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Original Article

Seroprevalence of Human Hydatidosis in Ardabil Province, North-West of Iran

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Abstract

Background: Cystic Echinococcosis is considered a cosmopolitan cyclozoonotic parasitic infection. This study aimed at evaluating the seroprevalence of human hydatidosis using ELISA test and find the role of mutable factors such as age, sex, occupation, residency in the broadcast of the parasites in rural Ardabil Province, North-West of Iran.

Methods: The study population was 950 asymptomatic individuals selected randomly from urban and rural populations of Ardabil province, North-West of Iran by randomized cluster sampling in 2019-2020. Immunoglobulin G antibodies against *Echinococcus granulosus* spp. were analyzed by ELISA test. Data were analyzed using SPSS software and Multivariable logistic regression model.

Results: Overall, 42 (4.4%) of the participants had anti *E. granulosus* antibodies in this region. High titer antibodies were most prevalent in people age group of >70 yr old, rural areas, females and people having history of contact with dog that showed significant difference. There was no significant association between the presence of *Echinococcus* antibodies and sex, occupation, having history of eating unwashed vegetable.

Conclusion: This is the first description of the seroprevalence of *E. granulosus* infection in the population in Ardabil Province, North-West of Iran. Obtained rate of hydatidosis approves the importance of diagnosing human cystic echinococcosis in these regions and it is expected that the authorities be careful to screen the disease.



Introduction

Cystic echinococcosis (CE) is a near-cosmopolitan zoonosis parasitic infection, rendered by larval stages of cestodes belonging to the genus *Echinococcus* (family Taeniidae) (1, 2). Two various forms of cysts including echinococcosis and alveolar echinococcosis that caused by the two major species of medical and public health importance, *E. granulosus* and *E. multilocularis*, which cause cystic echinococcosis (also known hydatidosis) and alveolar, respectively are serious life-threatening diseases. The burden of the disease is very high and causes wide damages in terms of human and veterinary affairs (3, 4). Adult parasites are found in the intestines of carnivores such as dog and wolves as primary definitive hosts. Eggs, passed in feces, infect a large number of mammalian intermediate hosts including sheep, pigs and cattle. Larval stages (hydatid cysts) develop in internal organs mainly liver and lungs and occasionally other organs. The life cycle is completed when offal containing these cysts with viable protoscoleces is consumed by dogs. Humans become infected directly or indirectly via ingestion of eggs excreted with dog faeces (4).

Echinococcosis/hydatidosis is one of the most important zoonotic diseases prevalent in different parts of Iran (5). Previous investigations showed the presence of infection among dogs (6-10), livestock and man (11-14). Human infection is common in countries where sheep and cattle rearing constitute an important industry (15). Cystic echinococcosis is considered endemic throughout the Mediterranean region and has been reported from all the countries of Middle East, via different studies on human and animals, for example, Iran (5, 15). The diagnosis of human hydatidosis is based on ultrasound imaging, immunological methods, and clinical symptoms. Enzyme-linked immune sorbent assay (ELISA) is one of the optimal serological tests for

diagnosis of hydatidosis. Parasite-specific antibodies are detected by this method (16).

Ardabil Province has a high density of stray dogs. Due to the lack of data about human hydatidosis prevalence in our different areas, the present study was carried out to assess the prevalence of human hydatidosis in Ardabil Province, North-West of Iran using ELISA test. Moreover, the epidemiological factors associated with the spread of the disease were determined.

Materials & Methods

Study Area

Ardabil Province is located between 38.2514 °N and 48.2973 °E in northwest of Iran. It covers an area of approximately 17,800 km². Ardabil capital stands about 70 km from the Caspian Sea. Neighbouring is the Caspian Sea and the Republic of Azerbaijan. Population estimated 1,270,420 million (2016). The province is divided into 10 counties: Ardabil (capital), Bilasavar, Germei, Khalkhal, Kowsar, Meshginshahr, Namin, Sarein, Nir, and Parsabad (https://en.wikipedia.org/wiki/Ardabil_Province) (Fig.1).

Samples

The study population was 950 (470 males and 480 females) asymptomatic individuals referred to clinical and health centers in Ardabil Province, North-West of Iran and four counties of Ardabil Province including Parsabad, Nir, Meshkinshahr and Khalkhal, using randomized cluster sampling in 2019-2020. Although based on the statistical calculations the size of sample was estimated as 912, but for more caution, overall 950 people were included in the study.



Fig. 1: Left: map of Iran highlighting the study area, Ardabil Province, Right: map of Ardabil Province showing the capital, Ardabil, and different counties of the province

A written epidemiological questionnaire and informed consent form including the demographic characteristics i.e., age, gender, residency, occupation, contact with dogs, eating uncooked vegetables and history of disease were completed by the individuals. The study was approved by Ethical Committee of Ardabil University of Medical Sciences (IR.ARUMS.REC.1397.264).

