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Original Article

Seroprevalence of Human Cystic Echinococcosis and Risk Factors in Nahavand, Hamadan, Western Iran

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Abstract

Background: Hydatid cyst is a global infectious disease caused by the larval stage of *Echinococcus granulosus*. The annual incidence of human cystic echinococcosis (CE) in Iran varies from 0 to 61 in 100,000 cases, depending on geographical conditions, epidemiological factors, and diagnostic methods. Studies showed that *E. granulosus* infection is one of the most important health problems in most parts of the country.

Methods: This descriptive cross-sectional study was conducted on 400 cases referring to Shahid Ghodoosi and Shahid Alimoradi hospital laboratories in Nahavand, Hamadan, Western Iran. IgG antibodies against hydatid cysts were evaluated by the commercial enzyme-linked immunosorbent assay (ELISA) kit, and socio-demographic data were collected using a questionnaire. Then, data were statistically analyzed by SPSS software.

Results: Out of 400 subjects in this study, five (1.25%) were positive for CE IgG antibodies by the ELISA method. Two (40%) females and three (60%) males were reported positive for CE IgG via the ELISA Hydatid test. Rural residents consisted of the majority of positive cases (80%). Nearly all of the hydatid-infected subjects were categorized in the age group above 50. Moreover, housewives and self-employed businesses were identified as the two occupations with the highest antibody titers against *E. granulosus*. In addition, no significant statistical difference was observed in variables.

Conclusion: Although the frequency of hydatid cyst infection in Nahavand is lower than the average proportion confirmed by previous studies in the country, due to the numerous records of hydatid cyst disease in the region, the establishment of health programs is regarded essential to control this parasitic infection.

Keywords: *Echinococcus granulosus*, Seroprevalence, Epidemiology

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Introduction

Hydatidosis as one of the most important zoonosis diseases with a worldwide distribution is caused by the larval stage of dog tape-worm, *Echinococcus granulosus* (1). It is mostly prevalent in rural areas where livestock production is considered the main occupation. This zoonotic disease annually leads to human and animal health issues as well as economic losses (2,3). Humans are infected as intermediate hosts by accidental ingestion of *E. granulosus* eggs, whereas in the wildlife cycle, dogs and other carnivores are considered the final host, and sheep and other herbivores are regarded as intermediate hosts (4).

The liver and lungs are the two most affected organs by *E. granulosus* larval stage; however, other organs such as muscles, spleen, soft tissues, bone marrow, heart, brain, and other points can also be impacted by the larval stage (hydatid cysts) (5,6).

Countries with temperate climates, including the Mediterranean, southern and central parts of Russia, Central Asia, China, Australia, South America, and North and East Africa reported the highest prevalence of cystic echinococcosis (CE) in human and animal hosts (7).

The prevalence of hydatidosis in different regions of Iran varies depending on geographical conditions, epidemiological factors, and diagnostic methods. Annual incidence is reported from 0 to 61 in 100 000 cases. It is estimated that about 1% of surgeries are performed due to hydatid cysts over a year (1).

CE diagnosis is controversial and generally made by clinical signs and imaging techniques, including ultrasound, computerized tomography, and magnetic resonance imaging. Even though these techniques are complicated and unavailable in some poor areas, they do not always provide a good prognosis for early detection, and the interpretation requires experience in order to prevent the misdiagnosis of the cyst by a neoplasm (8).

Availability, cost-effectiveness, and no need for advanced equipment made serological tests a preferable method for diagnosis and screening in comparison to imaging techniques (9). Regarding the fact that Nahavand is located in the south of Hamadan, west of Iran, based on the study conducted by Bahrami et al in Hamadan, the frequency of hydatid cyst infection was 0.4% (10).

Nevertheless, several seroepidemiological studies have been conducted in different parts of the country, and few studies have been performed outside the state capital. Since most of the inhabitants are engaged in agriculture, and frequent contact with dogs is undeniable, studies to identify the extent of this zoonotic disease in these areas sound essential. Accordingly, this study aimed to provide the opportunity to raise awareness of the active condition of hydatid infection in the region in order to adopt preventive policies in the management and control of the infection among humans, livestock, and canids.

Materials and Methods

This descriptive cross-sectional study was performed in 2021 from January to December in Nahavand, Hamadan province in western Iran. Blood samples (about 5 mL) were collected from 400 participants referred to Nahavand hospital laboratories (Shahid Ghodoosi and Shahid Alimoradi) using the probability sampling method. Then, basic sociodemographic characteristics, including age, sex, education, job, close contact with dogs, washing vegetables, and living in rural or urban regions were recorded via survey questionnaires, and meanwhile, the informed written consent was signed either.

