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Seroprevalence of Hydatidosis in Kaboodarahang, Hamadan Province, Iran, in 2016 - 2017

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Abstract

Background: Cystic echinococcosis (CE) or hydatid cyst as a significant zoonotic disease caused by *Echinococcus* spp. is endemic in Iran. The present study was carried out to clarify the status of hydatidosis in Kaboodarahang, Hamadan province.
Objectives: This descriptive cross-sectional study aimed to investigate the prevalence of the hydatid cyst seroepidemiology in Hamadan province, Iran. Hamadan province was chosen due to its wide range of livestock breeding and human exposure to stray dogs. We studied the seroprevalence of the parasite and infection risks.
Methods: In this cross-sectional study, overall 1232 serum samples (524 from males and 708 from females) were collected by randomized cluster sampling in 2016 - 2017. The sera were analyzed by the enzyme-linked immunosorbent assay (ELISA) using IgG ELISA kits. Before taking the specimens, a questionnaire was filled out for each enrolled patient. The data were analyzed using the Chi-square test and multivariate logistic regression for risk factors analysis.
Results: The seropositivity was 1% (12 cases including four males and eight females). No significant difference was seen according to gender (P = 0.51). There was no significant association between CE seropositivity and age group, occupation, and region, but the association between keeping a dog (in the house or workplace) and a positive IgG test was statistically significant (P = 0.02).
Conclusions: Although the seroprevalence of CE was relatively low in Hamadan province, yet, due to the importance of the disease and zoonotic feature of the organism, all preventive measures should be taken into consideration.

Keywords: Hydatid Cyst, Kaboodarahang, Seroprevalence Study

1. Background

Human hydatidosis or human cystic echinococcosis (HCE) is a chronic parasitic disease caused by the larval stage of *Echinococcus granulosus*, which has an important effect on public health in human populations. This cyclozoonotic disease starts with the accidental ingestion of the parasite's egg. It can be transmitted by infected feces of dogs via soil, vegetables, water, food, etc. (1, 2). The disease is widely distributed in regions where sheep rearing is a major industry. This multi-host disease is one of the most important public health infection diseases in Iran. It causes a huge economic burden on governments (3). Epidemiologically, Iran is classified into two regions of hyperendemic (the northern part) and endemic (the southern part) in terms of CE (1). Some studies showed that the rate of the disease is high in the northwest of Iran (4). Studies on final hosts in the western regions of Iran showed that stray dogs, jackals, and foxes are infected in rates of 3% - 20% (5). Geographically distinct strains of *E. granulosus* exist with different host affinities. The penetration of larvae through the mucosa leads to blood-borne distribution to the liver and other sites, where the development of cysts begins. Most primary infections in humans consist of a single cyst (6). The detection of cystic echinococcosis (CE) is mainly confirmed by a combination of previous exposure history, serological testing, and imaging methods. Various serological methods have been developed and implemented for immunodiagnosis of CE in recent years, including indirect hemagglutination (IHA), immunoblotting, enzyme-linked immunosorbent assay (ELISA), indirect fluorescent-antibody (IFA), latex agglutination test, and immunochromatography test. For the improvement of these assays, different antigens from Echinococcus, protoscolices, worm eggs, or hydatid cyst fluid have been defined, purified, and evaluated in the aforementioned sero-

logical tests (7, 8). Serology-based methods have been innovated for the total screening of population in endemic areas but the sensitivity and specificity of the diagnostic antigens are of importance (9, 10). Several immunochemical tests such as ELISA and IFA have been developed for determining anti-Echinococcus IgG in sera for the diagnosis of HCE in Iran, which are available commercially.

2. Objectives

The present investigation was performed to describe the seroprevalence of *E. granulosus* infection in a population from Kaboodarahang, Hamadan province, Iran. This area is an important region for livestock breeding and the risk of infection transmission via stray dogs to humans. The study performed by the detection of anti-Echinococcus IgG in serum. In this study, we reported the diagnosis of hydatid cyst exposure by ELISA for patients.

3. Methods

A descriptive cross-sectional study was done on serum samples of enrolled cases from Kaboodarahang city, Hamadan province, Iran, in 2016 - 2017 to investigate the situation of hydatid disease among the population. Hamadan province is located in the west of Iran. Kaboodarahang is one of the relatively large cities of the province with an estimated population of 155000 people that lies on wet mountains in 34.7989°N, 48.5150°E (www.iga.ir).

3.1. Serum Collection

A cluster sampling method was used for collecting the serum samples. First, according to the prevalence formula and P = 1.4%, the d value was calculated as 0.025 with 95% confidence interval for 1232 samples. The samples were obtained from 18 clusters (according to villages and size of population) from December 2016 to September 2017. In this research study, a total number of 1232 serum samples including 524 from males and 708 from females were collected. The serum specimens were transferred to the Parasitology and Mycology Department of Hamadan University of Medical Sciences. Filling out the questionnaires and gathering the samples were performed by the staff well trained for these tasks. The compiled data were classified according to age groups, gender, occupation, education level, and consumers of not washed vegetables. This study was approved by the Ethics Committee of Hormozgan University of Medical Sciences (HUMS) numbered HUMS.REC.1395.64. Informed consent was obtained from all the enrolled cases.

