Sample collection and area of study

The present cross-sectional study was performed on referred people to rural health service centers of Zahedan during 2019–2020. A total of 551 samples were collected from seven rural health service centers of Zahedan in an easy and accessible way. The number of samples in each center was determined based on their household size. The number of collected blood samples from seven health centers including, Sarjangan, Shuro, Allahabad Doomak, Garage, Haji Abad Cheshmeh, Roshanabad, and Chah Zard was 121, 31, 126, 50, 110, 46, and 67 respectively. Blood samples were taken from the people with informed consent. People who did not want to continue cooperating and had cyst removal surgery were excluded from the study. After selecting each person, first, a checklist containing information such as age, sex, occupation, place of residence, etc., was completed in person and face to face, and the blood sample was taken from the same person at the same time.

Indirect ELISA test with recombinant antigen B subunit B8/1

In this study, the indirect ELISA method using recombinant antigen B subunit B8/1, which was produced and evaluated (sensitivity 93% and a specificity of 92%) in previous studies [7], was used to estimate the seroprevalence of human hydatidosis. The antigen was coated with 1.5 µg/ml of recombinant antigen B (100 µl/well) in 0.5 M of carbonate-bicarbonate (pH 9.5) coating buffer and incubated at 4°C, overnight. Then the plates were emptied and washed with PBST (phosphate buffer saline containing 0.05% Tween 20) solution, five times. In the next step, blocking was performed for one hour with 10% skimmed milk powder, and after washing, as in the previous step, 100 µl of the patient's serum with a dilution of 1:100 was added, and incubation was performed at room temperature for one hour. After re-washing, as in the previous step, 100 µl of Goat Anti-human IgG-HRP conjugate (Sigma, Cat. No: DB9573) with a dilution

of 1:2500 was added, the plates were incubated for one hour at room temperature. Washing was performed five times again. Then, 100 µl of tetra methyl benzidine (TMB) substrate (Sigma, Cat.No: DB9510) was added to the wells and placed at room temperature and in the dark for 10 minutes. The reaction was terminated by adding 50 µl of 0.2 M sulfuric acids. Finally, the ELISA reader, read the results at 450 nm and 630 nm (reference wavelength), and after defining cut-off, positive samples were determined. Positive control and negative control serum (to determine cut-off) were used in each plate. The cut-off was calculated from the average absorption of negative control samples plus two times the standard deviation. It should be noted that the positive samples were examined in duplicate after screening

Statistical analysis

SPSS (version 22) software and Chi-square test were used to analyze and determine the association between relative risk factors and seropositivity to hydatid cyst.

Ethical considerations

The present study was approved with ethical approval code of IR.ZAUMS.REC.13 98.317.

Results

In the present study, 22 out of 551 samples had total IgG antibodies against recombinant antigen B of hydatid cyst. The seroprevalence of hydatid cyst was 4%. Also, 2.7% and 1.3% of seropositive cases, respectively, were women and men. The most positive cases (2.18%) were reported in the age group of 10–30 years, and among the studied centers, Allahabad Doomak rural health center had the most seropositive cases (1.45%). The highest seropositivity (1.6%) was reported in homemakers. Also, a significant relationship ($P < 0.05$) was only reported between the seropositivity to hydatid cyst and the presence of dogs in the residence place of the subjects. No significant relationship ($P > 0.05$) was noted between other related risk factors, including sex, age, rural health center, wash vegetable method, job, and seropositivity to hydatid cyst. Table 1 shows demographic data, risk factors and hydatid cyst seropositivity among the referred people to rural health centers of Zahedan.

Table 1. Demographic informations and relative hydatid cyst seropositivity in humans referred to rural health centers of Zahedan

Risk factors and demographic data		Frequency	%	Seropositivity		P-value
				frequency	%	
Age groups	<10	26	4.8	2	0.36	0.6
	10–30	293	53	12	2.18	
	31–50	179	32.5	7	1.27	
	>50	53	9.7	1	0.18	
Sex	Female	381	69	15	2.7	0.54
	Male	170	31	7	1.3	
Rural health center	Sarjangal	121	22	5	0.9	0.58
	Shuro	31	5.6	0	0	
	Allahabad Doomak	126	23	8	1.45	
	Garagheh	50	9.1	3	0.54	
	Haji Abad Cheshmeh	110	20	3	0.54	
	Roshanadad	46	8.3	1	0.18	
	Chah Zard	67	12	2	0.36	
Presence of dog in environment	Yes	208	37.7	20	3.63	0.0001
	No	343	62.3	2	0.36	
Vegetable washing method	Water	419	76	16	2.9	0.43
	Salt and detergent	132	24	6	1.1	
Job	Rancher	45	8.1	4	0.73	0.16
	Farmer	39	7.2	1	0.18	
	Student	45	8.1	1	0.18	
	Homemaker	301	54.6	9	1.6	
	Other	121	22	7	1.3	