For determination of previous contact to *E. granulosus*, in clinical and health centers, blood samples were obtained from all the individuals and transferred to laboratory of Medical Parasitology, School of Medicine, Ardabil University of Medical Sciences, until examination for immunoglobulin G (IgG) antibodies. Afterward, the blood samples were centrifuged for 10 min at 1900 g at 4 °C. Then serum was transferred into another tube and stored at -80 °C until ELISA examination.

ELISA test

Human anti-*E. granulosus* IgG assay was carried out on sera by enzyme-linked immuno-

sorbent assay (ELISA) in 96 well micro plates. For anti-*E. granulosus* IgG assay, the VIRapid® HYDATIDOSIS (Vircell, Granada, Spain) test was performed according to the manufacturer's recommendations, and each test was read independently by 2 different operators. The OD was registered at 450 nm with an ELISA reader (Tecan, Switzerland).

Statistical analysis

All data were analyzed using SPSS software ver. 22 (Chicago, IL, USA) and Multivariable logistic regression model. Results were considered statistically significant when the *P*-value was less than 0.05.

Results

The cut-off was calculated as 0.35. The result of seroprevalence study of human hydatidosis was detected as 4.4% (42 cases) by ELISA test in this area (Fig. 2).

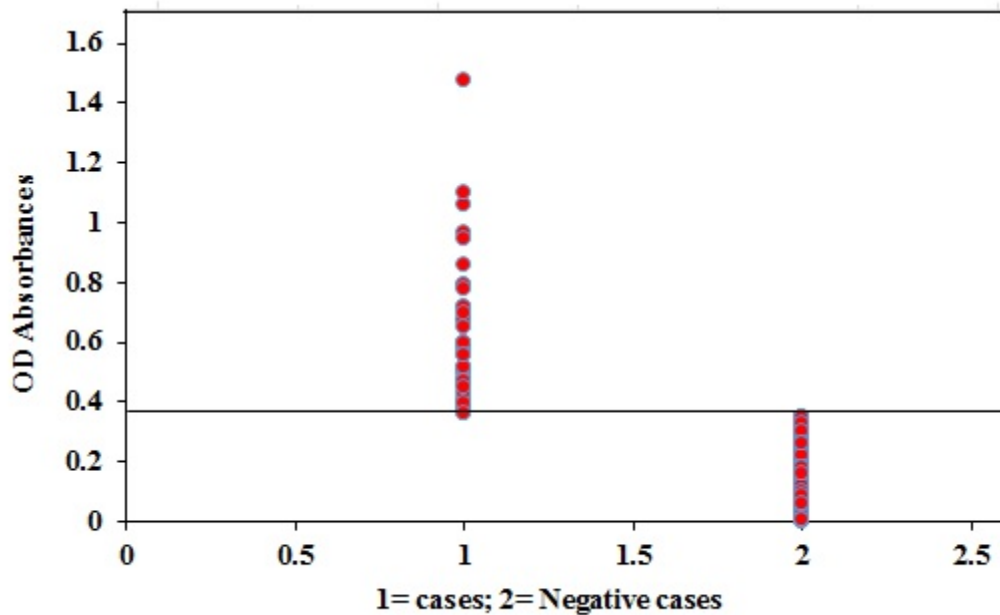


Fig. 2: Analysis of sera from subjects and normal controls by IgG-ELISA

The seroprevalence rate was higher among the people age group of >70 yr old (n=7, 50%) but there is no statistically significance.

Other age groups' infectivity is obvious in Table 1.

Table 1: Distribution of positive cases of hydatidosis using ELISA according to age group (yr) in Ardabil Province, North-West of Iran

Age group (yr)	Sample taken (No.)	Positivity (No.)	Seroprevalence (%)
1- 19	75	2	2.66
20- 29	320	12	3.75
30- 39	249	6	2.40
40- 49	165	4	2.42
50- 59	83	5	6.02
60- 69	44	6	13.63
> 70	14	7	50
Total	950	42	100

Seropositivity was more prevalent in females (5.2%) than in males (3.6%), and showed no significant difference.

Rural areas prevalence was significantly higher (5.6%) than the urban area (3.7%) ($P<0.05$). There was no significant association between CE seropositivity and people job and eating unwashed vegetable in the study. As

regards occupations, freelance workers had the highest rate of infection (5.1%). The rate of the disease was 10.8% in people having history of contact with dog ($P<0.05$). The prevalence of disease in people with history of unwashed eating raw vegetables was higher than other people. However, there was no statistically significance.

Discussion

Cystic echinococcosis (CE) has a worldwide distribution. CE is an emerging neglected disease and one of the major Human and animal health problems in the Middle East and Iran for a long time is regarded as an endemic area for hydatidosis (17). Globally the burden of disease in the regions with developed sheep and cattle industry is very high comprising Oceania, Europe, China and Central Asia, parts of Africa, and the Americas (18). The overall seropositivity of CE among general population in the Middle East estimated 7.44% (19). In a systematic review and meta-analysis study, the weighted prevalence of human hydatidosis in Iran was 4.2% (20). The prevalence of cystic echinococcosis (CE) and alveolar echinococcosis (AE) in Iran from 1990 to 2017 was 5% and 2% respectively (21). In a 15-year retrospective epidemiological study, during 15 years 501 CE surgical cases in southwest Iran were recorded that annual incidence was 33.4% (22).

