

Research Paper

Serological Investigation of Human Cystic Echinococcosis and Associated Risk Factors in the At-risk Population of Northeast Iran in 2018-2019

Mitra Salehi¹, Akbar Solati^{2*}, Ahmadreza Atarodi^{3*}, Jafar Hajavi⁴, Hossein Nezami⁵

1. Department of Parasitology, Faculty of Para Medical Sciences, Iran University of Medical Sciences, Tehran, Iran.

2. Department of English, School of Medicine, North Khorasan University of Medical Sciences, Bojnurd, Iran.

3. Social Development and Health Promotion Research Center, School of Medicine, Student Research Committee, Gonabad University of Medical Sciences, Gonabad, Iran.

4. Department of Basic Sciences, Faculty of Allied Medicine, Gonabad University of Medical Sciences, Gonabad, Iran.

5. Department of Epidemiology and Statistics, School of Health, Gonabad University of Medical Sciences, Gonabad, Iran.



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**ABSTRACT**

Background: Hydatidosis is a leading zoonotic parasitic disease causing economic losses and public health concerns worldwide. The current study aims to determine the seroepidemiological status of hydatid cysts (HCs) and assess the relationship between risk factors and this disease in high-risk individuals in Gonabd City, Iran.

Methods: This cross-sectional study was conducted in Gonabad health centers in 2018-2019. Sampling was performed using the census method. A total of 393 serum samples were collected from 259 men and 134 women at risk for HC. A questionnaire was prepared for all participants. Demographic information, such as gender, age, place of residence, education level, participation of children in animal husbandry, the way of washing vegetables, and work experience in animal husbandry were recorded. The levels of immunoglobulin G (IgG) antibodies were measured by enzyme-linked immunosorbent assay (ELISA).

Results: The infection rate was 9.7% for men and 10% for women, showing no significant correlation between gender and hydatidosis. Among the affected population, the prevalence rate was 10.4% in undergraduates and 16.3% in the age group of 20-30. Additionally, individuals with less than ten years of experience in animal husbandry had a prevalence rate of 11.7%. Those who had contact with dogs had a prevalence rate of 7.9%, while individuals who did not use disinfectants to wash vegetables had a higher rate of 12%.

Conclusion: This study highlights the significant prevalence of HCs in at-risk individuals. This indicates the need for effective preventive measures to reduce the burden of infection. The recommendations include washing vegetables with disinfectants to eliminate any potential sources of contamination. Additionally, obtaining identification certificates for livestock dogs and collecting stray dogs can help control the spread of the disease. It is essential to implement a comprehensive public health strategy to address this issue effectively.

Keywords: Echinococcosis, Enzyme-linked immunosorbent assay (ELISA), Prevalence, Iran

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*** Corresponding Authors:**

Akbar Solati, Associate Professor.

Address: Department of English, School of Medicine, North Khorasan University of Medical Sciences, Bojnurd, Iran.

Phone: +98 (939) 9910325

E-mail: great1351solwati@gmail.com

Ahmadreza Atarodi

Address: Social Development and Health Promotion Research Center, School of Medicine, Student Research Committee, Gonabad University of Medical Sciences, Gonabad, Iran.

Phone: +98 (938) 9532886

E-mail: a.atarodi.b@gmail.com

Introduction

H ydatidosis is a disease caused by a parasitic tapeworm called *Echinococcus*. This disease can affect both animals and humans and is most prevalent in areas where people raise livestock and keep livestock dogs. The disease is particularly burdensome in Asian countries, such as Lebanon, Jordan, Syria, Iraq, Saudi Arabia, and Iran, where it is considered a major endemic zoonotic disease [1-7].

Dogs are the main carriers of *Echinococcus granulosus* (*E. granulosus*) and are crucial in spreading the infection in affected countries. Humans can accidentally become infected by ingesting parasite eggs in the feces of these infected dogs [8, 9]. Despite efforts to control and prevent hydatid disease, it still poses a significant risk to both humans and animals [10]. This disease, known as hydatid cyst (HC), causes substantial economic losses and public health issues for domestic animals and humans [11, 12]. The annual economic loss due to this disease is estimated to be around \$232.2 million [13].