Discussion

Knowing the prevalence of hydatidosis in different areas is very effective in controlling and preventing this disease. Different studies have evaluated the prevalence of this disease by different methods, including serology, imaging, and surgery. The serodiagnosis of human cystic echinococcosis (CE) is complex. The main pitfalls of the serodiagnosis tests are unsatisfactory performance of the available tests and the hardness of the preparation and standardization of the antigens [8]. Up to date, several antigens were used for this purpose. Hydatid fluid (HF) antigen is one of the most important of them that cause false-positive

results in serological tests due to heterogeneity and carbohydrate epitopes common between parasite and non-parasite antigens. Also, the cysts' size, number, and stage can lead to false-negative results of the antibody detection tests against HF antigen [8,9]. Two main components of HF are antigen B and Arc 5. In most studies, an ELISA test using antigen B has been performed to evaluate the seroprevalence of the CE. This antigen has limitations, and in any region, antigen B of the same area should be used to assess individuals. Also, common epitopes between antigen B and *Taenia* species, including *Taenia saginata* and *T. solium*, can be lead to false-positive results in serological tests. Overall HF and purified components,

including AgB and Arc5 are heterogeneous and lead to false-positive and negative results in serodiagnostic tests. As you know, serodiagnosis tests based on antibody detection can not differ between the present and past infection and remain positive after treatments and ten years after cyst removal [8,12]. Nowadays, recombinant antigens with their high sensitivity and specificity are more accurate for CE serodiagnosis and population screenings and able to solve mentioned problems [7–9,11–13]. Therefore, in the present study, to investigate the seroprevalence of hydatid cyst in patients referred to Zahedan rural health centers, recombinant antigen B subunit B8/1 was used, and to overcome the false-positive results, patients who had a history of cyst surgery were excluded. Therefore positive cases were detected in the current study are more valid. In the present study, the seroprevalence of hydatid cyst in patients referred to Zahedan rural health centers is 4%. In this regard, our study was in line with studies conducted in different regions such as Karaj, Arak, Isfahan, East Azarbaijan, and Shiraz [14–18]. The seroprevalence of hydatid cyst in rural areas of Zahedan is lower than the studies in Khuzestan, Lorestan, Mazandaran, and Kohgiluyeh and Boyer Ahmad [19–22]. Due to the hot and dry climate, the southeastern regions do not have enough pastures and animal husbandry. Therefore the intermediate host for this parasite has less variety. In addition, the parasite's eggs are more sensitive to high temperatures and low humidity, and all of these factors may explain the lower seroprevalence of hydatid cysts in people in these areas.

In the present study, the seroprevalence of hydatid cyst in women is higher than men without significant difference, in this regard, our study was in line with similar studies in Kashan, Jordan, Iraq, and Egypt [23–26]. Also, in the current study, seropositive cases were higher among homemakers than in other occupations. CE is associated with exposure to parasite eggs in various ways, such as contact with soil and contaminated hands and food. Homemakers are highly exposed to the source of contamination when washing vegetables, fruits, and other activities. In rural areas, it is expected that due to more men dealing with soil and certain occupations such as animal farming and agriculture, the prevalence of the disease to be higher. Still, in the present study, the seroprevalence of hydatid cyst was higher in women and homemakers. In this respect, it is consistent with the other studies

[27,28].

In this study, the highest frequency of people with hydatid cyst antibodies was observed in 10–30 years. In terms of age, seropositivity to hydatid cyst is observed in all age groups, and none of the age groups are immune to this disease. In other studies, different age groups, including 60–90 years in Ardabil, 40–59 years in Moghan, 20–29 years in Khorramabad, 30–39 years in Yasuj, 29–39 years in Kerman were reported [20,22,28–30]. Because of the long incubation period of this disease, it is more difficult to identify the actual age group that is at risk.

Based on the present study results, a significant relationship was observed between the seroprevalence of hydatid cyst and the presence of dogs in the residence place of the subjects. In this respect, the present study was consistent with similar studies in west Azerbaijan, Meshkinshahr, and Hamedan [31–33]. Dogs are the main factor for spreading the parasite's eggs in the human's living place. In rural areas, dogs coexist with humans because of guarding herds, farms, rural houses, and husbandry occupation. According to the significant relationship between the seroprevalence of hydatid cyst and the presence of dogs in the habitat of the subjects, it seems necessary to investigate the infection in dogs in rural areas of Zahedan.

In the present study, the seroprevalence of hydatid cyst was lower in people who used salt and detergent to wash vegetables than water, although this difference is not significant. In this respect, our study was consistent with similar studies in Hamedan and Mazandaran [33,34]. One of the most critical ways of transmitting the disease is the consumption of vegetables contaminated with the eggs of *Echinococcus granulosus* parasite. Therefore careful washing can essentially prevent the occurrence of hydatid cyst disease. Besides the serological tests, ultrasound imaging techniques based on the WHO Informal Working Group on CE classification can be helpful for a reliable diagnosis. Still, in pulmonary echinococcosis, this method has a false-negative result [11]. Therefore, cyst stage (CE1–CE5) and cyst location could play a role in serological and imaging outcomes, and both methods have restrictions and limitations to CE diagnosis [13].

In this study, some seropositive patients had a simple cyst in favor of a hydatid cyst in the liver. Also, performing ultrasounds for all seropositive individuals was impossible due to Covid-19

pandemic conditions, noncooperation of patients and long-distance between villages and towns. In this regard, future studies should be conducted on seropositive cases by ultrasound method and classify the stage of the cyst based on WHO classification.

The present study's findings indicated human hydatid cyst in rural areas of Zahedan is a health problem; moreover, the control and prevention principles and analysis of various epidemiological aspects of this disease should be considered.

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