3.2. ELISA Test

About 5 mL of the blood sample was taken from each participant. The sera were isolated and before the ELISA test, all samples were diluted 1:101 according to the kit manufacturer's protocol. The ELISA tests were performed using ELISA kit Echinococcus (Pishtazteb, Tehran, Iran). The wells of a 96-well plate were coated with *E. granulosus* antigens. Serum samples were added to the plate and the protocol was according to catalogue No. PT-Hydatid-96 kit.

Our serologic method in the study was based on the indirect ELISA test. In the ELISA technique, the wells of microplates (Ariagene, Tehran, Iran) were covered by a given amount of Echinococcus antigens. Serum samples were diluted initially and added to the microplate wells. Sera and probable antibodies were allowed to react with *Echinococcus* antigens. If specific antibodies were present in the sera, they would attach to the solid phase that contained parasite antigens. After washing the microplate wells and removing unattached antibodies, a secondary antibody labeled HRP (anti-human IgG-horseradish peroxidase (HRP) conjugate) was added to the wells. The cutoff value was determined according to the below equation: Cutoff = N.C. mean OD (450 nm) + 0.25 that was calculated for all samples (ELISA reader, BioTek, USA).

The IgG levels of 10% higher than the cutoff value were considered positive and lower levels were taken as negative. Any value between these values is indeterminate and it should be repeated.

3.3. Statistical Analysis

The data were analyzed via SPSS software (V. 18) (Chicago, IL, USA). Moreover, the chi-square test was used for the analysis of qualitative data. For controlling the confounder variables, the logistic regression analysis was implemented. The results would be considered statistically significant if the P value were less than 0.05.

4. Results

The serology test showed 1% seropositivity (12 cases). Table 1 indicates the seropositivity of hydatidosis in Kaboodarahang according to sex.

Table 1. The Seroprevalence of Hydatid Cyst Disease Among the Study Population from Kaboodarahang, Hamadan, According to Gender in 2016 - 2017[a]

Seropositivity	Male	Female	Total
Positive	4 (0.76)	8 (1.13)	12 (1)
Negative	520 (99.24)	700 (98.87)	1220 (99)
Total	524	708	1232 (100)

[a] Values are expressed as No. (%).

There was no significant association between seropositivity and gender in our study (P = 0.517, Chi-square = 0.420).

Table 2 shows the seroprevalence of the disease according to age groups. We divided the enrolled population into three groups. The minimum and maximum ages of the cases were 10 and 96, respectively.

Table 2. The Age Groups of the Study Population from Kaboodarahang, Hamadan, and the Seroprevalence of Hydatid Cyst Disease[a]

Age Groups	Seropositivity		Total
	Positive	Negative	
Up to 25 years	0	143 (100)	143
25 - 40 years	5 (1.2)	413 (98.8)	418
More than 41 years	7 (1)	664 (99)	671
Total	12 (1)	1220 (99)	1232

[a] Values are expressed as No. (%).

Statistical analysis indicated that there was no significant association between CE seropositivity and age group (Chi-square = 1.654, P = 0.437).

In our study, we took different samples from people with various occupations and some of these careers had direct contact with a definitive or intermediate host of hydatid cyst disease. Table 3 shows the relationship between seropositivity and occupation.

Statistical analysis showed no significant association between seropositivity and occupation in the study population (P = 0.36).

We analyzed our data seeking an association between seropositivity of CE and education level of the study population. We did not find any significant relationship between education level and positivity, as shown in Table 4 (P = 0.84).

The only variable that was statistically significantly relevant to seropositivity was 'keeping a dog in the house' (Table 5). Data showed that the relationship between keeping a dog and being seropositive among the population was statistically significant (P = 0.02). Furthermore, when the odds ratio is larger than unity, the exposure variable considerably increases the risk of the outcome variable to cause the disease. The odds ratio was 3.796 in our study [CI = 1.13 - 12.67, 95%].

