Serological survey of *Echinococcus granulosus* in nomads of southwest Iran using the ELISA method during 2015-16

Razieh Kasaei¹, Mehdi Tavalla², Hosein Etebar²

¹Department of Parasitology, School of Medicine, Student Research Comittee, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran; ²Department of Parasitology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

**SUMMARY**

Echinococcosis is one of the diseases common to humans and animals worldwide and its metacestode form *Echinococcus granulosus* is the cause of disease. Diagnosis based on clinical symptoms is a problem. However, using serological methods such as ELISA, specific antibodies can be determined. Based on the medical and economic importance of the disease, this study aimed to undertake a serological survey of human echinococcosis in Behbahan nomads using the ELISA method during 2015-16. In a descriptive study, 180 blood samples were collected and analysed from the tribes in the four geographic regions of Behbahan city. The obtained sera were assessed for the presence of IgG antibodies against hydatid cyst. Demographic data were collected using a questionnaire and the results were analysed by SPSS software. In all, 175 persons (97.2%) tested negative while five (2.8%) were positive. The most frequent was in people over 50 years with three persons (1.7%). In the 40-50 years age group, two subjects (1.1%) tested positive. 62 (34.4%) of the subjects were female and 118 (65.6%) were male, among which four men (2.2%) and one woman (0.6%) were infected. Overall, the most infected were men. Of the infected persons, three lived in the East, one in the South and one in the West of the city. All subjects had a history of contact with dogs. According to the results of this study, the prevalence was high in the area and therefore disease control programmes are recommended.

**Keywords**: echinococcosis, nomads, ELISA, Behbahan, Iran.

**INTRODUCTION**

Hydatid disease echinococcosis or hydatidosis is one of the most important zoonotic parasitic diseases in the world including Iran caused by the larvae of the cestode *Echinococcus granulosus* in humans and herbivorous animals [1]. The disease is well distributed in most parts of the world, especially in countries where animal husbandry is common [2]. It is an endemic public health problem in some Mediterranean, Asian, South American and North African countries and Australia [3].

Iran is an hyperendemic area [4] as the disease has been reported in all provinces in the country with the highest infection rate in humans (4.5 in 100,000) from Khorasan province and the lowest (0.1 in 100,000) from Hormozgan province. The average of surgical cases for the whole country is estimated as 1.2 in 100,000 [2].

In humans, the disease is mostly limited to areas where there is constant contact with domestic and wild canines and herbivores with humans. Due to poor hygiene associated with nomadic life, increasing cattle and sheep rearing using traditional methods, access of dogs to infected viscera and offals of slaughtered and dead sheep, the life cycle
of the parasite is continuously established [4]. In rural and tribal areas, many flock dogs are contaminated with *Echinococcus granulosus* and thus infect pastures with faecal droppings containing the parasitic eggs. The sheep are infected with the cyst by grazing in these pastures. Also, humans are infected (as accidental host) when they ingest infected vegetables and water and or direct contact with infected dogs [5]. Therefore, rural dwellers who are herdsmen are easily infected due to more contact in comparison with people who live in urban areas [6]. Usually, in human the liver and lungs are the infected organs. Clinical and pathological signs of the disease depending on the location, size and effects of space-occupying cysts differs [1]. Often, the time between infection and appearance of symptoms vary from a few months to even years [7]. Echinococcosis is a malignant disease among humans because diagnosis and treatment requires time and enormous cost and is sometimes futile and fatal [8]. In addition, large hydatid cysts in the liver and lungs of sheep and cattle can result in significant economic loss to the meat industry through condemnation of the infected organs [9]. Therefore, there is need for comprehensive and continuous evaluation of the disease in the country [8]. The most striking echinococcosis clinical specification is that resulting infection may be completely without symptoms [10]. The treatment of choice is surgery and may not achieve because of high medical expenses, problems and complications [11].

Diagnosing the hydatid infection is complex and requires multiple paraclinical, lab investigations (serological methods) and imaging techniques (X-ray, ultrasound, CT and MRI), which usually confirm the clinical suspicion [12]. But imaging methods are sometimes limited by the small size of the lesion and the atypical images which are not easy to be distinguished from abscesses or neoplasms [13]. Meanwhile, radiological techniques are often too expensive or are not available in many areas where hydatidosis is highly endemic [14]. Also the early stages of disease are asymptomatic [15]. Since diagnosis of this disease by clinical symptoms and scanning alone is often difficult and confusing, some reliable and sensitive serological tests are required to corroborate the evidence reached [16]. The serological tests apply in order to confirm or refute the clinical suspicion of hydatidosis [12]. Also this tests for diagnosing hydatid infections in people living in areas where the disease is endemic are useful because of the low cost and ease of performance [14]. Therefore, early detection of the disease using methods of sensitive immunology has lead to better treatment and reduced deaths [7].