Furthermore, in Iran, a significant number of livestock are affected by HCs [14, 15]. The prevalence of HCs in Iranian dogs ranges from 5% to 49% [16, 17]. The incidence of HCs in humans is reported to be 0.6-1.2 cases per 100000, indicating a high rate of infection [18]. A 10-year study in Tehran City, Iran identified 203 cases of human HCs, while another study conducted in Khorasan Razavi Province, Iran reported 1759 cases over 20 years [16, 19]. In provincial medical centers, the incidence of HC surgery cases is estimated to be 1.18-1.3 per 100000 [15]. Serological techniques, such as enzyme-linked immunosorbent assay (ELISA), are valuable for diagnosing HCs in endemic areas, especially when clear symptoms are absent. These tests can detect the presence of the disease and differentiate it from other cystic lesions with high sensitivity and specificity [20, 21]. Additionally, serological tests are beneficial for monitoring patients' progress after treatment or surgery [22, 23].

The conditions of animal husbandry and the presence of herd dogs and the increase in the population of stray dogs endanger the health of humans and animals in Gonabad City, Khorasan Razavi Province. To the best of our knowledge, no similar study was found in the reviews to determine the seroepidemiological status of HC and assess the relationship between risk factors and this disease in high-risk individuals in this area. Therefore, the current study was conducted to achieve this goal.

Methods

Study area

This analytical cross-sectional study was conducted in Gonabad City in Khorasan Razavi Province in eastern Iran with about 5902 square kilometers in geographical location is 34° 20' 20" north and 58° 42' 3" east. This city has been evaluated with an average rainfall of 166 mm per year except for arid, semi-arid, and semi-desert areas. According to the 2016 census, the population of this city is 88753 people.

Sampling and sample size

Sampling was performed using the census method, and individuals with livestock and herding dogs, as well as those at risk were identified, by referring to health centers. Data were collected between April and March 2020 utilizing both a questionnaire and observation method. The sample size was determined to be 380 using the Equation 1 based on a confidence level of 95%, a margin of error of 5%, and a prevalence rate of 4.42%. To account for potential sample loss, the sample size was increased to 401.

$$1. n = \frac{z^2 p(1-p)}{d^2}$$

Participants

After the research proposal was approved by the university's student research committee and ethical clearance was obtained, residents under the coverage of healthcare centers were selected for the study. First, information on all comprehensive healthcare centers and health houses under the supervision of the [Gonabad University of Medical Sciences](#), along with the population under their coverage in urban and rural areas, was obtained. Then, six centers were selected among the available centers. The people at risk in each center were identified with the help of designated personnel of each center and invited to participate. The number of samples from each center was proportionally calculated based on the specified sample size and the total sample size. Once the participants were briefed on the study's objectives, they were requested to fill out the questionnaire and provide consent. The inclusion criteria included shepherds and individuals who have contact with dogs. On the other hand, the exclusion criteria included individuals without contact with dogs or a livestock occupation. Out of the 401 individuals who visited health centers, 393 completed the questionnaire and consent form and were eligible for the study. The questionnaire was designed to gather demographic de-

tails, including gender, age, place of residence, education level, involvement of children in animal husbandry, vegetable washing method, and work experience in animal husbandry. Subsequently, 5 mL blood samples were collected, with the serum samples promptly separated and stored at -20°C for further analysis.

Statistical analysis

We utilized a commercial human hydatid detection kit (Echino ELISA kit) according to the manufacturer's instructions to test for immunoglobulin G (IgG) antibodies against HC. Analytical statistics were reported using the chi-square test and data were analyzed using SPSS software version 20. The collected data was entered into the SPSS, and after ensuring the accuracy of the data entry, frequency distribution tables were used to describe the qualitative variables. For quantitative variables, appropriate statistical measures, such as Mean±SD were used. Additionally, the chi-square test and independent t-test were employed to analyze data. A significance level of 0.05 was considered in this study.

Results

The present study included 393 cases, with 259 men (65.9%) and 134 women (34.1%) (as livestock farming is considered a profession, the number of men is expected to be higher). Among the participants, 62 people (15.8%) lived in urban areas and 331(84.2%) in rural areas. The study showed that 10% of men and 9.7% of women tested positive for IgG. No significant relationship was observed between sex and the presence of HC antibodies. Additionally, 12.9% of the urban residents and 9.4% of the rural residents had anti-HC antibodies, with no significant relationship between residence and the presence of antibodies. In terms of education level, 338 participants (86.0%) had education below a diploma, 44(11.2%) had a diploma, and 11(2.8%) had a university education. Among them, 10.4% of individuals with education below a diploma, 6.8% with a diploma, and 9.1% with a university education tested positive for IgG. No significant relationship was observed between education level and hydatidosis.