5. Discussion

Our study showed that about 1% of the participants were serologically positive for hydatidosis. Echinococcosis is a neglected widespread zoonotic parasitic disease and the best available and cost-effective method for diagnosis in large sample studies is ELISA (11). To find the seroprevalence of hydatid cyst, 1232 serum samples were collected from a population from Kaboodarahang rural areas, Hamadan province, Iran. The ELISA test with B antigen (the major protein secreted by the *E. granulosus* metacestode that is involved in key host-parasite interactions during infection) has been used in most of the studies (20 studies) (12) because this method is acceptable, easy, efficient, and affordable with a high level of sensitivity and specificity. In addition, since preparing the B antigen is easy, using the local area antigen gives highly accurate test results (9). Our study showed that the seroprevalence of the disease was low, similar to some other studies. Farrokhzad et al. in 2004 reported a rate of 0.2% in Tehran by the IFA method (10) while Zibaei recorded an amazing rate of 15.4% in Khorramabad in 2013 (2). Some studies showed that the most prevalence of the disease was obtained in the west and southwest of Iran with the highest prevalence obtained in Lorestan (13) and we gained the same data in our study. Many studies carried out in the south of Iran have reported the seroprevalence rate of 1% - 6% approximately (14). However, the highest rate in Iran reported from Mashhad (4). These differences seen in the studies performed in various regions of the country may be due to the classification of age groups, the difference in the type of the study population, and the geographical distribution in each study.

Our data about the consumption of vegetables among the study population from Kaboodarahang, Hamadan, showed that there was no significant difference between people who washed vegetables with water and those who washed with disinfectants (P = 0.5).

Among the study population, 301 cases had a history of contact with house dogs, sheepdogs, or stay dogs, but only were two cases seropositive that was not statistically significant (P = 0.52).

Other variables evaluated in our study were age, gender, and occupation. Our data showed no significant differences between the seroprevalence of CE and the above mentioned variables, which is consistent with the study of Rokni in Qom (15) although Nourjah et al. in 2004 showed that women had a higher prevalence of hydatid cyst and underwent more surgery (13) and the study of Shafiei et al. indicated that the seroprevalence of hydatid cyst was higher in the middle-aged group, which are not in agreement with our study (2). Another study in Zanjan showed that the infection is more common among over 50-years-old (16). Some studies insist on the higher rate of infection in housewives because of their more chance of contact with the source of infection (13, 15). The only significant variable in our study was keeping a dog in the house or workplace as a sentinel. Some studies consider this item

Table 3. The Seropositivity of Hydatid Cyst Disease in The Kaboodarahang Population, Hamadan, According to the Occupation[a]

Seropositivity	Occupation					Total
	Employee	Student	Housewife	Rancher	Other	
Positive	2 (3.33)	0	6 (0.9)	3 (0.8)	1 (1.33)	12 (1)
Negative	58 (96.67)	46 (100)	669 (99.1)	373 (99.2)	74 (98.67)	1220 (99)
Total	60	46	675	376	75	1232 (100)

[a] Values are expressed as No. (%).

Table 4. The Seropositivity of Hydatid Cyst Disease in the Kaboodarahang Population, Hamadan, According to Education Level

Seropositivity	Education Level			Total
	Illiterate	> Diploma	Bachelor's Degree or Higher	
Positive	4 (0.85)	7 (1)	1 (1.61)	12 (1)
Negative	464 (99.15)	695 (99)	61 (98.39)	1220 (99)
Total	468	702	62	1232 (100)

[a] Values are expressed as No. (%).

Table 5. The Relationship Between Keeping a Dog in the House and Seropositivity of Hydatid Cyst Disease Among the Study Population From Kaboodarahang[a]

Keeping a Dog in the House	Seropositivity		Total
	Positive	Negative	
Yes	8 (1.9)	421 (98.1)	429
No	4 (0.5)	799 (99.5)	803
Total	12 (1)	1220 (99)	1232

[a] Values are expressed as No. (%).

as an important factor in the transmission of the disease and the higher seroprevalence in some regions (17). Our study data showed that the relationship between keeping a dog and being seropositive among the population was statistically significant (P = 0.02). Furthermore, when the odds ratio is larger than unity, the exposure variable is considered to increase the risk of the outcome variable significantly. The odds ratio was 3.796 in our study [CI = 1.13 - 12.67, 95%]. The results obtained in the study can guide health policy-makers to pay more attention to the relationship between human, stray dogs, and livestock to keep the prevalence low. We suggest researchers study stray dogs to gain the more precise rate of the infection in definitive hosts because they keep the infection in the environment. In addition, vegetables used by inhabitants should be examined for Taenia eggs.

The limitations of the study including the high price of ELISA kits, the large sample size, and the need for coordination among health centers to take samples took time to finish the project.

5.1. Conclusions

Hydatidosis is considered an important disease in terms of endemicity and severity of signs after infection and rehabilitation following surgical cares although we detected a few infected cases. Most researchers showed that the incidence of the disease has decreased in the last decade. Vegetables contaminated with parasite eggs can be one of the routes of transmission in Iran but in our study, we did not find it an effective factor. Contact with infected dogs can intensify the transmission cycle. Although the seroprevalence of CE is relatively low in Hamadan province, yet, due to the importance of the disease and zoonotic identity of the organism, all preventive measures should be taken into consideration.

Supplementary Material

Supplementary material(s) is available here [To read supplementary materials, please refer to the journal website and open PDF/HTML].

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Footnotes

Conflict of Interests: The authors whose names are listed in the paper certify that they have no affiliations with or involvement in any organization or entity with any financial interest.

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