Blood specimens were transferred to the Parasitology research laboratory at Hamadan University of Medical Sciences. Then, sera were separated after centrifugation at 2000g for 10 minutes and kept at -20°C until antibody detection experiments.

The commercial enzyme-linked immunosorbent assay (ELISA kit), Pishtaz Teb Company (*E. granulosus* IgG / PT-Hydatid-96) was employed for IgG antibody identification against *E. granulosus*. Briefly, serum samples (1:100 dilutions in PBST) were added to flat bottom 96-well microplates, and the plates were washed after 30 minutes. Afterward, horseradish peroxidase-conjugated anti-human antibody (100 µL ready to use solution) was added to the plates and then washed after 30 minutes. Next, chromogen-substrate was added (100 µL to each well), and 100 µL of stop solution terminated the enzymatic process after 15 minutes. Finally, the absorbance (at 450 nm) was measured using a microplate reader (Bio-Tek, ELx800). Sera from surgically confirmed hydatid cyst cases were recruited as positive controls and negative control samples were employed for the cut-off point determination. The samples 10% higher and the samples 10% lower than the cut-off were considered positive and negative samples, respectively (10). Then, statistical analysis was carried out using SPSS version 16, and the chi-square test or Fisher's exact test was performed at a significance level of 0.05.

Results

Out of 400 subjects in this study, five (1.25%) were positive for CE IgG antibodies, and the seroprevalence in men (60%) was higher than that in women (40%). The age range of participants was determined from 3 to 90, whereas the mean age was 58 (±19.9). The majority of seropositive hydatid disease cases were categorized in people aged over 50 in rural areas; however, no significant statistical difference was observed. Most of the subjects (208) resided in urban regions, while 192 subjects lived in rural areas, and among five positive cases, one (20%) was a resident of the city, and four (80%) resided in rural areas. According to the results, three (60%) of positive participants were uneducated, and two (40%) studied until secondary school. Furthermore, self-employed businesses and housewives were the two occupational groups with the highest positive cases (60% and 40%, respectively). Moreover, three (60%) of the positive subjects kept dogs in their habitation, while two (40%) subjects did not have any contact with dogs. Three out of five seropositive participants washed vegetables just with water, and two subjects used disinfectants to wash vegetables (Table 1). Additionally, age, sex, educational level, keeping dogs, residential areas, and vegetable washing had no significant influence on the risk of hydatid cyst infection ($P>0.05$).

Discussion

In the present study, the serum prevalence of hydatid cyst infection was 1.25% among 400 patients referring to health centers of Nahavand in Hamadan. Consistent with the present study, in a project conducted by Ilbeigi et al in Isfahan, the prevalence of anti-IgG cystic echinococcosis was 1.1% among 635 participants (11). Likewise, in the study by Bahrami Moghadam et al in Hamadan, the seroprevalence was 0.4% in the 1000 serum samples (10). Other seroepidemiologic studies in different parts of Iran reported various frequencies. For example, Shafei et al in North Khorasan Province in northern Iran reported a 3.96% prevalence among 932 referred individuals (12). In another study on 220 municipal workers in the northwest of Iran by Asadi et al, the seroprevalence of cystic echinococcosis was estimated at 2.3% (13). The ELISA test was used in most studies due to its high performance, acceptable sensitivity and specificity, and cost-effectiveness. Apart from the differences in the type of coated antigens in the ELISA test, disparate results can be attributed to the varieties in the number of stray dogs, the extent of contact with dogs, climate changes, as well as the manual preparation of food, vegetables, and water sources (14). Livestock rearing in the area and roaming dogs as the final host of parasites have accelerated the transmission of infection (15). As the main occupation of people is farming and stock raising in this area, free-roaming domestic dogs are living in this area, and keeping dogs as pets is becoming widespread as well. Dog-transmitted zoonotic diseases have significant impacts on human public health.

Regarding age, subjects aged 60 and over exhibited

Table 1. Demographic Features and Relative Seropositivity to Hydatid Cyst in People Referred to Hospitals in Nahavand