The results of the independent t-test showed no significant difference in the mean age between individuals with a positive IgG result and those with a negative IgG result. Additionally, the results of the chi-square test indicated no significant statistical relationship between the IgG result and variables, such as gender, place of residence, level of education, having children, consumption of raw vegetables, owning a dog, number of years of work experience in animal husbandry ($P<0.05$).

In this investigation, 365 people provided information on their period of working in animal husbandry and 28 people refused to provide information. The presence of antibodies was observed in 11 cases (4.12%) with a history of animal husbandry for 40-55 years, and in 11 cases (7.11%) with less than ten years, and no significant relationship was observed between the history of animal husbandry and HC ($P=0.506$) (Table 1). According to the observed results, in the analysis of a variable, none of the studied variables show a significant difference. Therefore, these variables cannot be used in multivariate analysis.

Discussion

The present study showed that 10% of high-risk individuals in Gonabad City had antibodies against HC. Serological tests are inexpensive methods that are widely used and can be performed on a large number of samples in a short time. Previous studies conducted by the ELISA method in different parts of Iran revealed a rate of 1.2%-21.4% for disease [4, 19]. In some studies conducted in the different parts of Iran (applying seroepidemiological and ELISA methods), the prevalence of human hydatidosis was reported as follows, 1.6% in Qom Province, 7.2% in Yasuj City, 3% in Zanjan City, 2.4% in Kashan City, 1.8% in Meshkinshahr City, 1.2% in Ilam City, 2.3% in Golestan Province, 13.8% in Khuzeestan Province, 7.5% in East Azerbaijan Province, 13.7% in Fars Province, 8.2% in Kerman Province and 8% in Kermanshah Province, Iran [13, 24-30].

The mean prevalence rate of hydatidosis in Iran is estimated to be 5%, and its prevalence rate is 12% in the farmers, which is consistent with our results [30]. A study conducted by Ebrahimipour et al showed the highest (15.2%) and lowest (0.7%) rates of the disease in the Northeast and Southeast, respectively and their results are consistent with our results [31].

In an investigation in Fars Province conducted by Sarkari, the prevalence of hydatidosis was evaluated at 6.7%, and inconsistent with our results, no significant relationship was observed between the age, level of education, having a dog, residence, and prevalence of HC [32]. In another study conducted in East Azerbaijan Province by Mehrabani et al et al, the highest rate of HC surgery was observed at the age of 40-60 years, which was the same as our results, in which the highest prevalence of the antibodies was at the age of 20-30 years (16.3%) and 50-60 years (12.3%) [33]. The same results were achieved in the Hamadan and Kermanshah Provinces conducted by Barati et al and Vajdani et al, which declared the highest rate of HC antibodies in the age

Table 1. Relationship between IgG titer and studied variables

Variables		Mean±SD/No. (%)		P
		Positive	Negative	
Age (y)		41.46±16.76	44.83±17.53	0.241**
Gender	Male	26(10.0)	233(90.0)	0.916*
	Female	13(9.7)	121(90.3)	
Residence	City	8(12.9)	54(87.1)	0.393*
	Village	31(9.4)	300(90.6)	
Education	Below diploma	35(10.4)	303(89.6)	0.76*
	Diploma	3(608)	40(93.2)	
	University degree	1(9.1)	10(90.9)	
Children	Yes	19(12.5)	133(87.5)	0.175*
	No	20(8.3)	221(91.7)	
Using of disinfectant for washing vegetables	Yes	25(9.1)	251(90.9)	0.37*
	No	14(12.0)	103(88.0)	
Dogs	Yes	28(9.7)	261(90.3)	0.795*
	No	11(10.6)	93(89.4)	
Age (y)	<20	4(10.0)	36(90.0)	0.58*
	20-30	7(16.3)	36(83.7)	
	30-40	8(10.0)	72(90.0)	
	40-50	7(7.9)	82(92.1)	
	50-60	8(12.3)	57(87.7)	
	>60	5(6.6)	71(93.4)	
Work experience in animal husbandry	<10	11(11.7)	83(88.3)	0.506*
	10-25	4(8.0)	46(92.0)	
	25-40	7(10.6)	59(89.4)	
	40-55	11(12.4)	78(87.6)	
	>55	3(4.5)	63(95.5)	

*Chi-squared test, **Independent samples t-test.



group of 25-40 years and age >40 years with no significant relationship between age and hydatidosis [34, 35]. In Khorramabad City, Zibaei et al reported a prevalence of 5.4% anti-hydatid antibodies, with the highest rate of antibodies in the age group of 20-30 years, which is similar to our results [36]. Mahmoudvand et al reported a rate of 2.84% hydatidosis in the neighborhood province, Lorestan [37].