The annual incidence in Iran from various cities was as follows: Hamadan 1.33/100000, Kashan (Isfahan) 3/100000, Babol 1.18/100000 and entire of Iran 0.61/100000 (15). Ardabil Province is among the biggest territories for rearing domesticated farm animals that this specific situation contributes to the distribution of the parasite in this region (23). Many studies have shown the rate of human hydatidosis in several parts of Iran (15). Accordingly the rate of infection has been reported as Zanjan 3% (24), Golestan 2.34% (25), Meshgin Shahr 1.79% (26), Qom 1.6% (27), Arak 3.46% (28), Isfahan 1.1% (29) and Alborz 3.4% (30). In this study, the seropositivity of human hydatidosis using ELISA test in Ardabil Province, North-West of Iran and suburb area, was found 4.4% (42 cases) that show more or less similar rate with other parts of Iran.

In the current study, the highest rate of infection was in age group of >70 yr old (50%)

which is in contrast with other results (30). Moreover, other studies have shown the 60-69 yr old as the highest infected age group in Isfahan (29), 60-80 yr old in Hamadan (31), and 60-90 yr old in Meshgin Shahr (26). The highest rate of infection in the age group of >70 in the present study and comparison with other studies in the world maybe because the infection occurred at a young age and secretion of hydatid cyst antigens occurred at an older age or because people in this age group of >70 yr in the study area are more in contact with dogs and their surroundings (32, 33).

In this study, infected females were more prevalent than males but showed no significant difference. Although in several studies in Iran demonstrated more seropositivity in males than females (26, 29, 30). Moreover, in Iran, females were more infected than males, e.g., in Golestan Province (4.7% vs. 2.3%) , and in Zanjan 3.2% vs. 2.7% (24, 34).

Comparing the results of the present study and other studies, the higher prevalence of hydatid cyst among women in Ardabil Province may be related to the fact that women are exposed to hydatid cyst infection due to their way of life and activities in the region; both at home and outdoors, more than men (35). Among patients who had hydatid cyst operations in Milad hospital, the highest rate of infection was observed in females (56.5%) (36). The higher prevalence of hydatid cyst was observed in women in Northern Iran from 2005 to 2015 (37). In addition, similar results were seen in East Azerbaijan Province during 2009-2011 (38).

Sources of infection with hydatidosis are varies such as eating uncooked meat, eating unwashed vegetable, contact with domestic animal/dog, soil, etc. Because of different cultures and geographical conditions, hydatidosis risk may differ between males and females (15). In this study rural areas prevalence was significantly higher (5.6%) than the urban area (3.7%) which might be a result in their closer contact with animals and poor personal hy-

giene. This finding is in concordance with other studies that reported 6.98, 1.46% prevalence for rural and urban life in Arak Province respectively (28). People living in rural areas of Mazandaran Province, northern Iran showed highest rate of hydatid cyst infection than urban area (39).

People living in rural areas were probably more in close contact with the contaminated environment and had a lot of contact with the dog's living environment however, Rakhshanpour et al, have shown a predominance of CE in urban populations (2.1%) (27). The majority of the hydatid cyst patients were living in rural areas (38). Similar results were seen in Meshkinshahr District, Ardabil Province, Iran but they showed no significant difference (26).

According to occupation, freelance workers had the highest rate of positivity (5.1%), but in some other studies, farmers, employees, housekeepers encompassed the highest rate of infection (28, 30). Greengrocer, rancher, farmer and housekeepers had low rate of hydatid cyst infection compared with other occupation in Mazandaran Province but showed no significant difference (39).

In this study, the rate of hydatid disease was 10.8% in people having history of contact with dog and higher than other people, that this result is an agreement with some other studies performed in Iran (26, 39-41). In the present study, although the prevalence of disease in people with history of unwashed/raw vegetable eating was higher than other people but there was no statistically significance. However, in some other studies, there was significant association between history of unwashed eating raw vegetable and rate of infection (28, 39, 40).

Overall, due to relatively high prevalence (4.4%) of hydatidosis among people referred to clinical and health centers in Ardabil Province, it is necessary to pay attention to control strategies such as preventive measures and public health education.

The limitation of this study might have been the sampling and the method of antibody detection used.

Conclusion

The prevalence of hydatidosis is an issue of high importance in this region and demonstrated that CE is a public health problem in Ardabil Province, North-West of Iran. There is an urgent need for increasing the people's awareness about the disease in exposed populations. Implementation of infection control and prevention programs can help to control and reduce infection in humans and livestock in the region and the country.

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Conflict of interest

The authors declare that there is no conflict of interest.

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