Characteristics	Groups	Positive Cases No (%)	Negative Cases No (%)	Total No (%)	*P* value
Age	0-9	0 (0)	6 (1.5)	6 (1.5)	0.418
	10-19	0 (0)	12 (3)	12 (3)	
	20-29	0 (0)	52 (13.1)	52 (13)	
	30-39	0 (0)	71 (21.3)	71 (17.8)	
	40-49	0 (0)	76 (18)	76 (19)	
	50-59	2 (40)	66 (16.7)	68 (17)	
	>60	3 (60)	112 (28.3)	115 (28.7)	
Gender	Male	3 (60)	151 (38.2)	154 (38.5)	0.377
	Female	2 (40)	244 (61.7)	246 (61.5)	
Habitation	Rural	4 (80)	188 (47.6)	192 (48)	0.199
	Urban	1 (20)	207 (52.4)	208 (52)	
Education	Uneducated	3 (60)	77 (19.5)	80 (20)	0.319
	Elementary school	0 (0)	78 (19.7)	77 (100)	
	Secondary school	2 (40)	68 (17.2)	70 (17.5)	
	High school	0	52 (13.1)	52 (13)	
	College education	0	121 (30.6)	121 (30.25)	
Job	Unemployed	0 (0)	42 (10.63)	42 (10.5)	0.443
	Self-business	3 (60)	120 (30.3)	123 (30.75)	
	Housewife	2 (40)	176 (44.5)	178 (44.5)	
	Employed	0 (0)	57 (14.43)	57 (14.25)	
Contact with dog	Yes	3 (60)	84 (21.2)	87 (21.75)	0.071
	No	2 (40)	311 (78.3)	313 (78.25)	
Manual vegetable washing	Water	3 (60)	84 (21.2)	87 (21.75)	0.664
	Disinfection	2 (40)	311 (78.7)	313 (78.25)	

the highest prevalence of infection, but no significant relationship was reported. It is undeniable that the risk of infection increases with aging; however, other studies demonstrated that much younger age groups are involved in hydatidosis. For instance, in the study conducted by Zibaei et al in Khorramabad, southwest of Iran, the majority of CE-positive cases were aged between 20-29 (16). Likewise, Mohammadzadeh Hajipirloo et al in the northwest of Iran suggested that the most affected age group is 20-30 (17). According to Rokni, CE is a chronic disease that continues to grow slowly, and the highest rate of hydatid cases is reported to be between 20-40 (1). Since hydatidosis develops years after the original infection (16), one addressing reason for the prevalence of CE in people over 60 in this study can be attributed to the late onset of the disease in the inhabitants of this region, the effectiveness of public health education, as well as the entire adherence to health standards in the younger generation (18).

In the present study, most cases of antibody-positive titer cases were found in the group who did not go to school. Hence, the level of education is directly related to health awareness. In other words, extensive knowledge about hydatidosis as well as the parasite life cycle can substantially prevent the upcoming consequences of infection.

Furthermore, seropositivity was higher in men (60%) than in women (40%). This is expected in men due to their job encounters, social approach, and close contact with dogs. Similar results were documented by Zibaei et al (16) in which 60% of males versus 40% of females were infected. In addition, Andrabi et al (19) reported that 6.31% of men versus 2.54% of women had IgG antibodies against *E. granulosus*. On the contrary, Rafiei et al (20) stated that seroprevalence in women was greater than in men (37.5% in males versus 62.5% in females). However, according to studies in Khuzestan, East Azerbaijan, and Sistan and Baluchistan provinces of Iran, the number of hydatid cysts surgical operations was higher in male than in female cases (21-23).

The results indicated that the prevalence of hydatid cysts in rural areas (80%) is higher than that in urban regions (20%). Based on the Ziaei Hezarjaribi et al in Mazandaran, living in rural areas could be considered as one of the risk factors which increases the rate of infection up to four times (24). Both stray and sheep dogs stay near humans in rural areas, so dog-human contact is generally inevitable, and high antibody titers in rural areas are feasible. Free-ranging dogs are not dewormed. They may occasionally be fed the remnants of slaughtered animals in local butcheries; thus, the infection remains active in these areas (25).

Housewives and self-employed businesses were identified as the two occupations with the highest antibody titers against *E. granulosus*. In Ebrahimpour and colleagues' study,

farmers, housewives, and students have shown infection with hydatid cysts (26). Although cystic echinococcosis is regarded as a job-related infection in sheep farmers, dog owners, and shepherds (27), the presence of antibodies against *E. granulosus* in each job category is expected since Iran is an endemic area for CE (28)

In this study, 60% of positive serologic cases reported the ingestion of undisinfected vegetables. In a study on 1296 serum samples in Jolfa, northwestern Iran, Sakhaei et al found a significant relationship ($P<0.0001$) between raw vegetable consumption and positive serological results of hydatid cyst (29). Contact with unwashed and raw vegetables contaminated by parasite eggs could be considered one of the major leading factors for Hydatid cyst infection.

Conclusion

Overall, the results of this study revealed that although the seroprevalence of echinococcosis is lower than the average prevalence confirmed by previous studies in the country, the disease is more likely to be observed in any group of the community. Preventive measures in these areas should include reducing the contact of stray dogs with human populations, preventing local slaughtering, and avoiding feeding on the offal of slaughtered animals.

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Authors' Contribution

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Competing Interests

The authors declare that they have no conflict of interests.

Ethical Approval

The present study was approved by the Ethics Committee of Hamadan University of Medical Sciences (Ethics Committee reference number: IR.UMSHA.REC.1399.711).

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