In a study conducted by Heidari et al, the rate of hydatidosis was higher in men than women in Ardebil Province, northwestern Iran [26] while the rate of the disease was higher in the women population in North Khorasan Province in studies conducted by Salehi et al [18, 19]. In contrast, no significant relationship was found between sex and hydatidosis in our study (P=0.916).

Rafiei et al estimated the prevalence of 13.7% for hydatidosis in Nomads in southwest Iran with humid and warm weather [27]. In our study, the prevalence of hydatidosis in Gonabad City with warm weather was achieved at 10% in the at-risk population.

A study conducted by Zeinali et al showed that Khorasan Razavi Province with an incidence of 91 per 100 000 people has the highest rate of HC infection [38] and Gonabad is located in the Province. In previous studies, it has been shown that the rate of hydatidosis is higher in rural areas than in urban [39, 40] but in contrast, our results revealed that the rate of the disease is higher in urban areas. The reason may be related to the high number of stray dogs in the city and adjacent to urban and rural areas. Also, agriculture and livestock have boomed around Gonabad City. In this study, no significant relationship was found between habitat and the presence of hydatidosis ($P=0.393$), which is similar to the results of the studies conducted by Gholami et al in Ahvaz City, Iran [39]. In another study, a HC was reported from the arm of a 40-year-old woman [40]. In a study in Fars Province, out of 1500 people, 131 people were infected with HCs [41]. In a 19-year study in Qom Province, 156 people were infected with HC [42].

In the present study, no significant relationship was observed between keeping a dog and having a HC, and this is similar to the results of a study conducted by Zeinali et al in Meshkinshahr City (Ardebil Province) [38] and by Kamali et al in Khuzestan Province [43]. Although, it has been shown that dogs play a crucial role in the development of HCs, and the incidence of HC disease in people in contact with dogs is 9.7% [44].

Our results elucidated the higher incidence of hydatidosis in populations with a lower level of education, which is consistent with other studies [40, 45]. It was declared in the previous studies that contaminated vegetables with dogs feces can cause HCs [44, 45] and the people who did not use disinfectants to wash vegetables have a higher percentage of anti-hydatid antibodies [15]. In our study, the relationship between the way of washing vegetables and hydatidosis is mentioned with no significant correlation between them ($P=0.37$). The present study evaluated the relationship between work with livestock and hydatidosis and the results revealed no significant relationship between the history of animal husbandry and HC ($P=0.506$). Also, in this study, for the first time, the level of children's participation in animal husbandry was measured, which showed that the people with whom the children participated had a higher prevalence of IgG antibodies, which may be explained

by playing with dogs in leisure time and lack of attention to health factors by children.

Conclusion

The high prevalence of stray dogs, the absence of health certificates for herding dogs, inadequate cleanliness practices, and dogs having access to infected animal remains contribute to the high contamination levels in Gonabad City. To prevent the spread of this disease, it is recommended to gather stray dogs and provide identification cards for herding dogs. Implementing measures, such as public health education, regular treatment of herding dogs, ensuring proper vegetable hygiene, and keeping dogs away from densely populated areas are effective in reducing and preventing this disease.

Ethical Considerations

Compliance with ethical guidelines

The study was approved by the Ethics Committee for Research at the [Gonabad University of Medical Sciences](#) (Code: IR.GMU.REC.1396.166). Informed consent was obtained from the participants before the study.

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Authors' contributions

Study design: Mitra Salehi and Hossein Nezami; Sampling: Jafar Hajavi; Serologic assay: Jafar Hajavi and Hossein Nezami; Data collection and drafting the manuscript: Akbar Solati and Ahmad Reza Atarodi; Critical revision of the manuscript: Mitra Salehi; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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