Complement fixation test (CFT) was the first immunological test used for serodiagnosis of CHD. Since then, a wide number of immunological tests have been developed for the detection of hydatid antibodies and of late hydatid antigens in the serum. The hydatid anti-based serological tests include indirect haemagglutination (IHA), indirect immuno-fluorescence (IFA), immunoelectro phoresis, counter-current immuno electrophoresis (CIEP), radio-immunoassay (RIA) and ELISA. Development include enzyme-linked immunoelectrotransfer blots (EITB), enzyme-linked immunoelectrodiffusion assay (ELIEDA), time-resolved fluoroimmunoassay (TR-FLA) and immunoblot [16].

Routine laboratory diagnosis of CE is dependent on detection of specific antibody response [13]. The most suitable of immunoglobulin for the detection of echinococcosis is IgG, because its level in the blood is high long after surgery or drug therapy [17]. In 1975, Farag used ELISA for the diagnosis of hydatid cyst. ELISA is a quantitative serological method used for the diagnosis of hydatidosis and optical density (OD) is measured with the reader after adding substrate [18]. Other advantages of ELISA include high sensitivity and specificity, application to a large number of samples at a time, and it is suitable for seroepidemiological studies [17]. Sensitivity of the ELISA method was determined to be 92.5% and the specificity was found to be 97.3%. Positive and negative predictive values were 92.5% and 97.3%, respectively [13]. ELISA is a useful technique for population screening, where corrections of the results can be made through statistical calculation [12].

Fotiou and colleagues in 2012 reported a 1.1% prevalence of the disease in Greece [19]. Shambesh and colleagues reported a prevalence of 11.2% in Libya [20].

Until now, many researchers from various provinces of Iran have reported the prevalence of human echinococcosis using the ELISA method [17]. Rafiei and colleagues reported a 13.8% prevalence of human hydatid cyst in Khuzestan province us-
ing ELISA [21]. In a study by Heidari in Meshkineshrar in Ardabil, the prevalence was 1.79% [22]. In another study by Garedaghi and Bahavarnia, the prevalence in East Azarbaijan province was 1.28% [17]. Mirzanejad-Asl and Fasihi Harandi reported the a prevalence of 9.2% in Mohgan plain in Ardabil province [10]. In Another study by Esmaeili and Arbabi among the adults in urban and rural areas of Kashan, the positive cases were 3.05% [1]. Afkali and colleagues reported 1.2% positive cases in Ilaam [23].

According to literature, hydatid cyst infection is one of the most important zoonotic diseases. Public health and economic status are directly effective in controlling its infection, and based on recent studies, Iran is among echinococcosis endemic areas in the world, hence the necessity to carry out this research among tribes in the region [24].

**RESULTS**

In this study, 45 samples were selected from each geographical area of North, South, East and West of Behbahan making a total of 180 samples. 118 (65.6%) of the subjects were male and 62 (34.4%) female. Also according to age, 37 persons were less than 30 years, 40 persons between 30-40 years; 39 persons were 40-50 years and 64 persons were more than 50 years.

Of the 180 subjects 175 (97.2%) had negative test result while 5 (2.8%) were positive. Most people were over 50 years old (3 persons; 1.7%) and most positive results were reported in the 40-50 years age group (2 persons; 1.1%) (Table 1). Among the subjects there were 4 male (2.2%) and 1 female (0.6%) with positive test results with the most frequent in men (Table 2).

According to questionnaire, 119 persons had contact with dogs while 61 persons reported no contact. The results showed that all 5 positive samples had contact with the dog. In terms of geographic location of infected people, three persons were from the East and one each from the South and West.

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**Table 1 - Frequency of positive and negative tests according to the patients age.**

<table>
<thead>
<tr>
<th>Age</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 30 years</td>
<td>37</td>
<td>0</td>
</tr>
<tr>
<td>30-40 years</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>40-50 years</td>
<td>37</td>
<td>2</td>
</tr>
<tr>
<td>More than 50 years</td>
<td>61</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>175</td>
<td>5</td>
</tr>
</tbody>
</table>

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**ELISA procedure:** 5 ml sera were placed in four wells with two wells each cut off for positive and negative control; 5 ml serum was placed in the rest of the wells. Then 100 ml serum diluent was added and after shaking, and homogenizing the wells were then covered and incubated at 37°C for 45 minutes. The wells were then drained and rinsed 5 times and after draining, 100 ml conjugated solution was immediately added to the wells, and incubated at 37°C for 30 minutes. After 5 washes, 100 ml substrate was immediately added to the wells and then placed for 20 minutes at room temperature in the dark. Finally, the reaction was stopped after adding 50 ml solution. The optical density of wells was read at 450/620 nm wavelength of.

Data and results were entered into SPSS software and T-test and chi-square were used for result analyses.
By comparing average age with percentage contamination, there was no significant difference at 5% (p=0.236). Between the positive cases and their genus, no significant difference (p=0.491) was obtained. Also geographical area had no effect on test results (p=0.272).

### DISCUSSION

Hydatidosis is one of the most important parasitic diseases in the world. In Iran, animal and human hydatidosis has high prevalence. Dogs as definitive host play a very important role in the transmission of disease in Iran. In a study, prevalence in different parts of Iran in herd dogs was reported to be from 5 to 49% [25]. Similar studies have shown that 20% of infection was in dogs in Khuzestan province [21]. Due to animal husbandry among nomads, the definitive host (dog) is great and so the cycle of transmission of infection is easily possible.

This study is the first serological survey of human echinococcosis in Behbahan nomads. In this study, the prevalence among nomads was estimated to be 2.8%. Infection was 2.2% in men and 0.6% in women. This could be due to men’s involvement in more farming and pastoral activities leading to continuous contact with dogs. Of course, most of the studies in Iran reported higher infection rates in women more than in men. In our survey, there was no significant difference observed between the disease and gender. The highest percentage of positive cases was in the “above 50 years” age group, which is consistent with other studies. Also, there was no significant difference observed between positive cases and age group. All the infected people came in contact with dogs.

In terms of geographic region, 1, 1 and 3 persons from South, West and East, respectively were infected but no significant difference was observed between geographical area and the level of contamination.

In a survey, Rafiei and colleagues reported echinococcosis infection rate of 13.8% in Khuzestan province; 18.2% in Izeh; 17.3% in Masjed Soleiman, 12.4% in Shoush and 1.9% in Behbahan. However in this study, the infection rate in Behbahan is higher (2.8%). The highest contamination levels reported in their study was in shepherds (15.8%) and 18.5% in farmers; these results are consistent with our study. Also in Rafiei’s study, there was no significant correlation between positive cases and gender, job and dog keeping [21]. The prevalence rate in Mirzanejad-Asl and Fasibi Harandi’s study in Moghan plain in Ardabil province was 9.2% which is not in agreement with this study perhaps, due to the use of more samples in their study. The most infection in this study was in people over 50 years at a rate of 9.5%, which is consistent with this study. This can be explained that having been infected at an early age and the antigen of the cysts is secreted at an older age, or because this age group have more contact with dogs [10]. In a study of Garedaghi and Bahavarnia in East Azarbaijan province, the highest infection rate was dependent on rural population and was reported to be 1.8%. Based on the results of this research, Sarab with 2.17% had the highest percentage of positive cases. Most of the rural population lived in this city and were mostly involved in agriculture and animal husbandry which is consistent with the results of this study, as our population was nomadic farmers involved in animal husbandry [17]. In a study by Aflaki and colleagues in Ilam province, the contamination level was 1.2% i.e 0.56% in the urban population, 1.57% in the rural population and 10.77% in the nomadic population which is the largest percentage of infection. This result is consistent with our study [23]. In another study by Heidari and his colleagues in Meshkinshahr in Ardabil contamination level 1.79% was reported. Infection in men was 2.6% which was more than in women with 1.68%. In terms of job, farmers and ranchers with 3.17% had the highest percentage of contamination, which is consistent with the current study [22].

Due to the high prevalence rate reported, prevention and disease control are essential and public education should be prioritized for the region’s health.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>61</td>
<td>1</td>
</tr>
<tr>
<td>Male</td>
<td>114</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>175</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 2 - Frequency of positive and negative tests according to the patients gender.
Conflict of interest: The authors declare no conflict of interests.

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REFERENCES


